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

I n t r o d u c t i o n

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 a f t e r 2 0 0 1 .

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 h a v e b e e n d e v e l o p e d s i n c e 2 0 0 1 [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 c o m p e t e n t a u t h o r i t i e s w h e n a p p r o p r i a t e .

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 s p e c i f i c s e c t o r s , s u c h a s t h e o r g a n i c s e c t o r .

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?

Croatia

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

* 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

> Current validated methods are not aplicable to NGT products

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

○ Yes
◉ No

*   Please explain why not

> Current validated methods are not aplicable to NGT products

* 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

> Current validated methods are not aplicable to NGT products

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

○ Yes
◉ No

*   Please explain why not

> Current validated methods are not aplicable to NGT products

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

◉

Yes

No

*   Please describe the traceability strategy, including details on the required financial, human resources and technical expertise required

> Only within plant varieties registration as described under question 6. And 7. Traceability trough laboratory testing could not be achieved since current validated methods are not applicable to NGT products.

*   What best practices can you share?

> At the end of 2018, we have sent a questionnaire to all applicants which have registered varieties on our national list and asked them if they have used genetic engineering techniques including NGT.

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

    Yes

    ◉ No

*   Please explain why not

> Expected costs are included in costs of performing official controls.

* 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:

    ☑ **Agri-food sector?**

    ☑ **Industrial sector?**

    ☑ **Medicinal sector?**

Agri-food sector

> We do not have any experience concerning NGT product releases.

Industrial sector

> We do not have any experience concerning NGT product releases.

Medicinal sector

> We do not have any experience concerning NGT product releases.

* 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**

> We have implemented question in application form in which applicant are obligatory to declare is variety created using genetic engineering techniques including NGTs (question 11. in attached questionnaire form-Application for addition to the Nathional variety list).

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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/APPLICATION_FOR__ADDITION_TO_THE_NATHIONAL_VARIETY_LIST.pdf

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

> Total number of projects are 13 during the period 2015/2020
> Total amount of financing is around 1.885.000,00 eura
>
> PROJECT TITLE
> 1. Sustainable production of bioethanol and biochemicals from agricultural waste lignocellulosic raw materials
> 2. Investigation of substrate and editing specificity in tRNA synthetases and the mechanism of antibiotic action
> 3. Aminoacyl-tRNA synthetases as gatekeepers of the standard genetic code
> 4. Non-canonical roles of aminoacyl-tRNA synthetases
> 5. The role of autophagy receptors in selective removal of mitochondria – AutoMito
> 6. Interactome identification of SSB paralogous proteins in multicellular prokaryotes, Streptomyces coelicolor
> 7. Exploring novel molecular mechanisms of alternative electron partitioning in photosynthesis
> 8. The Role of Ribosomal Proteins L5 and L11 in Tumor Suppression
> 9. Center of Excellence for Virus Immunology and Vaccines (CERVirVac)
> 10. Elucidating the HPV E6/E7 Oncogenic Functions at Different Anatomical Sites
> 11. Genomic and epigenomic changes in auto- and allopolyploids: case studies on dalmatian pyrethrum, shallot and anemone
> 12. Molecular aspects of disease pathogenesis in AHCY deficiency
> 13. Epigenetic regulation regulation of immunoglobulin G glycosylationlist
>
> The Summary of Croatian projects are under question 12 (CROATIAN LIST OF PROJECTS BY NGT)

5

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

> N/a

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

> Challenging in the context of traceability and monitoring of products, and other issues related to the implementation of current legislation.

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

○ Yes
◉ No

* 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

> The use of NGTs have great potential for improving drug discovery both target and molecule recognition and development. The use of NGTs have the potential to deliver new drug targets through the exploration of differences in gene expression, for example a normal cell and one that is transformed.

* 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

> In the field of developing vaccine against COVID-19

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

* 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

The maximum file size is 1 MB

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

> The use of NGTs bring opportunities in the field of drug discovery both target and molecule recognition and development.

* 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

> The use of NGTs have great potential for improving drug discovery both target and molecule recognition and development. The use of NGTs have the potential to deliver new drug targets through the exploration of differences in gene expression, for example a normal cell and one that is transformed.

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

> In accordance to the   EU legislative for specific field such as environment, human, animal or plant health .

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

    ⦿ Yes
    ◯ No

*   Please explain under which conditions

> It is not under our scope of activities.

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

    ⦿ Yes
    ◯ No

*   Please describe and provide concrete examples/data

> It is important to protect intellectual property within, We believe, well defined EU patenting framework. One good example are patented tobacco lines that do not have glycosilation machinery and can be used for antigen expression in, for example, for Covid-19 vaccine production. Example:
> CRISPR/Cas9-mediated knockout of six glycosyltransferase genes in Nicotiana benthamiana for the production of recombinant proteins lacking β-1,2-xylose and core α-1,3-fucose.
> Jansing J, Sack M, Augustine SM, Fischer R, Bortesi L.
> Plant Biotechnol J. 2019 Feb;17(2):350-361. doi: 10.1111/pbi.12981. Epub 2018 Aug 11.

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

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

As stated previously there are challenges in the context of traceability, laboratory testing and risk assessment practices. Currently implemented practices are not applicable to products obtained through NGTs.

* 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

The use of NGTs have great potential for improving drug discovery both target and molecule recognition and development. The use of NGTs have the potential to deliver new drug targets through the exploration of differences in gene expression, for example a normal cell and one that is transformed.

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

 As stated previously there are challenges in the context of traceability, laboratory testing and risk assessment practices. Currently implemented practices are not applicable to products obtained through NGTs.

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

- ◉ Yes
- ○ No

* Please explain under which conditions

In accordance to the currently EU legislation.

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

- ○ Yes
- ◉ No

* Please explain why not

The European patenting framework is, We believe, very well established. The above mentioned CRISPR /Cas9 lines are a very good example.

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 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

Contact

SANTE-NGT-STUDY@ec.europa.eu

ZAHTJEV ZA PRIZNAVANJE SORTI POLJOPRIVREDNOG BILJA
Application for Addition to the National Variety List

1. Podnositelj zahtjeva
   Applicant

Ime *(First name):*	
Prezime *(Last name):*	
Tvrtka: *(Organisation):*	
Adresa: *(Address):*	
Poštanski broj *(ZIP code):*	Mjesto *(Place):*
Država *(Country):*	
Telefon *(Phone Nr.):*	
Telefaks *(Fax Nr.):*	
E-mail *(e-mail):*	

2. Punomoćnik *(Uz zahtjev priložite punomoć o zastupanju u postupku priznavanja sorti poljoprivrednog bilja)*
   Procedural representative *(Please attach the completed form of the authorization of the procedural representative)*

Ime *(First name):*	
Prezime *(Last name):*	
Tvrtka: *(Organisation):*	
Adresa: *(Address):*	
Poštanski broj *(ZIP code):*	Mjesto *(Place):*
Država *(Country):*	
Telefon *(Phone Nr.):*	
Telefaks *(Fax Nr.):*	
E-mail *(e-mail):*	

3. Oplemenjivač/i sorte
   The original breeder(s) of the variety
☐ Oplemenjivač sorte je podnositelj zahtjeva *(breeder of variety is applicant)*

☐ Oplemenjivač sorte nije podnositelj zahtjeva *(breeder of variety is NOT applicant)*

Oplemenjivač 1 Breeder 1		*Oplemenjivač 2 Breeder 2*	
Ime *(First name):*		Ime *(First name):*	
Prezime *(Last name):*		Prezime *(Last name):*	
Tvrtka: *(Organisation):*		Tvrtka: *(Organisation):*	
Adresa: *(Address):*		Adresa: *(Address):*	
Poštanski broj *(ZIP code):*	Mjesto *(Place):*	Poštanski broj *(ZIP code):*	Mjesto *(Place):*
Država *(Country):*		Država *(Country):*	
Telefon *(Phone Nr.):*		Telefon *(Phone Nr.):*	
Telefaks *(Fax Nr.):*		Telefaks *(Fax Nr.):*	
E-mail *(e-mail):*		E-mail *(e-mail):*	

4. Održivač sorte
   Maintainer

Ime *(First name):*	
Prezime *(Last name):*	
Tvrtka: *(Organisation):*	
Adresa: *(Address):*	
Poštanski broj *(ZIP code):*	Mjesto *(Place):*
Država *(Country):*	
Telefon *(Phone Nr.):*	
Telefaks *(Fax Nr.):*	
E-mail *(e-mail):*	

5. Vrsta kojoj sorta pripada
Species to which the variety belongs
 Latinsko ime vrste *(Latin name):*_____
 Hrvatsko ime vrste *(Croatian name):*_____

6. Ime sorte / denominacija *(upotrebljavajte velika slova)*
 Variety denomination (using capital letters)
 Oplemenjivačka oznaka *(Breeders reference):*
 Predloženo ime sorte *(Proposed name of the variety):*

7. Predloženo ime sorte je *(Proposed designation is)*
  ☐ Zamišljeno ime *(Fancy name)*   ☐ Kod *(Code)*

8. Podaci o upisu sorte na sortnu listu i oplemenjivačko pravo sorte
 Information regarding national listing and plant breeder's rights of the variety
  ☐ Sorta je prijavljena ili je upisana na sortnu listu u drugoj državi *(The variety has been applied or has been listed to the National Variety list in any other country)*

Država *(Country)*	Ime ili predloženo ime sorte *(Variety denomination)*	U postupku upisa *(Application pending)*	Upisana *(Already listed)*
		☐	☐
		☐	☐
		☐	☐

  ☐ Sorta nije upisana i nije prijavljena za upisivanje na sortnu listu u niti jednoj drugoj državi *(The variety has NOT been listed and not applied for National variety list in any other country)*

9. Podaci o zaštiti sorte *(The variety protection data)*
  ☐ Sorta je zaštićena u drugoj/im državi/ama, navedite u kojoj/im i godinu zaštite *(The variety has been protected in other countries, in which countries and year of protection):* _____
  ☐ Sorta nije zaštićena niti u jednoj državi *(The variety has NOT been protected in other country)*

10. Postupak ispitivanja različitosti, ujednačenosti i postojanosti (DUS)
 Technical examination of the variety (DUS)
  ☐ je završen, navedite državu/e *(has already been completed, give the country):*_____
  ☐ se trenutno provodi, navedite državu/e *(is conducted, give the country):*_____
  ☐ nije proveden *(has not been done)*

11. Sorta je stvorena uz pomoć genetske tehnologije ili sadrži genetski modificirane organizme
 The variety is or includes genetically modified organisms (GMO)
  ☐ DA *(Yes)*   ☐ NE *(No)*
 Ako DA, priložite dozvolu za puštanje GMO u okolinu. *(In case of YES enclose premission to release GMO into the environment)*

12. Porijeklo sorte *(Origin of the variety)*
 _____

13. Upotreba sorte *(Use of the variety)*
 _____
  ☐ Čuvana sorta/domaća i udomaćena sorta *(Conservation variety)*
  ☐ Sorta razvijena za uzgoj pri određenim uvjetima *(Variety developed for growing under particular conditions)*

14. Prilozi *(Enclosures)*
  ☐ tehnički upitnik *(Technical Questionnaire)*
  ☐ punomoć za zastupanje *(Authorization of the Representative)*
  ☐ izvješće o DUS testiranju *(DUS Test Report)*
  ☐ dozvola za puštanje GMO-a u okolinu *(Permission for releasing GMO in to environment)*
  ☐ ostalo, navedite priloge *(other, specify):*

15. Izjava *(Statement)*
 Prijavljujem se za postupak priznavanja sorti poljoprivrednog bilja Republike Hrvatske *(I apply for addition to the National Variety List in the Republic of Croatia.)*
 Izjavljujem da su svi podaci u prijavi i prilozima potpuni i točni *(I declare that all data stated in the application and attachmentsare, to the best of my knowledge, complete and correct).*
 Suglasan/a sam da se Zavod može savjetovati i izmjeniti podatke sa drugim sortnim uredima *(I agree that , the Administration may consult and exchange data with other variety offices).*
 Izjavljujem da ću platiti sve troškove i pristojbe u postupku priznavanja sorti poljoprivrednog bilja u predviđenim rokovima.
 (I agree to pay all the costs and fees variety addition to the National Variety List).

          Mjesto:_____   Datum:_____
          (Place):   *(Date):*

                        Potpis podnositelja zahtjeva:_____
                        (Signature of the person entitled):

                              M.P.

List of NGT-related research projects in Croatia suported with nationaly funding

A total of 13 projects have been funded in the amount of 1.885.000,00 € over the last five-years period from 2015/2020.

Project title and Abstract

1. **Sustainable production of bioethanol and biochemicals from agricultural waste lignocellulosic raw materials**

The aim of this project was the development of sustainable bioprocesses for the production of bioethanol and biochemicals [lactic acid (LA) and physiologically active oligosaccharides (PAO)] from agricultural waste lignocellulosic raw materials. Studies of bioethanol and LA production were performed by the semi-solid or solid state fermentation in the new constructed horizontal rotating tubular bioreactor (HRTB). The most suitable mic-roorganisms for bioethanol and LA production were identified and improved by genetic methods. Studies regarding suitable purification methods for all three bio-products were performed in order to obtain pure compounds for their further practical use. The sustainability of bioethanol and LA pro-duction was evaluated through the realization of Life Cycle Assessment (LCA). The scale-up procedure was based on the developed integral biopro-cess mathematical model and other scale-up criteria. Project outcome was a new integrated bioprocesses for the transformation of agricultural waste lignocellulosic raw materials into high added value products.

2. **Investigation of substrate and editing specificity in tRNA synthetases and the mechanism of antibiotic action**

Proteins are the working horses of the living cell. They are built from twenty amino acids that are attached to one another in a specific order. Aminoacyl-tRNA synthetases (aaRSs) are enzymes that dictate amino acid incorporation into proteins following the information encoded in genes. There are 20 aaRSs in the cell each specific for one amino acid. These enzymes face the difficult task of selecting the designated amino acid from a pool of similar amino acids. Protein functionality strongly depends on the accuracy of amino acid incorporation, making the fidelity of protein synthesis crucial for cell viability.This project aims to delineate the evolutionary and mechanistic basis of aaRSs fidelity. AaRS enzymes are very accurate enzymes that double check that the correct amino acid is chosen. First in the active site of the enzyme and then through additional enzymatic proofreading. We will investigate which characteristics of aaRSs allows them to be fast and at the same time accurate, and to which degree their enzymatic properties may have evolved to prevent binding of substrate-mimicking antibiotics into the active sites.

3. **Aminoacyl-tRNA synthetases as gatekeepers of the standard genetic code**

The genetic code provides the basis for translation of genetic information into functional proteins. Codon assignments are established by aminoacyl-tRNA synthetases (aaRS), which couple amino acids to their cognate tRNAs. Mistakes in translation are generally kept low, in part by inherent aaRS hydrolytic editing. The extent to which mistranslation occurs presents a fundamental question in basic research with impact on medicine and biotechnology. AaRS quality control mechanisms also act as gatekeepers of the standard genetic code and prevent infiltration of natural but non-coded amino acids. Consequently, they present an obstacle for synthetic amino acid translation in rational protein design. Here we propose to investigate the synthetic and editing mechanisms of three aaRSs responsible for translation of amino acids that build the protein core: leucine, valine and isoleucine. We will compare the catalytic principles and gained specificity of the enzymes' synthetic and editing sites against proteinogenic and non-coded amino acids. The cellular toxicity and stress responses of induced non-canonical mistranslation will be addressed. The goal is to improve our understanding of the basis for selection of the natural amino acid alphabet, and how violation of the coding principles influences cell physiology. Mechanistic insight will extend our ability to apply fluorinated amino acids in protein rational design. A possible link between altered translational fidelity and adaptation to environmental stress will be sought. This research couples biochemistry and chemical biology through the interest in reassignment of the genetic code. The

assembled expertise through the international and national collaborators will strongly enhance the capacity of our group, which is well-recognized in the field of protein translation, to conduct this research.

4. Non-canonical roles of aminoacyl-tRNA synthetases

In collaboration with foreign and domestic partners, we will study newly discovered unusual or non-canonical roles of aminoacyl-tRNA synthetases (aaRSs) in transmission of genetic information and in other cellular processes. As housekeeping enzymes, aaRSs catalyze the specific coupling of amino acids with their cognate tRNAs to produce aminoacyl-tRNAs, which serve as starting materials for protein biosynthesis. Recent studies performed by us and the others revealed that these enzymes are much more versatile than initially thought. In all three domains of life they form macromolecular assemblies with other aaRSs and various non-synthetase proteins. These associations affect synthetase functions and their localization in the cell. In archaea we have identified a multi-synthetase complex, which associates with the ribosome and our objectives are now to investigate the consequences of complex formation with respect to the function of individual components and the protein synthesis machinery in general. By characterizing the effects of complex formation on protein synthesis, we will begin to resolve how such assemblies control the efficiency and accuracy of translation. In support, recent genome wide analyses of gene structure and codon usage strongly suggest that such complexes may be critical for channeling substrates during protein synthesis and optimization of translation. They may also act as a repository for aaRSs. In response to cellular changes, aaRSs are subsequently liberated from the complex to participate in non-canonical tasks beyond translation, such as protein degradation or regulation of transcription and metabolism. Our preliminary in vivo screens, revealing interesting protein interactors of aaRSs, support the role of aaRS complexes in protein homeostasis. Finally, aaRS homologs, full-lengths or truncated, take a part in non-ribosomal synthesis of natural products, emphasizing diversity of functions displayed by aaRS super-family. Seryl-tRNA synthetases (SerRSs), studied in our laboratory for many years, will be further employed as excellent model system, because these enzymes display unique structural and functional characteristics. We have recently described the structure and partly the function of these truncated SerRS-like enzymes, which do not aminoacylate tRNAs, but transfer activated amino acids to specific carrier proteins, representing a link between programmable protein biosynthesis and template-independent peptide synthesis. The objective of this project is to resolve structural, functional and mechanistic diversities of SerRS super-family in fundamental biological processes and possibly highlight and exploit their biomedical values.

5. The role of autophagy receptors in selective removal of mitochondria – AutoMito

Autophagy is cellular degradation pathway essential for cell survival, development and differentiation. Its digestive property has been shown important in different pathologies including cancer, neurodegeneration and pathogen removal. Discoveries of specific autophagy receptors have opened a new chapter in the autophagy field. Of great interest is the removal of damaged or excess mitochondria through specialized autophagy- mitophagy. Together with colleagues, I have identified and characterized mitochondrial proteins Nix/Bnip3L and Bnip3 as a mitophagy receptors for recruiting the autophagic machinery to damaged mitochondria and help their elimination (Novak et al, 2010; Zhu et al, 2013). We demonstrated that receptors mediate mitochondrial clearance during reticulocyte differentiation. Our goal is to further study autophagy receptors and proteins associated with mitochondrial clearance to better understand the role of mitophagy and its mechanism. We will study: (1) phosphorylation and dimerization of receptors and the consequences of this events on mitophagy progression; (2)factors that regulate tight connection of apoptosis and mitophagy; (3)potential interplay between mitophagy receptors and other mitophagy-related proteins, like E3-ligase Parkin and kinase PINK1; (4)the effects of mitophagy on cancer development. To reach our goals we will use established methodology in our laboratories including protein identification and interaction techniques (Western blot, IP, GST-pull down, RNAi), immunofluorescent microscopy, mass spectrometry, ITC and NMR. All experiments will be performed using cultured cells.

6. Identifying interactome of paralogous SSB proteins in a mullticellular prokaryote, *Streptomyces coelicolor*

Streptomycetes are best known as producers of clinically important antibiotics and for their complex life cycle. Single-strand DNA-binding (SSB) proteins have a key role in DNA replication, repair and recombination in all domains of life. Various bacteria possess paralogous SSBs, but their biological role is poorly understood. We reported that two *S. coelicolor* SSBs have adopted different cellular functions through evolution. While SsbA is indispensable for survival, SsbB has a key role in chromosome segregation during spore formation. Recently, we found that a specific mutant with deleted *ssbB* increases antibiotic production. To get a better insight into biological functions of paralogous SSB proteins, this project aims to identify SSB-interactomes, to analyse their binding domains and to elucidate the importance of disulphide bridges for SsbB functionality. Once identified by B2H system or TAP technology, the most promising interactants will be analysed by applying *in vivo* and *in vitro* techniques. Genetic constructs carrying mutations in gene encoding protein partner (if not essential) or double mutants with *ssbB* gene will be examined phenotypically and morphologically. The co-localization of selected SSB partners will be examined by fluorescence microscopy. To obtain information on the binding mechanisms of SSB-protein interactions, the affinity of the interacting proteins will be analysed by several approaches, such as semi-quantitative assays (EMSA), spectroscopic techniques (CD, fluorescence, MST), and calorimetric techniques like ITC. Comparative proteomic analysis will be applied to examine temporal synthesis of SsbB and its protein partners during early log and programmed cell death stage of growth in *S. coelicolor* and *S. rimosus* with elevated antibiotic production. Expecting that most of the SSB partners are conserved in *Streptomyces* spp., proposed analyses could uncover time-specific interaction, potentially important for antibiotic production.

7. Exploring novel molecular mechanisms of alternative electron partitioning in photosynthesis and Development of Young Scientists Careers-Education of New PhD's

In photosynthesis, final electron transfer from ferredoxin to NADP+ is accomplished by the flavo enzyme ferredoxin:NADP+ oxidoreductase (FNR). FNR is recruited to thylakoid membranes via integral membrane thylakoid rodanese-like protein TROL and soluble Tic62. TROL protein has recently been characterized by our group. FNR also represents a link between light-driven reactions and general metabolism (e.g. carbon fixation, nitrogen metabolism and fatty acid and chlorophyll biosynthesis). In this project, we address the fate of electrons downstream of photosystem I when TROL is absent. We will employ gene knock-out and knock-in approaches in the model plant Arabidopsis thaliana to study in vivo functions of TROL and its domains. We hypothesize that the interaction of FNR with TROL is dynamic, similar to FNR-Tic62 association, and in synchrony with changing environmental light conditions. Further, we propose that thylakoid lumen-exposed rhodanese domain of TROL is involved in perception of redox signals, while proline-rich swivel preceding FNR membrane attachment motif is responsible for the dynamic FNR biding and/or recruitment of FNR into different membrane domains. We will use elaborate biochemical and biophysical approaches to quantify free radical formation and electron flow and partitioning in various TROL mutants. We will aim to demonstrate that TROL-FNR branch point is integrated into plant stress responses. Finally, we want to demonstrate that the dynamic binding and release of FNR from TROL can control the flow of electrons prior to activation of the pseudo-cyclic electron transfer pathway. This mechanism may have been overlooked in present models of electron flow in oxygenic photosynthesis.

8. The Role of Ribosomal Proteins L5 and L11 in Tumor Suppression

The critical role of p53 in tumor suppression is supported by the observation that approximately 50% of all human cancers have mutation within this gene. The ability of p53 to detect many stresses and appropriately respond by regulating target genes that induce various biological responses is essential for prevention of malignancies. It is unknown how such diversity of cancer-related stress signals can be integrated by the p53 tumor suppressor. Although it was largely accepted that common to all p53-activating stresses is DNA damage, we reported that genetic disruption of ribosome biogenesis elicits a p53-dependent checkpoint response independently of DNA damage (Volarević S et al, Science, 2000; Šulic S et al, Genes&Dev, 2005; Panić L et al, MCB 2006). Several ribosomal proteins (RPs) have been suggested as transducers of p53-activating signals in response to inhibition of ribosome biogenesis. Our recent work has shown that RPL5 and RPL11 are necessary for p53 up-regulation not only in response to ribosomal, but also in response to genotoxic stresses (Barkić et al, MCB 2009; Donati G et al,

Oncogene 2011; Bursać S et al, manuscript in preparation). Moreover, we identified seven different heterozygous mutations in the coding regions of RPL5 and RPL11 in human colon cancer, which are the first identified cancer-associated mutations in these genes.

Taking into account these observations, we propose a hypothesis that RPL5 and RPL11 act as tumor suppressors and that this function is largely mediated via p53. To test this hypothesis, we propose the following specific objectives:

1. To determine the molecular mechanisms whereby RPL5 and RPL11 activate p53 in response to ribosomal or genotoxic stresses.

2. To test the functional significance of colon cancer-associated mutation in RPL5 and RPL11 genes in p53 activation in cultured cells.

3. To test whether RPL5 and RPL11 genes are indeed bona fide tumor suppressors.

9. Center of Excellence for Virus Immunology and Vaccines (CERVirVac)

The Center of Excellence is focused on establishing a base for further development of viral immunology and vaccinology in Croatia. The project studies the interplay between viruses and the immune system and aims to design effective vaccines and/or vectors for the prevention and treatment of emerging infectious diseases and cancer. The work packages (WPs) 1 and 2 are dealing with novel immunoregulatory viral genes and their role in viral pathogenesis and immunosurveillance. In WP3, the focus is put on the study of cytomegalovirus (CMV)-based vaccine vectors for cancer and for several human pathogens. The proof-of-concept studies of WP4 aim to create, by the use of rational design approach, novel mumps virus mutants that can serve as a vaccine and / or as a vector for vaccines. WP5 and WP6 are devoted to the study of human immune responses to two important pathogens (hantavirus and hepatitis C virus) and viral mechanisms that regulate the immune response. The emphasis is put on the characterization of memory NK-cell response in hantavirus-infected human subjects.

WP7 is aimed at improving the production and quality of live virus vaccines and vectors, in accordance with the standards declared by regulatory agencies.

In addition to the proposed research, an essential role in this project is given to two horizontal groups of activities: 1) training of young researchers and networking with other excellent institutions in the European Research Area and 2) dissemination and exploitation of the research results generated by the Center.

10. Elucidating the HPV E6/E7 Oncogenic Functions at Different Anatomical Sites

Human Papillomaviruses (HPVs) cause over 600,000 cancers annually. These occur at diverse anatomical sites, and include anogenital and head and neck (HN) cancers. Of these the most important is cervical cancer, which is the leading cause of cancer-related death in women in many parts of the world. HPVs encode two oncoproteins, E6 and E7 that are directly responsible for the development of HPV-induced malignancies. They do this cooperatively by targeting diverse cellular pathways involved in the regulation of cell cycle control and apoptosis. Most importantly, there is a continued requirement for E6 and E7 expression throughout tumorigenesis, with loss of either protein resulting in a cessation of transformed cell growth. Therefore, these viral proteins represent excellent targets for therapeutic intervention and understanding the molecular mechanisms underlying their respective functions is critical for developing such antiviral therapies. In recent years there has been dramatic increase in the number of HPV-associated cancers at other anatomical sites, especially in the HN region. Cancers found in this area largely contain HPV-16 sequences and the time frame between initial infections and cancer progression is much shorter in comparison with the anogenital cancers, indicating potentially major differences in how these viruses induce cancer development at different anatomical sites. Determining if this is a reflection of different modes of virus-host interactions in these sites will be the main focus of this project.

11. Genomic and epigenomic changes in auto- and allopolyploids: case studies on dalmatian pyrethrum, shallot and anemone

Polyploidy or whole genome duplication (WGD) is the main operating mechanism of speciation and biodiversity in plants, which leads to the creation of new genetic variants in natural populations, drives evolution, increases genetic diversity and complexity of organisms and plays an important role in the

domestication of plants. Although numerous studies have been conducted so far, we still don't know enough about the changes that are taking place at the level of the genome after polyploidization and hybridization. Auto- and allopolyploidization lead to genome instability and its reorganization. Improper segregation of chromosomes in meiosis is the main problem for the formation of functional gametes and successful sexual reproduction of recently created autopolyploids (neo-autopolyploids). On the other hand, evolutionary old autopolyploids have developed mechanisms that eventually lead to proper chromosome segregation. The exact mechanism of the transition from the unstable to the stable genome condition after polyploidization is far from being fully understood. This project will investigate the meiotic processes that lead to the (non)proper segregation of chromosomes as well as the expression of genes involved in chromosome pairing and homologous recombination in autotetraploid pyrethrum plants (Tanacetum cinerariifolium). Stabilization of meiosis through a few generations is important for commercial production of autotetraploids since they could give a higher yield of secondary metabolites. Genomes of allopolyploid species are extremely dynamic in terms of the interaction between their subgenomes that lead to their homogenization/diploidization. Homogenization is carried out through genomic rearrangements (inversions, translocations, transposition) and deletion of parts of the genome, especially those rich in repetitive DNA, as well as changes in methylation and gene expression. With this project we want to determine the mechanisms at the genome and the epigenome level which are involved in reorganization of subgenomes in sterile allotriploid shallot (Allium x cornuntum) and fertile allotetraploid species Anemone multifida and allohexaploid species A. baldensis. The research proposed in this project will provide a better insight into changes at the genome and epigenome level in species important for human consumption (shallots), organic farming (pyrethrum) and the production of new horticultural varieties (anemone).

12. Molecular aspects of disease pathogenesis in AHCY deficiency

-adenosylhomocysteine hydrolase (AHCY) catalyzes the hydrolysis of Sadenosylhomocysteine (SAH) to Adenosine (Ado) and Homocysteine (Hyc). Recently, several studies pointed out connections of AHCY with cancer from various standpoints: as a player that possibly regulates cancer phenotype, as a druggable candidate, or, as a promising biomarker. Metabolome-wise, connections between adenosine and cancer have been established, showing stimulative effects on cell proliferation, and other important roles in inflammation or immunity. Implications of AHCY in hepatic pathology are well documented, and culminated in the recently reported case of hepatocellular carcinoma in an adult with AHCY deficiency. Also, we have preliminary data showing that low AHCY activity and depletion of adenosine induces DNA damage and cell cycle arrest in hepatocellular carcinoma cells. In summary, involvement of AHCY in molecular mechanisms of cancer is undisputable. However, the molecular basis for the connection between AHCY and cancer is not quite obvious. In order to answer these questions we will deploy a multi-omics approach, in combination with basic molecular and cellular biology procedures focus on the role of AHCY and its mechanism of action on cell cycle, cellular proliferation and DNA damage response in histological and serum samples of liver disease patients, in relevant cancerous and AHCY deficient cell lines, and a unique mouse animal model. In particular, we will investigate the implications of adenosine, besides homocysteine, the primary product of AHCY hydrolytic activity on the cellular metabolism. By answering these questions we hope to establish the mechanism that connects AHCY activity, DNA damage and regulation of cell cycle through adenosine levels. The outcomes of underlying research may lead to a refinement of therapeutical procedures for cancer as a result of AHCY malfunction, and a potentially new approach for targeted cancer therapy based on adenosine depletion.

13. Epigenetic regulation of immunoglobulin G glycosylation

IgG is one of the main effectors of the immune system. Contrary to antigen binding properties which are defined by nucleotide sequence for its Fab part, effector functions of IgG are mostly regulated by Fc glycosylation, which is regulated by complex interplay of dozens of genes in the IgG glycosylation pathway and different environmental factors. Different clones of B lymphocytes have defined patterns of glycosylation, indicating temporal stability in the glycosylation pathway. DNA CpG methylation is the most stable epigenetic modification and is thus the most prominent candidate for "fixation" of the IgG glycosylation profile in different clones. Our past and current genome wide association studies (GWAS) are identifying complex genetic network which regulates IgG glycosylation. In this project we will use several complementary experimental approaches to identify genes and epigenetic regulatory

mechanisms which regulate IgG glycosylation. IgG glycosylation is relevant in numerous diseases, including autoimmune diseases, inflammatory diseases and cancer. Several epigenetic drugs are already used to treat cancer, and many others are developing. Understanding of the role of the epigenetic regulation in IgG glycosylation will provide new targets for the development of new epigenetic drugs and new biomarkers for patient stratification.