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Member State questionnaire on new genomic techniques to contribute to a Commission study requested by the Council

Fields marked with * are mandatory.

Questionnaire on new genomic techniques to contribute to the study requested by the Council

endorsed in the Joint Working Group of GMO competent authorities on new genomic techniques on 15 January 2020

Introduction

With this questionnaire the Commission is collecting contributions from Member States competent authorities to respond to the Council's request[1] for "a study in light of the Court of Justice's judgment in Case C-528/16 regarding the status of novel genomic techniques under Union law" (i.e. Directive 2001/18 /EC, Regulation (EC) 1829/2003, Regulation (EC) 1830/2003 and Directive 2009/41/EC). The scope of the study goes beyond new mutagenesis techniques, as there are other new techniques, for which the Council seeks clarification. Therefore, the study covers all new genomic techniques, which have been developed after 2001.

For the purpose of the study, the following definition for new genomic techniques (NGTs) is used: techniques, which are capable to alter the genetic material of an organism and which have emerged or have been developed since 2001[2].

Unless specified otherwise, the term "NGT-products" used in the questionnaire covers plants, animals, micro-organims and derived food and feed products obtained by NGTs for agri-food, medicinal and industrial applications and for research. GMO competent authorities are invited to seek input from other competent authorities when appropriate.

The questionnaire is meant to provide information primarily, but not exclusively, at national level. Please substantiate your replies with explanations, data and source of information as well as with practical examples, whenever possible. If a reply to a specific question only applies to a specific NGT, please indicate this in the reply. With regard to agri-food applications, replies may include considerations on specific sectors, such as the organic sector.

Please indicate which information should be treated as confidential in order to protect the commercial

interests of a natural or legal person. Personal data, if any, will be protected pursuant to Regulation (EU) 2018/1725 [3].

[1] Council Decision (EU) 2019/1904, OJ L 293 14.11.2019, p. 103-104, https://eur-lex.europa.eu/eli/dec/2019/1904/oj
[2] Examples of techniques include: 1) Genome editing techniques such as CRISPR, TALEN, Zinc-finger nucleases, mega nucleases techniques, prime editing etc. These techniques can lead to mutagenesis and some of them also to cisgenesis, intragenesis or transgenesis. 2) Mutagenesis techniques such as oligonucleotide directed mutagenesis (ODM). 3) Epigenetic techniques such RdDM. Conversely, techniques already in use prior to 2001, such as Agrobacterium mediated techniques or gene gun, are not considered NGTs.
[3] Regulation (EU) 2018/1725 of the European Parliament and of the Council of 23 October 2018 on the protection of natural persons with regard to the processing of personal data by the Union institutions, bodies, offices and agencies and on the free movement of such data, and repealing Regulation (EC) No 45/2001 and Decision No 1247/2002/EC, OJ L 295, 21.11.2018, p. 39–98

Instructions

Please note that the survey accepts a maximum of 5000 characters (with spaces) per reply field. You might be able to type more than 5000 characters, but then the text will not be accepted when you submit the questionnaire. You will also receive a warning message in red colour below the affected field.

You have the option to upload supporting documentation in the end of each section. You can upload multiple files, up to the size of 1 MB. However, note that any uploaded document cannot substitute your replies, which must still be given in a complete manner within the reply fields allocated for each question.

You can share the link from the invitation email with another colleague if you want to split the filling-out process or contribute from different locations; however, remember that all contributions feed into the same single questionnaire.

You can save the draft questionnaire and edit it before the final submission.

You can find additional information and help here: https://ec.europa.eu/eusurvey/home/helpparticipants

Participants have until 30 April 2020 (closure of business) to submit the questionnaire via EUsurvey.

QUESTIONNAIRE

* Which Member State are you representing?

Sweden

A - Implementation and enforcement of the GMO legislation with regard to new genomic techniques

* **1. Have you been consulted by companies/organisations/research institutes for regulatory advice or another issue on products developed or to be developed by NGTs ?**

- (●) Yes
- ( ) No

* Please provide details on the request

At least the Swedish Board of Agriculture and Vinnova, Sweden's innovation agency, have been approached by researchers and companies due to the ECJ-ruling. They experience that the ruling has created a situation of uncertainty and has hindered marketing plans and research which was described in the document "Consequences of the EC-ruling according to Swedish companies and research groups, from 20-12-2018.

* **2. Have you taken specific measures (other than inspection) related to the application of the GMO legislation to NGT-products?**

- ( ) Yes
- (●) No

* Please explain why not

No new measures have been taken due to the ruling besides that the new interpretation of what constitutes a regulated GMO has been implemented in our work.

* 2 bis. Have you encountered any challenges or limitations, including administrative burden or costs?

- (●) Yes
- ( ) No

* Please describe

After the ruling, the Swedish Board of Agriculture has received one field trial notification for a plant mutated without the insertion of any foreign DNA. We found it difficult to do a risk assessment on these plants and to decide on appropriate safety measures since the plants contained mutations that might as well have occurred spontaneously. We have previously regarded organisms that have been mutated in a way that could also happen spontaneously, and where no foreign DNA is present in the end product, as GMOs that are not covered by the scope of Directive 2001/18/EC. If an organism with a spontaneous mutation is not considered a GMO and is therefore considered safe, we believe that an identical organism with the same mutation produced by a "new" technique of mutagenesis is equally safe.

* How could these challenges or limitations be overcome?

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*

3. Have you adapted your inspection practices to cover all NGT-products and to ensure the enforcement of traceability requirements?

- ○ Yes
- ◉ No

* Please explain why not

There are no commodities in Sweden produced by NGTs as far as we know. We are aware of that it might not stay that way in the future and that NGT-products from third countries, where these products are not regulated as GM-products, might e.g. become mixed into non-GM-products that are imported into the EU. However, the competent authorities do not find it efficient to implement a new control regime when we do not know what to look for or how to perform the controls or analyses with any kind of legal certainty due to limitations regarding what types of GMOs that can be detected. When it comes to small modifications, it is not possible to determine whether they are the result of spontaneous mutations or if they were induced by traditional or new (genome editing) techniques. The ENGL-report “Detection of food and feed plant products obtained by new mutagenesis techniques” gives an in depth description of the limitations regarding detection of plant products obtained by genome editing where no inserted recombinant DNA is present in the final plant.

* 3 bis. Have you encountered challenges or limitations, including administrative burden or costs?

- ◉ Yes
- ○ No

* Please describe

Since we have not adapted our inspection practices (as described above), this answer is theoretical. The challenge and limitation is that we are supposed to determine the possible presence and amount of GMOs in non-GMO products when we cannot, through analysis, differentiate some of the NGT-products from non-regulated GMOs.

* How could these challenges or limitations be overcome?

New technological methods and innovations should be used in order to ascertain the future availability of local and regional varieties that contribute to greatest possible utilization of production resources and the adaptation of agriculture to climate change.

Sweden welcomes a revision that achieves a more proportionate, predictable and transparent regulatory framework that is adapted to the fast technological development of the gene editing field. We welcome the study and hope it will provide guidance on how to deal with the problems that exist. The legal framework must focus on potential risks regardless of technology.

* **4. Do you have experience or information on traceability strategies, which could be used for tracing NGT-products?**

- ○ Yes
- ◉ No

* 4 bis. Have you encountered any challenges or limitations, including administrative burden or costs?

- ○ Yes
- ◉

No

* Please explain why not

Since we are not using traceability strategies, we have not encountered challenges or limitations with them.

* **5. What other experience can you share on the application of the GMO legislation, including experimental releases (such as field trials and clinical trials), concerning NGT-products in the:**

- [x] **Agri-food sector?**
- [ ] **Industrial sector?**
- [ ] **Medicinal sector?**

Agri-food sector

One application for introduction of a variety produced with an NGT in the national variety catalogue was withdrawn following the ECJ-ruling. One of the reasons was that the applicant did not want their product to be considered as genetically modified and the applicant will therefore not commercialise the product in the EU. The variety testing for that specific crop is not offered in Sweden and the applicant would have to perform the testing in another member state. Since the crop is considered a GMO, this would also require an application and authorisation for a GM field trial in that member state to perform the variety testing. That would have entailed an increase in the administrative burden and cost.

* **6. Have plant varieties obtained by NGTs been registered in national catalogues?**

- ( ) Yes
- (•) No

* **7. Do you require specific information in national catalogue when registering plant varieties obtained by NGTs?**

- (•) Yes
- ( ) No

* Please specify

The applicant is required to submit the CPVO technical questionnaire where the method used for the production of the plant variety should be described. Information on whether the variety is a GMO is also required in that questionnaire. Since all plants produced with NGTs should be considered as GMOs according to the ECJ-ruling, we do not foresee the need for separating GMOs produced with NGTs from traditional GMOs in the national variety catalogues under the current circumstances.

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

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B - Information on research and innovation

* **8. Have you supported with national funding programmes NGT-related research projects/programs (ongoing or finalised in the last 5 years), including on identification or traceability?**

- (●) Yes
- ( ) No

* Please provide an overview of the project/program including title of project, a brief summary with scope and objectives, the amount of national funding received and possibly specify if the receiving entity is public or private

Vinnova is Sweden's innovation agency and the national contact authority for the EU framework programme for research and innovation (https://www.vinnova.se/en/). The agency has funded approximately 6 million SEK to the following NGT related research projects since 2015:

- Prevention and treatment of cerebral palsy through genetic and epigenetic modulation
- RNA-centric view on oligodendrocyte lineage development
- Validation of a gene technology for the production of industrial enzymes
- CRISPR for product development using hybrid aspen and eucalyptus as models

Mistra, the Swedish Foundation for Strategic Environmental Research (https://www.mistra.org/en), has funded the research program "Biotechnology for Sustainable and Competitive Agriculture and Food Systems": https://www.slu.se/en/Collaborative-Centres-and-Projects/mistra-biotech/. The program is directed towards studies of traditional and modern methods for plant breeding and animal husbandry for the domestication of species and for the selection of desired traits while attempting to eliminate undesired traits. The program included standard procedures for gene transfer in the initial studies but it changed to include a more comprehensive use of gene editing (CRISPR/Cas9). The research funding was received by the Swedish University of Agricultural Sciences (SLU) which is a public university. Mistra has provided 94 million SEK in funding.
The Swedish Research Council (https://www.vr.se/english.html) has compiled a list of projects related to NGTs for the last 6 years. You will find the list in a separate document.

According to the Swedish Foundation for Strategic Research (https://strategiska.se/en/), genetically modified organisms are used in a number of projects that they finance. However, they do not request the level of details in the applications to determine in which projects and to what extent the projects include techniques invented after 2001.

SweCRIS is a searchable national database where you can see how participating research funders have distributed their money to Swedish recipients: https://www.swecris.se/betasearch/?q=*&view=cards&lang=en&sort=start_desc. The research funding bodies who supply data to SweCRIS are both public and private. Thirteen research funders have provided data for the database. When searching that database you will find that more projects related to NGTs have been financed in Sweden than what we have mentioned and referred to here.

* 8 bis. Please highlight the potential challenges encountered when supporting/funding NGT-related research and any consequences from these challenges.

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* **9. How do you see NGT-related research evolving?**

In 2019, there were more than twice as many research projects funded by the Swedish Research Council that include new genomic technologies compared to 2014. Since the CRISPR/Cas9 gene editing technology

was developed, the number of scientific articles in PubMed increased from 129 in 2012 to 4601 in 2019. During the first two months of 2020, 887 scientific articles related to CRISPR/Cas9 have been published in PubMed.

When it comes to genetically modified plants, public research and research in plant breeding companies may move to countries outside of the EU to avoid fulfilling requirements in Directive 2001/18/EC.

In the short-term, for the next 5 or more years, a divergence of research activities is envisaged in countries prepared to adopt NGTs as additional tools, to be used in the further development of society, and those that classify NGTs as intrinsically uncertain and prone to unacceptable risks. Those countries, which allow the full use of the entire spectrum of NGTs, will probably develop their industries and reach a considerably increased competitiveness compared with others. While assumptions on risks, risk assessment and principles of uncertainty serve society well, there has to be a point at which society needs to decide to accept a specific technology as essentially beneficial. The existing rules and regulations refer to the situation that existed when the basic techniques emerged in the mid-1980s through the 1990s.

* **10. Have you identified any NGT-related research needs from private or public entities?**

- (●) Yes
- ( ) No

* Please specify which needs and how they could be addressed

There are a number of areas where NGTs could contribute to a better society and where both public and private entities would benefit from research. Firstly, in the area of public health, NGTs could be used in the development of new drugs for a wide range of diseases including those caused by resistant bacteria, and emerging viral agents. In terms of food and nutrition, the use of NGTs could lead to better crop yields, reduced pesticide use and improved or additional nutritional content in agricultural produce.

There is a major need for information that explains the benefits and risks of using NGTs to the general public. The information provided should be factual, based on the latest research results and be provided in a neutral manner. Similarly, information describing the above, but in greater depth should be made available for people involved in making policy decisions in the public sector (politicians and civil servants) and the private sector (company leadership).

* **11. Could NGT-related research bring opportunities/benefits to science, to society and to the agri-food, medicinal or industrial sector?**

- (●) Yes
- ( ) No

* Please provide concrete examples/data

Since the development of genome editing techniques, especially CRISPR/Cas9 in 2012, site-directed mutagenesis has been used in basic research in e.g. plant and animal sciences, in medicine, evolutionary studies and microbiology.

New vaccines as well as other treatments could be developed faster or more efficiently. Genetically modified microorganisms could be used for the food industry and for environmental purposes, for example in water treatment and remediation of soil.

In clinical trials genome editing has been used for treatment of different types of cancer, sickle cell-anemia, beta-thalassemi, HIV and retinitis pigmentosa.

Genome editing has also been used to breed e.g. hornless cattle, pigs resistant towards porcine reproductive and respiratory syndrome and African swine fever and pigs that are more suitable as organ donors.

In the agri-food sector, NGTs could be used to develop crops with resistance to common diseases such as blight and mildew. These diseases cause a major loss of income for farmers and can result in reduced harvest leading to food shortages. Agriculture today uses chemical pesticides to combat pathogens often through widespread spraying as a preventative measure. The result is that unnecessarily large areas are sprayed, resulting in negative effects on the environment and, in many cases, residual amounts of pesticides in the food we eat.
The use of NGTs could thus lead to an increased yield, lower financial risk for the growers, decreased use of pesticides and decreased levels of pesticide residues in our food. NGTs could also be used to introduce traits into plants for nutritional purposes to achieve a balance in the diet that we consume today. As an example, a project with CRISPR/Cas9-modified potato, which has a low glycemic index and works as a prebiotic, can be mentioned (see the document “Consequences of the EC-ruling according to Swedish companies and research groups” from December 20, 2018 compiled by the Swedish Board of Agriculture) .

Site-directed mutagenesis has been used in a number of plant species, for example button mushroom, cassava, rapeseed, maize, orange, sugarbeet, sugarcane, papaya, soybean, tomato, groundcherry, barley, kiwi, wheat, grape, grapefruit, lettuce, alfalfa, potato, melon, watermelon, strawberry, grapefruit, sorghum, coffee, cocoa, apple, banana, cooking banana, rice, hemp, Camelina, poppy and durum.

Among the traits that have been introduced are, except for higher yield and resistance towards biotic stress as mentioned above, also abiotic stress, improved fruit quality, non-browning properties and extended shelf life. One advantage of site-directed mutagenesis compared to older breeding techniques is that a single trait can be changed in an otherwise robust cultivar. Genome editing has also been used for rapid de novo domestication of e.g. wild tomato and groundcherry. Domestication and breeding over millennia for yield and productivity has led to reduced genetic diversity. As a result, beneficial traits of wild species, like aroma in tomato, have been lost. By editing specific genes in a wild species, it is possible to combine agronomically desirable traits with useful traits in wild species. Landraces of rice have also been edited to better resist lodging.

The work being done for domestication of Lepidium campestre, which includes the use of CRISPR/Cas9, would bring an alternative crop for oil seed production and the possibility to cultivate at higher latitudes. This species is biennial and would, if used as a catch crop, also decrease the need for land.

Potato that produces naturally stable starch for food applications is also being developed using CRISPR /Cas9, thus avoiding the otherwise usually necessary chemical modification to make it stable. We are now dependent on import of specific types of starch and if these could instead be produced in Sweden, this could contribute to an increased competitiveness and employment in Swedish agriculture and industry and have a positive effect from a climate- and environmental point of view.

Considering all the possible benefits mentioned here, the use of NGTs may effectively help meet the UN Agenda 2030 goals for good health and no hunger/poverty.

* **12. Could NGT-related research bring challenges/concerns to science, to society and to the agri-food, medicinal or industrial sector?**

- (●) Yes
- ( ) No

* Please provide concrete examples/data

Effects on the ecosystem and various ethical aspects need to be discussed in an open and transparent way. At the same time, it is important that such discussions are based on scientific facts so that sound and well balanced decisions can be taken.

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

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C - Information on public dialogues and national surveys

* **13. Have you or other institutions/bodies/entities organised national dialogues concerning NGTs?**

- (●) Yes
- ( ) No

* Please describe briefly the content, methodology and conclusions

Such initiatives have not been taken by any competent authority. However, Mistra Biotech (a research program focusing on the use of biotechnology in crop and livestock breeding for sustainable and competitive agriculture) has published a number of articles and held seminars on the subject. More information is found here: https://www.slu.se/en/Collaborative-Centres-and-Projects/mistra-biotech/news-mistra-biotech/?page=0. An example of a seminar is ”Will new plant breeding techniques have a future in the EU?” that the Royal Swedish Academy of Agriculture and Forestry arranged and that was live streamed. During the seminar, the consequences of the ECJ-ruling for research, plant breeding and agriculture in the EU were discussed: https://www.ksla.se/aktivitet/will-new-plant-breeding-techniques-have-a-future-in-the-eu/.

* **14. Have you or other institutions/bodies/entities organised national surveys, which assessed public opinion on NGTs?**

- ( ) Yes
- (●) No

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

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D Information on ethical aspects

*

15. Have any national bodies or expert groups discussed or issued opinion on the ethical aspects of NGTs?

- ○ Yes
- ◉ No

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

E - Information on opportunities and benefits from the use of NGTs and NGT-products

* **16. Could the use of NGTs and NGT-products bring opportunities/benefits to the agri-food, medicinal or industrial sector?**

- ◉ Yes
- ○ No

* Please provide concrete examples/data

We would like to refer to the document “Consequences of the EC-ruling according to Swedish companies and research groups” from December 20, 2018 compiled by the Swedish Board of Agriculture. The document was primarily based on information given by actors in the plant agricultural sector. When considering the research projects financed (see question 8) within the other sectors, there are several projects that would benefit society if proven successful.

* **17. Could the use of NGTs and NGT-products bring opportunities/benefits to society in general, such as for the environment, human, animal and plant health, consumers, animal welfare, as well as social and economic benefits, in the short, medium and long term?**

- ◉ Yes
- ○ No

* Please provide concrete examples/data

NGTs are in many cases faster, cheaper and more precise tools for the development of new products. NGT: s could bring many opportunities for, in particular, small markets and the production of sustainable food both in the short and long term perspective. There is a need for new products to meet the threat of for example climate change induced increases in diseases for plants. For more examples see answer under question 11.

* Under which conditions do you consider this would be the case?

See our answer to the previous question.

* **18. Do you see particular opportunities for SMEs on the market access to NGTs?**

- ○ Yes
- ◉ No

* Please explain why not

No, not in the crop breeding sector, which is in focus in the current ECJ ruling. But similar problems could also be manifested in other sectors. Several of the “new” techniques of mutagenesis were a means for SMEs to develop new and improved crops for release on the market. The “new” targeted methods of mutagenesis are more efficient than older random methods. An added benefit was, prior to the ECJ-ruling, that the time, costs and uncertainty connected to the application and approval process for market release of a regulated GMO could be avoided. It has been estimated that the average cost for market release of a GMO in the EU for food and feed use is in the range of 6-15 million Euros. Additionally, it takes 4-8 years for a decision on an application for market release and only one GMO has been approved for cultivation since the Directive came into force. SMEs will therefore not be able to go through with this process. That in turn entails that SMEs will not obtain any benefits from the development of these modern techniques unless there are opportunities on other markets than in the EU. Additionally, avoiding having to label something a GMO is most likely an advantage considering that some people are against genetically modified products.

* **19. Do you see benefits/opportunities in patenting or accessing patented NGTs or NGT-products?**

- (●) Yes
- ( ) No

* Please describe and provide concrete examples/data

we refrain from commenting on patents

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

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F - Information on potential challenges and concerns of NGT products

* **20. Could the use of NGTs and NGT-products raise challenges/concerns for the agri-food, medicinal or industrial sector?**

- (●) Yes
- ( ) No

* Please provide concrete examples/data

The lack of specific methods for detection results in legal uncertainty when performing controls to analyse for the possible presence of GMOs and when determining if products should be labelled or not according to the labelling requirement for GMOs.

* **21. Could the use of NGTs and NGT-products raise challenges/concerns society in general, such as for the environment, human, animal and plant health, consumers, animal welfare, as well as social and economic challenges, in the short, medium and long term?**

- (●) Yes

No

* Please provide concrete examples/data

All types of genetic modifications could potentially entail risks. The risk assessment should be viewed on a case-by-case basis. But when it comes to targeted mutagenesis, this should, if anything, be considered safer than random mutagenesis since fewer off-target effects are expected. It is therefore more likely that the producer knows which molecular changes that have been made and what to expect regarding possible effects on the organism, the environment and human health. However, when determining if there are any specific risks, we have to look at the molecular changes that have been made in an organism. Molecular changes produced with targeted mutagenesis do not carry any other risks than identical molecular changes produced by random techniques of mutagenesis, although only organisms produced with the latter are considered safe according to Directive 2001/18/EC.

* Under which conditions do you consider this would be the case?

-

*** 22. Do you see particular challenges for SMEs on market access to NGTs?**

Yes
No

* Please explain under which conditions

For a more thorough explanation of this, see our answer to question 18.

*** 23. Do you see challenges/concerns in patenting or accessing patented NGTs or NGT-products?**

Yes
No

* Please describe and provide concrete examples/data

we refrain from commenting on patents

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

The maximum file size is 1 MB

G - Final question

*** 24. Do you have other comments you would like to make?**

Yes
No

Please provide your comments here

The requirement to provide a specific identification method for a GMO when applying for market release makes it impossible for the applicant to comply with as it is never possible to provide the method. This is the case with organisms created with an NGT that has caused mutations that cannot be distinguished from spontaneous mutations or from mutations produced with “old” techniques of mutagenesis.

The Swedish Board of Agriculture (SBA) thinks that the principle of proportionality is disregarded in cases where two organisms containing the same mutations (and therefore should be considered to carry the same risks) are regulated differently because they were produced with different techniques. In such a case, the status as a regulated GMO would mean disproportionate costs for the applicant. Even if we did not have the current problem regarding the applicant not always being able to provide the mandatory specific identification method when applying for market release, the legislation would still be misleading to the consumers, according to the SBA. This would be the case when one product produced with a “new” technique of mutagenesis has to be labelled as a GMO while another identical product produced with an “old” technique would not.

Should the Commission conclude that the GMO-legislation need to be revised, Sweden welcomes a revision that achieves a more proportionate, predictable and transparent regulatory framework that is adapted to the fast technological development of the gene editing field.

The SBA has recently done an assessment of the legal status of diploid cybrid plants and determined that they were not GMOs. The diploid cybrids were produced in several different steps, with different techniques and over several generations. The SBA came to the conclusion was that since none of the genetically modified DNA inserted in a previous generation was present in the end product, it cannot be considered genetically modified.
If previous generations should be taken into consideration when determining the legal status of a modified organism, then those generations should also be taken into consideration in the risk assessment. In that case, the risk assessment must consider a different organism, that potentially carries completely different risks, than the organism that would be released into the environment.

A problem that we can foresee in the future is having to determine whether different techniques of mutagenesis have appeared or been mostly developed before or after 2001.

Finally we would like to add that the funding agency Vinnova states that the issue of policy and legislation being challenging to new technologies is not new to them and that it affects many sectors. However, when it comes to NGTs, they find that the issue seems extra polarized.

Please upload any supporting documentation for this section here. For each document, please indicate which question it is complementing

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Contact

SANTE-NGT-STUDY@ec.europa.eu

Dnr 4.6.17-16859/2018

Swedish Board of Agriculture

20-12-2018

Consequences of the EC-ruling according to Swedish companies and research groups

In order to collect information on the consequences of the EU Court of Justice (ECJ) ruling, the Swedish Board of Agriculture (SBA) have contacted a number of organisations, companies and research institutes with an interest in plant breeding, plant research and agriculture. We requested information regarding if the ruling will affect them and in that case how and to what extent. The organisations, companies and institutes that answered are listed in Table 1 together with a short introduction about them. The information that we received is summarised in this document.

Additionally, we have added information from a cost analysis performed by the Swedish National Reference Laboratory (NRL) for GMOs, which is a part of the National Food Agency. The analysis contains a cost estimate for necessary investments and analyses.

Some key points:

- The use of targeted mutagenesis within the EU is likely to be very limited when regulated as GMO. This, since the EU GMO legislation entails long and expensive authorisation procedures.
- It is less likely to receive funding for research within the EU when the chances for a practical use of the results are reduced because the resulting product is considered GMOs.
- Research project using targeted mutagenesis to achieve new or improved crops or food products have already been changed or paused. Collaboration with research institutes and businesses abroad will decrease.
- Products with an improved environmental profile will not reach the market. It will be more difficult to achieve a sustainable and resilient agriculture and forestry in a changing climate without this important tool for plant breeding.
- Made investments in patent, staff, research, product development and knowledge will be lost. Production and marketing of some products will most likely be redirected to countries outside the EU.
- Countries that do not regulate organisms produced with targeted mutagenesis as GMO will have advantages in both commercial and research opportunities compared to the EU.

Consequences of the EU Court of Justice ruling on new techniques of mutagenesis (case C-528/16)

In what context are the new breeding techniques important?

Among the answers, it was pointed out that the access to different methods is necessary for successful breeding. The methods are combined in different ways depending on for example the genetic regulation of the desired traits. A spectrum of methods are used, including traditional selection of (phenotypic) traits, selection based on germplasm (e.g. genomic selection), transferring of genes (genetic modification) and new techniques of targeted mutagenesis. It was pointed out that all of these tools are necessary in a plant breeder's tool box to, in the best way possible, solve the difficult problems that we are facing. Genome editing is considered to have great potential to increase breeding efficiency when it comes to improvement of plant traits and increasing the genetic diversity.

Targeted mutagenesis does not replace conventional cross breeding but it is rather a complementing tool for efficient and successful plant breeding. The greatest benefit of this technique is obtained when handling one or a few traits in already high-performing varieties. The technique enables fine adjustment of gene expression to optimise the phenotypic effect in the crop (Mumm, 2013). With genome editing such as CRISPR/Cas9, it is possible to mutate only a few determined base pairs in the genome. This can be done without integrating any external DNA in the genome. In this way, genetic variation in specific characters can be introduced into elite varieties without simultaneously transferring genetically linked DNA or other unwanted DNA. This means that a number of generations of backcrossing to a parental line can be skipped in contrast to what is the case with conventional cross breeding or breeding through random mutagenesis.

According to the European Seed Association (ESA), two generations of back crossings are often enough when targeted mutagenesis is used, while seven are needed for conventional breeding (Information material from P. Jorasch). However, this presumes that a suitable elite material is already available, that you have enough knowledge about gene sequences, their biological functions, and that you have identified suitable targets for mutagenesis.

A research team from Brazil, Germany and USA has recently demonstrated domestication of a new tomato relative over the course of only one generation through CRISPR-editing of six different loci that control for example fruit size, fruit number and content of nutrients (Zsögön *et al*., 2018). However, in practice, a number of generations (up to five) of greenhouse testing of the material, is needed to control the quality of the introduced trait. This is also needed to ensure that the crop has an added value for growers and the industry.

In summary, targeted mutagenesis as a method has great potential to shorten the time to produce new varieties with specific traits when using elite material as starting material. However, this remains to be demonstrated in practice.

Ongoing projects involving “new” techniques of mutagenesis

Specific ongoing projects are described below and have been divided by crop. In addition, there are other ongoing projects within the area of new breeding techniques in both the industry and academia. There are also ongoing collaborations with international companies who use methods of genome editing such as oligonucleotide directed mutagenesis (ODM) and CRISPR/Cas9.

Potato

There is an ongoing project regarding the production of new potato varieties for the Swedish starch industry. This involves mainly the use of CRISPR/Cas9 without introducing any new DNA into the genome. In this context, amylose potato with modified starch quality is mentioned. The potato works as a prebiotic and has a low glycemic index. Additionally, naturally stable potato starch for food applications is being developed, thus avoiding the otherwise usually necessary chemical modification to make it stable.

Starch producers in Sweden and northern Europe uses potato as raw material. Unlike maize, potato does not have the natural variation in starch quality necessary to obtain starch with special characteristics, e.g. high amylose content. We are therefore dependent on import of these types of starch. Such production in northern Europe would be important for both food production and the production of bioplastic from high amylose potato, which can replace a part of the fossil oil that is used for the production of plastic today. This would contribute to an increased competitiveness and employment in Swedish agriculture and industry production and have a positive effect from a climate and environmental point of view.

Breeding for resistance to potato late blight is also carried out by using CRISPR/Cas 9 to knock out genes whose expression contribute to facilitate infection. Positive preliminary results suggest that this could be a good way of obtaining good food potato with lasting resistance. This has, despite great efforts, not yet been achieved through traditional breeding, due to difficulties of combining many traits. With genome editing, all other agronomic and quality traits can be retained which is important in potato breeding since backcrossing is not possible. Resistance to pathogens is important to decrease the use of pesticides in potato cultivation, which makes up a large part of the general pesticide use in agriculture.

Another project aims at decreasing glycoalkaloids in potato which leads to a decreased content of unhealthy substances and thereby increased food safety.

Modified potato protein is also mentioned in the context of ongoing projects. This leads to an increased extractability of food graded highly nutritional protein.

Studies on autophagy; basic research on recycling of cellular components, is also carried out and could potentially lead to increased yield and resistance traits.

Lepidium Campestre

One of the ongoing projects regard domestication and breeding of *Lepidium Campestre* for improved cultivation properties, oil content and oil composition. For this, a combination of crosses and traditional selection, genomic selection and genome editing with CRISPR/Cas9 have been used.

Due to limitations in the natural variation of some critical traits, genome editing has proven to be indispensable, especially to improve the fatty acid composition and to reduce the levels of substances that make the pressed seed cakes (a bi-product from the oil production) unsuitable for feed.

L. Campestre grows in the wild in e.g. Sweden and has the potential to be grown as an oil crop farther north than what is possible with existing oil crops. Growing this species as a new oil crop could contribute to the survival of the agribusiness in the whole country, especially in the northern parts.

Barley

In barley, breeding is carried out for resistance to net blotch, a common and important disease that is usually treated with chemicals. Resistant varieties are not available. The project involves trying to mutate the genes that increase the susceptibility to the fungus by the use of CRISPR/Cas9.

Another ongoing project regards resistance to virus spreading bird cherry-oat aphids. The aphid is an increasing, climate related problem and is treated with pesticides. Resistant varieties are not available.

Rapeseed

Development of non-glaucous rapeseed lines is carried out through genome editing to improve pathogen resistance and to reduce spraying with chemicals.

Projects starting from 2019 involving “new” techniques of mutagenesis

Potato

One project aims at an efficient use of an industrial side stream for circular bio-based economy for food graded protein of premium quality. The breeding goal of another project regards starch content, granular size and structure by elucidating mechanisms of initiation and differentiation of starch synthesis.

Rapeseed

The mentioned projects regard:

-Elimination of anti-nutrient factors in the pressed seed cakes for both feed and food use.

-Characterisation of the rapeseed enzyme DGAT, a key enzyme for quality and quantity of the seed oil.

Cost Action PlantEd

The newly EU financed project PlantEd (Genome editing in plants- a technology with transformative potential) aims at networking and research coordination between 70 participants from 24 countries.

Consequences of the ECJ ruling on ongoing and future projects

The preconditions of the PlantEd project are changed to the worse if Europe loses expertise in plant editing. There will be decreasing opportunities for Swedish research to benefit from this and other networks if research and development in the area are moved to countries outside the EU.

One university department states that there has been no immediate change of ongoing projects. It is however likely that the ruling will entail a loss of interest from the industry regarding involvement and financing. This probably also applies to other financiers, similar to what is the case with projects involving traditional genetic modification. Financiers increasingly require a direct and apparent social relevance and practical application of proposed projects. The use of GMO techniques could be considered to be of low social relevance, even if they are considered to be the most effective and relevant tools.

For another research group, with a focus on plant breeding for the food sector, the ruling has had more direct consequences. In some project proposals, the original idea of using genome editing has been changed to using less optimal methods and some projects have not started at all.

If the ruling is to be followed with no regard to the development of technologies since 2001, one company will be forced to terminate ongoing projects. This will lead to the loss of a large portion of the knowledge, products and technology that have been developed.

For research groups primarily working with basic research, the ruling will not change the direction of their project much. Since the funding is not aimed at developing new crops, the researchers can basically work as before. However, in a few cases, there were plans for field trials with genome edited plants. If such trials are now considered as GMO trials it is doubtful that they will be executed, since the administration is both time consuming and expensive.

If new breeding techniques are considered to give rise to GMOs, it will, in the best-case scenario, mean both increased costs and project delays. In the worst-case scenario, many projects will no longer be economically feasible.

Consequences for analysis according to the Swedish NRL

The National Food Agency (NFA) in Sweden states that the strategies and costs for analysis are determined completely by whether information on the actual genetic change is available or not. In cases where an application for approval of a genome edited crop exists, the technique for analysis (quantitative real time PCR), which is already in place at the NFA, could be used in most cases. If there are only a few mutations, it could be difficult to develop a PCR method that is specific enough. However, that burden lies upon the applicant who has to provide a functioning detection method, in accordance with the application procedure. When the method is collaboratively tested, and is performing according to the demands, the method ought to be able to become implemented in the same way as the existing methods for detection of transgenic GMOs. The cost to detect a specific transformation event is, according to the NFA, approximately 100 EUR/sample.

In cases where there is DNA sequence information available for an unauthorised genome edited crop, but where an application for approval is missing, it would be possible to develop PCR based methods for certain genetic changes. If the changes regard single nucleotides, DNA sequencing methods are likely necessary. Traditional sequencing technique (Sanger) would not work for samples where there is DNA from both genetically modified and conventional crops. Next generation sequencing (NGS) is a technique which is being increasingly used and the technique could be used to detect and identify known genome edited crops, even in samples with different genotypes. The drawbacks of the technique are high costs for analysis per sample, long response times and the need of access to high quality reference genomes. Interpretation and analysis of NGS-data also requires competence in bioinformatics and systems to handle the large amount of data that the technique entails, which today is lacking at the NFA. The simpler NGS instruments today cost about 100,000 EUR and a rough estimate of the cost is more than 1,950 EUR/sample. In addition, the recruitment of bioinformaticians will be necessary which means approximately 73,000 EUR per year in salary. If the modification only comprises one or a few nucleotides, it will also not be possible to determine whether the modification is a result of a spontaneous mutation or if the modification has been induced by traditional or new (genome editing) technique.

If no information on the mutation is available when it comes to an unauthorised genome edited crop, the prospects for detecting the mutation is almost non-existent. Additionally, no realistic possibilities for analysis are available today. The development of PCR methods is not possible if no DNA sequence information is available. The use of whole genome or exome sequencing to identify unknown mutations in the genome of higher plants would require constantly updated pan-genomic reference databases, which is considered unrealistic. The cost of analysis per sample would be immensely high and in the case of a potential find of a mutation, it would still not be possible to determine whether the mutation arose spontaneously or was induced.

Analysis and control

At the moment, no effects are seen when it comes to trading of tree based products in relation to analysis and control, but this could change.

Future problems are predicted with international exchange of plant material, when the outside world does not intend to regulate mutagenesis where no external DNA is present in the end product. If there is no reason to label research material as regulated material in the country of origin, we have no possibility to control if mutagenesis with methods that are regulated in the EU has taken place at some point. This creates a legal and credibility problem given that the quality of products is currently guaranteed by analysis.

When research and plant breeding are moved outside of the EU, there is a risk that the crops that were originally developed in EU countries will be imported back into the EU. This could partly be the case for seeds for cultivation within the EU but also for consumer ready products. Since there are no detectable differences between crops that have been developed with modern and traditional plant breeding techniques, it is questioned how the control of such import would be possible. There is a risk that food and ingredients produced with the new techniques of mutagenesis, will still be on the EU market, in spite of the efforts of the EU to limit the use of these techniques.

If a genome edited crop would after all be produced, the current EU legislation for traceability and labelling of GM crops would obstruct commercialisation since it will be very difficult to produce a method to identify and distinguish mutations when only one or a few nucleotides have been changed. In practice, the ruling therefore means a ban on genome edited crops.

Environmental consequences

One of the university departments cannot see that the use of genome editing, where no foreign or recombinant DNA is integrated in the plant, would cause environmental effects that differ from mutagenesis obtained with “older” techniques. On the contrary, these techniques are a more efficient way of obtaining desired effects with a higher degree of precision.

It will become more difficult to produce new plant varieties with higher quality, improved resistance, improved uptake of nitrogen and phosphorous and the production will be delayed if the new breeding techniques cannot be used. The use of chemicals in agriculture will continue for a longer time and might even increase (with increasing leakage to water) as compared to if we would have been able to use targeted techniques to improve the resistance of plants to diseases etc. The agriculture in Sweden as well as in the rest of the EU will thereby become less sustainable, from both an ecological and economical point of view.

There is also a risk that we will be stuck with crops developed without the goal of favouring ecosystem services and the environment, goals which we have in

Swedish plant breeding and in EU countries with higher demands on environmental sustainability.

The loss of investments in plant breeding due to the ECJ ruling could result in a less climate-adjusted agriculture that cannot persevere in a changing and erratic climate. To be able to meet the challenges that we are facing in the environmental area and with a changing climate, we need varieties with increased resistance to different pests, an improved use of plant nutrients, efficient use of water, tolerance to drought and flooding and adjustments to changes in cultivation systems. In a warmer climate, the risks of pests and fungal infections are increased. Additionally, we need a greater access to perennial crops which can be adapted to our cultivation technique and give a high yield. This could also increase the content of humus and bind more carbon to meet the climate goals and decrease the leakage of plant nutrients.

One of the ways to increase the biological diversity is to use a more diverse set of crops. The loss of investment in plant breeding might risk obstructing this development. By decreasing the efficiency in agriculture, more land will be needed to cultivate the food and feed that we need. A decreased efficiency in forestry will decrease the possible biomass that is available to develop the bio-based economy. Both of these effects will likely have negative consequences for the climate and the environment.

When it comes to potato, the use of chemicals for cultivation and production can be decreased substantially with the development in modern plant breeding. The crops that have been developed with new methods of mutagenesis have a significantly lower impact on the climate and environment than the current potato varieties. A decreased use of chemicals will not be accomplished without the use of new techniques to improve the potato crop. Today, we use a couple of thousand tons of fossil-based chemicals for the modification of our food starch. This use would become unnecessary with the new potato varieties. When it comes to cultivation, there are ongoing projects to decrease the amount of plant protection products by half. In development projects that use targeted mutagenesis, an effect has been achieved that is 100 times larger than the tons of chemicals that have been saved. A large part of the work on sustainability of the company has been done on development of new potato crops with the help of new techniques of mutagenesis.

Difficulties of reaching specific, political sustainability goals without using the new techniques of mutagenesis have also been addressed. In relation to this, the FN’s Agenda 2030 and the 1.5℃ goal in the Paris Agreement, the EU sustainable development strategy and the Swedish government’s food strategy have been mentioned. The latter clearly points out that plant breeding is a strategic investment that is needed to create long-term competitiveness in Swedish agriculture.

Economic consequences

Economic effects on businesses

One company states that more than 10 million EUR invested in research and development of crops and techniques during the last years will now be lost due to the ruling. In addition, many years of competence building will be lost due to the ruling. Market release of GM starch in not an option due to the high costs for trials, production, control and monitoring. More important though is the lack of tolerance for GM labelled products on the EU market.

Manufacturing of naturally storage stable starch is a breakthrough that dramatically changes the use of potato starch compared to existing raw materials. The effect of the ruling is that it has marginalized potato as raw material for industrially manufactured foods. That will result in an enormous financial impact on their business. They now need to consider moving the manufacturing of the product outside the EU, either physically or through license agreements.

Another company states that in both short and long term, there will be less opportunities to sell projects or to develop products that could be marketed.

The feed industry in Sweden and in the EU is a large importer of vegetable produce. The asynchrony between approval of GM commodity in the EU and exporting countries is causing significant problems for the feed industry today. The problems will increase when more countries decide not to regulate genome-edited crops as GMOs.

Economic effects for research institutes

Turning academic progress into product requires companies in the sector, which have both the possibility and willingness to invest. These will now gradually disappear. This will affect the possibilities to obtain research funding for more research in agriculture and forestry, both nationally and from the EU. According to previous experience, project applications involving GMOs have not been prioritised because they would not lead to any practical applications in Europe.

One of the universities state that a high percentage of the staff is fully or partially financed through projects that include genome editing. They might not be able to keep all staff and will lose competence. The budget of the department for projects involving genome editing is approximately 850,000 EUR for 2019. The same university has so far purchased equipment for approximately 180,000 EUR due to genome editing projects specifically. Even though some of the equipment could be used for other purposes, the main application relates to work with genome editing.

A DNA-free genome editing project in potato is carried out by what is equivalent to 3.5 full time positions a year. The project is financed until 2021 but unless the legal situation is changed, it is not likely that this prominent research will continue in Sweden for long.

Economic effects due to GM notification requirement

The cost of obtaining an approval for market release of a GM crop or product in the EU is in the range of 6-15 million EUR to meet all regulatory requirements. When genome editing falls within the scope of Directive 2001/18/EG, this means that the costs of developing plants with new properties where such techniques are used in practice become so high that companies within the EU abstain from it. To this can be added the uncertainty in the decision-making process regarding whether the EU will allow cultivation of varieties that have genome edited traits.

The direct economic cost of the regulatory process for GMOs in the EU is very extensive and affects the use of the new methods of mutagenesis, since only the largest and most resourceful companies can afford to complete the process. According to information from Monsanto in 2011, it costs on average about 100 million USD to bring a genetically modified crop to the market, using maize as an example (Mumm, 2013). Of that, the cost of complying with regulatory requirements and reaching market approval for a GM crop has been estimated on average 6,788,000 EUR (3,820,000 – 10,388,000 EUR). Much of the costs result from the requirements of field trials to evaluate environmental effects and phenotypic comparisons with corresponding conventional lines (Food Chain Evaluation Consortium, 2010). Estimations from EuropaBio result in similar figures with costs for GMO approval in the EU of around 7-10 million EUR per event (EuropaBio, 2011). A survey from 2007 of four major international seed companies showed that regulatory compliance in ten different jurisdictions varied between 6 million USD to over 15 million USD for insect resistant or herbicide tolerant GM maize (Kalaitzandonakes *et al*., 2007). Another survey from 2011 of six major international seed companies showed that the regulatory costs specific for the US market are on average over 35 million USD, or about a quarter of the total R & D costs (McDougall, 2011). A review of approximately 50 different studies from all over the world, about regulatory compliance costs for one event on one market, showed that this varies widely across countries. On average, the cost is 7.8 million USD, with the lowest cost being 53,000 USD and the highest 14.8 million USD (Phillips, 2013). With the ECJ ruling, the costs for a genome edited crop would be of the same magnitude.

Marketing of genome edited products is only economically feasible for the big multinational companies. However, in 2012 and 2013, BASF and Monsanto announced that they will discontinue their R & D activities on plant biotechnology in Europe as a result of the restrictive application of GMO legislation (AgbioInvestor, 2018).

Application is also restrictive to the extent that only one GM crop is currently authorized for cultivation in the EU and a relatively small number of GM products (about 65-70) are approved for import. In the EU, it usually takes between 4 and 8 years to get market approval for import of a GM product and often much longer to obtain authorization for commercial cultivation of GMOs.

If crops produced with targeted mutagenesis are regulated as GMOs, there are only small chances to obtain marketing approval for the cultivation of these

crops or for use of the products. It also means that the time required to obtain marketing approval for these will be equivalent to what has been the case for conventional GMOs.

Socioeconomic effects

The ruling will have negative effects on the national economy when it comes to both plant and animal production. It is counter-productive to make it more difficult to use a technique with high precision and with several benefits compared to “older” techniques. Genome editing is a brilliant example of technical development being the most important factor to be able to deal with challenges regarding food supply, resource management, climate adaptation and the environment.

A socioeconomic analysis of the costs of refraining from genetically engineered crops in Swedish agriculture was published in 2011. The analysis indicated that the lack of acceptance of genetically modified herbicide tolerant sugar beet, herbicide tolerant rapeseed and potato blight resistant potatoes entails missing out on a potential socio-economic gain of approximately 27 million EUR per year and a reduced use of cultivable land of 10,000 hectares. For the whole EU, this would amount to about 2 billion EUR and saving an area of about 645,000 hectares per year (Fagerström & Wibe, 2011). It is likely that the potential gains for genome edited crops would be equivalent to this.

The EU Commission's Research Service, the Joint Research Centre (JRC), has estimated that each year a GM crop or GM product is delayed can cost anywhere from 700,000 to 70 million EUR in lost income (Food Chain Evaluation Consortium, 2010).

Plant breeding is of central importance for development of a sustainable and viable farming. It creates values in the chain from farm to fork for farmers, the food industry and consumers and for growth and competiveness in Sweden. Additionally, we will have to increase the production to meet a growing demand. We also have to increase the domestic production of protein feed and protein crops and varieties that are adjusted to the conditions of different regions. If research and plant breeding is limited, the range of protein crops is also decreased.

Plant breeding creates possibilities for innovation and job opportunities, which in turn results in possibilities for export of safe products with an added value. The ruling limits the possibilities of choosing the new techniques for plant breeding and this has already created doubts about new projects. Is it worth spending time, effort and money on something that might not reach the market? It is stated that we are losing momentum on a matter that should be pursued forcefully to tackle the challenges we have in front of us.

The decision regarding the regulation of targeted mutagenesis is likely to affect the establishment and maintaining of international companies on the European market. There is a risk of losing collaborators, companies and researchers from academic institutions as they now choose to cooperate with institutions outside

the EU. In the long term, this may lead to losing promising young researchers who choose to place their research in countries where genome edited organisms are not regulated as GMOs.

Effects on trade

All imports of seeds for sowing from countries where cultivation of genome edited crops is not regulated will become difficult. It cannot be excluded that there could be seeds modified with new methods of mutagenesis in all seed lots imported to the EU, regardless of the plant species. There might be a need to set up special inspection programs for this. Since it will not be possible to determine the potential presence of genome edited unauthorised seeds by genetic analysis, you may need a system for certification of the entire seed production chain. This will obviously become more expensive for the importer. Since the cultivation of genome edited plants will not be allowed, unless it has been authorised, the requirements for seed purity will be high. It should be emphasized again that, currently, there is no control system that can determine whether a single point mutation has occurred naturally or by genome editing. The same scenario as described above is the case for trade with plant based commodities.

Loss of competitiveness within the agriculture sector

The competition is increased at a global level where the United States, and also to an increasing extent the major Asian economies, over time will have a large impact on the ability of the EU to compete. The EU imports approximately 32.5 million tons of soybean and soy flour from mainly Argentina, Brazil and the United States, and around 5 million tons of rapeseed and rapeseed oil from mainly Australia, Canada and Ukraine. A genome edited rapeseed has already been marketed in Canada and the United States and genome edited soybean and maize are in the pipeline (BioVox website). China is also important when it comes to research and development of gene edited crops. China is primarily invested in rice and is almost dominating the patents related to CRISPR/Cas9 technology.

When Swedish and European farmers do not have access to varieties with the same traits as in other countries, they have weakened their competitiveness in agriculture and forestry internationally. This effect will not only be noted in the cultivation of major crops such as wheat, rye, maize and rapeseed but also for different specialty crops, not the least for vegetables and other crops where not many examples of the use of GMO technology have been seen. In animal production, we will lose competitiveness through increased costs for feed in the EU compared to countries that do not regulate gene edited crops.

One company risks finding itself in a worse position, competitively, due to the ECJ-ruling, compared to farmers outside of the EU. This is due to both wanted traits not being acquired with new techniques of mutagenesis, and to increased costs for analyses and control of imported raw material for feed.

One association states that if the EU chooses not to benefit from new genome editing techniques, we will lose our competitiveness in the long run. We will be dependent on imported commodities both when it comes to food and seeds for sowing. We will also lose control of plant breeding. Food from other regions of the world will most likely be cheaper and have a more secure production.

As developments are taking place at a rapid pace, smaller regions such as northern Europe will not be interesting for the major plant breeding companies outside the EU. This means that crops adapted for northern Europe will fall behind in development. One consequence of this is that countries in northern Europe will lag behind in competitiveness, a trend that is already a fact today. When plant breeding can no longer be maintained in an efficient and modern way in the future, these parts of the world will fall behind even further. For the countries in the north, there is a risk that the consequences will be greater than for the countries in central Europe.

The United States, Canada and additional countries have decided that genome edited plants are not regulated as GM plants. Overall, this means more disadvantages for European plant breeding. So far, European plant breeders have, for their overseas development and marketing of varieties in crops such as maize and rapeseed, been dependent on licenses from biotechnology companies that have patented GMO properties in these crops. With that comes costs for controls and separation of GM and non-GM crops. Now we are faced with the same situation in a variety of other crops such as wheat, oats, potatoes and sugar beet, if we still want to be able to market varieties in these crops successfully outside the EU.

Loss of competitiveness within the research community

Plant breeding is an international operation. Exchange of research results and genetic material is a major part of any practical research. The ruling brings about obstacles that will weaken the collaboration of Swedish and European researchers with non-EU countries. Difficulties of predicting the consequences are expressed but also a fear that the consequences will be considerable.

There is a great risk that all research and knowledge in the area will be transferred to non-EU countries. As a consequence, Swedish and European initiatives will be transferred to companies in non-EU countries that are able to apply the knowledge that has been developed. Modern plant breeding technology will in time replace traditional plant breeding and the conclusion is that the EU will soon be dependent on the competence and financial interests of other countries. There is a risk that the multinational companies will have patented most applications and we will be dependent on these companies for access to applications interesting for Swedish conditions.

As described previously, several research projects involving genome editing have changed direction or been put on hold. Even if projects would be resumed, the research would be delayed compared to that of international competitors. With the competition that prevails, this could mean that we will lose our head

start towards other researchers who can now publish and patent their results before the Swedish researchers. In the long run, the consequences will be less opportunities for financing. Furthermore, there will be other conditions for licensing of technology and its applications.

One large research group states that they had plans to apply for research funding to develop new varieties for practical use. This concerned, for example, investments in forestry, agriculture and horticulture in north of Sweden. The research group had begun to contact stakeholders, but the plans were brought to an end since it is not plausible that they, at this stage, could convince commercial actors and research financiers about this. The GMO legislation has, for many years, meant that they were prevented from translating their basic research findings into farm- and forestry practice. They had hoped that this situation could partially be resolved with the use of genome editing. It is still unclear to what extent their international collaborations will be affected. In the short term, it does not mean any dramatic differences, but a research centre in the EU is of course less attractive as a partner now than it would have been otherwise. There were also plans of a lectureship to better utilize the opportunities that they thought would be realised but which will not happen now.

Consumer´s choice

Consumers are making increasingly specific demands for vegetables and other foods. This could regard the absence of substances that may cause allergies, such as gluten, or an altered content of other specific components which have positive or negative health effects. Higher demands are made on food safety, i.e. a guaranteed supply even in the case of disturbances in food production. In these respects, it will be difficult for the agricultural sector to live up to the wishes and demands of the consumers, unless plant breeding has access to all new methods and technologies.

Chemically modified starch has to be declared, in accordance with current legislation, with an E-number in the ingredient list of foods. The consumer demand for clean label (E-number free) products in the EU is strong and will be further enhanced over time. Currently, in the UK, 44% of the sales of modified starch for food comprises of clean label. It is based on raw materials other than potatoes (mainly maize) because, due to plant cell structure, it is possible to develop naturally storage stable starch using traditional plant breeding. Due to a lacking acceptance of GM labelled products, it is considered impossible to sell GM labelled starch. It is also stated that there is a risk that the debate is distorted, so that the new techniques are questioned on completely partial and unscientific grounds.

Legal uncertainties

In almost all the responses that the SBA received, it was stated that, for a legislation to be meaningful, there has to be reasonable possibilities to control compliance. This, among other things, will result in that the trust in EU as an institution and its ability to develop its legislation will become damaged.

In one of the responses it was written that the decision of the court has created legal uncertainty for the researchers. Could researchers bring plants with them when they move outside the laboratory or the country? What will the research groups do in the future when they get research material, which might have been produced with genome editing, sent to them from colleagues or stock centres in other countries? Would they commit crimes if they would handle such material outside of their GMO facility, if they do not even know whether the material is genome edited or not? How would they communicate this to their employees? Many questions remain unanswered. For many researchers, the ruling is perceived as very problematic and it creates a great deal of uncertainty in their daily operations.

Table 1. Organisations/institutes that that have contributed with information

Organisation/Institute	**Purpose**
Föreningen Foder och Spannmål	An industry association for companies manufacturing and trading with *e.g.* feed, cereals, seeds, fertilizers and plant protection products. The association currently has about 60 members, both from individual companies and cooperative associations.
Lantbrukarnas Riksförbund	The Federation of Swedish Farmers is an interest and business organisation for the green industry with approximately 140,000 individual members.
Lantmännen Lantbruk	Lantmännen is an agricultural cooperative and Northern Europe's leader in agriculture, machinery, bioenergy and food products. It is owned by 25,000 Swedish farmers, has 10,000 employees with business in about 20 countries and a turnover of approximately 4 billion EUR/year. Lantmännen Lantbruk, which is the agricultural sector of Lantmännen, has 10 plant breeding programs for the production of new varieties for the benefit of farmers, the industry and consumers.
Mistra Biotech	A research program focusing on the use of biotechnology in plant and livestock breeding to contribute to environmentally, socially and economically sustainable Swedish agriculture. The program started in 2012 and involves about 60 researchers specialised in natural science, ethics and social science.
Sveriges Stärkelseproducenter	An economic association and owner of *e.g.* the company Lyckeby Starch AB. The association has a turnover of approximately 180 million EUR per year and has about 600 employees. It is owned by approximately 800 farmers and about half of those cultivate starch potato. Sustainable enterprise is an important part of the business strategy and the association is world leading within the development of sustainable starch potato.
Sveriges Utsädesförening	A non-profit association for issues regarding plant breeding and seeds for sowing. The association has about 250 members spanning everything from plant breeding research to plant breeding, seed production, agriculture and an involved general public.
Swedish University of Agricultural Sciences,	The department has around 100 employees and is a part of the research centre Umeå Plant Science Centre

Department of Forest Genetics and Plant Physiology	(UPSC)*. The department conducts research in plant physiology, ecophysiology, plant molecular biology, forest genetics and forest biotechnology.
Swedish University of Agricultural Sciences, Department of Plant Biology	The department develops fundamental knowledge about developmental processes and defence in plants for application in agriculture and forestry.
Swedish University of Agricultural Sciences, Department of Plant Breeding	The Department of Plant Breeding carries out research, pre-breeding and the production of varieties in a number of crops with the goal to contribute to a sustainable production of food, feed and bio-based materials. A great deal of effort is invested in the development of modern breeding methods such as genome editing. The department is also highly involved in the spreading of information and in questions regarding the policy of different breeding techniques.
Swedish University of Agricultural Sciences, Grogrund	Grogrund is a knowledge centre initiated by the Swedish government. The centre gathers the academia and business to develop skills to, in accordance with the objectives of the national food strategy, ensure the availability of varieties for sustainable and competitive agricultural and horticultural production in Sweden.
SweTree Technologies AB	A plant and forest biotechnology company providing products and technologies to improve the productivity and performance properties of plants and wood for forest owners and fibre related industries
Umeå University, Department of Chemistry	The department has more than 200 employees. Research at the department includes three major areas: biological chemistry, environmental and biogeochemistry and technical chemistry. The department is a part of Umeå Plant Science Centre (UPSC)*.
Umeå University, Department of Plant Physiology	The department has around 100 employees and is a part of the research centre Umeå Plant Science Centre (UPSC)*. Their main activity is academic research in experimental plant biology, with the goal of understanding all aspects of plants in relation to the environment that they live in.
*Umeå Plant Science Centre, UPSC	UPSC is a "centre of excellence" and one of the most prominent research environments for plant research in Europe. About 40 research groups work in UPSC with

	large international element with over 40 nationalities represented. Virtually all groups use genetically modified plants in some part of their research. In recent years, many groups have also started to use genome-editing techniques. The main part of the research at UPSC is basic research, but some projects have a more applied nature. In 2014, researchers at UPSC were the first ones to ask the Swedish Board of Agriculture about their views on the regulatory status of genome editing.

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2019

Synthetic biology for novel chemical-producing anaerobic pathways

Genome editing, CRISPR

3 920 000 SEK

Microbial metabolic pathways for anaerobic production of a desired chemical provide by definition the most efficient stoichiometric biotechnological option when thermodynamically feasible. This project will design, test and build new anaerobic pathways for microbial products that hitherto can only be made aerobically. Starting with proof-of-principle anaerobic production of 2,3-butanediol with yeast, general pathwaydesign principles will be derived that can be applied to economically efficient and sustainable production of chemicals. The support from VR, together with strategic resources from KTH, will be used to recruit two sequential post-docs for a total of 4 years. The project will start with fine-tuning the community-based genome-scale models for anaerobic metabolism and the design and implementation of the molecular strategies for the extensive CRISPR-Cas9-based genome editing. The quantitative physiology of the resulting pathways and yeast strains will subsequently be tested in controlled, anaerobic bioreactors. Where required, laboratory evolution will be used for further improvement of the growth coupled, redox-cofactor balanced and ATP-yielding product formation pathways. Sequence-based reverse metabolic engineering of the evolved strains will be used to elucidate the underlying molecular mechanism, thereby creating both new knowledge and portable genetic elements that can be implemented for a wider range of products.

Evolution of memory molecules

CRISPR

3 674 000 SEK

Learning and long-term memory (L&M) are complex phenomena and the relationships between specific memories and molecular events in neurons are still shrouded in mystery. Our approach to unravel L&M mechanisms utilizes their evolution.

Our pioneered bioinformatics approach which combines sequence-based phylogenies with comparisons of chromosomal locations of genes is a powerful way to untangle complex gene relationships and distinguish orthologs and paralogs across species. This strategy is used to resolve the large families of receptors involved in L&M, especially glutamate and GABA-A receptors, and several gene families that regulate these. Special focus is on protein kinases involved in memory consolidation, primarily PKMzeta. Gene expression patterns are mapped in zebrafish brain and compared with rodents, particularly in the hippocampus. Evolutionary comparisons have allowed us to identify multiple promoters in the PKMzeta gene whose roles will be explored using a reporter system. An evolutionarily conserved short open reading frame in the PKMzeta mRNA, with potential regulatory roles, will be investigated by mass spectrometry. L&M genes will be knocked out in zebrafish using the CRISPR/Cas9 system and the consequences on spatial and social memory will be

investigated from the second year. By extending the evolutionary perspective on L&M, our understanding of the mechanisms will be improved and will facilitate future intervention to enhance or erase memories.

FROM OMICS TO PATIENT-SPECIFIC SIMULATIONS

CRISPR

3 800 000 SEK

One of the main strengths of biomedical engineering is to go from measurements to clinical impact using advanced data analysis. Several such important showcases exist within image analysis, done with advanced machine learning methods such as deep learning. These showcases should now be expanded to also integrate other types of clinically available data, such as omics measurements of all genes and protein activities. In a VR-financed postdoc at Harvard Medical School, I have developed and validated a new cutting-edge analysis technique, to deal with such protein activity data. The method is based on executable models, which makes use of and adds to biological knowledge. However, this method can today only deal with ~100 proteins, and not the >10,000 proteins available in full omics data. For this reason, I will in this project do three key expansions of the method 1) develop a framework based on pairwise models to reach - for the first time – executable models of omics scale for clinically relevant data, 2) use state-of-the-art validation data from an inducible CRISPR-Cas9 system specifically developed for this project, 3) derive patient-specific models to simulate clinical scenarios where all available patient data serves as model input. My unique set of collaborators involving both world-leading theoretical, pharmaceutical, and clinical implementation environments ensure that my award-winning models can make a difference in both research, drug-development, and healthcare.

Molecular and evolutionary studies of chromosomal gene clusters active during male reproductive development

CRISPR

3 160 000 SEK

Chromosomal clustering of co-expressed genes may confer a selective advantage as it enables coordinated gene regulation at the chromatin level. In the proposed project we aim to study this conceptually new mode of gene regulation. We will use male reproductive organs, the stamens, of the flowering plant Arabidopsis thaliana as a model system for our studies, and focus on tapetum-specific clusters. We suggest using three recent technical advances to study the biological function of the chromosomal gene clusters, how they are regulated, and how they have evolved. To study the function of the clustered genes we aim to generate mutants of all genes in one cluster using genome-editing techniques that build on CRISPR/Cas9. To study the mechanism of clustered gene regulation we will use the INTACT method, to purify tapetum nuclei from floral tissues. The purified nuclei will be used in subsequent ChIP-seq experiments to analyze epigenetic marks associated with either repression or activation of gene activity. To enable evolutionary studies we intend to generate tapetum-specific transcript profiles from Brassica rapa using Spatial Transcriptomics. The data generated can subsequently be combined with available genomic data to analyse clustering tendencies of B. rapa genes. Together with the functional and mechanistic studies, this will increase

our understanding of both the evolutionary forces that drive gene clustering in flowering plants and how pollen development is regulated.

DNA replication and repair: a clearer picture on timing and therapies

CRISPR

3 000 000 SEK

To battle diseases such as cancer we need to understand the processes that drive human cell proliferation. But how do human cells multiply? Before a cell divides it has to faithfully duplicate its genome and transition between distinct biochemical states known as cell cycle phases. All these events are tightly interconnected and highly dynamic, and thus timing is paramount.

Decades of pioneering research, mainly using unicellular organisms or in vitro systems, have led to a prototype model of DNA replication initiation, revealing a complex multi-step process involving many factors directly associated with cancer risk. However, recent efforts to extrapolate this model to human cells revealed several contradictions, forcing us to go back to the drawing board. Here I propose to use the VR starting grant to set up a team of three researchers and pioneer a new research direction focused on how human cells start DNA replication and ensure genome integrity. I plan to do this by combining a new rapid protein depletion method state-of-the-art microscopy and DNA sequencing techniques. In parallel I plan to perform CRISPR screens and mass spectrometry studies to find factors controlling DNA replication initiation and genome stability. Insights gained from these studies will define how, when and where DNA replication is started with unprecedented precision in time and space and will provide clues on how to advance current cancer therapies and prevent drug resistance

Assembly and function of NO-reducing heme-copper oxidases

Site-directed mutagenesis

4 132 000 SEK

Bacterial NO-reductases (NOR) are integral membrane proteins that reduce the toxic gas nitric oxide to dinitrous oxide: $2NO+2e+2H+\rightarrow N2O+H2O$. This reaction is a step in denitrification, an anaerobic respiratory process that reduces nitrate stepwise to dinitrogen. NORs are divergent members of the super-family of oxygen-reducing, proton pumping heme-copper oxidases (HCuOs), to which the mitochondrial cytochrome c oxidase (mCcO) also belongs. Despite belonging to this family, the NORs function very differently from other HCuOs in not conserving the free energy available from NO reduction by creating a proton gradient over the membrane. The closest relatives to NOR among the HCuOs are the cbb3 oxidases, which have a high oxygen affinity and are often found in pathogenic bacteria. The cbb3 oxidases can also reduce NO in contrast to the mCcO, and NORs can reduce O2. The project aims at elucidating the structure-function relationships in the bacterial NORs and cbb3 oxidases, as well as the assembly process and the specific roles of the metal ions in the active site. We expect to gain insight also into the evolution of the heme-copper oxidase family and the interplay between assembly and functional properties. Our studies involve a broad set of techniques; e.g. site-directed mutagenesis, protein purification optimisations, cryo-EM structural investigations, reconstitution into membrane mimetic systems, and time-resolved optical spectroscopy of single catalytic turnovers.

Bio-modifying technologies in Change: Cultural Perspectives on Responsible Research and Innovation in CRISPR- and stem cell technologies

CRISPR

3 990 000 SEK

This research project investigates how ideas of “responsible research and innovation” (RRI) is (re)produced at the intersection between laboratory and surrounding society, and between the researchers´ everyday work practices and society´s discourses on modern biomedicine.

Two technologies are studied in this research project: CRISPR technology and neuro-transplantation with stem cells.The goal is to investigate (A) how biomedical researchers relate responsibly to the biomodifying technologies researched and to (B) the “economy of hope” that they may create. How (C) RRI is extended to new areas and dimensions of research, as well as to new stakeholders is also examined.

The ways in which (D) the extended area of responsibility affects the research group as an educational and career environment for doctoral students and post-doctoral researchers will therefore be looked into as well. Our empirical starting point is the laboratory, and we examine how practices and prerequisites of it relate to surrounding actors.The laboratory will be explored through ethnographic fieldwork. Fieldwork starts early 2020 extending through late 2021. Authoring academic publications and presenting at conferences overlap with fieldwork starting early 2021, extending into late 2022.Relating everyday practice of the laboratory to discussions on RRI helps highlight tacit dimensions of ethics-in-practice, and is of great importance to the arrangement of biomedical research environments.

Experimental validation and systematic review of sexually antagonistic genes

Genome editing, CRISPR

4 910 000 SEK

Sexually antagonistic (SA) selection occurs when alleles favoured by selection in one sex are selected against in the other. Unresolved, this intralocus sexual conflict is predicted to have consequences for the maintenance of genetic variation, and the evolution of sexual dimorphism. Evidence for SA genetic variation comes from numerous taxa, in both laboratory and wild populations. But despite this evidence, there are only two specific examples generally known of SA loci. My project aims to systematically survey fly and human genomes for specific examples of SA loci. I will take two approaches. First, candidate SA alleles from a recent genome-wide association study in the fruit-fly will be recreated using CRISPR/Cas9 gene editing, validating their effects in assays of sex-specific fitness. Validated loci will then be investigated in more detail to pinpoint which fitness components contribute to the overall effect. Key predictions from evolutionary theory regarding their role in the maintenance of genetic diversity and the evolution of sexual dimorphism, will then be tested. Second, we shall carry out the first systematic review of the biomedical literature to identify specific examples of SA genes in humans, which are "hidden" from evolutionary biologists due to discipline-specific terminology. This will provide further evidence for role of sexual antagonism in humans, give insights into how interdisciplinary communication may break down, and how that can be rectified.

Nanotube-Assisted Direct Cargo Delivery To The Cytosol For Precise And Efficient Live Cell Manipulation And Sensing

CRISPR

4 200 000 SEK

Being able to interfere with the cell machinery while monitoring the resulting effects on a whole population of cells, over time, at the singe cell level, would bring significant breakthrough in, for instance, understanding diseases.Today, there are a lot of molecular tools available to perturb and sense cells. In order to perturb and sense cells with efficacy, these tools need to reach the cell interior in great quantities within a short period of time. However this is not possible with currently available delivery technologies. Indeed, electroporation leads to poor cell viability and cargos delivered using liposomes are trapped in cellular compartments, leading to slow delivery and poor delivery efficiencies.Here, we aim at using nanotubes for achieving direct and immediate delivery of cargos in living cells. The overall purpose is to establish a platform enabling minimally invasive and efficient cell perturbation, combined with longitudinal and reliable individual cell interrogation, over a population of live cells.Cargos for cell perturbations will include siRNA, mRNA and CRISPR-Cas9. Cargos for individual cell sensing will consist of molecular beacons (sensing the transcriptional state of cells) and functionalized nanodiamonds (for live cell superresolution microscopy imaging).The project will enable novel studies of live cell populations at the individual cell level that would not be possible without achieving direct and efficient cargo delivery to the cytosol.

Bio-modifying technologies in Change: Cultural Perspectives on Responsible Research and Innovation in CRISPR- and stem cell technologies

CRISPR

3 990 000 SEK

This research project investigates how ideas of “responsible research and innovation” (RRI) is (re)produced at the intersection between laboratory and surrounding society, and between the researchers´ everyday work practices and society´s discourses on modern biomedicine. Two technologies are studied in this research project: CRISPR technology and neuro-transplantation with stem cells.The goal is to investigate (A) how biomedical researchers relate responsibly to the bio-modifying technologies researched and to (B) the “economy of hope” that they may create. How (C) RRI is extended to new areas and dimensions of research, as well as to new stakeholders is also examined. The ways in which (D) the extended area of responsibility affects the research group as an educational and career environment for doctoral students and post-doctoral researchers will therefore be looked into as well. Our empirical starting point is the laboratory, and we examine how practices and prerequisites of it relate to surrounding actors. The laboratory will be explored through ethnographic fieldwork. Fieldwork starts early 2020 extending through late 2021. Authoring academic publications and presenting at conferences overlap with fieldwork starting early 2021, extending into late 2022.Relating everyday practice of the laboratory to discussions on RRI helps highlight tacit dimensions of ethics-in-practice, and is of great importance to the arrangement of biomedical research environments.

Long-range gene regulation and 3D organization of the Glioblastoma Genome: mechanisms underlying tumor heterogeneity and high invasiveness

Genome editing

6 000 000 SEK

Glioblastoma (GBM) is the most lethal and aggressive, but also the most common, of all primary brain tumors. Most genetic variants that predispose to cancer are located in non-coding regions enriched in putative enhancers, whose systematic rearrangements have a high impact on gene expression in cancer. Despite the number of susceptibility loci identified in glioblastoma, a functional understanding of how the non-coding regulatory genome contributes to the pathogenesis is missing. Therefore, I aim to study how reprogramming of regulatory regions and topological changes in 3D chromatin organization determine gene dysregulation in glioblastoma, and how this subsequently contributes to malignancy, heterogeneity and invasiveness. To this end, I will bring knowledge from different biological and technical backgrounds into the glioblastoma field, employing next generation sequencing approaches, genome editing and live-cell imaging in both GBM mouse models and human glioma cell lines. The planned research will shed light on how the regulatory and topological architecture of the genome influences malignancy and integrates cues from the neurons in the tumor microenvironment to orchestrate gene expression in glioblastoma. The expected outcome will open up for new therapeutic opportunities in the field of glioblastoma by modulating the regulation of certain genes or the neuron-glioma cell interaction.

Investigation of hepatocytes and liver macrophages in nonalcoholic fatty liver disease progression

CRISPR

6 000 000 SEK

Non-alcoholic fatty liver disease (NAFLD) is emerging as the most common liver metabolic disorder worldwide. Liver lipid overload and subsequent immune cell activation contribute to irreversible progression of liver steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and cancer. Current efforts to combat these diseases have been halted by limited understanding of the underlying regulatory networks that define their development. To address the key mechanisms,

I have previously explored (Nat Med 2016, Nat Com 2019) and continue to investigate the liver and immune cell samples from multiple cohorts including a French cohort (Bart Staels) of 104 NAFLD to NASH fibrosis patients; and a Swedish cohort (Bo Angelin) of lipid disorder patients. The purpose is to understand how dysregulated hepatocyte metabolism and liver macrophage inflammation cause NAFLD progression.The proposal combines next generation sequencing (NGS), interactome/posttranslational proteome, CRISPR(i) depletion/inhibition/library screening and in vivo enhancer manipulation in mouse liver disease models and primary human cells, aiming to explore the remodeling process in both hepatocytes and liver macrophages during NAFLD development. We hope to identify novel transcription regulatory mechanisms and to validate transcriptome and epigenome targets in the hepatocytes and liver macrophages to better understand NAFLD development, with ultimate goal to develop better diagnostic and intervention strategies.

Development of a proof of concept for a cell-based method for potency determination of Botulinum toxin

CRISPR

2 164 657 SEK

We will within this project develop a proof of concept assay to replace Botulinum Toxin type A (BoNT/A) testing in animals. Our assay is a cell based assay, based on human embryonic stem cells. These cells are sensitive enough against the low concentrations of BoNT/A in medical products and is an adequate model to reflect all aspects of BoNT/A mechanism of action. We have preliminary data showing that we can differentiate these embryonal stem cells into neurons suitable for the assay, in a robust and reproducible manner. We will use CRISPR/Cas to insert reporter genes into these cells, that will give a readout proportional the amount of toxin the cells are exposed to. As an alternative approach we will develop affinity reagents that are capable of detecting and differentiat between cleaved and non-cleaved SNAP-25. Testing of BoNT/A is performed at production batches as well as final drug products and for stability testing of drugs. Our goal is to have an assay able to replace all aspects of the mouse testing, but even if our assay will not replace animal test for all these aspects it will highly reduce the number of animals needed. The goal is to produce a proof of concept assay with analytical parameters such as precision, accuracy and sensitivity determined, that can form the basis of a commercial, GLP approved release assay.

Bacterial fitness mechanisms of the versatile pathogenic variants of Escherichia coli and the emerging opportunistic pathogen Acinetobacter baumannii

CRISPR

3 600 000 SEK

The purpose is to increase our understanding of the fitness properties and mechanisms by which the very versatile extra-intestinal pathogenic variants of Escherichia coli (ExPEC) and the emerging nosocomial pathogen Acinetobacter baumannii express their virulence-associated properties.Specific aims:To investigate the molecular basis of novel c-diGMP phosphodiesterases in regulation of ExPEC motility and adhesion.To clarify molecular links between c-di-GMP and filamentation of ExPEC cells.To clarify links between citrate utilization and expression of virulence by ExPECTo investigate if strains with properties of NMEC are represented among isolates from cases of asymptomatic bacteriuria.To perform genomic studies of A. baumannii collections with respect to antibiotic resistance development.To explore a CRISPRbased approach subtyping A. baumannii isolates. To claraify if antibiotic resistance genes are spread via bacterial membrane vesicles.To clarify the role of colony morphotype switching in A. baumannii biofilm formation.To clarify the role of Csu fimbriae in colony morphotype switching and in A. baumannii biofilm formation.To clarify functions of the newly defined C-di-GMP signalling netork in A. baumannii.To elucidate surface properties of A. baumannii that contribute to resistance to desiccation.

Gene Knock Up via 3'UTR targeting to treat Alzheimer's disease

CRISPR

2 400 000 SEK

Current genetic tools do not allow researchers to upregulate the levels of a given protein while retaining its cell-type-specific regulation. This inability is likely a key limitation in creating side effect free regenerative treatment for neurodegenerative disease. We developed two approaches for increasing endogenous gene expression via 3'UTR editing while retaining its spatiotemporal expression pattern: conditional Knock Up (cKU) utilizing the Cre/lox system, and CRISPR-Cas9 mediated gene Knock Up (KU) in wild-type mouse embryos and human cells. Both approaches resulted in upregulation of endogenous neurotrophic factor GDNF levels and in enhanced dopaminergic function without side effects, suggesting that similar approach may be useful for defining which genes, when, where and how should be upregulated to treat Alzheimer's disease (AD). Failure in proteostasis and possibly neurotrophic function is believed to drive pathogenesis in AD. We propose to use gene Knock Up tools to explore whether upregulation of endogenous genes which regulate proteostasis and neurotrophic function result in side effect free treatment of AD in mouse models.

For this we will identify gRNA-s which can be used for gene Knock Up of treatment relevant genes via 3'UTR editing and test AAV mediated treatment pipeline in AD mouse models. To gain deeper insight into AD etiology we will also use cKU tool to explore how adult onset increase in mutated beta-amyloid protein influences AD progression in mice.

Dissecting the molecular and regulatory mechanisms of Chaperone-mediated autophagy as an effective means to target pathological proteins

CRISPR

4 800 000 SEK

The main goal of this proposal is to provide detailed knowledge of chaperone-mediated autophagy (CMA), a selective cellular degradation process, as a basis for the development of novel therapeutic therapies. Proteins that promote disease development and maintenance are often deregulated and stabilized in cells. As pathogenic accumulation of these proteins sustain and progress illnesses, they are indisputable targets for treatment. CMA, a unique type of autophagy, have been shown to selectively target several mutant and pathogenic proteins for degradation, providing a strong rationale to activate CMA as novel therapeutic strategy. CMA activation is, however, very poorly understood due to the incomplete knowledge of its signaling mechanisms, the lack of proper activators and a quantitative assay to monitor CMA. We have now developed a new method to assess the activity of CMA in live cells. By this method, we performed both small-molecule and CRISPR/Cas9 screenings to uncover pharmacological and genetic regulators of CMA. This discovery platform gives us a unique experimental system to address a long-standing question in cell biology; How is CMA activated and regulated? This proposal will provide detailed molecular understanding of a fundamental process and advance current knowledge of CMA in medical conditions. Our work will lay the foundation for new therapeutic avenues and uncover novel drug targets, that may have implications on how to successfully target CMA in patients.

Identifying and characterising new targets, drugs, and small molecules for prevention and treatment of coronary artery disease

CRISPR

4 800 000 SEK

My research program aims to translate robust associations from largescale genome-wide association studies for coronary artery disease into functional understanding and novel treatments. Candidate genes will be systematically characterised for a role in disease-related traits using large-scale, in vivo, image and CRISPR-Cas9-based zebrafish model systems. Phenome-wide association studies in UK Biobank data and further characterisation in zebrafish larvae will help prioritise promising targets for therapeutic intervention.

For these targets, we will explore drug-gene interaction databases to identify targets that are already acted on by existing medication, for any indication. For such existing drugs, we will use Swedish register data to examine if the drug can be repurposed to prevent or treat coronary artery disease. For targets without existing drugs, we will screen customselected small molecules that are predicted to bind the target in zebrafish as well as in humans for an effect on disease-related traits in zebrafish larvae. For small molecules that induce desirable effects in zebrafish larvae, we will examine binding efficiency and target specificity across the entire human proteome.My research will: 1) increase our understanding of cornary artery disease pathophysiology; 2) provide existing drugs that can be repurposed for prevention and treatment; and 3) provide a set of drug targets and small molecules acting on them that can be taken forward towards clinical trials.

Pancreatic islet hormone secretion and type 2 diabetes -focus on microRNAs in human gene expression networks

CRISPR

4 800 000 SEK

Deregulated secretion from the α- and β-cells within the pancreatic islets of Langerhans is part of the pathogenesis of type-2 diabetes (T2D). We hypothesize that microRNAs (miRNAs) are key in the adjustment of βcell function and have discovered novel miRNAs involved in the posttranscriptional regulation of gene expression essential for insulin secretion. However, there are still many open questions on their role in diabetes pathogenesis and in α-cell function. We therefore aim to: 1) Decipher miRNA networks to disclose central miRNAs in T2D development. We will examine miRNA expression in human islets and sorted cells and combine this with unique islet data from the same donors to generate novel islet-specific global gene expression networks using up-to date bioinformatics techniques.2) Investigate the function of groups of miRNAs in α-and β-cells using state-of the art techniques including patch-seq and CRISPR/Cas9.3) Measure miRNAs in serum samples from well characterized cohorts, which we expect will give possibilities for personalized prediction of T2D. Finally, in aim three we anticipate finding miRNA inhibitors (antagomirs) that can adjust the differential expression of miRNAs in T2D and thereby lead to improved insulin and glucagon-secretion. Altogether we expect the suggested investigations to increase not only the understanding of the complex disease T2D, but also to reveal new methods in diabetes therapeutics and diagnostics.

Defining regulators of hematopoietic stem cells – novel paradigms for stem cell expansion and therapeutic targeting of leukemic cells

Gene editing, CRISPR

7 200 000 SEK

The molecular principles regulating hematopoietic stem cells (HSCs) remain incompletely defined. Through development of innovative approaches to study regulation of both human and murine HSCs we aim to characterize modifiers of self-renewal and differentiation and to define novel therapeutic approaches for treatment of leukemia, either by enhancing stem cell transplantation or by selectively targeting leukemic cells. To this end, my laboratory has developed paradigms for genomewide functional screens in human HSCs, employing both shRNA and CRISPR gene editing tools, and identified new modifiers of self-renewal and differentiation. Within this research program we will specifically focus on development of ex vivo HSC expansion strategies by targeting candidate genes from the screens in human cells and assess expansion using genetic barcoding and transplantation assays in immundeficient mice. We will further study functional and mechanistic aspects of two novel candidate genes (NCAPD2 and SDPR) in both normal and malignant hematopoiesis using mouse models. Finally we will exploit the concept of synthetic lethality between members of the cohesin complex as a strategy to selectively eradicate leukemic cells. The aim is that our work will advance clinical trials for expansion of transplantable HSCs to treat patients with leukemia. Moreover we hope that these studies will define new therapeutic strategies to selectively target leukemic cells that can be exploited clinically.

New roles of oligodendroglia in multiple sclerosis

CRISPR

9 000 000 SEK

Multiple Sclerosis (MS) is characterized by an auto-immune attack targeting myelin, a lipid rich insulating membrane produced by oligodendrocytes (OLs), leading to the symptoms associated with the disease. The adaptive immune system is currently thought to be the most likely etiological component for MS. We have recently identified novel mouse and human OL cell states in development and disease (Science 2016, Developmental Cell 2018, Nature Medicine 2018, Nature 2019). In particular, we found that OLs and OL precursor cells (OPCs) can transition to cellular states with immunological properties (imOLs and imOPCs). In this project, we will determine the role of imOPCs and imOLs in the origin and progression of MS and investigate the molecular mechanisms that mediate their functional interaction with immune cells. We will use bulk and single-cell transcriptomic and epigenomic technologies to characterize the dynamics of imOLs/imOPCs in the EAE mouse model of MS and generate transgenic mice to investigate the function of MHC II and related pathways in OLs.

We will also unveil the transcriptional and epigenetic landscapes of human OL lineage cells in MS. We will then identify epigenetic molecules that can be targeted to modulate OL function, by performing CRISPRbased screenings. This proposal explores new facets of MS involving OLs, and as such, is bound to give novel insights on the biology of the disease, uncover new therapeutic approaches.

Unravelling the Notch code in hepatic portal vein mesenchyme: Master controller of portal triad development and architecture?

CRISPR

2 400 000 SEK

During liver development, Jag1-expressing portal vein mesenchyme (PVM) induces hepatoblasts to become cholangiocytes and form bile ducts, after which the hepatic artery forms, yielding the classic "portal triad" structure. We recently published a Jag1 mouse model for Alagille syndrome, faithfully recapitulating bile duct paucity. In exciting preliminary studies, we discovered that this model has underdeveloped PVM and in contrast, we identified a Notch3 mutant with PVM expansion and concomitant increase in bile ducts and hepatic arteries. This phenotype mirrors infantile myofibromatosis in which NOTCH3 is mutated, characterized by mesenchymal tumors. We hypothesize that a Jag1/Notch3 axis controls PVM development, underlying portal triad growth.Using Jag1 and Notch3 mutant mouse models, 3D biliary organoid culture, CRISPR Notch-null reporter cell lines and Alagille patient samples, we aim to identify which PVM cell types control portal triad development, elucidate the trans- or cis-signaling mechanisms employed by Jag1/Notch3 in PVM, test the role of the PVM-generated extracellular matrix, and perform rescue experiments in vivo. We will use single cell RNA sequencing, micropatterns, histology, and ultrasoundguided in utero nano-injection to address our aims.The Notch pathway is implicated in multiple facets of liver disease, from the Alagille syndrome, to NAFLD, to cancer. Results from our studies could thus have farreaching implications, with rapid translation potential.

Regulation of immunoglobulin gene diversification: with relevance for primary immunodeficiency and lymphoid malignancies

CRISPR

9 000 000 SEK

The aim of this project is to understand the complex molecular mechanisms involved in the regulation of immunoglobulin (Ig) gene diversification processes, i.e. V(D)J recombination, class switch recombination (CSR) and somatic hypermutation (SHM). We have developed a series of next generation sequencing technology-based strategies to study the in vitro and in vivo V(D)J recombination as well as CSR and SHM patterns in human B-cells. Furthermore, inducible pluripotent stem cells reprogrammed from healthy individuals´ and patients' fibroblasts have been generated, and will be further differentiated in order to recapture different B cell development stages. Moreover, single cell RNA sequencing and CRISPR-based technologies will be applied to help to identify key factors driving B cell development as well as Ig gene diversification. Combining these approaches, and taking advantage of our unique collection of patient samples with rare primary immunodeficiencies, we hope to delineate the complex molecular mechanism involved in Ig gene diversifications. Finally, we hope to address the question whether illegitimate CSR and SHM events are associated with B cell genome instability and lymphomagenesis. The somatic mutation and translocation patterns will be systematically characterized in B-cell lymphoma in relation to DNA repair defects and chronic viral infection status (exemplified by HBV virus).

Identification and functional characterization of innate immune checkpoint regulators in acute myeloid leukemia

CRISPR

4 800 000 SEK

Acute myeloid leukemia (AML) arises from hematopoietic stem and progenitor cells and is associated with exceedingly poor survival. For the disease to develop, the AML cells need to escape immune surveillance, but the mechanistic basis for this is unknown. The goal of this project is to provide a more effective treatment for AML by unleashing the innate immune system against the AML cells. I will pursue this goal by first identifying and mechanistically characterizing cell surface proteins on AML cells that suppress Natural killer (NK) cells and macrophages. To identify such cell surface proteins, which serve as innate immune checkpoints, I will pioneer unique in vivo and ex vivo CRISPR screens that we have developed within my research group. I will then characterize the function and expression patterns of the identified innate immune checkpoint regulators, and evaluate therapeutic effects of inhibiting them in leukemia mouse models. In particular, I will develop tools to target a chemotherapy-resistant cell population of leukemia cells with the ability to self-renew, a population termed leukemic stem cells. I will perform this project together with two postdocs and one PhD student. Successful completion of this research program will increase our understanding of how AML cells suppress the innate immune system, and will provide initial validations of therapeutic potential, findings that may translate into new and more effective antibody-based therapies for AML patients.

Molecular Mechanisms underlying Pericyte Dysfunction in Brain Pathology

CRISPR

4 800 000 SEK

Pericytes are perivascular cells that undergo pathological changes in brain disease. One of their key functions is the secretion of molecules and microvesicles that contribute to disease progression. This collaborative project identifies drugable molecular targets that regulate the secretome of pericytes in ischemic stroke and glioblastoma multiforme using a stepwise approach in 3 phases over 4 years. We identify key regulators of pericyte pathology by combining transcriptomic profiling of pericytes with proteomic analyses of pericyte-derived microvesicles using transgenic mouse models and human samples. We then corroborate and pinpoint relevant signaling pathways in cell culture models. Selected candidate genes are validated in loss/gain of function studies using CRISPR/Cas9 and lentiviral technology and their functional impact on the pericyte secretome and disease progression verified in several transgenic mouse lines.This type of study is highly warranted as it will provide the fundamental basis for the development of novel treatments modifying pericyte function in pathology.Decoding the molecular regulation of the pericyte response to stroke will allow to target pericytes potentially leading to therapies that e.g. halt the inflammatory cascade in stroke, whereas knowledge on the molecular regulation of pericyte recruitment and signaling in glioblastoma may advance therapies that control immune evasion, tumor growth and perivascular infiltration.

CRISPR/Cas based gene activation in filamentous fungi for antibiotics discovery

CRISPR

3 150 000 SEK

Genomic data shows that filamentous fungi have the biosynthetic potential to produce a vast number of natural products, however the responsible biosynthetic gene clusters (BCGs) are transcriptionally silent under laboratory growth conditions. In this project we seek to harness the untapped resource of natural compounds in fungal genomes by developing new high throughput and generic methodologies for activation of silent BGCs in the native host, followed by initial testing for antibiotic properties. By doing so we hope to accelerate discovery of much needed novel antibiotics for treatment of resistant Gram-negative infections. The generic gene activation method(s) we will develop would be useful for academic and biotechnological purposes.We plan to whole genome sequence undomesticated filamentous fungi, and select highpotential BGCs based on bioinformatic analysis. Simultaneously we will adapt the CRISPR/Cas technology to activate silent BGCs in filamentous fungi. It has been shown that fusing nuclease-deficient Cas enzymes to activator or repressor domains enables stable and efficient transcriptional regulation. After using the developed CRISPR/Cas technology to activate selected BGCs, culture broths will be screened for novel compounds using LC-MS and subjected to preliminary plate-assay screens against clinically relevant Gram-negative bacteria. Furthermore, I will adapt the CRISPR/Cas technology for two distinct fungal species in collaboration with Prof Stenlid in Sweden.

2018

Sexual conflict in the genomic era: using experimental evolution to characterize sexually antagonistic regions of the genome

Genome editing, CRISPR

3 150 000 SEK

A longstanding challenge in evolutionary biology has been to explain what maintains genetic variance in fitness. Sexually antagonistic (SA) genetic variation - i.e. tradeoffs in which alternative versions of genes pose opposite fitness effects in males and females - may be one of the most widespread explanations, but remains poorly understood. The proposed research aims to clarify the nature and prevalence of SA genetic variation. In the first two years I will utilize a novel experimental evolution approach developed by Professor Agrawal at the University of Toronto (UofT) to identify and characterize the SA regions of the fruit fly genome. The Agrawal lab at UofT has the necessary expertise, resources and infrastructure to facilitate the proposed genomic and transcriptomic approaches. In the third year I will return to Sweden with a panel of genetic lines and a highly-credible list of SA loci to investigate. I will use CRISPR genome and ‘epi-genome’ editing to investigate how males and females differentially regulate alternative SA alleles - a crucial theoretical prediction for SA genetic variation. This work will be cohosted by Professors Mannervik and Theopold at Stockholm University - experts in CRISPR technologies and gene regulation. This cross-disciplinary approach will give unique perspective to the role of SA genetic variation in evolution, wildlife management and medicine, and provide me with a highly competitive set of skills among modern evolutionary biologists.

Real-time characterisation of neuropeptide binding to a membrane receptor involved in pain and ischemic stroke

Site-directed mutagenesis

3 150 000 SEK

ASIC1a is a pH-activated ion channel with crucial roles in neuronal signaling. As some of the most prevalent neuronal disorders such as chronic pain, ischemic stroke and psychiatric diseases are associated with local acidosis, ASIC1a has emerged as promising drug target, yet no compounds are currently available that specifically target ASIC1a. Big Dynorphin is a neuropeptide known to modulate ASIC1a with high affinity. The details of this interaction, however, remain largely enigmatic. The aim of the proposed project is to directly characterize the binding of Big Dynorphin (BD) to ASIC1a in real time. I will use a unique in-house developed high-sensitivity fluorescence electrophysiology setup and establish a protocol for a FRET-based ligand-binding assay. Together with site-directed mutagenesis, this approach will identify the BD binding site on ASIC1a and define the key interactions. Further, this will allow direct analysis of binding affinity and kinetics under pathological conditions; all in intact membranes and with unprecedented (microsecond) temporal resolution. This information will aid future design of a new generation of ASIC1a-selective drugs to, for example, treat pain without the typical downsides seen with opioids. The technology developed for this project will further enable high sensitivity ligandbinding studies of other membrane proteins and thus have significant scientific impact beyond the scope of this proposal.

Uncovering MLLT3 regulation to improve hematopoietic stem cell self-renewal

CRISPR

3 150 000 SEK

Hematopoietic stem cells (HSCs) sustain life-long blood formation because of their ability to self-renew and differentiate into all blood cell types (referred to as “stemness”). Transplantation of HSC is a life-saving therapy for multiple blood disorders; however, shortage of immunologically matched donors limits the number of patients that can be treated. Expansion/generation of HSCs in culture would greatly improve transplantation therapy, but has so far been unsuccessful due to poor understanding of the underlying biology of human HSC stemness. The Mikkola lab recently identified MLLT3 as a novel human HSC regulator that is highly enriched in human HSC but downregulated during culture. MLLT3 is critical for HSC self-renewal and rescuing its levels in culture expands HSCs by protecting their “stemness” program. Despite these studies, nothing is known about the mechanisms controlling MLLT3 expression in HSC, which is crucial to safely implement it in clinical practice. To understand how MLLT3 expression is regulated in human HSCs, we propose three complementary strategies: 1) CRISPR/dCas9-mediated genome activation to dissect the role of MLLT3 candidate enhancers; 2) combined bioinformatic and experimental approaches to identify MLLT3 upstream regulators, and 3) compound screening to identify extrinsic regulators of MLLT3 expression. Success in any of these approaches could help generate/expand human HSCs and thus, increase the availability of HSCs for transplantation.

Understanding the genetics of nonalcoholic fatty liver disease: from genetic association to function

CRISPR

3 150 000 SEK

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases and results in serious public health problems. Globally, about 25% of the adult population is estimated to suffer from NAFLD. Patients with NAFLD are at increased risk of cardiovascular disease and type II diabetes. Aim: Genome-wide association (GWA) studies have been used to identify associations between common genetic variants and NAFLD.

In this proposal, we will characterize NAFLD-associated loci in in vitro systems using large-scale CRISPR-Cas9 gene editing coupled to single cell RNA-seq (CROP-seq).Work plan: We will create a HepG2 cell line stably expressing dCas9-krab followed by introduction of pooled sgRNA virus vectors for gene perturbation. Perturbed cells will undergo singlecell sequencing and putative causal genes will be followed up with functional molecular biological methods with regards to glucose and lipid metabolism. Significance: The proposed research project holds the promise to substantially advance knowledge on the pathophysiology of NAFLD. By performing detailed follow-up analyses of loci hypothesized to be involved in NAFLD, causal genes and novel mechanisms of action will be established. Our work will benefit the scientific community, and lead to important new insights about NAFLD and its complications; type 2 diabetes and cardiovascular disease. Stanford provides an unparalleled scientific environment, imperative for the development of the applicant.

Large scale, time- and cost-effective production and commercialization of human ribosome

CRISPR

950 000 SEK

Ribosome, the protein factory of the cell, is central to research and innovation in life science including drugs and diseases, cell-free expression of toxic proteins or incorporation of unnatural amino acids. The conventional ultracentrifugation-based ribosome purification is lengthy, time and money consuming. Purifying eukaryotic ribosomes, particularly the mitochondrial ones is highly challenging due to low density of the ribosomes. Ribosomes are not commercially available.Under VR grants (2006-4530 & 2013-8778) we succeeded to establish a fast and easy, affinity tag based method for purification of bacterial ribosome from Escherichia coli (Ederth et al., NAR, 2009) and Mycobacteria tuberculosis (Ge et al., in review). In this proof-of-concept project, we aim to develop a similar method for tag-based purification of the eukaryotic (both cytosolic and mitochondrial) ribosomes from human (HeLa / Hek293) cells. Using CRISPR-CAS technology, His/ FLAG tag will be introduced at the chosen r-protein gene loci. Then single-step affinity purification of the cyto- and mito- ribosomes will be developed from the genome edited cells. This time and cost effective method will revolutionize research and innovation (drug development) with ribosomes. Biopharmaceutical companies will be interested for checking cross-reactivity of the antimicrobial drugs. The tagged ribosomes, the engineered cell-lines and the ribosome purification service will be commercialized through RTTF-Technologies.

Developing a unique high throughput platform for identification of essential cellular factor for virus replication Cycle

Mutagenesis

25 200 000 SEK

Emerging and re-emerging Zoonotic pathogens are challenging human and animal health all around the world, but nonetheless for most of these bio-threats there is no good antiviral treatment available. The identification/characterization of cellular factors exploited by the pathogens are essential for development of novel antiviral drugs. To improve the situation, we propose to develop a powerful and unique screening platform that enables to rapidly identify host cell factors that are essentially required by pathogens for infection and replication. By using the power of our developed haploid embryonic stem cells (ESCs) library, where every single gene is reversibly mutated, we will establish an in vitro infection model system, which will facilitate characterization of the essential cellular genes for virus replication. On the top of this, we have recently developed a novel mutagenesis system that allows to map host factor interactions at amino acid resolution, which in normal screens are impossible to find. In addition, we have developed 3D human organoids, which can be the ultimate model for validation of the identified cell factors.

Furthuremore We will adapt our already developed microfluidic system to these organoids, which will give us an unique possibility to investigate the function of our identified cellular factors. These tools, in concert, will give us a powerful platform to define and characterize the most essential cellular factor for the virus infection.

Developing new tools for breeding blast resistant wheat

CRISPR

4 332 811 SEK

In February 2016, the first observation ever of wheat blast caused by Magnaporthe oryzae pathotype Triticum (MoT) beyond South America was recorded in Bangladesh. The extent of the wheat blast outbreak in Bangladesh was considerable and rapid, with 15,000 hectares affected between February to March 2016, and is estimated to have affected at least 45,000 farmers, the majority of whom are resource-poor smallholders. Most importantly this first introduction of a highly virulent form of MoT, in heavily populated South Asia represents a serious threat not only for Bangladesh, but also for regional food security in S. Asia, home to 300 million undernourished people and whose inhabitants consume over 100 million tons of wheat each year. The speed by which MoT however spread across Bangladesh in 2016 was alarming, indicating that if blast were to further spread in South Asia, inaction to mitigate the disease could lead adversely affect the food and income security of over a billion people.

There is thus an urgent need to characterize these isolates to identify their morphology, virulence, fungicide resistance and genome evolution at various geographical locations. This work which includes disease surveillance, marker development and participatory trials would help researchers and breeders to identify solutions for the sustainable wheat production in the region in presence of this new highly virulent pathogen. This will be critical to avoid a major potential disaster.

The role of FT-like genes in the regulation of the annual growth cycle in trees

CRISPR

3 200 000 SEK

The goal of this project is to understand the role of the Populus FT-like genes in the regulation of the annual growth cycle in trees. We have previously shown that FT-like genes not only function as regulators of flowering time, but can also regulate other photoperiodically regulated processes such as growth cessation and bud set in trees. We were also the first to show how sub- and neo-functionalization of FT paralogs in sugar beet has evolved to provide new ways to control the biannual life strategy. We now want to understand the role of the two FT-like genes in Populus trees (Aspens and Poplars). Our preliminary data, based on CRISPR-Cas9 experiments, show that the paralogs have subfunctionalized. One of the genes, at the end of the growing season, controls growth cessation, bud set and entry into winter dormancy, while the other gene controls the release of winter dormancy and/or bud flush in the spring. Together, the two FT paralogs therefore control all major aspects of the annual growth cycle in Populus trees, and are probably also instrumental in the regulation of Populus flowering time. We seek resource for a graduate student that will make a detailed analysis of the function and regulation of the two paralogs in order to get a mechanistic understanding of their roles and sub-functionalization. This will be important for our understanding of how trees adapt to growth at different latitudes, and how this adaptation will be influenced by climate change.

CRISPRi for elucidating stress regulation in yeast

CRISPR

3 300 000 SEK

Phenotypic traits are affected by an interplay of genes, thus redirecting cellular fluxes may require alteration of the expression of many genes. Transcription factors (TFs) control the expression of target genes, forming a refined regulatory network. My aim is to gain novel insight in how the regulatory cascades of a cell influence its phenotypic traits.I will individually alter the expression of 10-20 TFs involved in stress response of Saccharomyces cerevisiae through the CRISPRi technology. In CRISPRi, an RNA guided protein, dCas9, fused to an activator or repressor is targeted to a specific locus by a co-expressed RNA. By targeting a dCas9-fusion to a promoter, the expression of a TF is gradually altered, while still allowing native regulation. By altering the expression of a TF, the whole underlying regulatory network is altered and even small perturbations can greatly impact the regulatory network and cell fitness.

For further understanding, I will combine the CRISPRi-based change in expression with determination of physical binding of the TFs, through Chip-Sequencing.The generated CRISPRi strain library will be screened for increased inhibitor tolerance, a key feature of industrial production hosts. Transcriptomic analysis will be done on selected strains for identifying changes in transcription leading to increased tolerance. A thorough understanding of cellular regulation is needed for the development of cell factories for biochemical conversion of renewable carbon.

Plant Biotech in sub-Saharan Africa – technology transfer and new policies

Genome editing

800 000 SEK

Sub-Saharan Africa (SSA) with 70% of its population still living rural area is the region with the highest prevalence of undernourishment. To abolish hunger and feed a growing population without depleting natural resources, new ideas and tools are required. As part of sustainable agriculture, biotechnology can play a key role to improve food production in SSA. However, the limited biotechnical capacities and the current policy in many SSA countries for regulating these technologies hamper the realization of their full potential in the region.We therefore want to (i) improve knowledge-exchange and capacity building to better implement plant biotech in SSA, especially the emerging technique of genome editing, and (ii) explore the implications of converging and/or diverging plant biotechnology policies in the EU and Africa.We focus on cassava and potato, two important staple crops in SSA facing different challenges: Cassava is nutrient-poor and potato is easily devastated by late blight. Genome editing is a promising way to increase Provitamin A and combat vitamin A deficiency, affecting millions of people. Better late blight resistance would decrease yield-losses and fungicide use, and thus increase income while reducing impact farmers' health.At the same time, it is important that biotech policies and regulations in SSA are supportive rather than restrictive or prohibitive.

One contribution of this proposal is to increase the dialogue between researchers and policy-makers.

Regulation and functional role of translational stop codon readthrough

Gene editing, RNAi

2 820 000 SEK

Translational readthrough (TR) refers to when the ribosome continues beyond a stop codon and synthesizes alternative protein isoforms. Comparative genome analyses indicate that TR is a universal mechanism that increases the proteome of cells, tissues and organisms. Some studies suggest that TR may be genetically programmed, and also regulated by external stimuli. A few studies in cell culture have shown that TR of specific mRNAs do occur at relatively high frequency, but almost nothing is known about the functional roles of TR in whole animals. We will use the favourable genetic model Drosophila to address this.

The aim is to gain comprehensive understanding of the underlying mechanisms of regulated TR, and of its role in biological processes in vivo. We will focus on a set of ten transcription factors with predicted TR, involved in developmental decisions and regulatory switches. The functional importance of transcription factor isoforms generated by TR will be studied by a combination of genetic tools, such as the CRIPSR/Cas9 gene editing technology, transgenesis, RNA sequencing, RNAi and chemical library screens, and advanced imaging. We will also perform whole genome analyses of TR using ribosomal profiling, and identify genes that are co-regulated by the same external stimuli. Together this will answer essential biological questions on TR and the downstream phenotypic consequences. It may also serve as a start for drug design against certain types of genetic diseases.

Establishment of epigenetic landscapes in the Drosophila embryo

CRISPR

3 270 000 SEK

Creation of different cell types from an identical DNA sequence is a remarkable property of genomes and a fundamental question in biology. Cell type-specific gene expression is initiated by transcription factors and maintained by epigenetic mechanisms. Although epigenomes of many cell types have been sampled, how differences in epigenetic information between cells arise is poorly understood. We will use the unique experimental advantages of the Drosophila embryo to identify how tissue-specific epigenetic patterns are established in vivo. Chromatin immunoprecipitation (ChIP) and precision nuclear run-on (PRO) experiments coupled to high throughput sequencing will be combined with transgenic expression assays, reporter genes and CRISPR/Cas9based epigenome engineering. Using these approaches, we will 1) Determine the mechanisms by which Dorsal (NFkB) uses the CBP coregulator to activate transcription and histone acetylation 2) Investigate how the Snail repressor interferes with CBP to quench histone acetylation 3) Identify factors that recruit Polycomb proteins to methylate histones. 4) Identify the DNA sequences that establish epigenetic patterns in different tissues. Our studies provide a link between the information for cell specification that is hard-wired in the DNA with epigenetic regulation. These in vivo experiments provide mechanisms for cell differentiation and formation of epigenetic landscapes that cannot be obtained by cell culture based or biochemical assays.

DNA-PK-dependent strategies to promote genome stability

Genome editing

2 400 000 SEK

The eukaryotic linear chromosomes require mechanisms to protect the DNA ends. Loss of telomere protection has been associated with aging, genome instability and cancer progression. The DNA dependent-Protein Kinase catalytic subunit (DNA-PK) plays a critical role in preventing genome instability, and inhibitors of DNA-PK are under clinical test for cancer therapy. The purpose of this research is to resolve the molecular mechanisms by which DNA-PK protects the telomeres and contributes to the overall genome stability in normal and malignant cells. The project involves one project leader, one PhD student and one postdocs. In the first and second year, we will combine a candidate approach with a mass spectroscopy-based screen to identify DNA-PK's targets involved in telomere metabolism. By the end of the third year, we will validate the targets using a panel of genetically modified mouse cell lines. In parallel, we will elucidate the conservation of the telomeric role of DNAPK in human telomere metabolism using different human cell lines. We will combine standard molecular and cell biology methods with genome editing, protein-protein interaction assays, immunofluorescence, telomere overhang assay and metaphase analysis, as well as telomere visualization by electron microscopy. This study will reveal how DNA-PK works at the chromosome ends and we believe this will facilitate the generation of more effective treatment strategies for patients with tumors reliant on DNA-PK function.

Development of gene therapy targeting amyloid-β and α-synuclein on cell and mouse models for Alzheimer's disease and Parkinson's disease

CRISPR

2 400 000 SEK

Gene- and immunotherapy against amyloid-beta (Ab) and alphasynuclein (a-syn) have begun to be explored for Alzheimer's disease (AD) and Parkinson's disease (PD). We showed both ex vivo and in vivo that CRISPR/Cas9 can disrupt expression of the amyloid precursor protein gene (APP) with the Swedish mutation (APPSwe) and also reduce Ab generation in patient cells. Moreover, we have showed that antibodies against toxic Ab and a-syn oligomeric can prevent brain pathology in transgenic mice.We will now study the use of CRISPR/Cas9 also against presenilin 1 mutations. Moreover, we will use adenoassociated virus (AAV)-vectors to target APPSwe alleles in neurons from induced pluripotent stem cells derived from patient fibroblasts. AAVmediated CRISPR/Cas9 will also be applied to APPSwe knock-in mice, to study whether behavior and brain pathology can be reduced compared to vector-only treated mice.We will also deliver our oligomer-selective Ab and a-syn antibodies mAb158 and mAb5 via AAV-vectors to APP and a-syn transgenic mice. The mice will be assessed and compared to vector-only treated mice with respect to behavior and brain pathology.This program aims at developing AAV-based delivery of CRISPR/Cas9 and antibodies targeting Ab and a-syn. CRISPR/Cas9 trials are underway for cancer disorders and should have a clear potential also for familial forms of AD and PD. Furthermore, AAV-based antibody delivery could further enhance immunotherapy efficacy for the same disorders.

Deciphering the role of programmed DNA breaks in aging

Genome editing, CRISPR, mutagenesis

7 200 000 SEK

Emerging evidence indicates that, during transcription, physiologically programmed DNA double-strand breaks (pDSBs) transiently form inside gene regulatory regions, such as promoters, as a consequence of topoisomerase activity. Although functionally important, these pDSBs can occasionally be misrepaired and result in mutations.

Here, we hypothesize that, during organismal aging, pDSBs lead to progressive accumulation of such mutations inside promoters, which in turn contributes to set the transcriptional changes characteristic of aging. To test this hypothesis, we pursue three main objectives: first, we identify fragile promoters, by charting the landscape of pDSBs in cells from the same subjects at different ages, using our powerful method, BLISS, for genome-wide DSB localization (years 1–2). Second, we identify pDSBdependent mutations, by performing deep sequencing of fragile promoters in DNA derived from normal individuals spanning a broad age range, including centenarians (years 2–3). Third, we assess the effects on transcription of individual pDSB-associated mutations, by performing CRISPR-based genome editing and single-molecule RNA FISH (years 3–4). This is a pioneering effort that aims to link physiologic DNA breakage with aging, using a unique cohort of samples and methods. As such, our project has the potential to uncover novel mechanisms of aging and lead to a better understanding of the extraordinary lifespan of centenarians.

Mutational heterogeneity in non-coding regions of tumor genomes

CRISPR

4 800 000 SEK

The overall goal of this proposal is to improve detection of driver mutations in cancer through better understanding of how mutations arise and distribute across tumor genomes, so called mutational heterogeneity. In particular, we want to determine the basis of heterogeneous mutation patterns in UV-exposed tumors, where locally elevated mutation rates in non-coding promoter regions have posed challenges in recent times.

To this end, we will generate the first high-resolution maps of UV damage (pyrimidine dimer) formation in the human genome. We will also develop new bioinformatics methods for describing mutational processes in tumors, which will be generally applicable to human cancers. This combined computational and experimental project, that involves one postdoc and two PhD students during five years, will provide insight into mutational processes in human cancers to facilitate separation of functional cancer driver mutations from non-functional passengers, a key challenge in cancer genomics.

Studies of primary human immunodeficencies affecting cytotoxic lymphocyte function

Genome editing

4 800 000 SEK

Cytotoxic lymphocytes provide essential immune protection. Although recent advances have provided molecular diagnoses for severe cases of primary immunodeficiencies caused by impaired lymphocyte cytotoxicity, a majority of patients cannot be explained by current insights. My team has elucidated fundamental mechanisms of lymphocyte cytotoxicity and developed diagnostic assays. In identifying specialized human adaptive NK cell and tissue-resident memory CD8+ T cell subsets, we recently uncovered novel epigenetic diversification processes. Yet, many molecular aspects of cytotoxic lymphocyte differentiation and function remain unclear. I outline efforts to dissect the molecular mechanisms and regulatory regions that control human cytotoxic lymphocyte diversification and function. Our objectives will be addressed using a combination of unique PID patient samples and cutting-edge technologies, including single-cell analyses, genome-wide epigenetic and architectural interrogation, and genome editing, all combined with advanced computational analyses. These complimentary approaches promise to identify and validate novel disease-causing mutations. Generating dynamic maps of cytotoxic lymphocyte circuitry, we can provide fundamental insights to human lymphocyte cytotoxicity. Taking full advantage of a vast clinical network, cutting-edge techniques, and strong collaborations, our ground-breaking studies may benefit patients through improved diagnosis, directing targeted therapies.

Neural cell dysfunction in aging and neurodegeneration

Genome editing, CRISPR

4 000 000 SEK

The main aim of my research is to gain cellular, molecular and functional insights and understanding of neural aging and disease. In this project we will use innovative technologies in the fields of stem cell biology, reprogramming and genome editing to study and model human neural aging and neurodegenerative disorders.To generate isogenic human neural cells predisposed to neurodegeneration and aging, we will use CRISPR/Cas9 genome engineering to introduce mutations known to cause frontotemporal dementia and Alzheimer's disease or knock out longevity genes in human pluripotent stem cells. Targeted lines will then be differentiated to neurons as well as astrocytes using novel protocols I took part in developing or developed in my lab. Next we will in detail analyze how these mutations and genes affect neural cell structure, molecular properties and function.Furthermore, we will isolate newborn neurons from different regions in the brains of young, middle aged as well as aged mice and perform global transcriptomic analysis to understand the effect of aging and the aged environment on neurons.Results from these studies have the potential to be extremely important for how we perceive neural aging and degeneration and be the basis for novel therapeutic intervention.

Transposable elements and the human brain - implications for psychiatric disorders

Gene editing, CRISPR

4 800 000 SEK

The complexity of human brain development differs markedly from other mammals and is thought to underlie the emergence of human cognition. However, the genetic basis for this complexity remains largely unknown. In this project we combine epigenomics and gene editing to unravel the role of transposable elements (TEs) in this process. The project is based on our recent findings describing that the transcriptional co-repressor TRIM28 binds to TEs in neural progenitor cells (NPCs) and mediates transcriptional silencing by depositing H3K9me3. Since the H3K9me3 mark can spread into the neighbouring chromatin, TRIM28-bound TEs have the capacity to serve as "hubs" that mediate silencing of nearby genes. Considering that the composition of TEs varies much between species, this mechanism has the potential to mediate species-specific transcriptional networks. In this proposal we will use human, chimpanzee and orangutan NPC cultures to perform direct comparative epigenomic studies. We will identify TEs that are bound by TRIM28 in either a human-specific or primate-conserved manner and then use CRISPRbased screens to identify TEs that contribute to forebrain expansion. By deleting individual TEs in human NPCs we will mechanistically dissect how TEs contribute to NPC functions. These experiments will elucidate the role of TEs in the role of human brain evolution and may in the long run provide novel diagnostics and therapeutic approaches for neurodevelopmental and psychiatric disorders.

Epigenetics of Multiple Sclerosis: towards a better understanding of pathogenic mechanisms and improved disease management

Genome editing

4 800 000 SEK

Multiple Sclerosis (MS) is a leading cause of incurable progressive neurological disability in young adults. We propose that epigenetic mechanisms such as DNA methylation, that regulate gene expression without affecting the genetic code, mediate the processes that cause MS and that aberrant epigenetic states can be corrected to develop alternative therapeutic modalities. We will exploit the stable and reversible nature of DNA methylation to: (i) Identify novel modifiable mechanisms that trigger MS and predispose for disease progression, (ii) Develop alternative therapeutic strategies based on reversing causal epigenetic states, and (iii) Characterize epigenetic marks that can serve as specific biomarkers. Our unique MS biobank combined with cuttingedge methodologies to capture relevant cells and measure their functional states from the full methylome and transcriptome data provides a starting point to identify aberrant epigenetic changes in MS. This will be complemented with studies of the functional impact of these changes using innovative in vitro epigenome screens. We will then utilize in vivo animal models to dissect their molecular mechanisms and test the therapeutic potential of targeted epigenome editing. Our findings may set the stage for a paradigm-shift in studying and treating chronic diseases based on preventing and modulating aggressive immune responses and promoting brain tissue repair through inducing self-sustained reversal of aberrant epigenetic states.

Targeting cancer stem cells in high-grade brain tumours

CRISPR

2 400 000 SEK

Relapse is a major concern in cancer therapy. Cancer stem cells have emerged as the major driving force governing tumour recurrence. Designing treatments that specifically target the cancer stem cells and not the normal stem cells is a major challenge. The aim of this proposal is to increase the survival of patients with central nervous system tumours while decreasing side-effects from treatment. The specific aims are: (1) To identify genes involved in the development/progression of paediatric brain tumours.(2) To determine the role of the identified candidates and design/identify drugs or epigenetic modulators that targets them.(3) To validate the role of the identified candidates in vivo and test the efficacy of new drugs/modulators to target the tumours. We will use primary cancer stem cell cultures established from paediatric brain tumours and xenograft mouse models for our analyses. Methods used in the project include next-generation sequencing of DNA, DNA methylation, RNA, miRNA, chromatin accessibility and CRISPR/Cas9 manipulation of the genome and epigenome. The unique model systems developed by the applicant, expertise in methods and key team members and collaborators certify the successful completion of the project. The applicant’s strong record at performing science that have impacted and implemented new avenues for clinical diagnostics will be a solid basis for translating the findings into new opportunities for improved therapy.

Migration towards the next generation-a better understanding of mammalian germ cells and germ cell tumors

CRISPR

4 800 000 SEK

The proper differentiation of primordial germ cells (PGCs) to functional gametes is crucial for precise transmission of the genetic information to the next generation and assuring normal fertility of the individual. During this intricate process, PGCs have to accomplish several challenges including a lengthy migration, regain of pluripotency and erasure of epigenetic memory. Despite decades of research, little is known about how PGCs coordinate proliferation, genetic and epigenetic reprogramming of directional migration across diverse microenvironments. Further investigation will not only uncover the mechanisms underlying the origin of germ cell tumors (GCTs) but also fully harness the potential to derive functional gametes for regenerative medicine. Within this proposal, we aim to combine genetics lineage tracing with LCM-seq (laser capture microdissection with single-cell RNA-seq) to profile PGCs along their migratory route. Comparing leading, lagging and stray PGCs, we will be able to reveal the fail-safe mechanism that prevents lagging and stray PGCs from forming tumors. Moreover, CRISPR/Cas9 functional screening together with ex-utero embryo culture will identify signaling pathways for migration to reconstitute PGCs in vitro towards the late gonadal stage for disease modeling. We will apply single nucleus RNA-seq to probe the origin of GCTs in comparison with migrating PGCs. Finally, the impact of maternal diet and hyperandrogenism on oocytes will also be investigated.

DNMT3A in acute myeloid leukemia - exploring mechnisms for leukemogenesis and developing targeted treatment

Gene editing

4 800 000 SEK

In this project, we are developing a novel treatment strategy against acute myeloid leukemia (AML) with mutant DNMT3A. In addition, we will define the mechanisms by which DNMT3A mutations drive leukemogenesis. We build on our previous experience with early drug development, epigenetics and DNMT3A mutations and on our participation in the important discovery of clonal hematopoiesis. Clonal hematopoiesis is a condition found in a substantial proportion of older individuals without hematologic disease. The condition is predisposing for hematoloical malignancies and is dominated by DNMT3A mutations. We aim to develop a novel treatment approach that inhibits growth of DNMT3A mutated clones and thereby preventing the development of AML, specifically targeting DNMT3A mutated stem cells. It is still unknown how DNMT3A mutations drive leukemia development. Thus, we will define aberrant and proleukemic effects of the R882 DNMT3A mutant on DNA binding, DNA methylation, epigenetic regulation, regulation of long non-coding RNAs and on the proteome. We specifically aim to define genes that mediate DNMT3A driven leukemogenesis. Such discoveries can identify novel treatment targets for the disease. For our studies, we use leukemia samples from large cohorts of AML patients, DNMT3A mutant cell and mice models as well as high-through-put techniques for sequencing of DNA, RNA, novel proteomic techniques and cutting edge gene editing methods for in-depth analysis of key regulatory events.

Identification of novel restriction factors against alphaherpesviruses

CRISPR

6 300 000 SEK

The first line of defense against infections can occur through cellextrinsic or cell-autonomous mechanisms. It has been known for many years that natural killer cells and the type I interferon (IFN) system are the central mediators of early cell-extrinsic antiviral defense. However, little is known about cell-autonomous antiviral defense, where restriction factors (RFs) represent one class. We have performed genome-wide CRISPR screens to identify candidate RFs against herpes simplex virus (HSV)1, one of three human alpha-herpesviruses, which are neurotropic (preliminary results). In this project we will first characterize the mechanism of action of a panel of the novel RFs. Second, we will use gene-modified mice and stem cell-derived neurons to explore the physiological importance of the novel RFs. Finally, through access to more than 500 exomes from patients with severe CNS disease caused by alphaherpesviruses we will look for naturally occurring loss-of-function mutations in the novel RFs, and characterize impact on control of infection. Strong preliminary data have been generated, unique reagents are available, and world-leading collaborations are in place. The required methodological expertise ranging from virology and molecular biology via animal science and stem cell biology to functional studies is in place. If successful, the results will lead to identification and characterization of novel RFs, as well as demonstration of their physiological importance.

YAP/TAZ regulation of lung epithelial progenitor cells during repair

CRISPR

6 000 000 SEK

Idiopathic Pulmonary Fibrosis (IPF) is a chronic lung disease characterized by progressive extracellular matrix deposition and shortness of breath. Two newly approved therapies are not curative and result in slowing IPF progression but are accompanied by severe side effects. 5 year mortality is around 50%, worse than many malignancies. Understanding the exact mechanisms involved in IPF progression may reveal new drug targets.

Recently, we identified dysregulation of a development pathway, Hippo signaling, and hyperactivation of its nuclear effectors YAP/TAZ in IPF, in particular in the epithelium. In this project, we will study the regulation of YAP/TAZ under the prototypical pro-fibrotic stimuli TGFβ and its interplay with Hippo signaling. We will perform co-immunoprecipitation followed by mass spectrometry proteomics to identify protein-protein interactions in the cytoplasm and nucleus to identify novel YAP/TAZ binding partners which regulate epithelial cell behavior. We will explore the functional consequences of these proteins using CRISPR/Cas9 gene editing in isolated primary lung epithelial progenitor cells in 2D and in 3D models of organoids and acellular lung scaffolds from normal and fibrotic murine lungs. The work in this proposal will be a significant step forward for further understanding the role of YAP/TAZ and Hippo signaling in the adult lung epithelium and will provide new opportunities for designing novel therapies which might revert pulmonary fibrosis.

A precision medicine platform to identify novel redox dependent metabolic vulnerabilities in lung cancer

CRISPR

6 000 000 SEK

I propose to build a precision medicine platform at Gothenburg University (GU) using state-of-the-art mouse models to identify novel redox dependent metabolic vulnerabilities in lung cancer. Specifically, I plan to identify metabolic vulnerabilities in KRAS-driven lung cancers by combining a high-throughput screening platform using lentiviralCRISPR/Cas9 strategies and genetically engineered mouse models. In these models, endogenous tumors develop from healthy cells in an authentic microenvironment in the lungs of mice with an intact immune system; and produce non-small cell lung cancer. We will use lentiviralCRISPR/Cas9 strategies to inactivate any gene-of-interest at tumor onset or later during tumor progression and metastasis. We will also develop patient-derived xenograft models with matching patient derived lymphocytes to model immunotherapies. We will use this platform for a range of lung cancer experiments in my own group and in collaborative efforts at GU. We have recently shown that activation of Nrf2, the endogenous antioxidant pathway, accelerate lung cancer progression at the cost of a drugable metabolic dependence on exogenous glutamine.

Here I propose to utilize my knowledge and experience of building a precision medicine platform combined with the latest technologies in lung cancer research to establish an extensive translational lung cancer group in Sahlgrenska Cancer Center where we will define novel redox dependent metabolic vulnerabilities in lung cancer.

CRISPR-Cas gene therapy for monogenic blood disorders

CRISPR, genome editing

6 000 000 SEK

AIMS: We aim to develop a CRISPR-Cas-based gene therapy platform for monogenic blood disorders. BACKGROUND: Monogenic diseases of the hematopoietic system can present as immunological and malignant diseases, hemoglobinopathies, or coagulation defects. CRISPR-Cas gene editing system can correct the disease-causing mutation in harvested patient stem cells ex vivo. After correction, the cells can be infused back to the patient which cures the disease in an optimal case. WORKPLAN: To increase safety and efficiency for mutation correction, we have optimized the editing process in various ways. We have attached different DNA repair proteins to Cas9 and timed the Cas9 activity with cell cycle, as precise mutation correction only happens in specific phases of the cell growth and division. Furthermore, we have shown that transient p53 inhibition is necessary for efficient gene correction and cell survival.In subsequent studies, we will combine all these improvements into a single gene editing platform. We will extensively test the platform for off-target cutting and efficiency in hematopoietic cells and mouse models, with the aim of translating it to clinical trials within the next 5 years. SIGNIFICANCE: If successful, the method can treat a wide variety of diseases and mutations. It will require only light conditioning chemotherapy and will not induce graft-versushost disease, making it superior to allogeneic bone marrow transplant, the standard therapy for severe blood diseases.

Deciphering the transcriptional landscape of mast cell differentiation in normal and aberrant hematopoiesis

CRISPR

6 000 000 SEK

Systemic mastocytosis (SM) is a severe, currently incurable hematologic neoplasm, driven by the accumulation of aberrant mast cells in several tissues. The goal of the present proposal, for which I am principal investigator, is to decipher the mast cell differentiation in normal and perturbed hematopoiesis. The long-term aim is to improve the diagnosis and treatment of SM.A single cell transcriptional landscape of human hematopoiesis will be generated during the first 18 months. Microfluidicsbased single cell RNA sequencing of healthy bone marrow and bioinformatics analysis, performed partly in collaboration with prof Göttgens' laboratory (University of Cambridge), will reveal the mast cell differentiation trajectory. Single cell sorting and culture will verify mast cell-forming potential throughout the trajectory.In parallel, my PhD student will use a deep single cell RNA sequencing protocol (improved Smart-seq2) to compare normal and perturbed mast cell differentiation. Mast cells and their progenitors are index sorted and sequenced from SM patients and controls.

Associations between genetic mutations, aberrant CD2/CD25/CD45RA expression, and gene expression will be revealed.

A mouse model-based CRISPR-Cas9 knockout screen is used to verify which genes that promote mast cell differentiation throughout the project period. The projects described will uncover genes that perturb mast cell differentiation, which can lead to new therapeutic options for SM.

Assessing the conservation of transcriptional networks driving pancreas and nervous system differentiation

CRISPR

3 150 000 SEK

Both, pancreas and nervous system contain highly specified cell types with shared secretory activities but distinct physiological functions i.e. metabolism vs. signal transduction. Intriguingly, many genes are shared across the two tissues. In this project, I intend to molecularly decode the conservation of gene regulatory networks (GRNs) of transcription factors, crucial for pancreas and spinal cord development. In addition, I aim to dissect the conservation of gene regulatory regions for key genes, controlling spatio-temporal gene expression. This will provide a regulatory map to help understand disease-causing mutations in noncoding regions controlling appropriate cell biogenesis in development. The project will be carried out in the laboratory of Prof. Anne GrapinBotton, whose work has had a major impact in the field of pancreas development. Her lab uniquely developed pancreas organoid systems that will provide the throughput for GRN validation. The data on the nervous system will be provided by our collaborator Dr. James Briscoe (Neurobiologist, PI at "The Francis Crick Institute", London). In the first and second year, I will establish transcriptomes and map regulatory regions. In the second and third years, I will analyze the data, experimentally validate selected GRNs and draft a manuscript. Methods include, cell culture of mouse/hESC derived pancreatic progenitors and organoids, single cell RNA-seq, ATAC-seq, enhancer analysis and CRISPR/Cas9 mutagenesis.

Delineating the molecular determinants and evolutionary dynamics of tumorigenesis and resistance

Genome editing

3 150 000 SEK

To increase the success rate of treating and preventing cancer it is imperative to understand the evolutionary aspects of the disease. Recent innovations, including organoid culturing systems and genome editing makes it possible to study tumor evolution in a natural environment in vitro, and innovations in cellular barcoding, where each cell gets a unique DNA barcode inserted into its genome, enables studying the process on a subclone basis. Being at Stanford, affiliated with two labs that are on the forefront of growing and transforming organoids into tumors and modeling tumor evolution, enables me to have a unique perspective on tumor evolution during tumorigenesis and in response to drugs.

For example, we have preliminary results showing that independent gastric organoid clones from different patients evolve similar chromosomal lesions in response to a TP53 mutation, and that the establishment of these mutations in the entire population occurs quickly, often within 3 months. The proposed three-year project delineates the evolutionary dynamics of tumorigenesis and resistance, which are the two areas where we believe the combination of organoids and cellular barcoding are most likely to produce novel and important results. If it turns out, as our preliminary results indicate, that there is large degree of determinism in tumor evolution, this would lead to more accurate models of tumor evolution, as well as enabling improved methods to diagnose and predict disease outcome.

Genetic variation exposes regulators of blood cell regeneration in vivo in humans

Genome editing

4 800 000 SEK

We will explore an innovative, population-genetic approach to find regulators of blood cell formation. Unlike traditional studies in vitro or in animal models, we will use genetic variation to find DNA sequence variants and genes that influence blood cell formation in vivo in humans. Building on our previous work, unique sample materials, mathematical modeling, and the latest genomics and genome editing techniques, we will: Develop high-resolution association data and analysis methods to find sequence variants influencing human hematopoiesis, including stem- and progenitor stages. Identify DNA sequence variants and genes influencing specific stages of adult and fetal/perinatal hematopoiesis. Define the function, and disease associations, of identified variants and genes. Led by our lab, this large-scale effort will involve researchers at Lund University and several collaborating institutions. It will be carried out in strong research environments, and has been preceded by significant preparatory work, including a pilot study on 7,000 samples. It will provide a first detailed analysis of how natural genetic variation influences development in the human hematopoietic system, potentially exposing novel regulatory mechanisms that can be utilized for the treatment of malignant blood disorders.

Identify and explore bacteria-produced mosquitocidal molecules

CRISPR

3 150 000 SEK

New tools for the control of mosquito-borne diseases are acutely needed as over 50% of the worlds' population today live at risk of acquiring diseases transmitted by mosquitoes, for example by Anopheles gambiae (malaria) and Aedes aegypti (dengue, Zika, etc). The goal of this proposal is to gain a deeper knowledge on the biology of a Chromobacterium species (Csp_P) that mediate mosquitocidal activity. Specifically, I aim to identify and explore Csp_P-produced mosquitocidal molecules, and thereby lay the groundwork for the development of novel strategies for vector control.To reach the goal of this project, a multidisciplinary set of bioinformatic, biochemical, in vitro and in vivo methods will be used.

The first two years will include bioassay-guided HPLC-based product discovery, comparative genomics, RNA sequencing, mutagenesis, and be conducted at the Johns Hopkins University, USA. The final year will include viral vector competence studies on mosquitoes at Umeå University. The research environment both at the international host and home institute, including technical capabilities, infrastructural resources, expertise and intellectual environment, is ideal for the proposed studies. Expected outcome is the discovery of promising mosquitocidal molecule(s), and a better understanding of mosquito-bacteria interactions, of value for the development of new strategies for vectorborne disease control.

Dissecting the role of neutrophils in auto-antibody mediated autoimmune disease

CRISPR

3 150 000 SEK

Autoimmune diseases are a common source of pain and disability in society but little is known about the mechanisms that drives the disease. This project aims to understand fundamental interactions between the innate and adaptive immune system: how neutrophils generate targets for antibodies and how this contributes to autoimmune inflammation. The long-term goal for my research is to gain insight how an autoimmune disease turns chronic and this pathological process can be inhibited.The initial work is ongoing at Drs. Wermeling and Klareskog labs at KI to learn CRISPR screens, involving molecular biology, next generation sequencing and bioinformatics. Interactions between antibodies and neutrophil will also be studied using a unique library of patient-derived monoclonal antibodies developed at the Rheumatology Unit, KI. This summer I will move to Dr. Kaplan lab at NIH to apply the CRISPR screen and antibody assay in a functional setting, studying neutrophil behavior and extracellular trap (NET) formation in particular using various in vitro and in vivo models.The Dr. Kaplan lab is world-leading in neutrophil biology and a very creative environment, which is important form my development as an independent scientist. Additionally, this project would give me the opportunity to create an international professional platform, which is essential for my future career when I return to Sweden.

2017

Beyond additivity, unlocking the contribution of gene-gene and gene-environment interaction to individual variation

CRISPR

3 150 000 SEK

Technological advances have recently fueled the ascent of personal genomics and the promise of precision medicine. The success of medical genetics in realizing this promise will depend on its capacity to predict the disease risk of individual patients based on their genotypes - a grand challenge. Such individualized prediction requires a shift in focus, from average effects of alleles in a population, to their effects in the genetic and environmental context of particular individuals. I will address this problem by taking advantage of a synthetic outbred population of Drosophila, developed by my host lab at Princeton University. The lab has also developed a unique pipeline, allowing me to sequence the genomes and transcriptomes of thousands of flies. During year one I will use this new resource to rear thousands of genetically unique flies, expose one group to a high sugar diet, and contrast the ensuing genetic architectures underlying variation in lipid homeostasis. During year two I will use CRISPR to engineer genotypes in order to validate findings and test individual predictions. During year three I will use the lab of Helgi B Schiöth to do further functional work, to understand the biological mechanisms underlying previous findings. This research project paves the way for precision medicine by unlocking the context dependence of allelic effects, while also addressing why certain individuals are genetically predisposed to metabolic disorders such as diabetes and obesity

Phenotypic implications of microRNA-mediated dosage compensation in birds

CRISPR

3 150 000 SEK

Across taxa, dosage compensation mechanisms have evolved to resolve the otherwise deleterious expression imbalance of sex chromosomal genes between the two sexes. However, how dosage compensation is achieved in birds remains a mystery. The goal of this project is to provide novel insights into the molecular mechanisms underlying avian dosage compensation. Specifically, we aim to: (i) confirm the hypothesis that mir2954, a conserved avian microRNA, equalizes the expression of dosagesensitive genes on the Z chromosome between the sexes, and (ii) investigate the phenotypic importance of dosage compensation in birds by knocking out this purported molecular mechanism of dosage compensation. The experimental plan entails mir-2954-3p knockout in cell cultures and in embryos using CRISPR/Cas9 (at The Roslin Institute), followed by RNA sequencing and microscopic investigation of embryos. RNA sequencing and associated bioinformatics analyses will be performed at the Heidelberg University (24 months). Investigations of developing embryos will be conducted at Uppsala University (12 months).

The proposed study will employ state-of-the-art technologies at cuttingedge research institutions in the field of cellular/molecular biology and genomics, and aims at solving a fundamental question in evolutionary biology. I am therefore confident that my administrating lab and I will substantially benefit from these established collaborations and from the resulting acquired knowledge and skills.

Virus infections in silkmoths

RNAi, RNA interference

1 020 000

The largest number of insect species described as affected by viruses is found in Lepidoptera (moths and butterflies). Among the commercially important species heavily affected by virus infections is the tasar silkmoth, Antheraea mylitta, which is yearly harvested in India by thousands of tons. Therefore, the aim of this project is to analyze the tasar silkmoth anti-viral defense in general exploring signal pathways and proteins active in the response. Candidate genes will be analyzed with knockdown using RNA interference (RNAi). We also want to compare a newly described iflavirus found in the Chinese oak silkmoth, ApIV with a similar virus in A. mylitta.Wealth in India is unevenly distributed and the farmers engaged in silkworm production (sericulture) are typically poor. The annual income from sericulture is about 10 kSEK for a sericulture farmer, accounting for 60% of the total revenue. Virus infections are abundant, normally ending up in losses of about 10-20%, but sometimes as much as 80%, having major implications on family income for these poor farmers who are dependent on the harvest of pupae. We will in this project merge the knowledge from three countries in Asia with long experience in silkmoth practice with knowledge on viruses and RNAi in Sweden so that we in the end have a good foundation for continuation of the research.

Glutathione transferases provide resistance to chemical stress in rice (Oryza sativa)

Gene editing

1 170 000 SEK

The immediate goal is to characterize two enzymes from rice, GSTL2 and GSTU4, both implicated in protection of rice plants from different kinds of environmental stress including drought, salt, metals and other pollutants. The enzymes could also provide tolerance to herbicides use in rice fields to combat weeds. The enzymes will be obtained from synthetic genes and produced in the laboratory for biochemical experiments with commonly used herbicides. We want to enhance the activity of the enzymes by engineered mutations such that their protective effect is augmented. By elucidating mutations necessary for generating more resistant varieties, rice can be modified via gene editing, which will follow the present project.The work will be done in close collaboration between groups in Stockholm and Cairo. The principal investigator in Stockholm will direct the work and be responsible for the multivariate analysis underlying the molecular engineering. Screening of sets of mutants for enhanced activities will also be done in Stockholm. The purification of enzymes and activity measurements will be made in Cairo. Regular reciprocal visits as well as workshops between the two sites will make all participants engaged in all aspects of the work.The ultimate goal is to obtain new rice cultivars in which designed GST enzymes afford increased resistance to herbicides and other forms of environmental stress. Such varieties could better withstand climate change and increase the rice production.

Yeast-based production of isoprenoid plant hormones

CRISPR

3 600 000 SEK

Isoprenoids represent a large group of natural products that play an important role in our everyday life as food and cosmetic ingredients or pharmaceuticals. Their production is however often not sustainable as many of them are produced by plants that only contain trace amounts of the isoprenoid of interest and their cultivation therefore requires large areas of land. A more sustainable solution is the production by microbial fermentation, where the product pathways are transferred from plants to yeast.Isoprenoid precursors are in yeast produced by the mevalonate pathway. Thus, efficient isoprenoid cell factories need to have an efficient mevalonate (MVA) pathway. In this study, we aim to systematically evaluate heterologous MVA pathways and enzymes to identify novel routes with a superior flux and by this develop yeast strains with increased precursor formation. In the second part of the project, we will then use these improved cell factories for the production of an industrially relevant plant hormone, abscisic acid (ABA). This will also require the full elucidation of a fungal ABA pathway that has so far only been partially characterized. State-of-the art synthetic biology techniques including CRISPRi and genetically encoded biosensors will be used to tackle the challenges in order to develop novel cell factories for the production of plant hormones.

Osmoregulation in the euryhaline barnacle Balanus improvisus: functional characterisation of aquaporins

CRISPR

2 850 000 SEK

This application outlines how to provide ground-breaking knowledge about molecular mechanisms in osmoregulation in an exceptionally euryhaline crustacean, the barnacle Balanus improvisus. This is the only barnacle species living in Swedish waters that can invade the brackish environment of the Baltic Sea. Our earlier publication concerning ion transport via the Na+/K+ ATPase, have recently been complemented by our characterization of its eight aquaporin genes. Aquaporins are poreforming integral membrane proteins that facilitate transport of water and small molecules through cellular membranes, and they are of fundamental functional importance to cells/organisms.We will functionally characterise the aquaporins in B. improvisus via studies of tissue localisation by in situ hybridisation, gene expression via RNA-seq, 3D structure determination as well as heterologous expression and functional analysis in a surrogate system (yeast). Furthermore, we will shed light on different species' evolutionary limitations by comparative studies of aquaporins and osmoregulatory genes in barnacles with variable salinity tolerance. To enable hypothesis testing of aquaporin functionality in vivo we will also develop procedures for CRISPR-cas based gene modifications and apply this to the deletion of individual aquaporins. The information gathered will be instrumental in providing better understanding of barnacle ecology and evolution.

Structural intermediates of cryptochromes and photolyases visualized by serial femtosecond X-ray crystallography.

Gene editing

3 800 000 SEK

Cryptochromes and photolyases are closely related, ancient proteins, which exist in all kingdoms of life. Cryptochromes provide light-input to the circadian clock and enable birds and insects to sense the magnetic field of the earth. Photolyases repair DNA with blue light. These diverse functions are controlled by photocycle intermediates. For understanding with molecular detail these transient structures must be resolved. I propose to uncover the structure of these intermediates for the fruit fly cryptochrome (DmCRY) and photolyase (DmPL6-4) with serial femtosecond crystallography (SFX) at an X-ray Free Electron Lasers (XFELs). I propose a stepwise plan to obtain well ordered, photoactive microcrystals and to solve the transient structures on pico-microsecond time scales. My young research group has in-depth experience with light sensor proteins (Takala, Nature 2014; Björling, Science Advances 2016, Berntsson, Structure, in press) and SFX (Edlund, Sci. Rep. 2016). We have recorded excellent preliminary data for this research project.

Our cryptochrome snapshots will reveal structural details with atomic resolution on how light signals are guided to the circadian clock and how a bi-radical is stabilized, which is crucial for magnetoreception. By uncovering the chemical mechanism of DNA-repair in photolyases, we will map out an ancient mechanism, which is fundamental to all life on earth. This may also enable engineering of photolyases for lightdependent gene editing.

Evolution of mosquito preference for human odour

CRISPR

2 100 000 SEK

Female mosquitoes are major vectors of human disease and the most dangerous are those that preferentially bite humans. These mosquitoes have evolved a remarkable innate preference for human odor that helps them find and bite us. The main purpose of this project is to further dissect the chemical and molecular basis of this behavior. The overall aim of the project is to elucidate the contribution of individual odorant receptors (Ors), ‘tuned’ to behaviorally active salient human and animal odorants, to the host seeking behavior of the anthropophilic African malaria mosquito, Anopheles coluzzii. For this purpose, the expression of select Or genes will be silenced using CRISPR-Cas9, and the resulting mutant lines assessed using electrophysiological recording and high-resolution behavioral analyses. Moreover, by linking Or gene sequences with odor-evoked responses we will gain further insight in the molecular basis of this behavior. For this, we will identify single nucleotide polymorphisms among previously functionally characterized Or homologs of the related species An. arabiensis and An. quadriannulatus, which display opportunistic and zoophilic host preferences, respectively. As a whole, the project will lead to an increased understanding of the molecular genetic basis of preference for humans in disease-vectoring mosquitoes, and lay a foundation for our continued efforts to identify novel tools for integrated vector control.

From sequence to function - evolution of pheromone receptors in a molecular perspective

Mutagenesis

4 100 000 SEK

Lepidoptera with their intriguing pheromones provide many emerging models for studies of both major transitions in communication systems and divergence of pheromones in closely related species with an established set of pheromone components. Whereas recent studies have shed light on how new signals can emerge, almost nothing is known about how matching specific receptors have evolved in a phylogenetic perspective and how differences in selectivity are encoded. An overwhelming number of candidate olfactory receptors (ORs) have become available through transcriptome and genome sequencing but their functional characterization is lagging behind. What is the evolutionary trajectory leading to the current functions of different ORs? (1) First, we will validate high-throughput methods necessary for characterization of ORs. (2) Secondly, we will perform a crossphylogenetic study of pheromone receptors in representative moth and butterfly species, to explore how changes in the chemical composition of the signal have been matched by specificity changes in olfactory receptors. (3) Special attention is given to the co-option of receptors within a given clade for new functions. (4) We will then by mutagenesis and protein modelling study how sequence variation is translated into differences in function and specificity. (5) As a final touch, we will address the Holy Grail in olfactory research and make an attempt to determine the atomic structure of an olfactory receptor.

Evolution of intrinsic barriers under gene flow

CRISPR

3 300 000 SEK

Hybrid zones where incipient species are in contact by gene flow are focus sites for studies of barrier mechanisms. Hybrid zones established after a period of isolation (secondary zones) show intrinsic barriers formed from genetic incompatibilities evolved during the isolation. However, intrinsic barriers are usually not associated with hybrid zones evolved in the face of gene flow (primary zones). As expected, multiple strong and extrinsic barriers have evolved in primary hybrid zones between ecotypes of the snail Littorina saxatilis, but, we recently also found support for a strong intrinsic barrier. In this project we aim for a detailed study of the significance of this barrier, its genetic background and how it has evolved. The study will benefit from the genomic resources developed for this species including an annotated reference genome and a gene map. We will identify the genomic regions and genes involved in the deformation by resequencing snails with and without the deformation from transects sampled over hybrid zones. To be able to eventually test candidate mutations and epistatic interactions involved, we will also optimize CRISPR/Cas9 knockdown for use in the snail system. The project will describe a potentially very important (but hitherto neglected) mechanism contributing to formation of barriers under gene flow, and so shed new light on mechanisms of speciation with gene flow.

Decision-making for children in a state of medical indeterminacy

Gene editing

7 455 000 SEK

Scientific uncertainty in medical care often tests the boundaries of parental authority in caring for their children. Many medical treatments have not been carefully, scientifically validated for their safety and efficacy. Other treatments often have questionable benefits for children or, even if they ameliorate a specific condition, may be carried out in a degrading way. Many innovative treatments offer unrealistic hopes to parents, while others, such as gene editing, may violate children's rights, such as rights to bodily integrity and privacy. In all of these cases, treatments are frequently offered to parents without the information needed to enable them to understand the scientific complexities and uncertainties involved.The proposed project seeks to address the question of how pediatric care decisions are made by parents, clinicians, and in some cases children themselves, especially in a state of rapid medical advances and scientific uncertainty. Thus, the proposed research will focus on a comprehensive review Swedish law (including gaps in the law), which will then serve as a framework for comparative legal analysis in Northern Europe, as well as analysis of obligations under international law.

The proposed research builds on the researchers´ previous work, commissioned by the Council of Europe, to survey the rights of children in health care in a European context. The proposed research is also essential to identify reforms needed to protect children in medical care.

Development of a novel alpha-synuclein lowering strategy based on the CRISPR-interference technology

CRISPR

4 800 000 SEK

Our objective is to develop a novel alpha-synuclein (a-syn) lowering strategy using CRISPR-interference and destabilizing domains. The combination of these novel molecular tools will enable us to develop a tunable and reversible transcriptional repression strategy of a-syn in the brain. Full knock-down of a-syn in iPSC is detrimental for their neuronal function and survival and thus partial and tunable mechanisms of gene repression is important for clinical treatment strategies of synucleinopathies, such as Parkinson's disease (PD). Our aim is to demonstrate efficient and reversible transcriptional repression of a-syn both in in vitro (HEK293 cells and PD patient-derived iPSC) and in vivo (humanized a-syn mouse model and human iPSC grafting paradigms) with this new approach. Tunable gene repression will be tested and demonstrated both regionally in the brain using well established AAV vectors, and globally in the CNS, by implementing a newly developed AAV vector capsid variant. Our final objective will be to test our construct in fully differentiated transplanted patient-derived iPSC by using the new global AAV gene delivery system, in order to demonstrate that our a-syn lowering strategy will have neuroprotective effects in a clinically relevant cell context. We will also study the implications of early versus late intervention in this paradigm, with the aim to show that disease modification by lowering a-syn levels will be most beneficial in the early phase of the disease.

Regenerative Strategies for Liver Disease

Gene editing, CRISPR

4 800 000 SEK

Abstract Our hepatocyte transplant program for the cellular therapy of liver disease and regeneration of liver function is the only such program in Scandinavia. We have identified a new type of stem cell in human placenta that, when transplanted into the liver, expresses liver genes at levels found in adult human liver and is able to correct otherwise lethal liver diseases. It has the ability to modulate the immune system and may be transplanted into patients without immunosuppression. We have an ethical permit to treat up to 10 patients with these stem cells. We will finalize regulatory procedures to begin patient transplants with these stem cells. Most diseases we treat are monogenetic defects. Gene editing technology enables us to correct mutations in a patient's cells (personalized medicine).

We will optimize CRISPR/Cas technology to correct the genetic defect in hepatocytes and liver, in vivo, and will investigate the safety and efficacy of this technology with our novel "liver-humanized" mice where it's liver is highly repopulated with disease-affected human hepatocytes from patients with liver-based metabolic diseases. Safety and efficacy studies with normal animals will not be sufficient. Our studies will be conducted, in vivo, in live animal and on actual disease-affected human hepatocytes, our "humanized" mouse model is perhaps the best and most relevant model available to investigate the safety and efficacy of CRISPR/Cas9 or other gene editing procedures.

Genetic variation exposes regulators of blood cell formation and blood cancer risk

Genome editing

9 000 000 SEK

We combine mathematics and population genomics to study blood disorders and blood cell formation. We are interested in how genetic variation predisposes to blood disorders. We are also interested in exploiting genetic variation to expose regulators of blood cell formation. Building on our previous work, unique sample materials, mathematical modeling, and the latest genomics and genome editing techniques, we will:A. Identify and functionally characterize DNA sequence variants and genes predisposing for malignant blood disorders, particularly multiple myeloma (continuation project). B. Identify and functionally characterize DNA sequence variants and genes influencing human hematopoiesis, including stem- and progenitor stages (new project). Led by our lab, these efforts will involve researchers at Lund University and several collaborating institutions. They will be carried out in strong research environments, and have been preceded by significant preparatory work. They will provide comprehensive, high-resolution analyses of how natural genetic variation influences development and disease risk in the human hematopoietic system, potentially increasing our understanding of, and abilities to control, malignant blood disorders.

Development of a novel cell-based assay for determination of skin sensitization potency of chemicals CRISPR

2 400 000 SEK

The aim of this project is to develop an assay that can be used to determine skin sensitization potencies of various chemicals. This is a critical toxicological endpoint required to be evaluated in different legal frameworks. At present the animal test “local lymph node assay” (LLNA) is the golden standard. Our hypothesis based on preliminary data is that this animal test can be replaced with an assay that determines keratinocyte secretion of an enzyme called ATX leading to dendritic cell activation. We will perform in-depth characterization of this signaling pathway using various techniques including Western blotting, confocal microscopy, proximity ligation assay and flow cytometry.

Chemical inhibitors, siRNA and CRISPR techniques will also be employed. We will test a wide range of substances with known skin sensitization potencies and validate our assay towards data from LLNA and clinical experience. We will also compare our assay to the performance of other cell-based assays regarding accuracy, sensitivity and specificity. Present data on other alternative methods suggest that these are quite unsuccessful for determining potency. Our 3-year project will be led by a group consisting of the PI with experience in developing high through-put cell tests, one expert in risk assessment, one dermatologist (and expert in allergic contact dermatitis) and one immunologist. The final goal is replacement of LLNA.

Ethical, legal and social issues of gene editing

Gene editing

4 800 000 SEK

This four year Bioethics research project will be performed at Uppsala University by a researcher with expertise in Genetics and Bioethics. The main aim is to analyse the ethical, legal and social issues (ELSI) of gene editing in humans to provide guidance for a responsible management of this technology. The methods used will be a mixture of literature review, document (e.g. guidelines) analysis and empirical methods to obtain stakeholder views and expertise regarding (ELSI of) gene editing. We will investigate the different roles and responsibilities of stakeholders to decide and govern the responsible use of gene editing, especially with respect to research monitoring and safety as well as for stakeholder engagement and translation of the use of gene editing in somatic cells from the resaerch context to the clinical context. We will also study different models of lay publics’ engagement in the debate, lay publics´ awareness and messages about gene editing in the popular media. A conceptual analysis of notions in genetics will also be performed to help contextualize the empirical data obtained. We will look at the notions of geneticization, paternalism, the technological imperative, and commercialization. Since Sweden is one of the countries where gene editing is already being conducted in human embryos in the research context, it is only responsible that ELSI research also be supported.

Functional analysis of m6A modification in breast cancer

TALEN

2 400 000 SEK

Breast cancer is the most prevalent female malignancy and is the second leading cause of cancer death. Chemical modifications of RNA provide a direct and rapid way to manipulate the existing transcriptome, allowing rapid responses to the changing environment. N6methyladenosine (m6A) is the most abundant internal modification of messenger RNA in eukaryotes, and its dysregulation has been implicated in several human disorders. Objective:

In this project, we propose to unravel the function of m6A RNA modification in breast cancer. Methods: Our project combines classical molecular and biochemical tools with cutting edge technologies (RNASeq, transcriptome-wide m6A profiling, TALEN) in stablished cell lines, mammary-like organoids, human breast cancer primary cultures and in mice in vivo. Organization: The project will progress over 3 years addressing the following specific aims: Aim 1: Elucidation of the m6A RNA regulatory function of ZNF217 in breast cancer. Aim 2: Identification of novel METTL3-interacting partners. Aim 3: m6A RNA methylation fingerprint of human samples. Importance: Overall, the integration of this data will identify transcripts that shape the breast oncogenic signature to unequivocally study the biological significance of m6A methylation in breast cancer pathogenesis, laying the foundation for new methods for predicting or augmenting drug response in these tumours.

Harnessing the BMP-SOX2 pathway to treat glioblastoma

CRISPR

2 400 000 SEK

Background. Despite the detailed information of oncogenic driver mechanisms in glioblastoma (GBM), no effective targeted treatment has been developed.Aim. The aim is to exploit the growth inhibitory pathway of bone morphogenetic protein 4 (BMP4) signalling to develop novel therapy against GBM.The extent of BMP-4 induced growth inhibition is determined in 50 human GBM cell lines and found to vary extensively both at the population and at the cellular level. Candidate genes interfering with the BMP4 response are identified from transcriptome analysis. Four such genes have been identified are functionally characterized.The contribution of cell cycle block, apoptosis, and cellular senescence to BMP4-induced growth inhibition is determined.Differential activation of BMP4 receptor downstream signals in responsive vs. refractory GBM cells is studied in order to find resistance mechanisms.The role of BMP4-induced transcription regulators (ID proteins, SOX2) in BMP4-regulated growth inhibition is determined.Genes regulating BMP4 responsiveness are identified by whole genome CRISPR-Cas9 gene targeting. In a preliminary experiment, the epithelial-to-mesenchymal transition regulator ZEB1 was identified and is currently functionally analysed.BMP4 regulatory circuits at the cellular level are identified by single-cell RNA sequencing.Significance. Our studies will show if delineate the BMP4 signaling growth inhibitory pathway, and demonstrate its usefulness for targeted therapy of GBM.

Metabol kontrol av beta-cellfunktion och insulinfrisättning

Genome editing

4 800 000 SEK

The soaring increase in the prevalence of Type 2 Diabetes (T2D) prompts major efforts to understand, prevent and treat this devastating disease. The past decade has seen remarkable advances in the discovery of risk alleles robustly associated with T2D risk. We have identified two risk alleles for T2D in genes encoding melatonin receptor 1B and transcription factor 1B mitochondrial. The functional basis of disease risk is unclear for most identified risk alleles. To resolve this, using a translational approach with cell and mouse models combined with human studies, we have implicated increased melatonin signalling in islets for the MTNR1B risk allele and mitochondrial dysfunction in b-cells for TFB1M in T2D pathogenesis. Now, to reach a deeper level of understanding and therapeutic implementation, we will use skin biopsies from risk allele carriers to make induced pluripotent stem cells (iPSC). iPSC will be differentiated into insulin-secreting cells, in which the molecular impact of risk alleles can be examined. Genome editing will be employed to correct or introduce pathogenic risk alleles. Balancing this high risk-high gain approach, we will continue studies with a more predictable outcome. We will elucidate mitochondrial control of b-cell function and mass, processes central to T2D pathogenesis. To this end, we examine TFB2M, a paralogue of TFB1M, DIMT1, a homologue of TFB1M of unknown function, and MICU2, controlling mitochondrial Ca2+ and, potentially, insulin secretion.

Genetic and epigenetic controls of germ cell development: studies of molecular mechanisms and novel treatments for infertility

CRISPR

4 800 000 SEK

About 10-15% of couples experience infertility. In this proposal, we plan to characterize novel molecular mechanisms underlining germ cells development, with the goal to reveal novel infertility related genes. Based on our solid research platform from the previous grant (2013-2017), we will focus on 3 aspects: (1) We will identify novel germ cell-specific genes from our RNA sequencing results of mouse germ cells. The functional roles of selected genes will be studied by large-scale generating knockout mouse models by our well established Crispr/Cas9 platform. (2) We will study epigenetic controls of infertility and fertility, such as ubiquitination and SUMOylation regulations of germ cell development. Our screening for novel germ cell-specific genes also reveals some epigenetic related molecules, which we will study extensively; (3)We will perform translational studies. For the “infertility genes” identified from mutant mouse models, we will analyze the genomic DNA and mRNA in about 1,000 premature ovarian failure (POF) samples and validate the functional significances of the mutations/variations found in POF. Our early results showed that a handful of novel germ cell-specific genes have been identified so far with important functions for fertility and infertility. We believe that the proposed research will lead to a better understanding of molecular mechanisms of fertility and infertility, and to open new aspects for developing novel treatments of infertility.

Defining molecular mechanisms controlling neurogenesis and retinogenesis and how it affects sensory systems.

CRISPR

4 800 000 SEK

To better understand the causes and consequences of diseases affecting the nervous system, it is an advantage to have knowledge about its normal development. The aim of our proposal is to provide novel information regarding the nature and sequential pattern of signals that control the generation of specific neurons, and how this affects the development of two sensory structures; the olfactory system and the eye. My research group has established unique intact chick embryo assays of neural differentiation and eye disease models in combination with timelapse imaging and analyses of mice mutants. The majority of our studies are in vivo gain- and loss- of function studies, in which Sox2 and Mab21L2 are two genes of particular interest that have been disrupted in chick and/or mouse. Techniques we use are: in ovo electroporation, tissue culture, fluorescent genetic reporters, CRISPR/Cas9 gene editing, immunohistochemistry, in situ hybridization quantitative real-time PCR and imaging. During this five year period we focus on providing results concerning signals that regulate the generation of specific neurons. These results can be used to direct stem cells to differentiate towards specific neural identities for screening purposes, cell therapy and as disease models. My previous findings regarding signaling interactions during neural development and cell fate decisions have been validated in mouse and human embryonic stem cells, which proves the relevance of our studies.

CRISPR-based studies of pathogenic neutrophil biology

CRISPR

2 400 000 SEK

The immune system is essential for our survival by protecting from infections agents, but can also cause potentially life-threatening diseases like allergies and autoimmune disease. Neutrophils are the most abundant immune cells of our body, and neutrophil deficiencies are linked to severe infections. This establishes their central role in the immune system. Neutrophils can also abundantly be found in inflamed joints of patients with the autoimmune disease Rheumatoid Arthritis (RA), and this has been suggested to have a direct role in initiation and progression of RA. Together this supports detailed studies of neutrophil biology in the autoimmune setting. Despite the important role for neutrophils in human disease, studies of their biology is majorly hampered by their short life span (hours), limiting the ability to experimentally manipulating them.

To circumvent this, we modify hematopoietic stem cells (HSC) using CRISPR, transfer these to mice, and study the neutrophils that continuously develop from the modified HSCs. This discovery platform gives us a unique experimental system that we use to study neutrophil behavior in mouse disease models. We will use the platform to ask specific questions related to how and why neutrophils migrate to inflamed joints, and how this can be stopped. Furthermore, we will study the regulation and function of specific posttranslational modifications in neutrophils that are linked to a more severe disease in RA patients.

High-throughput rapid manipulation of gene expression in the mouse: an efficient method to dramatically reduce the numbers of mice used in basic science (Continuation)

Genome editing, CRISPR

2 400 000

This research program describes a method to replace traditional mouse knockout technologies, dramatically reducing the numbers of mice used in science, while offering equal or superior results. The technology was developed by Elaine Fuchs´ laboratory at the Rockefeller University to manipulate gene expression in the developing skin. I performed guest research in the Fuchs lab to develop this technique further, and now show that this technology can also target the nervous system, lungs, eyes, gastrointestinal system and more. This means that we can reduce the numbers of mice used in developmental or cancer biology fields by more than 50%!The savings are especially pronounced when investigating multiple genes; in traditional triple knockouts only 1 in 64 offspring is triple homozygous from heterozygous parents. With 1 wild type, 3 single knockouts, and 3 double knockout controls this means 56 mice are wasted, byproducts of the breeding scheme. The suggested technology bypasses these problems, by specifically generating only the 8 required mice. Ultrasound-guided lentivirus nanoinjection into the embryonic amniotic fluid entails the injection of a lentivirus encoding shRNA, a gene of interest or CRISPR/Cas9 systems. Establishing the targeting of specific organs, the capacity to manipulate multiple genes at once, and harnessing the powerful potential of genome editing tools will make this technique a viable and attractive alternative, and save dramatic numbers of mice.

Targeting mechanisms for IGF-1R dependent and independent epigenetic gene silencing guarding proliferation and survival in multiple myeloma - Implications for biology and therapy

CRISPR

4 800 000 SEK

The overall objective of this proposal is to dissect a disease-specific global epigenetic pattern of clinical importance for multiple myeloma (MM), to increase understanding of how epigenetic abnormalities are important in development, drug resistance and stemness, and to evaluate the use of epigenetic modifiers as therapeutic targets. We have generated the first global analysis on the distribution of histone marks in MM and normal plasma cells and presented an initial proof-of-concept that silenced genes may be reactivated by use of epigenetic inhibitors. This proposal consists of three integrated projects. In the first we are systematically mapping the intrinsic network of epigenetic modifiers of histone i.e. Polycomb group (PcG), and of DNA collaborating to maintain gene silencing in MM. Epigenomic changes of pathogenetic and clinical importance in MM will be identified. In the second, components of PcG complexes and targets are functionally studied using knockdown by lentiviral vectors, CRISPR/Cas9 and selective small chemical inhibitors of clinical relevance in vitro and in vivo. Biological and therapeutical implications of reactivation of target genes, miRNAs and enhancer regions will be studied. The third project is focused on drug resistance mechanisms including analysis by masspectrometry the role of alternate usage of metabolic pathways, and strategies of combined targeting of epigenetic modifiers (PRC2, PRC1, DNMTs, MYC) to achieve proof of concept for use in vivo.

Targeting the glycoproteome – structure, function and precision medicine

Gene editing

2 400 000 SEK

The glycocalyx, the sugar coat surrounding every cell, is composed of complex carbohydrates linked to membrane lipids and proteins. It has been notoriously difficult to study the glycocalyx because of its´ structural complexity and the fact that the plasma membrane is the major site of contact with the cell surroundings and thus a major site for molecular interactions and signaling. Thanks to our recent progress in the mass spectrometric characterization of glycoproteins, especially proteoglycans, we will now challenge the full length sequencing of these complex glycans, their glycan attachment sites and core protein sequences of single cells, tissues and extracellular matrices. Of general interest for proteoglycans are their roles in embryogenesis, tissue organization and hemostasis, angiogenesis, cancer growth and dissemination, microbial and parasitic infections, protein deposition. Of specific interest is the site specific glycosylations of proteoglycans, which gives rise to either chondroitin sulfate, heparan sulfate or hybrid type modifications. Since these glycosaminoglycans induce contrasting biological effects, gene editing of such sites offers possibilities to study the biological functions of specific proteoglycan domains critical for optimized growth of e.g. induced pluripotent stem cells in cultures as well as in biological transplants. This will offer us unique possibilities to implement new knowledge into cell and tissue engineering and therapy.

Translational research in hepatitis C virus (HCV) and other microbial infections: Impact of inosine triphosphate pyrophosphatase (ITPase) activity on ribavirin-induced mutagenesis

Mutagenesis, CRISPR

2 400 000 SEK

Our group has evaluated the impact of genetic variations in the inosine triphosphate pyrophosphatase (ITPase) gene on HCV infection, and recently reported a novel ribavirin-like reduced risk of relapse among patients having reduced ITPase activity. Ongoing work in our group using the HCV culture system, has demonstrated that reducing ITPase activity by siRNA increased intracellular levels of ribavirin triphosphate (RTP), increased HCV mutagenesis as well as reduced virus production, and that RPT is a substrate of ITPase. During the coming grant period we plan to further evaluate the effect of ITPase on HCV and other microbial infections, as we hypothesize that inhibition of ITPase may point to novel antiviral and antibacterial strategies. We thus will: (i) further reduce ITPase activity using CRISPR/Cas9, (ii) evaluate mutagenesis in the HCV genome among patients treated with 4 weeks of ribavirin mono-therapy in the RibaC trial, (iii) evaluate whether the HCV polymerase can incorporate RTP or ITP into RNA, (iv) evaluate the impact of ITPase activity on hepatitis E virus (HEV) and Zika, (v) perform high-throughput screening of nucleotide-analogue libraries for discovery of potential ITPA-inhibitors, (vi) evaluate the functional consequences of reduced activity of the E. coli ITPase homologue, RdgB, on bacterial viability and on innate bacterial immunity as measured by resistance to T4 phage infection, and (vii) initiate development of an inducible ITPA transgenic mouse model.

Development of a novel oncolytic viral immunotherapy to treat lymphoma

CRISPR

3 150 000 SEK

Lymphomas account for the 5th most common cancer in the US and are among the 10 most common cancers worldwide. Low-grade B cell lymphomas, the most common lymphoma types, are generally incurable due to relapses and therapy-resistance, and there is an urgent need for novel and mechanistically distinct treatment strategies. This project will develop a unique type of cancer treatment combining oncolytic viral therapy with immunotherapy, and will take advantage of the inherent anti-tumor properties of Newcastle disease virus (NDV) that is considered safe and has real potential to overcome the limitations of current interventions. We will use state-of-the-art methodology, like CRISPR technology, CyTOF and RNA sequencing, to screen for novel immune stimulatory molecules that will potentiate the anti-tumor effects of NDV. The treatment approach will be evaluated in vivo in a lymphoma mouse model that is well-established in the lab of Dr. Brody. We will perform a detailed characterization of the immune response to treatment, which will substantially improve our understanding on the mechanisms underlying a successful treatment approach. The project will be performed at the Icahn School of Medicine at Mount Sinai, New York, in the lab of Dr. Brody, director of the Lymphoma Immunotherapy Program. The project is expected to provide a safe strategy that is more efficient than current therapies, and that can be translated into a novel clinical trial to treat lymphomas and other cancers.

The regulatory role of chromatin structure in cancer

CRISPR

3 150 000 SEK

I aim to investigate the roles of chromosome structuring proteins in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Recently it was shown that the genome is partitioned into insulated neighborhoods, which are chromosomal loop structures shaped by cohesin/CTCF protein binding. Genes encoding subunits of the cohesion complex are among the most frequently mutated genes in MDS and AML. In multiple tumor types, key oncogenes are associated with superenhancers that occur within the same insulated neighborhood as the oncogene in tumor cells but not in non-transformed cells. I will use ChIPseq and ChIA-PET data to identify such oncogene neighborhoods during year one. CRISPR/Cas9-based technologies will be used to perturb neighborhood boundaries to determine whether domain boundaries are essential for oncogene expression and the survival of malignant cells during year two. In year three I propose to investigate the mechanistic basis of two candidate therapeutic approaches for cohesin-mutated MDS and AML: spliceosome inhibition and hypomethylating agents.The proposed studies have the potential to reveal the mechanism of malignant transformation caused by disruption of the chromosome structuring protein cohesion and may ultimately aid the development and application of new therapeutic advances for cancer. The work will be conducted entirely at the host laboratory that holds both unique theoretical expertise and advanced technologies required for the proposed project.

Humanization of porcine embryos for whole human organ production

CRISPR

3 150 000 SEK

The goal of this project is to produce, in pigs, whole human organs that are universally immune compatible and ready for transplantation to human recipients. Organ failure is a major cause of death world-wide and transplantation is the only option for patients with end-stage organ failure. Although organ transplantation is often successful, complications arising from immune incompatibility and immunosuppressive therapies lead to high morbidity and mortality. Moreover, an acute shortage of immune-compatible donors means that patients often die while waiting for an organ – which in turn stimulates black-market organ trading in developing nations. To overcome these problems, we need to be able to produce large numbers of universally immune-compatible human organs. To approach this issue, I will first generate naïve human immunecompatible induced pluripotent stem cells and then use them to produce whole human organs in pigs. My foreign host is Professor Hiromitsu Nakauchi at the Stanford School of Medicine; who's pioneering advances in blastocyst complementation techniques, somatic cell nuclear transfer, and pig embryo manipulation has made the goals of this project possible.

2016

Why the long face? Development and evolution of the highly derived snout of pipefishes and seahorses

CRISPR

3 150 000 SEK

Understanding how biodiversity arises is fundamental in biology: why do some lineages comprise single species while others radiate explosively? Morphological key innovations—novel traits allowing niche expansion— facilitate species radiations. In vertebrates, key innovations enabling foraging adaptations are common, as seen in e.g. Darwin's finches and African cichlids. I will study the diverse syngnathid fish family, comprising 250 morphologically sepctacularly derived species of pipefishes, seahorses, and seadragons. Specifically, over 36 months, mentored by Bill Cresko (Univ. of Oregon) I will investigate the development and evolution of their snout-like face and mouth—a key innovation for pivot suction feeding. Since vertebrate craniofacial development is largely determined by deeply conserved genetic pathways, I will focus on variation in the regulation of gene expression. Studying natural populations and different syngnathiform species allows me to examine genetic variation within populations/species, and then expand the taxonomic scope to the family and order. By linking these taxonomic levels, I can add empirical data to the century-long debate whether the same processes driving microevolution also translate to macroevolutionary processes. I will take a novel multidisciplinary approach that integrates several fields of research, incl. developmental biology, population genetics, comparative genomics, and experimental functional validation using CRISPR/Cas9 in zebrafish.

Identifying modulators of Beclin 1 regulated autophagy using a CRISPR/Cas9 system

Gene editing, CRISPR

3 150 000 SEK

Autophagy is a degradative process that facilitates the recycling of nutrients, allows the cell to clear itself from damaged components, and is essential for the degradation of harmful proteins linked to neurodegeneration. To gain further insight into the mechanisms that governs autophagy, I propose a 3-year project that utilizes CRISPR/Cas9 gene editing to screen for novel regulators in a human cell line. The first part of the project will be performed at Cambridge University under the supervision of Prof. Rubinsztein, a leading researcher in the field of autophagy. The project will focus on finding regulators of Beclin 1, as cellular levels of this protein are tightly linked to autophagic activity. I will use CRISPR platforms to set up a genome-wide screen, and use FACS-sorting to isolate cells with significantly altered Beclin 1 levels. High-throughput sequencing will identify affected genes, and genes will be tested for their effect on the autophagic removal of mutant Huntingtin. Elucidating the genetic network of Beclin 1 is important to understand the essential process of autophagy, and could identify potential therapeutic targets to modulate autophagic clearance of disease-prone proteins. The 2nd part of the project will be undertaken at Gothenburg University, working with Prof. Zetterberg to validate the identified autophagy regulators in neurons, and evaluate the prospect of using autophagy genes as therapeutic targets and biomarkers for neurodegenerative diseases.

INTEGRATE– a paradigm for new and versatile cell factories

Gene editing, CRISPR

23 790 000 SEK

This project is a cellular engineering platform for the next generation of biologically produced fuels and chemicals. We will develop state-of-theart tools for genome engineering, pathway prediction and ranking, and enzyme engineering. As an application of these tools, we will engineer a versatile bacterial host, Cupriavidus necator, to make a versatile set of compounds, terpenes. C. necator can use syngas or formate as a substrate, the latter produced by electric reduction of CO2.The 6-year project will be done by an international team organized into three modules, each working simultaneously at the forefront of a key area of metabolic engineering. The host engineering module at KTH and Chalmers will engineer cell metabolism using modeling and CRISPR gene editing. The pathway discovery module at KTH and EPFL will use bioinformatics tools that identify new metabolic pathways to the target chemical. The biocatalysis module at KTH and Griefswald will use a novel enzyme engineering strategy to create enzymes with altered or improved activity. The project thus connects three areas of cell factory engineering that are often disparate. Added value arises from each module continuously integrating results from the others. The project will also disseminate its message and research results in a yearly international symposia for students. Importantly, the project as a whole is a pipeline that will be used in the future for quick development of other compounds.

Biofortification of cassava Genome editing

4 800 000 SEK

Cassava is one of the most important crops in the tropics with a remarkable tolerance to drought and ability to grow on poor soils. However, it has relatively low nutritional quality having dire implications for millions of people dependent on this staple crop. Particularly problematic is the low β-carotene level, which can lead to provitamin A deficiency with severe health and economic consequences, including early mortality, impaired physical ability and blindness. Efforts to breed for varieties with increased b-carotene have therefore been undertaken, but is difficult to combine with high starch content, which is a vital trait for farmers acceptance and the market value. Here we propose a new exploratory approach by cross-species comparative genomics and molecular network analysis in combination with functional testing by genetic transformation and the emerging technique of genome editing. We do this by generating transcript, smallRNA and metabolite data of specific varieties with different genetic background and β-carotene content to identify new candidate genes and key enzymes leading to increased β-carotene levels and sustained starch content. We will also identify molecular signatures associated to these traits to screen existing germplasms for more lines to be incorporated in breeding programs. The findings will serve either for marker assisted breeding or as engineering approach by gene-editing to overcome possible constrains in traditional breeding.

Adaptiv populationsdifferentiering hos växter CRISPR

4 200 000 SEK

An understanding of the ecological and genetic mechanisms governing plant adaptation to environmental conditions is of fundamental interest in evolutionary biology, but also for meeting the challenges of global change in applied fields such as conservation biology and plant breeding. In this project, we integrate ecological and genomic approaches in field and lab experiments in a study of the functional and genetic basis of plant adaptation. Our study systems are natural populations of the prime plant model organism, the selfing annual A. thaliana. Recombinant inbred lines, near-isogenic lines, and CRISPR/Cas-9 lines will be used in experiments designed to (a) identify traits and genomic regions subject to selection in contrasting climates in the field and under experimental conditions, (b) examine the functional and genetic basis of adaptive differences between populations and fitness tradeoffs, and (c) identify agents of selection in the native habitats. Recombinant inbred and near-isogenic lines were produced from a cross between two ecologically well characterized A. thaliana populations that exhibit striking adaptive differentiation. They therefore represent a uniquely suited genetic resource to study the functional and genetic basis of plant adaptation.

Mechanism and assembly of the water oxidation catalyst in photosystem II

Site-directed mutagenesis, mutagenesis

3 320 000 SEK

Photosynthetic water oxidation is the basis of higher life on Earth. The electrons and protons required for CO fixation are extracted by photosystem II (PSII) in a light-driven process from water. The field is now close to a molecular understanding of this process, but important questions remain. My group is in a unique position to make significant experimental contributions to solving them. We are the only group worldwide that can measure a unique property, the exchange rates with bulk

water, of the two substrate water molecules. Other techniques, such as crystallography and EPR, can identify water molecules at and near the cluster, but since there are more than two, they have no way of identifying the substrates. Using our unique technique, we will identify of the substrate water binding sites using Ca/Sr exchange and site-directed mutagenesis. We further combine our studies with parallel O-exchange advanced EPR (EDNMR) experiments to identify the binding site of the slowly exchanging substrate water. Room temperature x-ray free electron laser experiments will be used to obtain radiation damage free x-ray crystal structures of better than resolution from all relevant intermediates, including O-O bond formation.

Exploring How Vesicle Fusion is Regulated by Kinases and GTPases

Genome editing

3 100 000 SEK

Animal cells maintain tissue function by secreting regulatory factors to signal their activity and nutrient status, pathogen infections or injury. These regulated vesicle fusion events are typically much slower than the calcium-triggered release of synaptic vesicles and require, in addition to SNARE proteins, signaling regulators such as protein kinase C (PKC). Despite decades of investigation, the role of PKC in vesicle secretion remains incompletely resolved. Our goal is to explore the molecular mechanisms of secretion by investigating protein phosphorylation at secretory vesicles and the involvement of RhoGTPase. To identify phosphorylation targets of PKCB we established a PKCB knockout mast cell line using genome editing. Employing phosphoproteomics we identified 18,000 phosphopeptides and our goal is to follow up the 10 most promising hits, exploring their functions with genetic perturbations and single cell high-resolution imaging. Recent development of FRET biosensors has given us the opportunity to decipher the spatiotemporal activity for specific RhoGTPases. Our initial analysis showed that the RhoGTPase Cdc42 is strongly activated during mast cell secretion and a specific Cdc42 inhibitor was found to block mast cell secretion. ;In addition, we will use spatially resolved proteomics to identify novel regulators of vesicle fusion. Our study will provide systems level insights into the roles of phosphorylation and RhoGTPase activity during secretion.

Tackling the hard to get protein-protein interactions: Exploration of the prevalence, function, regulation and hijacking of short linear motifs

CRISPR

3 374 000 SEK

We will elucidate fundamental question regarding protein-protein interactions involving Short Linear Motifs (SLiMs) through investigation of their prevalence, function, cooperativity, regulation and deregulation by pathogen hijacking. SLiMs are 3-10 amino acid stretches that serve as binding sites for other proteins. SLiM-based interactions promote complex assembly, determine protein modification state, target proteins to cellular compartments and control protein stability. Consequently, SLiM-based interactions are crucial to cell signaling and protein regulation. In addition, several viruses and other pathogens mimic human SLiMs to take over the cell machinery for their own benefit.Like all large-scale analyses, the proposal leverages expertise from experimental (proteomic peptide phage display, next-generation sequencing & high-throughput affinity

measurements, cell based assays, lentiviral screens, CRISPR/Cas9 knock-outs) and computational (peptide libraries design and result interpretation) fields. The project will be performed in close collaboration with leading groups that provide complementary expertise. We will create novel methods, resources and databases of high value for the scientific community. The research will contribute to novel insights into SLiM-based protein-protein interactions and networks in health and in disease. On a personal level, I aim to consolidate a leading experimental group in the SLiM field.

Exploring development of virus resistance in the CRISPR-Cas immune system

CRISPR

2 900 000 SEK

The discovery of the CRISPR-Cas immune system in bacteria and archaea is an important part of understanding the ongoing struggle between viruses and their hosts, an interaction that affect the evolution and ecology of life on Earth. The system acquire immunity by storing fragments of viral DNA, known as spacers, in a chromosomal locus called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). The transcript of the locus is used to guide CRISPR associated (Cas) proteins to complementary targets and destroy them. The process of acquiring immunity, called adaptation, is only partially understood and this project aim at elucidating the process. The Cas1 and Cas2 proteins are key to adaptation, but they need support of nonCas host proteins. We will characterize the non-Cas proteins in order to develop complete understanding of the process. A part of the project is the development of a tool for directly detecting spacer incorporation. The tool is based on expression of fluorescent protein when a spacer is inserted, which can be detected in real-time on both single-cell and population level. The project will be performed in my research group at Uppsala University within a period of four years. Both conventional molecular biology tools and single cell technologies such as flow cytometry and microfluidics will be used. The results will make important contribute toward building a complete understanding of this elegant biological process and it role in nature.

Discovering methionine dependency factors to specifically target cancer cells CRISPR

3 000 000 SEK

A remarkable feature of many cancer cells is their strict dependence on the amino acid methionine. While normal cells can synthesize methionine to support their growth, many transformed cells cannot, and will die in the absence of the amino acid. This phenomenon has been known for decades, but has been difficult to exploit therapeutically since complete removal of methionine from the circulation to starve dependent tumors is not feasible in humans. To circumvent this problem, here we will utilize CRISPR-Cas9 genetic screening in a unique cell model combined with state-of-the art metabolic tracing methods to identify proteins that mediate methionine dependence in transformed cells. Such proteins must interact with methionine to suppress growth specifically in cancer cells, and are therefore of outstanding interest as cancer drug targets. We will also define a molecular signature of methionine dependency which can be used to identify individual tumors that might be vulnerable to targeting. This project builds upon previous efforts by our lab to establish advanced metabolic tracing methods, as well as our unique expertise in one-carbon metabolism and in computational techniques, and notably our recent discovery of a tractable cell system for genetic

screening. The project holds promise to crack open a long standing problem in cancer metabolism, and may identify novel drug targets affording an excellent therapeutic window.

Soat2 and Fsp27/Cidec; new therapeutic targets for cardiometabolic diseases

CRISPR

2 800 000 SEK

A role for acyl-Coenzyme A:cholesterol acyltransferase 2 (ACAT2) - encoded by the Sterol O-acyltransferase, SOAT2 gene - in the development of hepatic steatosis (HS) has been shown in mice genetically depleted of ACAT2 activity promotes the development of HS, but whether this affects insulin sensitivity (IS) is not known. Lipid droplet proteins play a role in HS. Cell death-inducing DFFA-like effector (CIDE) family proteins, including CIDEA, CIDEB, and CIDEC (in humans) or fatspecific protein 27 (FSP27; in mice) regulate intracellular lipid accumulation. CIDEC/FSP27 is barely expressed in normal livers but in AIM: We propose that inhibition of SOAT2 gene (or of its product ACAT2) and inhibition of hepatic & Fsp27/CIDEC expression are new strategies for amelioration of HS and IS. PROJECT PLAN: We are going to use a translational strategy. We will perform studies in human subjects treated with drugs affecting cholesterol metabolism, use human biobanks to search for genetic variants and epigenetic markers in SOAT2 and CIDEC genes. We will also use genetically modified mice, humanized-liver mice, human primary hepatocytes, and human hepatoma cells lines modified by CRISPR/Cas9 technology. New small molecules and anti-sense oligonucleotides will also be tested. ;RELEVANCE: Our data will increase the understanding of molecular mechanisms underlying HS and cardiometabolic diseases and validate new possible therapeutic tools

Exploring the role of prophages and the CRISPR/Cas system in successful clones of extensively drug resistant Klebsiella pneumoniae

CRISPR

2 100 000 SEK

Klebsiella pneumoniae (KP) is the second most common gram-negative pathogen isolated from bloodstream infections (BSI). European BSI data indicate that extensive drug-resistance (XDR) is increasing in KP. Although the resistance genes spread with horizontal gene transfer, the dissemination of resistance is still linked to a limited number of bacterial clones. Such clones have been defined by multi-locus sequence typing (MLST), identifying the sequence types (ST) 258, 14, and 147. However, little is still known about why these clones are successful. Herein we explore two explanations; prophages, bacteriophages integrated in the genome, and the presence of CRISPR/Cas, a system for regulating the uptake of foreign DNA, and potentially affecting virulence. A pilot study has identified frequent occurrence of prophages and also presence of CRISPR/Cas in some of the strains. The investigation will be expanded to several hundred strains of XDR-KP, and fitness experiments will be carried out.

Prophage knockout experiments will be carried out, and fitness with and without presence of prophages will be compared. Specifically, in vitro competition fitness experiments, using low-copy plasmids to introduce fluorescent dyes, followed by analysis with fluorescence-activated cell sorter, will be used. Sequencing of putative successful prophages will be done with long-read next-

generation sequencing. Finally, plasmiddependent phages will be used for in vitro cure of XDR-encoding plasmids.

Gene fusion discovery and circulating tumor DNA detection in upper aerodigestive system cancers

Genome editing, CRISPR

6 000 000 SEK

The overall aim of this proposal is to identify biomarkers in cancers of the upper aero-digestive system for therapeutics indications and for early detection We will employ a recent clinically proven targeted RNA-seq assay for detecting novel fusion using formalin-fixed paraffin-embedded samples to identify novel gene fusions in 56 potentially targetable genes, mainly kinases, on about 1500 squamous cell carcinoma of the head and neck (HNSCC) clinical samples. We will then validate whether the newly identified cancer gene fusions are truly tumorigenesis using an efficient genome-editing tool – the CRISPR-Cas9 system. For early detection of upper digestive system cancers, we will design an ultrasensitive assay for detecting circulating tumor nucleic acids and to evaluate the assay's performance (positive and negative predictive values) using plasma banked 0 to 5 years prior to clinical diagnosis based on a large longitudinal cohort. With the comprehensive survey for kinase gene fusions and on a large number of samples, based on our prior clinical experience, we will very likely identify several novel fusions. We expect these novel fusions, after validation, to define new subsets of HNSCC patients who are potentially targetable for better prognosis. From a preemptive strategy, a sensitive assay for early detection of cancer using circulating tumor nucleic acids will allow patients to receive best treatments at an early enough stage that might cure these cancers.

Affective disorders- A translational and prospective approach to dimensional etiology and treatment response

CRISPR

3 000 000 SEK

We aim to identify biological mechanisms underlying susceptibility to, and treatment response in bipolar disorder, depression and anxiety disorders. Our findings point to major impacts of inflammation in bipolar disorder. We also discovered that lithium has a major effect in protecting telomeres and impacts telomere biology in the brain. We have identified a number of vulnerability genes for bipolar disorder, genes that act across psychiatric diagnosis and genes that affect response to lithium. We focus on gene and epigene variations involved in treatment response, inflammation, cognition, mania, irritability, circadian regulation and fear. GWAS data is used to study pathways relating to inflammation, glutamate transmission and telomere biology. The relevance of gene variants will be validated by detailing changes in expression of vulnerability genes. Variants will be functionally analyzed using patient derived neuro-epithelial stem cells where drug and stress response phenotypes are recorded. Gene variants will also be introduced by CRISPR/Cas9. We will elucidate the relation between lithium, telomere biology and bipolar disorder by studying the role of inflammation, oxidative stress as well as effects on stem cells in a prospective study. Biomarkers can be used for predicting treatment outcome, as well as predicting side effects. An advantage of studying inflammation pathways in psychiatric disorders is the availability of inflammation modulating drugs that can be tested

Mapping of basal ganglia circuitry in Parkinson's disease using neuromodulation with novel viral vectors

Genome editing, CRISPR

6 000 000 SEK

The goal of this project is to elucidate the intricate functional interaction between the multiple neuromodulators in the striatum; dopamine, serotonin (5-HT) and acetylcholine. The interaction between these three neurotransmitters and the glutamatergic signaling from the stiatumprojecting thalamic nuclei determines the functional balance between the direct and indirect pathway of the basal ganglia circuitry and in the end effects the execution of goal directed movement. Dysregulation in this balance can lead to a number of pathological traits, one of the most well studies being the loss of voluntary motor control in Parkinson disease (PD) and the involuntary movements (dyskinesias) induced by the goldstandard medication, L-DOPA. In this project we propose to explore intrastiatal signaling mechanisms using advanced viral vector technologies (that we have developed in the lab) together with genome editing in the form of CRISPR interference and external regulation of neuronal activity using bi-modal chemogenetic receptors With these tools, we aim to clarify how a novel therapeutic target, the 5-HT6 receptor, modulates the L-DOPA-induced dyskinesias ;how the cholinergic interneurons in the striatum regulate dopamine release and motor function, how the intralaminar thalamic nuclei regulate the striatal circuitry and striatal motor function.Together these studies can enable novel application of 5-HT6 antagonists, already in a clinical use, in PD patients to alleviate dyskinesias.

Novel cancer drugs targeting loss of heterozygosity

Genome editing

4 000 000 SEK

The loss of genetic variation in human cancers relative to normal constitutional cells has been proposed, but not conclusively shown, to constitute a therapeutic opportunity. We have mined human genome variant to identify genes with common loss-of-function located in chromosome regions frequently lost in human cancers.This led to the identification of two genes fulfilling criteria for being pharmacological targets for LOH directed drug therapies. For one of these targets, relevant in colorectal cancer, we have already identified a drug which selectively kills tumor cells that lack the gene. This constitutes the first demonstration that anti-cancer drugs targeting LOH exist and has the potential to open new avenues in cancer therapy. The project will center on preclinical development of the two target genes. This involves of additional disease relevant cell models by genome editing (2016-17), development of companion diagnostics to identify eligible patients by clinical sequencing (2017), continued drug discovery efforts using an established in-house MTT assay format (2017-19), mechanistic studies of the identified compounds (2017-19), drug specificity evaluation (201719), demonstration in vivo efficacy of lead compounds in experimental animal models of cancer (2017-2020). The project team is composed of a postdoc, a graduate student and the applicant along with international collaborators and Chemical Biology Consortium Sweden (CBCS).

Development of immunotherapies targeting leukemia stem cells

CRISPR

2 100 000 SEK

Acute myeloid leukemia (AML) is associated with poor prognosis, hence new therapies are needed. This research project is based on the hypothesis that for optimal therapeutic effect, novel treatments for AML should target: (I) All leukemia stem cells (LSCs); (II) cell surface proteins that are essential for LSC survival, ideally involved in immune checkpoint regulation. With the aim to identify new immunotherapeutic targets, we will pioneer screens to identify cell surface molecules essential for LSCs. We will apply two screening approaches with readout of leukemiainitiating cells: CRISPR/Cas9 screens and cytokine screens with barcoded LSCs; combined with next-generation sequencing to assess the function of multiple clones of LSCs at a single-mouse level. The biological roles of the identified targets, especially in relation to immune cells, will be evaluated using leukemia cells transplanted into wild-type and immune deficient mice. Finally, immunotherapeutic approaches directed against the candidate cell surface targets will be investigated in leukemia mouse models. The proposed studies represent novel approaches to identify new therapeutic cell surface targets in AML and expand our knowledge regarding cell surface molecules regulating AML stem cells. Moreover, the project will provide proof of concept for therapeutic effect by targeting LSCs, findings that may translate into new immunotherapeutic opportunities in AML, a disease associated with dismal prognosis.

Midbrain dopaminergic neurons: From genes to development and Parkinson´s disease

CRISPR

6 000 000 SEK

In this project we propose to unravel the function of cell-type specific genes in mouse and human midbrain dopaminergic (mDA) neurons during development, in the adult and in Parkinson's disease (PD). Our project combines classical developmental and neurobiology tools with cutting edge technologies: Single cell RNA-seq, to identify cell types and their gene expression profile; and CRISPR/cas9 to rapidly and efficiently perform loss and gain of function experiments in human mDA cells in vitro in mice in vivo. The project will progress over 5 years from development to adult and disease, addressing the following fundamental issues: Is there a tissue-specific neural stem cell in the ventral. What is the function of the three unexpected ventral midbrain radial glia cell. How is neurogenesis and differentiation into mDA neuron subtypes regulated. How are adult mDA neuron subtypes specified and maintained. How is the development and maintenance of midbrain cell types coordinated What is the impact of PD mutations on human mDA neurons? Can we develop improved models of PD for drug discovery? Importance: These studies will provide an unprecedented knowledge of mDA neuron function in development and disease, which will enable improving models of PD and the future development of novel therapies for a disease with no cure, high incidence and very high prevalence in the ageing population in our society.

Monoamine networks in pancreatic islets – Control of ß-cell function and mass

Genome editing, CRISPR

2 800 000

Failure of pancreatic cells to release sufficient amounts of insulin is the main culprit in the development of type 2 diabetes. In this process, monoaminergic signaling plays a crucial role. In fact, pathogenetic roles for monoaminergic signaling in cells were recently highlighted by our work. For instance, functional signaling through the serotonin receptor 2b (HTR2B) in cells plays a critical role in regulation of insulin secretion in vitro. Moreover, carriers of a common variant of the melatonin receptor 1B (MNTR1B) gene are at higher risk of developing type 2 diabetes. The current project spans a 5-year period and aims to study the role of intraislet monoaminergic networks in development of type 2 diabetes. It utilizes cutting edge technology such as genome editing (CRISPR/Cas9) ;in vivo (mice), where the gene will be temporally and specifically inactivated in cells. Human derived induced pluripotent stem cells (iPSC) will be used to explore the diabetogenic properties of the MTNR1B risk allele; genome editing in iPSC will be used to reverse the diseased phenotype. Conversely, we will examine transgenic mice overexpressing cells. This work will deepen our understanding of monoaminergic control of islet function and allow screening of pharmacological compounds targeting monoamine signaling. This information can be utilized in a clinical setting to provide novel therapeutic strategies for the disease.

Massive aneuploidy: mechanisms and consequences of whole chromosome gain and loss in human malignancy

CRISPR

4 000 000

Aneuploidy, i.e., abnormal number of chromosomes, is one of the most common and characteristic features of tumor cells. We will investigate different aspects of aneuploid malignancies, with focus on high hyperdiploid (51-67 chromosomes) acute lymphoblastic leukemia (ALL) and anaplastic thyroid carcinoma (ATC), to elucidate how aneuploidy arises and how it affects the cell. In addition, we will use a genomics approach to identify novel therapeutic targets to improve patient survival. In high hyperdiploid ALL, we will utilize mutational data to determine the relative time frame since the extra chromosomes arose, to see whether all chromosomal gains occurred at the same time. Furthermore, we will perform whole genome sequencing on single leukemic cells, to determine whether there is variation in chromosome number, indicating chromosomal instability, and investigate the clonal architecture. Next, we will address the pathogenetic impact of the aneuploidy, by correlating copy number, gene expression, and protein expression levels. In ATC, we will study the genomic landscape, in order to unravel the tumorigenic process and identify novel therapeutic targets. Methods used include next generation sequencing, global protein analyses, SNP array analysis, and knock-out with CRISPR/CAS9. Taken together, this project will increase our understanding of the basic tumor biology of aneuploid malignancies and provide opportunities for the development of novel treatment modalities.

Treatment of liver failure by generation of fully functional human hepatocytes via a proliferative intermediate

CRISPR

6 000 000 SEK

Liver diseases are prevalent illnesses that affect around 29 million people in the EU. For end stage liver diseases, liver transplantation is currently the only option. However, transplants are not universally available and require the life-long use of immunosuppressive drugs. Thus, stem-cell based approaches have received much attention. Yet, stem-cell derived hepatocytes accumulate genetic mutations and exhibit dramatically reduced expression of hepatic markers, rendering them unsuitable for therapeutic purposes.We devised a different approach in which we dedifferentiate hepatocytes from patients with liver disease into fetal-like progenitor cells and stimulate their proliferation by mimicking molecular events that occur during liver regeneration in vivo. Importantly, after expansion, we redifferentiate these cells using a 3D culture system, resulting in physiological expression levels of hepatic markers. In order to bridge the gap to therapeutic utility, we propose to comprehensively characterize the molecular signatures of hepatocytes by integrating transcriptomic, proteomic and epigenomic data sets. Furthermore, we will assess genomic stability in long-term experiments. Finally, we aspire to correct mutations in patients with hereditary liver disorders using the CRISPR/Cas9-System, thus providing proof-of-concept that our appraoch can generate large numbers of genetically-corrected, functional hepatocytes that can be used for therapeutic transplantations.

Modulation of inflammatory responses to bacterial infections

CRISPR

2 700 000 SEK

Hospital-acquired infections are a significant source of increasing morbidity and mortality in intensive care units. Early diagnosis and monitoring of an infection are essential tools to identify patients at risk. However, this approach is dependent on the development of reliable diagnostic markers which, because of the disease complexity, constitute a serious scientific challenge. Novel concepts in diagnostics, also referred to as Precision Medicine, take the variability of each patient and the underlying individual complications into account and this may help to overcome the current problems in infectious disease diagnostics. In the present application we aim to develop novel diagnostic protein markers for hospital-acquired infections. To this end we will follow the principles of the Precision Medicine concept and define disease conditions that apply to a group of intensive care unit (ICU) patients suffering from lifethreatening secondary infections. We will establish novel animal models that that mimic the clinical situation of such ICU patients. Using microarray and proteomic analyses we will identify and further characterize proteins that could serve as potential diagnostic candidates. To test the patho-physiological functions of these proteins we will employ the CRISPR/Cas9 system to study their role in murine infection models. Finally, we will analyze plasma samples from ICU patients with hospitalacquired infections that are resembled by the animal models.

Genetics of platelet biogenesis and aggregation in humans and zebrafish, and relationship to cardiovascular disease

Mutagenesis, CRISPR

3 150 000 SEK

The overall aim is to understand platelet biogenesis, development and reactivity. The first two years of training will be in the host institute abroad, i.e., Framingham heart study (FHS), National Institutes of Health. I will work on two projects: 1) discovering novel genetic variants for platelet count (PLT), mean platelet volume (MPV) and platelet aggregation using whole-genome sequencing; 2) building a database for functional genomics of megakaryocytes and platelets, annotating putative causal genes and characterizing shared pathways among platelets traits and cardiovascular disease. In the third year, I will be working at the Swedish administrative institute, i.e., Uppsala University. I will further understand functional genes selected based on my work at FHS using CRISPR/Cas9-induced mutagenesis zebrafish models. The planned research has great potential impact on understanding the biological mechanisms of platelets and future therapeutic strategies. New anti-platelet therapeutic approaches are needed because of bleeding side effects. This work will benefit greatly from being conducted at FHS because researchers there focus on tackling cardiovascular disease research using multidisciplinary approaches and have access to large, cutting edge human datasets. I will gain deep knowledge of both human genomics and model organism systems by leading my own projects, in collaboration on other projects, and participating in on-site seminars, workshops, and lab externships.

2015

Defining the therapeutic potential of targeting the Nrf2 anti-oxidant pathway in lung cancer

Genome editing, TALEN

3 150 000 SEK

Lung cancer is the leading cause of cancer-related deaths worldwide. Non-small-cell lung cancer (NSCLC) accounts for ~85% of all lung cancers. 20-30% of human NSCLC tumors acquire mutations in the antioxidant transcription factor Nrf2 or its negative regulator Keap1 suggesting an important role for oxidative stress homeostasis to maintain cancer cell survival during lung tumorigenesis. Despite the high frequency of mutations observed in this pathway, little is known about its role in lung tumor initiation and progression. The host investigator has recently developed a rapid precise genome editing method in vivo that bypasses the need for time-consuming manipulation of the murine germline. I will combine the power of sophisticated Cre/loxP-based GEMMs and a novel precise genome editing platform designed to interrogate genes of interest in lung cancer with my expertise in oxidative stress biology.This study will integrate state-of-the-art gene targeting and biochemical methods with novel model systems, including lung organoids, to dissect the role of Nrf2-Keap1 pathway in lung cancer initiation, progression, maintenance and metastasis. Our ultimate aim is to define the therapeutic potential of targeting the Nrf2-Keap1 pathway in NSCLC and to characterize the stage specific role of oxidative stress pathways on cancer progression.The proposed project will be carried out at NYU Medical School 2016-2017 under the guidance of Assistant Professor Thales Papagiannakopoulos.

Exploring non-canonical miRNA pathways: nuclear Argonaute and cytoplasmic Drosha

RNA interference

3 150 000 SEK

In miRNA mediated gene silencing, small RNA molecules guide effector complexes to partially complementary mRNA targets resulting in posttranscriptional repression of gene expression. Isolated reports suggest that several well-studied components of the miRNA biogenesis and effector pathway may play additional roles beyond their canonical function in RNA interference. Argonaute (AGO) proteins were reported to localize to the nucleus where they are suggested to regulate transcription, while the nuclear RNase Drosha was shown to act as viral restriction factor in the cytoplasm. I aim to investigate non-canonical functions of miRNA binding proteins by utilizing panels of highthroughput screening based methods, such as tandem mass spectrometry to identify interacting partners, PAR-CLIP and RIP small RNA-Seq to identify RNA targets bound to AGO and Drosha. My goal is gain insights into the possible nucleo-cytoplasmic functions of AGO and Drosha. As part of the above aims, I also intend to characterize the novel idea that AGO proteins regulate chromatin. For this purpose I aim to develop photoactivatable-deoxyribonucleoside chromatin immunoprecipitation (PAD-CHIP). Nucleic acid binding proteins frequently appear chromatin associated in ChIP assays, however separating RNA-mediated chromatin association becomes challenging due to the use of long-range chemical crosslinkers. PAD-ChIP will improve the exciting ChIP protocols and move towards nucleotide resolution.

Epigenetic mechanisms involved in leukaemia stem cells selfrenewal

Mutagenesis, CRISPR

3 150 000

Acute myeloid leukaemia (AML) remains an incurable malignancy in over 70% of patients. Recent studies have demonstrated the central role of mutations in epigenetic regulators in the initiation and maintenance of AML. Most current therapies in AML are ineffectual at eradicating leukaemia stem cells (LSC). This project will aim to identify novel therapeutic targets to eradicate LSC. We will use sophisticated genomic screens to identify and characterise epigenetic regulators that can be targeted to eradicate leukaemia stem cells (LSC). The four specific aims of this project are: 1) to perform an shRNA screen against 1100 known and putative mammalian epigenetic regulators in LSC; 2) to use CRISPR/Cas mediated mutagenesis to identify the critical epigenetic domain(s) required to sustain LSC; 3) to characterise the mechanism by which the identified epigenetic target contributes to self-renewal in LSC; 4) to validate the therapeutic potential of the identified epigenetic target in murine models of AML. We anticipate that aims 1 and 2 will take 18 months and aims 3 and 4 will require the remaining 18 months of the fellowship. The Dawson laboratory has an international pedigree in cancer epigenetics and has recently identified a unique method to grow large quantities of LSC indefinitely in cell culture. Together with the epigenetics and functional biology expertise of the Castelo-Branco laboratory I will be ideally placed to fulfil this ambitious and innovative project.

Evolution, adaptive regulation, and functional impact of gene silencing by hairpin-RNA derived endogenous small interfering RNA pathways

RNA interference

3 200 000 SEK

As a major class of regulatory RNAs, small RNAs including microRNAs (miRNAs) and endogenous interfering RNAs (endo-siRNAs), have been of major research focus for the last decade. Recently I, with collaborators, characterized a new form of endo-siRNAs called hairpin RNAs (hpRNAs) that are recently evolved and restricted to the testis. We demonstrated, for the first time, a severe phenotypic effect of an hpRNA causing a male fertility defect, more than ten years since the original RNA interference (RNAi) publications. The second important finding was that hpRNAs evolve adaptively with their targets, indicating that they are under selection to maintain target pairing, which is unprecedented for animal small RNAs. This is an exciting finding indicating the first functional results in what promises to be an expanding field of discovery for this class of endo-siRNAs. In this proposal, I plan to use both computational approaches and molecular/genetic assays to gain a full picture of how this unique class of small RNAs works and its impact on the animal. A major aspect will be to computationally search for and characterize all hpRNA loci, both extending to all Drosophila species, and beginning the search in vertebrate species. A related aim is to perform comparative genomic analysis to fully characterize the evolution of this class across 12 sequenced fly genomes. In support of this, I have access to a very large set of small RNA-seq data, as well as experimental support from collaborators to follow up the expected computational findings. The outcome of this research will be expected to shed new light on this novel siRNA evolutionary pathway in Metazoan. The results will be of broad interest to the RNAi and evolutionary biology communities.

In Winters sex-ratio system, the recently evolved Dox gene impairs development of Y-bearing sperm in D. simulans and has consequently been repressed by the hairpin locus Nmy, which is homologous to Dox. Interestingly, the secondary structure of Nmy and its regulation of Dox resembles features that we characterized for hpRNAs and their targets in D. melanogaster. As an interesting case study, I will use both computational and molecular/genetic approaches to gain insights into evolution, regulation, and possible role of hairpin RNA mediated gene silencing in sex-ratio system.Currently, the mammalian endogenous siRNA pathway is notably absent and its extent is a key open question in the field of small RNA research. In this proposal, I also plan to use a deep sequencing approach to discover novel classes of endo-siRNAs and their targets in mammalian systems. The outcome of this study will build a better understanding of the human RNAi pathway which is currently far behind our knowledge of siRNAs in worm and Drosophila.As part of this proposal, other forms of small RNA mediated regulation, especially miRNA targeting, will be investigated. Although the basic mechanisms of miRNA targeting are well characterized, the study of its interaction with other regulatory systems is still in its infancy. Here I will expand upon my preliminary (unpublished) results demonstrating the widespread occurrence of RNA switches controlling miRNA targeting, in cooperation with RNA-binding proteins.Overall, the research proposed here will push the boundaries of knowledge in cutting-edge aspects of new (hpRNA) and well-studied (miRNA) small RNA regulatory systems and has the potential to usher in an entire new subfield of study.

MapToCleave: transcriptome-wide discovery of RNA hairpin fates

RNA interference

3 200 000 SEK

The emergence of genomic methods has over the last decade vastly increased our understanding of RNA splicing, in which transcripts are resectioned through cleavage to form new functional molecules. In a widely unknown - and vastly understudied - type of transcript biogenesis, RNA hairpin structures are cleaved into short molecules with gene regulatory function, the microRNAs (miRNAs). The human transcriptome contains more than 100,000 such RNA hairpins, more than half of which are located in mRNAs. These hairpins thus constitute critical crossroads for nuclear transcripts: they can either be cleaved into regulatory miRNAs or they can avoid cleavage and function as full-length transcripts, for instance through cytoplasmic transport and translation. Our lack of understanding of these transcript fates lies with technical limitations rather than biological importance. While the RNA field as a whole has been advanced by genomics methods, the field of miRNA biogenesis has hardly progressed the last ten years. The state-of-the-art methods are still limited to testing a single RNA hairpin in one experiment. To correct this, we propose to develop MapToCleave, the first method to profile hairpin cleavage transcriptome-wide.

Specifically, the method should be able to test thousands of RNA hairpins in a single experiment, by combining oligonucleotide synthesis, cell culture methods, next-generation sequencing and computational biology. We have finalized the pilot part of the project, in which 2,500 hairpins were successfully tested. Basically none of the negative control sequences were detected, while around 25% of trans-species fruitfly and mouse positive control hairpins were detected. The next step will be to upscale the method and improve the detection efficiency. Further, we will complement this method with profiling of nuclear transcripts levels and protein occupancy to develop a fully integrated and predictive model of hairpin cleavage. In summary, MapToCleave will be the first method to test the cleavage of thousands of distinct hairpins and thus lift the miRNA biogenesis field into the genomics era. Our model will explain why some hairpins are cleaved while others are left untouched, thus answering basic biological questions on transcript fates. Last, we will disseminate our findings via publications and an interactive web site, facilitating spinoffs to benefit other fields, such as improved gene prediction, RNA interference and RNA-based gene therapy.

Analysis of a protein complex that regulates chromosome pairing and recombination in germ cells

Gene editing, CRISPR

1 600 000 SEK

The synaptonemal complex (SC) is a conserved meiosis-specific chromosome–associated protein complex. The SC ensures the fidelity of the chromosome segregation process and generates genetic diversity in germ cells. Despite the central biological role of the SC, however, our knowledge of the function and structure of this protein complex is limited.The SC represents an exciting scientific challenge where a combination of ultrastructural, biochemical and genetic approaches need to be undertaken to unravel its critical meiotic functions. We have shown that the mammalian SC is composed of seven subunits, we have genetically inactivated several of its components and created a SC-null mouse strain. We will now in a systematic way study the assembly process that gives rise to the mature SC structure and how this structure promotes chromosome pairing and recombination between homologous chromosomes. Structural analysis of the SC will be undertaken by immuno-

electron microscopy, super-resolution microscopy and 3Delectron tomography in wild-type and mutant mice. The SC assembly process will be analyzed using in vitro binding studies and ex vivo complementary expression analysis. The individual roles of the SC proteins in assembly, chromosome pairing and recombination will also be evaluated in vivo using the CRISPR-Cas gene editing system.The proposed studies will uncover the fundamental nature of the mammalian SC, how this protein structure contributes to chromosome axis organization, alignment and recombination, biological activities that are critical for sexual reproduction and the maintenance of a euploid genome.

Proton transfer and nitric oxide reduction in heme-copper oxidases

Sie-directed mutagenesis, mutagenesis

3 000 000 SEK

Bacterial NO-reductases (NOR) are integral membrane proteins that reduce the toxic gas nitric oxide (NO) to dinitrous oxide (N2O) (2NO+2e+2H+->N2O+H2O). NORs are divergent members of the super-family of oxygen-reducing, proton pumping, heme-copper oxidases (HCuOs), to which the mitochondrial cytochrome c oxidase (mCcO) also belongs. Despite belonging to the same family, the function of the NORs is very different from other HCuOs in that NORs do not conserve the free energy available from NO reduction by creating a proton gradient over the membrane. The closest relatives to NOR among the HCuOs are the cbb3 oxidases, which have a high oxygen affinity and are found exclusively in bacteria, often pathogens. The cbb3 oxidases also show NO-reduction activity in contrast to the mCcO and NORs can reduce O2. The project aims at elucidating the structure-function relationships in the bacterial NORs and cbb3 oxidases, with special focus on proton transfer pathways, mechanisms and vectoriality. We expect to gain insight also into the evolution of the heme-copper oxidase superfamily. Our studies involve techniques from molecular biology to biophysics; e.g. sitedirected mutagenesis, protein purification optimisations, reconstitution into membrane mimetic systems, and time-resolved optical spectroscopy/electrometry of single catalytic turnovers.

Enzymes: from the test tube into the cell

Mutagenesis

3 200 000 SEK

Enzymes are key players in all cellular processes in every living organism. In spite of a wealth of knowledge, the functions of enzymes are still incompletely understood. Numerous application in biotechnology are based on enzymology and approximately half of all pharmaceutical drugs are directed against enzymes, but the design of enzymes for new purposes still presents a challenge and the interactions of targeted enzymes with the components in the intracellular milieu need further studies. Glutathione transferases (GSTs) are ubiquitous detoxication enzymes with multiple cellular functions and are excellently suited to explore various current aspects of enzymology. Genes encoding GSTs will be synthesized, enzymes with enhanced properties will be produced via information-optimized infolog mutants, and the functions of the enzymes characterized. Fluorophore-labeled GSTs as well as fluorogenic substrates will enable single-molecule characterization of the interactions of GSTs with alternative substrates as well as inhibitors, both in

vitro and in living cells. Insertion of GST proteins via endocytosis or via transfection from eukaryote expression vectors perturbs components of hormone signaling and studies of proteomes as well as single-cell transcriptomes, and we will explore these newly discovered phenomena in human cells. At the multicellular organism level, model studies will be performed with transgenic GSTs expressed in the fruit fly Drosophila melanogaster and in the thale cress Arabidopsis thaliana. In fruit flies the magnitude of the GST activity in relation to apoptosis and cell death under conditions of chemical stress will be explored, since there are indications that excessive activity may shorten the life-span. In Arabidopsis the utility of GSTs in the bioremediation of soil contaminated with explosives and other toxic agents will be demonstrated. In parallel, poplar GSTs are redesigned via computational predictions and mutagenesis for the ultimate goal of developing fast-growing trees for large-scale applications. In summary, the various functions of enzymes will be illustrated at all levels of complexity ranging from single-molecule kinetics, via enzyme evolution, intracellular effects on molecular networks, and their importance for longevity and biotechnical applications.

Regulation of aging by DAF-16/FOXO, its cofactors, and the chromatin landscape

RNAi

3 200 000 SEK

In these days of modern medicine, acute diseases are increasingly treatable, leaving aging and age-related diseases as our major health and lifespan determinants. Fortunately, aging is a very plastic process, so that a detailed understanding of its regulation could provide potent means to impair it and thereby enhance our quality of life. Aging regulation is conferred by various signaling pathways that in response to dire conditions induce stress responses, which increase the organism's durability, its longevity, and therefore its chances of survival. Central to this regulation is the transcription factor DAF-16/FOXO, which relays low insulin/IGF-like signaling and other pro-longevity stimuli into expression of stimulus-specific sets of stress response genes. But despite its importance, DAF-16/FOXO's mechanisms of transcriptional regulation remain largely elusive. Recently, we identified ~32 cofactors to DAF16/FOXO, and by focusing on one of them, the chromatin remodeler SWI/SNF, we could show that DAF-16/FOXO employs chromatin remodeling at its target promoters as a means to induce transcription. Following this discovery, we now want to further explore the interplay between DAF-16/FOXO, its cofactors, and the chromatin landscape in aging-regulation. We will use the model system Caenorhabditis elegans and a wide assortment of genetic, biochemical, and high-throughputsequencing techniques to explore our remaining ~30 DAF-16/FOXO cofactors and a recently described chromatin-associated DAF-16/FOXO activator and specificity determinant, SMK-1/SMEK, to better understand DAF-16/FOXO-driven transcriptional regulation and target gene choice. These efforts will be complemented by comprehensive searches for aging-relevant roles of chromatin remodelers and the epigenome, and how they influence DAF-16/FOXO function. Implementation: All aims of the grant are to be completed within four years. A postdoc will first prioritize the DAF-16/FOXO cofactors based on their stress resistance, lifespan, and gene expression phenotypes, to eventually pursue the best candidates by detailed mechanistic studies to publication. This same postdoc will join efforts with a separately funded PhD student to investigate the mechanism of SMK-1/SMEK function. Due to our preliminary data implying that SMK-1/SMEK functions as a part of Protein Phosphatase 4, we hope to identify the relevant substrates and complete this work already by the end of year 3. Methods used for all the above will be diverse but include RNAi and mutant screening for stress resistance and lifespan phenotypes, mass spectrometry-based proteomics approaches, mRNA-Seq, ChIP-Seq, and cytology.A second postdoc

with support by a part-time technician will investigate the role of the chromatin landscape at DAF-16/FOXO target promoters. A systematic survey of chromatin remodelers by RNAi or mutant screening and their further evaluation shall be concluded with publication by year 3. In addition, they will identify aging-related changes in histone marks by a challenging mass spectrometry-based strategy that we recently adapted for use in C. elegans and then determine their relevance for the aging process – globally and at DAF-16/FOXO target promoters. Methods applied will again range from genetic screening for age-related phenotypes to diverse biochemical or high-throughput sequencing approaches.Taken together, our work will substantially advance the mechanistic understanding of how DAF-16/FOXO fulfils its pro-longevity functions in concert with cofactors as well as the chromatin regions that it targets. And by gaining insight into DAF-16/FOXO target gene specification we may even learn to separate these beneficial functions from unwanted side-effects on development, fertility, or metabolism. Each would be fundamental steps towards the impairment of aging and age-related diseases in humans.

Mechanisms of Chromosome 1p36 Tumor Suppression

RNAi

4 000 000 SEK

The Kinesin KIF1Bb is a candidate 1p36 neuroblastoma tumor suppressor that regulates developmental apoptosis in the sympathetic nervous system. We observed a complete lack of KIF1Bβ expression in 1p36-deleted NB tumors, indicating that KIF1Bb is the pathogenic target of 1p36 deletions. To dissect fully the role of KIF1Bβ in tumor suppression, we generated conditional gene targeting of KIF1Bβ and observed failure in terminal differentiation in sympathetic neurons. Failure of apoptosis and lack of differentiation are hallmarks of cancer. However, the mechanism of KIF1Bb tumor suppression is unknown. Performing genome-wide RNAi screens, we found that KIF1Bb regulates DRP1 mediated mitochondrial fission to induce apoptosis. Importantly, inherited KIF1Bb loss-of-function mutations previously identified in NB fail to regulate DRP1, indicating that DRP1 is a pathogenic target. We observed that DRP1 is silenced in 1p36-deleted tumors with one exception. This tumor outlier carried a typical 11q-deletion, however CGH arrays showed 1p-deletion in 2011, but not in 2013. It was demonstrated that 11q-deleted tumors constitute a distinct group of unfavorable NB tumors that feature 1p deletions including the KIF1B locus. Distinguishing the two high-risk NB subsets, MYCN-amplified and 11q-deleted, would bear high clinical significance and potential for novel targeted therapies. While further research is needed to delineate the mechanism of KIF1Bb tumor suppression, this work opens up several avenues for investigation. For example, KIF1Bβ is regulated by the oxygen-dependent enzyme EglN3 that responds to a variety of metabolic signals and can be modulated with drug-like molecules. An intriguing possibility is that an increase in developmental apoptosis and differentiation is responsible for the spontaneous regressions frequently observed in neonates who present so-called Stage 4S neuroblastoma. Perhaps, in time, we can mimic this with EglN3/KIF1Bβ agonists. Specific Aims: Aim 1: To determine if KIF1Bβ is a bona fide neuroblastoma tumor suppressor gene in vivo.Aim 2: To understand how EglN3 regulates KIF1Bβ, and how this translates into cell death. Aim 3: Mechanism of KIF1Bβ tumor suppression. Significance:Neuroblastomas are the third most common cancer in children and account for almost 15% of childhood cancer fatalities, a number that reflects its aggressive nature and frequency of metastatic disease at diagnosis. Neuroblastomas constitute a group of tumors of ganglion cell origin that derive from primordial neural crest cells, which are the precursors of the sympathetic nervous system. We recently discovered that the genes called ‘EglN3’ and ´KIF1Bb´ play an essential role in

determining whether neural crest cells live or die. In particular, when these genes are lost, neural crest cells that should have died as part of the normal development of the fetus escape their death sentence. We have since learned that activating EglN3 kills neuroblastoma and other neural crest-derived tumors. In short, EglN3 appears to play a special role in the decision between life and death for neural crest tumors such as neuroblastoma. We are trying to understand the mechanisms by which EglN3 causes cell death because this understanding might, in time, allow us to induce neuroblastoma cells to die in patients. Performing a genome-wide screen, we identified genes that are required for EglN3 to induce apoptosis. One such gene we identified is the kinesin KIF1Bb. KIF1Bb is localized on human chromosome 1p36 in a region that is commonly deleted in neuroblastomas. In fact, this region has been thought to harbor a 'Neuroblastoma' gene for almost 2 decades. Our preliminary data strongly suggest that KIF1Bb might be such neuroblastoma tumor suppressor gene. Understanding the mechanism by which EglN3 and KIF1Bb induce neuronal cell death will allow the identification of additional proteins linked to developmental apoptosis and cancer.

Identifying targets and compounds for the therapeutic intervention of coronary heart disease using a zebrafish model system

CRISPR

5 875 226 SEK

The aim of my research programme is to identify novel drug targets and compounds for the therapeutic intervention of coronary heart disease (CHD). To this end, I have identified 93 positional candidate genes in loci identified as being associated with CHD in genome-wide association studies. Two PhD students will target these genes in a zebrafish model system using CRISPR-Cas9, and examine their effect on vascular infiltration by lipids, oxidized LDL cholesterol, macrophages and neutrophils, as well as on endothelial thickness and luminal diameter, before and after feeding on a control or high-cholesterol diet. Promising genes will be taken forward for additional characterization in adult fish, i.e. for RNA sequencing of macrophages, neutrophils, erythrocytes and endothelial cells, and for global metabolomic profiling of vascular plaque tissue from fish fed on a high-cholesterol diet. These experiments are anticipated to identify pathways by which putative causal genes influence atherogenic traits.Mutant models that already induce atherogenic traits at five days post-fertilization will be used for a chemical compound screen, aiming to identify small molecules that prevent or reduce early stage atherosclerosis. Promising compounds will be taken forward for additional experiments in high-cholesterol diet fed larvae.My research programme will: i) provide a framework for novel in vivo model systems to identify drug targets and chemical compounds aimed at decreasing atherosclerosis and CHD; ii) further increase our understanding of atherosclerosis and CHD pathophysiology by integrating results from imaging and -omics approaches; and iii) provide a new set of drug targets and compounds that can be taken forward for pharmacological and pharmacokinetic testing.

Translational epigenetics; from research theory to clinical practice

CRISPR

5 620 000 SEK

Complex immune disorders, such as allergy, are placing an increasing financial burden on health care systems. The increasing prevalence of many immune diseases and lack of a strong genetic component suggests a role for epigenetics, including DNA methylation, in their pathogenesis. However, despite publication of >100,000 complex disease methylomes, no DNA methylation markers are in common clinical use for the diagnosis or treatment of any complex disease, and clinical trials of compounds targeting DNA methylation have proven challenging. My previous research (Genome Research 2012, PNAS 2011, Genome Biology 2012, PLoS Genetics 2014 & Genome Biology, 2015) revealed that the majority of DNA methylation changes observed in many complex diseases are non-pathogenic and simply reflect disease state or tissue complexity. Thus, I propose that to truly understand and make clinical use of epigenetic changes in complex disease we must first understand their role in the biology of the unaffected normal tissue. The aim of this project is to use a knowledgebased approach to understand the significance of epigenetic alterations in complex disease, by first understanding the biology of DNA methylation in the corresponding healthy tissue. Specifically, I will elucidate the role of DNA de-methylation in normal human CD4+ T cell biology, using in vitro differentiation of CD4+ T cells as a model system and use this knowledge as a reference to study the pathogenic importance and predictive value of DNA methylation in seasonal allergic rhinitis (SAR).Using next-generation sequencing technology, I will determine the genome-wide profiles of 5-methylcytosine and 5hydroxymethylcytosine in human CD4+ T cells at several early and late time-points during in vitro differentiation into four T helper cell types (TH1, TH2, TH17, TREG). This will allow identification of loci undergoing DNA de-methylation in CD4+ T-cells during early (non-replicative) and late (replicative) phases of differentiation. The observed DNA demethylation events will be related to RNA abundance by RNA-Seq of the same samples. This 'time-series' approach is particularly powerful, as it allows direction of methylation changes and associated gene expression to be determined, as well as shedding light on how early (1 day) remodelling of 5hmC relates to later (6 day) changes in 5mC. To place changes in the DNA methylome during differentiation in a broader regulatory context, histone modifications associated with promoters (H3K4me3, H3K27me3) and enhancers (H3K27ac, H3K4me1) will be analysed in the same samples. Having identified the key functional DNA methylation remodelling events in normal CD4+ T-cell biology, I will design CD4+ T-cell-specific targeted tiling microarrays. Using these arrays, I will identify potentially pathogenic epigenetic changes in the CD4+ T-cells in SAR patients before and after reversal of the disease by specific immunotherapy and between monozygotic twins discordant for disease. I will then validate the disease relevance of the observed epigenetic changes in SAR by genomic editing of these regions in transgenic mice using CRISPR, and assessment of the effects on symptoms in an allergic mouse model.The proposed research is important as it will reveal 1) the role of DNA demethylation in CD4+ Tdifferentiation, a poorly understood process, key to the pathogenesis of many immune diseases, and 2) will allow changes in DNA methylation in complex immune diseases to be placed in a functional context for knowledge-based disease stratification. Thus the results will be of great interest to the fields of epigenetics, adaptive immunity and translational research.

Beyond GWAS of obesity: from genetic association to function

CRISPR

4 000 000 SEK

Over the next decades, dramatic increases of obesity and type 2 diabetes are expected in the U.S. and throughout the world. As insulin resistance is a usual intermediate step between obesity, type 2 diabetes and cardiovascular disease, discovery of more efficient ways of preventing, diagnosing and treating obesity and insulin resistance are of uttermost importance.In the past few years, our genome-wide association studies have identified more than 200 genetic loci that are robustly associated with adiposity and/or insulin resistance, but the causal genes and mechanisms are unknown for all but a handful of these loci, and their role in development of insulin resistance has not been studied systematically. This represents a gold mine for in-depth physiological and mechanistic studies, and as insulin resistance is a usual intermediate step between obesity, type 2 diabetes and cardiovascular disease, increased understanding of the links between conditions may lead to new approaches to prevention and treatment that could have a huge public health impact. To establish and characterize genes associated with insulin resistance, we plan a series of experiments in large human cohorts with functional follow-up using zebrafish and cell-based models.We will characterize suggested insulin resistance loci using detailed phenotypic information from large population-based samples (total N=13,811) assessed with dynamic measures of glucose and insulin metabolism, metabolomic, transcriptomic, epigenomic and proteomic profiling together with in silico data on gene regulation and transcription from public resources.Next, we will take about 30 candidate genes forward to our pipeline for efficient characterization in zebrafish using high-throughput visualization techniques and biochemical measurements. We use CRISPR-Cas9 to knockout the homologous genes of a selected number of genes based on results in aim 1 and study the effect of perturbing these genes on several traits related to insulin resistance.Finally, we will prioritize five candidate genes for IR development for mechanistic studies using model systems. We will use CRISPR-Cas9 for gene knockdown in adipocytes and hepatocytes to study glucose, insulin and lipid metabolism, gene expression and metabolic pathways.By performing detailed follow-up analyses of loci hypothesized to be involved in insulin resistance, we expect to establish causal genes and mechanisms of action for several of these loci. The in-depth characterization using in vivo and in vitro models will provide further evidence towards causality and the mechanisms of action, as well as a first evaluation of which could be viable drug targets. Our approach of integrating comprehensive characterization in humans with experiments in functional model systems provides a translational framework, which by design is more likely to yield findings relevant for human biology and medicine. Importantly, we have access to unique study materials, state-of-the art methodology, and have a strong track record of successful collaborations in this field. Our work is anticipated to benefit the scientific community, to lead to new important insights into insulin resistance, cardiovascular disease and type 2 diabetes.

Epigenetic regulation of multipotency in leukemic stem cells - with specific focus on enhancers

CRISPR

2 100 000 SEK

Background:Acute myeloid leukemia (AML) is a hematologic malignancy that is the most common type of acute leukemia diagnosed in adults and the second most common type in children. AML has a

poor prognosis in both, adults and children, with a long-term survival of only 15% and 50% respectively. Currently the majority of treatments for AML consist of cytotoxic drugs with low specificity. The relapse and consequently the poor prognosis of AML patients are likely to be caused by leukemic stem cells, which survive the drug treatment. Perturbed epigenetic regulation has been associated with AML. The histone methyltransferase Mixed Lineage Leukemia (MLL) methylates H3K4 at enhancers and transcription start sites. MLL is chromosomally rearranged in high-risk infant, childhood, adult and therapy related AML. The rearrangement causes a perturbed epigenetic regulation that may contribute to leukomogenesis. Hypothesis: Epigenetic aberrations in leukemic stem cells cause disturbed enhancer usage and consequently a leukemic transcriptome that leads to growth advantagesWork planWe will focus on perturbed epigenetic regulation at enhancers in AML with MLL translocations. The project aims to: A) Characterize the effect of perturbed epigenetic regulation on enhancer activity. B) Identify key epigenetic factors that regulate enhancer activity. C) Analyze the role of the identified epigenetic factors in drug resistance and their potential as drug targets.Primary leukemic (TIM3+) and normal hematopoietic stem cells (HSC), (CD34+ and CD38-) will be isolated from AML patients and healthy donors respectively, using fluorescence-activated cell sorting (FACS). The epigenetic landscape at enhancer regions in both stem cell populations will be analyzed, with respect to enhancer associated histone modifications, DNA methylation and chromatin structure. By analyzing the transcriptomes of normal and leukemic stem cells, potential aberrant expression of specific epigenetic regulators be identified. Finally their involvement in leukomogenesis and the potential of these factors as putative drug targets will be tested.MethodsWe will compare the presence, usage and function of enhancers in leukemic stem cells and their normal counterpart using ATAC-seq (for chromosome structure), RRBS (for DNA methylation) ChIP-Seq (for histone modifications), CAGE (for transcriptome and enhancer usage) and CRISPR-Cas9 (for deletion of epigenetic regulators and genomic enhancer regions). Time planYear 1: Performance of CAGE on leukemic stem cells and normal HSC to identify enhancers and the transcription profile of epigenetic regulators. Optimization of the ChIP-seq technology for small cell populations. Year 2: ChIP-seq and ATAC-sq assays on both leukemic stem cell and the main leukemic populations. Year 3: Integrated analysis of enhancers, transcriptome and epigenetic landscape in normal and leukemic cells that are obtained year 1 and year 2. Year 4: Functional analysis of potential epigenetic stem cell regulators in self-renewal and differentiation assays in normal and AML cells. Year 5: Analysis of re-sensitizing drug resistant AML cells, by manipulating the expression of key epigenetic regulators.Preliminary resultsAnalysis of DNA methylation in AML patients has shown an enrichment of aberrant DNA methylation in enhancer regions. Interestingly, these enhancers are significantly enriched for so called super-enhancers that are involved in AML caused by a MLL translocation. SignificanceThis project will provide detailed molecular knowledge of how the different altered epigenetic mechanisms in AML interact in leukemic stem cells. These results will create the possibilities to develop novel therapies that target the stem cell compartment that consequently may improve the treatment efficiency and decrease the risk for relapse. It has previous been demonstrated in other subtypes of leukemia that greater knowledge of the mech

Urothelial cancer in the context of normal urothelial differentiation

Genome editing, CRISPR

4 000 000 SEK

The major aim of this proposal is to test hypotheses regarding the development of bladder cancer from the perspective of normal urothelium development. As a source for hypotheses we have

several years of descriptive work of changes at the transcriptomic, genomic, epigenomic, proteomic, as well as from the gene mutation levels. In particular, our recent investigations have identified several transcription factors as determinants of bladder cancer molecular subtypes, factors also implicated in the differentiation of the normal urothelium. The overriding aim is to translate this knowledge into testable propositions. Our results have indicated that the nuclear receptor pair PPARG/RXRA, the transcription factor pair FOXA1/GATA3, and the anterior HOXA/HOXB genes, are involved in determining the molecular phenotype of the urobasal subtype of urothelial tumors, that the PLK1FOXM1 axis is a major determinant for the genomically unstable subtype, and that down regulation of PPARG/RXRA and FOXA1/GATA3, and strong up regulation of STAT3 regulated genes are important determinants of the basal like/SCC-like subtype. To obtain experimental evidence for the involvement of these factors in bladder cancer development, as well as of the development of the normal bladder, we intend to use the CRISPR systems both for genome editing and for efficient up or down regulation of specific genes. PPARG induced in vitro differentiation of normal urothelial cells will be studied using various constructs e.g., cells that do not express the PPARG/RXRA downstream targets FOXA1 or GATA3. These cell lines will also be used to elucidate the role of HOXA/HOXB in ligand induced differentiation. It is known that FOXM1 induces de novo methylation of the GATA3 promoter to evade differentiation. It is our intention to induce FOXM1 expression in normal cells and perform a whole genome screen for de novo methylation events at other locations. We will in addition use transgenic mice to study the importance of the factors both in tumor development and normal bladder function. The role of FOXM1 in bladder regeneration after induced injury will be studied in FOXM1+/+ and FOXM1-/- mice. We will investigate the importance of up regulation of STAT3 and down regulation of PPARG for induction of a basal cancer phenotype by constructing a mice chimeric for STAT3 up regulation and PPARG-/-. Taken together, our efforts are to investigate key genes for bladder cancer development in experimental systems. The project will be conducted in close collaboration with groups located at the Lund University Cancer Center at the Medicon Village, such as the group of Pietras (mouse models) and Axelson (cell work). Our group has long standing experience in immunohistochemistry (TMA), whole genome approaches to gene expression analysis and epigenetic changes, advanced bioinformatical analyses and genome annotation, as well as in biological and clinical interpretations. A prerequisite for proper treatment of cancer patients is a well-founded classification into clinically relevant molecular subtypes. Even though this will help the clinicians to administrate an efficient treatment, it will not by itself help us to understand the underlying biological mechanisms operating in these subtypes. The fact that the present proposal aims at establishing such mechanisms, and that several of the investigated gene regulatory systems is known to operate in other tumor types as well, a more generalized view of tumor development may be obtained. This may in turn foster a broadened approach to cancer treatment that extends beyond specific tumor types.

Regulation of Bone Metabolism by Hormones and Inflammatory Mediators. Studies using cell culture, mice and humans

CRISPR

4 000 000 SEK

A normal physiological balance between bone resorption and bone formation during bone remodeling is crucial for maintaining bone mass. A major health problem stemming from unbalanced bone remodeling is osteoporosis and accompanying fractures. Sweden has among the highest rate of osteoporotic fractures in the world (70,000 per year). In this project, we will study the importance of

two well known biological mediators (cortisol and vitamin A), as well as the effect of inflammatory mediators, for bone remodeling. Increased levels of cortisol and vitamin A are considered to be risk factors for osteoporosis and fractures. In clinical projects, we will assess how serum levels of cortisol and vitamin A are associated with bone mass, fractures, and biochemical markers of bone remodeling. The project also includes mechanistic studies to delineate how cortisol and vitamin A affect bone cells. Unbalanced remodeling leading to bone loss is commonly observed in patients with inflammatory diseases such as rheumatoid arthritis and periodontitis. We will study the role of the innate immune system in inflammation induced bone turnover.As we have summarized in a recent review paper in Journal of Internal Medicine, WNT signaling is an important regulator of bone mass. In a recent Nature Medicine paper, we have demonstrated, using mice with global or osteoblast specific deletion of Wnt16, the crucial role of WNT16 for cortical bone mass and fracture risk. The effect of WNT16 was due to inhibition of cortical osteoclast formation. We will study the role of WNT16 in osteoclastogenesis induced by cortisol, vitamin A and inflammation.It is possible this project may generate data which can open up new avenues for improving diagnosis and treatment of osteoporosis.Using DEXA, peripheral and high resolution computed tomography, biochemical analyses of serum proteins and available data from genome wide genotyping of MrOS population in Gothenburg, we will in CLINICAL STUDIES investigate:1. how serum levels of cortisol, retinol, retinol binding protein and WNT16 are related to bone mass, biochemical markers of bone remodeling, fractures and polymorphisms in genes encoding receptors for cortisol and retinoids and activating enzymes 2. how polymorphisms in genes encoding the receptor for cortisol and enzymes involved in activation of cortisol are related to bone mass, bone quality and biochemical markers of bone remodeling in patients treated with glucocorticoidsUsing peripheral and high resolution computed tomography, histomorphometry and immune histochemistry, we will in EXPERIMENTAL ANIMAL STUDIES in wild type mice, mice with cell specific deletion of glucocorticoid or retinoid receptors, mice with deletion of the Wnt16 gene and mice with transgenic overexpression of Wnt16 in osteoblasts investigate:3. how clinically relevant concentrations of vitamin A affect the skeleton of mice and the role of different cell specific retinoid receptors for the observed responses4. how glucocorticoids affect osteoclast formation5. the role of WNT16 in osteoclast formation induced by cortisol, vitamin A and inflammation6. pathogenetic mechanisms in osteoclast formation caused by inflammatory processes induced by local activation of Toll-like receptors in skull bones, alveolar jaw bones and jointsUsing osteoblast and osteoclast cell cultures and several molecular analyses such as qPCR, Western blots, ELISA, FACS, gene expression microarray, siRNA and Cas/Crispr we will in MECHANISTIC STUDIES investigate:7. how glucocorticoids and retinoids affect osteoclast differentiation and function 8. how Toll-like receptors stimulate RANKL and osteoclast differentiation9. the role of WNT16 in cortisol, vitamin A and inflammation induced osteoclast differentiation and function.

Novel mechanisms, models and therapeutic targets for inherited disorders CRISPR 4 000 000 SEK Purpose: To identify novel biomarkers and pathophysiological mechanisms in a set of inherited disorders using a combination of high throughput methodologies, unique patient/family materials and different biological model systems, including pluripotent stem cells. To improve diagnostics of the disorders investigated and identify/validate targets for the development of novel therapies.First part of this proposal will focus on the delineation of novel phenotypes and gene identification. To date we identified and investigated >100 families affected by different inherited phenotypes for which the genetic mechanisms and pathophysiology are unknown. The families are identified through collaborations within Sweden as well as with Pakistan. Samples (blood, viable fibroblasts) from selected patients and families will be analyzed in an established pipe-line using e.g. whole exome sequencing (74% yield hit-herto). In this pipe-line, we have recently identified strong novel

candidate genes (TBCD, MAST2, Claudin10b) associated with disorders of the central nervous system and the regulation of body temperature. These gene variants will be subject to functional analysis using different biological model systems. Analysis of each system involves methods such as e.g. CRISPR/Cas9 editing, quantification of mRNA and protein, high-resolution microscopy, growth, viability, cell and organ morphology. Additional candidate genes for e.g. familial oesophageal atresia and Menière disease are likely to be identified in patients/families that are now identified and sampled.Second part of this proposal focus' on induced pluripotent stem cells (iPSC) as a model to better recapitulate development and mechanisms of central nervous system disorders. We have generated iPSC from somatic cells of patients with inherited neurodevelopmental defects caused by defined genetic factors. We established protocols to differentiate iPSC into defined neuronal subtypes for in depth studies of neurodevelopment and disease progression of Mowat-Wilson syndrome, Dravet disease and Down syndrome. Neuronal iPSC derivatives will be investigated by high throughput analysis of the transcriptome, methylome and proteome as well as by different imaging techniques and functionality tests of neurons. Biomarkers identified will be validated for read-out assays and high through-put rescue screening in collaboration with the CBCS platform.Both parts of this proposal apply methods that are established in the applicants group at, Uppsala University, through service platforms (SciLife) or in on-going collaborations. The estimated timeline is 3-4 years. Significance: Inherited disorders comprise a heterogeneous group with many poorly described phenotypes and/or with unknown pathophysiology. There is a great need for better diagnostic tools as well as for biological model systems that are translatable to humans. The expected outcome of the proposal is the identification of novel phenotypic variants, disease associated biomarkers and mechanisms for several traits with a genetic background. Furthermore, iPSC based modeling of neuronal disease will provide an improved and predictable system for neuropathophysiology in humans. The project have an immediate clinical impact by improving diagnostic tools for several groups of patients as well as for novel insights in mechanisms of disease. Ultimately, some of the novel biomarkers identified may serve as candidates for rescue-screening and development of novel therapies.

The Zfp148/p53 interaction - a potential therapeutic target in atherosclerosis and cancer

Gene editing

4 000 000 SEK

A new paradigm suggests that local proliferation of tissue macrophages determines macrophage burden in atherosclerotic plaques, rather than infiltration of monocytes from the circulation. Because macrophages are key components in the plaque and are associated with plaque rupture, reducing macrophage burden is a logical approach to generate stable plaques and reverse the disease. The goal of this program is to reduce macrophage burden by unleashing the tumor suppressor p53 that arrests macrophage proliferation. Since activation of p53 eliminates cancer cells, the approach is equally applicable to cancer. We have shown that the transcription factor Zinc finger protein 148 (Zfp148) is a potent inhibitor of p53 activity; knockout of Zfp148 leads to phosphorylation of p53, expression of p53-target genes, and p53-indcued developmental defects. The defects are rescued on a p53-null background. The two proteins interact physically suggesting that Zfp148 inhibits p53 by direct interaction. We therefore postulate that therapeutic targeting of the Zfp148/p53 interaction reduces atherosclerosis and cancer by unleashing p53 activity, and will test this hypothesis in two aims. Our aims are (1) to define the effects of deletions or knock-in mutations in Zfp148 or p53 on atherosclerosis and tumors in a range of endogenous mouse models and xenograft mice; and (2) to

biochemically define residues in Zfp148 that are essential for the physical interaction with p53 and functionally validate these residues by gene editing in mice and human cancer cell lines. We will also perform a clinical correlation study of Zfp148 mRNA levels in atherosclerotic plaques. Because of our exciting preliminary data and state-of-the art models, we are convinced that we can reach our goal.

Switch recombination: a model system for DNA editing and repair in human lymphocytes

Mutagenesis

7 600 000 SEK

Immunoglobulin gene diversification: model systems for DNA editing and repair in human lymphocytes. The aim of this project is to understand the complex molecular mechanisms involved in DNA repair during immunoglobulin (Ig) gene diversification processes, i.e. V(D)J recombination, class switch recombination (CSR) and somatic hypermutation (SHM). Furthermore, to understand how these processes are involved in the steps leading to immunodeficiency and cancer formation in humans. We have developed a series next generation sequencing technology-based strategies to study the in vivo V(D)J recombination profile as well as CSR and SHM pattern in human B cells. Novel in vitro CSR assays, based on GFP expression, allowing both quantitative and qualitative measurements of substrate recombination, are also being developed. Furthermore, inducible pluripotent stem cells reprogrammed from patients' fibroblasts have been generated, allowing study of various DNA repair factors in defined B cell differentiation stages. Combining these approaches, and taking advantage for the unique collection of patient samples with rare chromosomal instability syndromes, we hope to delineate the DNA recombination/mutagenesis pathways involved in V(D)J recombination, CSR and SHM. We are also applying cutting-edged sequencing technologies to identify additional patients both with recognized and new diseases caused by mutations in DNA repair factors. Finally, we hope to address the question whether illegitimate CSR and SHM events are associated with lymphomagenesis. Several large-scale sequencing studies have already been initiated in order to characterize the DNA repairnome, the genome as well as the transcriptome of B-cell lymphomas. The somatic mutations patterns in the coding genome, the noncoding regulatory regions genome-wide, the somatic translocation events genome-wide, and their associations with DNA repair gene defect, B cell transcriptome will be systematically characterized.

Chromatin configuration regulates post-initiation control of transcription: means to inactivate HIV-1 in the genome Genome editing, CRISPR 2 100 000 SEK AIDS/HIV is a threat to human health at a global level, with 35 million people infected by the HIV-1 virus worldwide. No cure is available and current antiviral treatments have severe side effect. As a retrovirus, the HIV-1 genome is integrated into the genome of its host cells. The viral genome persists in a latent stage for several years, integrated in inactivated chromatin. However, the HIV-1 chromatin remains inducible. When induced, chromatin rearranges into an active conformation, resulting in viral activation and reproduction. We recently identified a protein complex that prevents changes in chromatin composition in a yeast model, and particularly at chromatin of viral decent. This process relies on RNA Pol II transcription and histone turnover. Here we propose to study this process in human cells. We hypothesize that preventing activation of HIV-1 can be clinically used to prevent development of AIDS. In this project we want to answer the following questions: 1) What are the changes to chromatin that initiate HIV-1 transcription after prolonged inactivation? 2) Can we prevent stable HIV-1 transcripts by interfering with transcription-mediated chromatin modifications?The first objective aims at uncovering the mechanism that allows transcription elongation of latent integrated HIV-1. After T-cell activation, transcription factors induce the RNA polymerase but a nucleosome

barrier prevents transcription elongation required for production of functional mRNA. At late stages of HIV-1 transcription, viral proteins are used to prevent this post-initiation control, but the initial stages of HIV-1 activation rely on host proteins. We hypothesize that our recently identified protein Leo1 has a conserved role in promoting chromatin transition by histone turnover also in human cells. In the second objective we will interfere with known and novel proteins to determine their relevance for postinitiation transcription control in general and at HIV-1 in particular. From previous studies we have a panel of candidate proteins. We will also attempt to identify new epigenetic factors involved in this process. Eventually, we will explore how small molecules can target these factors as potential drugs. We will use human cell lines with replication-deficient reporter HIV-1 integrated in the genome. In this system we can control virus activation and then follow concomitant changes in chromatin composition and transcription. Techniques include a variety of novel methods, including high resolution ChIP-exo (chromatin immunoprecipitation followed by exonuclease treatment and parallel sequencing) for chromatin characterization, physiological histone turnover measurement by RITE (recombination induced tag exchange) and genome editing by CRISPR-Cas9 to add the RITE cassette to histone H3.3. In addition to cell population studies, we will use single cell analysis, by proximity ligation assay (PLA) and flow cytometry. The initial characterization and set-up of the model is expected to be completed in 2 years, with follow up studies the following 2 years. A mediumthroughput siRNA screen for epigenetic factors will be done with GFP signal as a readout. The screen for novel proteins involved in the process will extend 2017-2019. Given positive results, we predict to initiate a small molecule drug screen in 2018. A post-doc and a PhD student will be recruited and the PI will be 80% involved in this project. The significance of this project comes from: 1) elucidation of cellular processes that governs transcription specificity. Little is currently known on how the post-initiation control of transcription works but this is a fundamental mechanism that controls transcient cellular responses and permanent regulation during differentiation; 2) the specific silencing of integrated HIV-1. Preventing transcription of HIV-1 will halt virus replication. Favorable outcome from these studies and identification of druggable targets to permanently.

The role of NADPH oxidases in regulation of colonic mucus secretion

CRISPR

3 150 000 SEK

Inflammatory bowel diseases (IBD) including Crohn’s disease and ulcerative colitis (UC) are complex multifactorial diseases caused by a combination of genetic and environmental factors resulting in a chronic inflammation in the gastrointestinal tract. There is currently no cure for these diseases and treatment strategies are restricted to unspecific antiinflammatory drugs. What triggers the onset of disease is currently unknown, but one common hypothesis is that the inflammation is directed towards the commensal microbiota. Despite the facts that the commensals contribute to our health by providing the intestine with nutrients and vitamins and protecting the host against pathogens, they need to be handled in a correct way to avoid induction of an inflammatory response. In the healthy intestine the bacteria are prevented from invading the tissue by a tight epithelium and secretion of a thick dense mucus layer that forms a physical barrier between the bacteria and the epithelium. A defective mucus layer that allows bacteria to access the tissue has been observed in patients with IBD, and is suggested to be one of the mechanisms that trigger disease onset. Although a defective mucus layer has been implicated in the pathogenesis of IBD, the underlying mechanisms responsible for defective mucus formation are unknown. A recent study on the subject of redox regulation of mucus secretion has however shed some new light onto the connection between IBD

and mucus secretion. The study showed that autophagy, a process responsible for recycling damaged organelles and proteins regulates mucus secretion via production of NADPH oxidase (NOX) mediated formation of reactive oxygen species (ROS). This novel connection between autophagy and mucus secretion is of great interest in the context of IBD as mutations in autophagy genes are associated with increased susceptibility to Crohn's disease. The finding that ROS supports the intestinal barrier may seem surprising due to the detrimental role ROS can play in cell health. However, during the last decade it has become clear that NOX enzymes, whose sole known function is generation of ROS (predominantly hydrogen peroxide (H2O2)) are critical regulators of cellular signaling. H2O2 transduces signals via oxidation of cysteines into sulfenic acid (SOH), a modification that functions as a regulatory switch determining the activity of a target protein. The exact mechanism by which ROS regulates mucus release is presently unknown, and the overall purpose of this project is to determine which signaling pathways in goblet cells that are regulated by ROS induced cysteine oxidation. By combining my knowledge in goblet cell functioning and mass spectrometry with Dr. Held´s expertise in redox and kinase signaling and mass spectrometry, and my co-mentor Dr. Stappenbeck's knowledge in mucosal biology we have all necessary tools to study this unexplored area. The proposed project is divided into three parts where we will first determine which of the five NOX isoforms are involved in the regulation of mucus secretion by knocking out the respective isoforms in a spheroid culture system developed by Dr. Stappenbeck using CRISPR/Cas9. Once we have identified which of the NOX enzymes that are regulating mucus secretion we will characterize the proteins that are modified by ROS using a novel mass spectrometry based method developed by Dr. Held. In the third part of the project we will study how an altered cellular redox state affects the goblet cell active kinome. We chose to specifically study kinases as this group of proteins is known to be regulated by ROS and both autophagy and mucus secretion are under strict regulation by kinases. The results obtained from this project will provide a better understanding of the cellular processes that regulates mucus release, and thereby the intestinal barrier. Increased understanding of how these processes are regulated in the healthy intestine, can in future studies be utilized.

2014

A new technology for deep brain mapping

TALEN

3 150 000 SEK

Thousands of new neurons are formed in the hippocampus every day, implicated in plasticity, learning and memory. General features of adult neural stem cells (NSC) are relatively well established, however, increasing evidence emphasize profound heterogeneity within the NSC population. The in vivo lineage tracing of adult born neurons in the hippocampus have yielded contradictive results. The major roadblock of lineage tracing is that of granularity and low throughput, and there are currently no available lineage tracing approaches applying unique marks to cells to be able to trace their individual fates.

This proposal describes a novel approach for tracing the evolutionary history of individual cells in the brain. The approach, TRACER, takes advantage of new technologies, such as deep sequencing and TALENs, and combines them to create a lineage tracer at the individual cell level. The aim is to develop the TRACER technology (which is currently being established in the McManus laboratory) to delineate cell ancestries on a large scale. I expect to map the lineages of adult NSCs in the brain in vivo as well as in neurospheres in vitro to reveal heterogeneity within the NSC population. In the

biggest picture, my work offers a way to distinguish the true stem cell from a large pool of cells that include its progenitors. This is a fundamental goal for the field of stem cell biology.

Sequence determinants of chromosome topology in human cells

CRISPR

3 150 000 SEK

Recent studies have shown that interphase chromosomes in human cells are partitioned into megabase-sized topologically associating domains (TADs). These domains are postulated to play critical roles in gene regulation by physically constraining the long-range interactions between enhancers and promoters. However, it is unclear how TADs form. I hypothesize that two classes of sequence elements are necessary for the formation of TADs, the CTCF binding sites enriched in TAD boundaries and the transcriptional enhancers, known to be critical for cell-specific gene expression. To test their role in chromosome topology, I will employ CRISPR/Cas9 tool to introduce mutations into human Embryonic Stem (ES) cell genome, and perform chromosome conformation capture assay (Hi-C) to determine the effects of sequence alterations on chromatin organization: (1) The CTCF binding motifs in several TAD boundaries will be identified and deleted individually or in combination in a human ES cell line. Hi-C will be performed to examine the resulting changes in the local chromatin domain, and RNA-seq to determine transcriptional effects; (2) Similarly, active enhancers in the same TADs will be deleted systematically to study their role in local chromatin interactions and transcriptional activation. Results from the proposed work will reveal sequence determinants of chromosome organization and advance our understanding of the functional relationships between chromatin structure and gene regulation.

Design of Blood Substitutes by Engineering Novel Electron Pathways in Fetal Hemoglobin

Site-directed mutagenesis, mutagenesis

3 200 000 SEK

Hemoglobin (Hb) molecules present outside the red blood cells undergo several redox activities in vivo. These redox reactions are likely involved with the pathogenesis of many disease states. An understanding of the fundamental underlying molecular mechanisms involved in toxicity of extracellular heme proteins is not only important for developing new therapies, but also underpins the development of safe hemoglobin-based blood substitutes.

The primary objective of the project is to develop a prototype hemoglobin-based blood substitute from a recombinant source that can deliver oxygen with a decreased oxidative toxicity. Focus will be put on: - Use of fetal hemoglobin (HbF) as a starting material. - Introduction of novel mutations in HbF to decrease its oxidative toxicity. - Random mutagenesis of HbF to stabilize the tetrameric form of the protein. - Introduction of surface located cysteine residues to allow chemical modifications. - Formulation of the Hb proteins into a suitable delivery system.

This will be achieved by introducing tyrosine residues in HbF at welldefined positions by site-directed mutagenesis. A group of lead proteins will be prepared and converted into candidate blood substitutes via chemical modifications of the globins. These prototypes will be examined using a combination of cell free and cell studies.

Mechanisms of cell plasticity during limb regeneration in adult vertebrates

Gene editing

3 200 000 SEK

The aim of this project is to reveal critical cell fate controlling mechanisms and understand how these mechanisms regulate regeneration processes in adult vertebrates. The animal model we primarily study is an aquatic salamander, the newt, which in contrast to mammals is able to regenerate complex structures, such as entire limbs. A hallmark of adult newt regeneration is that regenerative progenitor cells are formed both by stem cell activation as well as by the reversal of differentiation of terminally differentiated cells, which during normal homeostatic conditions are in a stable postmitotic arrest. By implementing novel transgenic and gene editing technologies we propose to compare how stem cell activation and dedifferentiation relate to each other, focusing on cell dynamics in skeletal muscle tissue during limb regeneration. In combination with massive parallel sequencing and molecular manipulations in newt/mouse cross-species comparative settings, using both in vitro as well as in vivo experimental paradigms, we aim to identify essential differences that allow dedifferentiation to occur in newt cells but prevent it in their mammalian counterparts. We expect our research to contribute to our understanding of the interspecies differences in regenerative capacities among adult vertebrates and to identify new strategies for how functional regenerative responses could be promoted in mammals.

Structural enzymology

Site-directed mutagenesis, mutagenesis

3 200 000 SEK

Structural biology of polyketide biosynthesis: One objective of this research program is the mechanistic characterisation of enzymes from the biosynthesis of polyketides by a combination of protein crystallography, site-directed mutagenesis, kinetics and other biophysical methods. So far, we have determined the structures of seventeen enzymes from these pathways, and characterized several previously unknown enzymatic reaction mechanisms. The focus of this part of the proposal is on the biosynthetic pathway of nogalamycin. The late steps of nogalamycin biosynthesis consist of the formation of a highly unusual carbon-carbon linkage of the carbon skeleton with a carbohydrate moiety and a final hydroxylation step. The purpose of the proposed studies is to elucidate the underlying enigmatic chemistry and the enzymatic mechanisms of these reactions. In a longer perspective, structural and mechanistic insights from these biosynthetic pathways are expected to facilitate engineering of enzymes and metabolic pathways for the production of novel antibiotics. Ubiquinone biosynthesis: Ubiquinone is an important component in the mitochondrial respiratory chain of living organisms. The enzymatic steps leading to its biosynthesis are however surprisingly little characterized, and a second objective of this research program is to elucidate the function and the structural basis of the reaction mechanisms of enzymes from this pathway.

Architectural control of cell polarity by signaling and non-coding RNA pathways

Gene editing, CRISPR

3 200 000 SEK

Generation of architectural polarity impacts all cell types, ranging from epithelial cells to neurons and astrocytes, relates to tissue organization and links to disease development when misregulated. Thanks to VR-MH grants, my group studies plastic changes of cell polarity in the context of cancer, by focusing on the process of epithelial mesenchymal transition. In this new and independent project we shift our attention to the establishment of cell polarity. Our working hypothesis proposes coordinate control of cellular architecture in the nucleus and cytoplasm. Such coordination may be mediated by long non-coding RNAs (lncRNAs) acting at the chromatin and transcriptional level or at the posttranscriptional cytoplasmic level. We propose that expression and localization of lncRNAs is controlled by liver kinase B1 (LKB1), a regulator of epithelial and neuronal polarity, and Smad4, a signaling mediator of transforming growth factor beta. The project is methodologically structured as follows: a) mammary epithelial, astrocytic and oligodendrocytic 3D cultures are faithful models of cell polarity, where b) LKB1 and Smad4 will be knocked out using the CRISPR-Cas9 gene editing system, and impact on c) 3D architecture and d) lncRNA expression profiles will be analyzed; e) selected lncRNAs will be functionally linked to downstream targets that control cell polarity. Our end goal is to understand coordinate control of cell polarity at the nuclear and cytoplasmic level.

Towards an understanding of aging and neurodegenerative disease using novel stem cell, reprogramming and genome editing technologies

Genome editing

6 000 000 SEK

In this project I will use novel technologies in the field of stem cell biology, reprogramming and genome editing to study aging and neurodegenerative disorders. I will study possible mechanisms for dysfunction and avenues for therapeutic intervention. To generate isogenic human neurons, specific genomic loci in human pluripotent stem cells will be modified to harbor mutations predisposing to neurodegeneration or to make conditional knockouts of age related genes. Targeted lines will then be directed to neurons using a rapid single step protocol. All generated lines and neurons will be analyzed for signs of pathology in vitro and in vivo by morphological imaging, functional and gene expression assays. In an alternative approach I will further develop the technology of directly converting fibroblasts to neurons and neural progenitor cells. To study aging and neurodegeneration in vivo and find possible novel therapeutic interventions I will use in vivo reprogramming to convert glial cells to neurons in the diseased and aged mouse brain. I will also study if genomic instability caused by retroelements is the underlying cause of age related neuronal dysfunction and decline in neurogenesis. Furthermore, I will characterize hypothalamic neurogenesis and investigate its role in brain aging and neurodegeneration as well as the potential role in organismal aging.

WNT16, a Novel Regulator of Cortical Bone and Non-Vertebral Fracture Risk

CRISPR

6 000 000 SEK

Sweden and Norway have the highest incidence of bone fractures in the world. Currently available osteoporosis drugs are effective in reducing vertebral fracture risk (mainly dependent on trabecular bone mass) while they are less effective in reducing non-vertebral fracture risk (mainly dependent on cortical bone mass) and most fractures actually occur at non-vertebral bone sites. Our recent human genetic studies demonstrate that the WNT16 locus is unique in being associated with cortical bone mass and non-vertebral fracture risk. I hypothesize that WNT16 might be a promising drug target for fractures at cortical bone sites. The overall aim of this proposal is to characterize how WNT16 regulates cortical bone mass. We will in functional in vivo studies characterize the skeleton of mouse models with altered WNT16 expression. The WNT16 producing and target cells will be identified using a combination of cellspecific WNT16 inactivated mouse models and a variety of in vitro systems. The novel Cas/CRISPR gene-targeting system will be used to identify intracellular WNT16 signaling pathways. Finally, the effect of WNT16 treatment in bone-related disease models will be evaluated. Our preliminary studies indicate that WNT16 is an unique osteoblast-derived regulator of cortical bone mass and fracture risk. This project will increase the knowledge of the regulation of cortical bone homeostasis and might open novel avenues for specific prevention or treatment of non-vertebral fractures.

High-throughput rapid manipulation of gene expression in the mouse: an efficient method to dramatically reduce the numbers of mice used in basic science

Genome editing, CRISPR

2 400 000 SEK

This research program describes a method to replace traditional mouse knockout technologies, dramatically reducing the numbers of mice used in science, while offering equal or superior results. The technology was developed by Elaine Fuchs? laboratory at the Rockefeller University to manipulate gene expression in the developing skin. I performed guest research in the Fuchs lab to develop this technique further, and now show that this technology can also target the nervous system, lungs, eyes, gastrointestinal system and more. This means that we can reduce the numbers of mice used in developmental or cancer biology fields by more than 50%! The savings are especially pronounced when investigating multiple genes; in traditional triple knockouts only 1 in 64 offspring is triple homozygous from heterozygous parents. With 1 wild type, 3 single knockouts, and 3 double knockout controls this means 56 mice are wasted, byproducts of the breeding scheme. The suggested technology bypasses these problems, by specifically generating only the 8 required mice. Ultrasound-guided lentivirus nanoinjection into the embryonic amniotic fluid entails the injection of a lentivirus encoding shRNA, a gene of interest or CRISPR/Cas9 systems. Establishing the targeting of specific organs, the capacity to manipulate multiple genes at once, and harnessing the powerful potential of genome editing tools will make this technique a viable and attractive alternative, which will save dramatic numbers of mice.

Quantitative Structure-Based Models for Ligand Interactions with GProtein Coupled Receptors

Site-directed mutagenesis, mutagenesis

4 143 000 SEK

This project deals with computational analysis and modelling of key interactions between membrane associated G-protein coupled receptors (GPCRs) of medical importance and endogenous as well as extraneous ligands. The main goals are to develop reliable structure-based models for quantitative modelling of ligand interactions with GPCRs of the neuropeptide Y, adenosine and angiotensin II families. We will provide quantitative predictions of the effects of site-directed mutagenesis experiments on these receptors and their ligand binding properties. In collaboration with medicinal chemists we will also engage in discovery of new ligands for these receptors both by virtual screening approaches and by rational design. Our basic strategy is to employ structural bioinformatics together with state-of-the-art computational modelling and simulation techniques, where we are also active in methodological development, in order to develop predictive schemes that can be tested and validated with experiments.

Non-coding RNAs in Neisseria meningitidis. "To silence or not to silence" ?

Site-directed mutagenesis, mutagenesis

6 000 000 SEK

Non-coding RNAs is becoming a major theme in pathogenesis research. Even though the genome sequence of Neisseria meningitidis is available for more than a decade, only 2 ncRNAs have so far been identified. My proposed research is to identify and characterise novel ncRNAs in this obligate human pathogen. Preliminary searches have revealed more than 700 putative ncRNA candidates. Further screenings will be performed to identify bona fide candidates. Expression patterns of these ncRNAs will be investigated by Northern Blotting on total RNA isolated from different growth conditions. In order to obtain the targets of these ncRNAs, RNA-seq, 2-D protein gel and Tandem Mass Spec. will be performed. The mode of action of these RNAs will be studied by molecular and biochemical approaches. Genetic methods such as knock-out and site-directed mutagenesis together with biochemical experiments such as in vitro transcription/translation will be carried out.

I will also be applying a novel technique using "Peptide-conjugated phosphorodiamidate morpholino oligomers", to silence expression of these specific genes in Neisseria thus disrupting its growth. Infection assays will also be used to further study the role of these RNAs and their targets in a more native environment. RNA-mediated gene regulation in human bacterial pathogens is an emerging field; I believe such knowledge will be essential for the better understanding and to the development of novel drugs to combat this deadly bacterium.