Institute of Molecular Biology "Roumen Tsanev"

Annual Report
2010

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- Expanding the Reparation Repertoire of the Cell: Discovery of a new gene involved in remotion of carbohydrate-derived moieties from DNA
- Study of the Interactions and Cellular Localisation of Tof1 Mrc1 and Csm3 Subunits of the Triple S-phase Checkpoint Complex Mrc1/Tof1/Csm3 by Means of Fluorescent Microscopy in the Living Cell (reported p.21)
- Protein stability of the pre-replication complex during DNA repair and apoptosis

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- Synthesis of non-protein amino acids and peptide mimetics and their analysis and characterization by capillary electrophoresis.
- Design, synthesis, and characterization of new biomaterials as specific carriers of therapeutic agents
- Assessment of biological responses to uranium mining at Senokos: a model for biomonitoring of the uranium mining impacted areas.
- Ecological status of marine ecosystems along the Bulgarian Black sea coast and relation to the structure and status of communities of macroalgae and sea grasses (*g*. *Cystoseira* and *g*. *Zostera*).
- Application of next DNA sequencing generation on microbial diversity in soils and ground water. Joint research project between BAS and the Academy of Sciences of the Czech Republic

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- Study of the influence of DNA/DNA and RNA/DNA duplexes' thermodynamic stability on the transcription of RNA in human (reported o. 21)

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- Changes of the human serum proteome in patients with heart failure
- Novel biodegradable nanostructured materials accelerating osteogenesis (reported p. 6)
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- Safety evaluation of manufactured nanomaterials by characterisation of their potential genotoxic hazard (NANOGENOTOX), Join Action of the EU Health Program
- Hydrophobic binding capacity of tumour specific galectins and anticancer effects of their synthetic ligands on tumour cell lines

Statistics

Finances and funding

The research in the Institute of Molecular Biology is carried out under extremely limited budget. The National budged for 2010 covers only part of the salaries of the researchers and the supporting personnel. Moreover, as seen in the table below the budget for 2010 has been cut by about but more than 13%:

Salaries in 2010 relative to 2009

2009		2010	
Annual budget	**Average salary monthly**	**Annual budget**	**Average salary monthly**
BGL 752062	BGL 554	BGL 653165 ↓	BGL 482 ↓
€ 376031	**€ 277**	**€ 326582** ↓	**€ 241** ↓

The extremely low salaries (which are in misbalance with the living costs in Bulgaria) and their reduction during 2010 have serious implications on the scientific activity of the institute. Although the institute belongs to those with a relatively high proportion of doctoral students and young researchers, the staff advances in age. The main reason is the decrease of applications for doctoral student positions, as well as reduced recruiting of young scientists. Moreover, the financial support from the State covers only the amount given in the table above. No support has been foreseen for overhead expenses. Basic costs, such as for electricity, heating, as well as for service of the scientific instruments and installations, chemicals supply, etc. are covered by the external grants.

Projects & funds *2010*. Amounts rounded to 1000 and given in BGL unless stated €.

Department/laboratory	National		International		Total
	Projects	Funds	Projects	Funds	
Medical and Biological Research	**3**	**98000**	**2**	**€ 39 000**	**175000**
Yeast Molecular Genetics	**5**	**64000**	**1**	**4000**	**68000**
Molecular Biology of the Cell Cycle	**1**	**138000**	**1**	**€ 20000**	**177000**
Molecular Design & Biochemical Pharmacology	**9**	**77000**	**6**	**-**	**77000**
Gene Regulation	**5**	**475000**	**2**	**€ 4 000**	**483000**
Structure and Function of Chromatin	**3**	**88500**	**-**	**-**	**88500**
Institute's project [a)]		**14708**			**147000**
TOTAL	**26**	**941000**	**13**	**274000**	**1 343000**
TOTAL in €		**€ 471000**		**€ 137000**	**€ 672000**

a) *Operational Programme for Human Resources Development*

Publications

In comparison with 2009 the scientific production is somewhat reduced. As already pointed out the main reason is the insufficient financial support.

Papers published and in press

Department	Number of papers			
	international journals	local journals	published reports	IF per paper
Medical and Biological Research	**5**	**2**	**-**	**1.805**
Yeast Molecular Genetics	**-**	**2**	**1**	**0.177**
Molecular Biology of the Cell Cycle	**4**	**1**	**-**	**1.693**
Molecular Design & Biochemical Pharmacology	**8**	**4**	**4**	**1.074**
Gene Regulation	**6**	**2**	**-**	**2.423**
Structure and Function of Chromatin	**2**	**1**	**-**	**1.850**
TOTAL	**25**	**12**	**5**	**1.504**
Total 2009	**40**	**15**	**-**	**1.154**

Education of researchers

Department	Number of PhD students		
	Enrolled after 2008 thesis under preparation	Enrolled before 2008 studentship prolonged	thesis defended in 2010
Medical and Biological Research	3	2	-
Yeast Molecular Genetics	-	1	-
Molecular Biology of the Cell Cycle	1	-	1
Molecular Design & Biochemical Pharmacology	2	3	-
Gene Regulation	2	3	1
Structure and Function of Chromatin	-	2	1
TOTAL	**8**	**11**	**3**

Awards

To young scientists

1. The Union of Scientists in Bulgaria awarded **M. Kirilova, PhD** (Department of Molecular Yeasts Genetics) with a certificate for excellent achievements by young scientists.
2. The World Science Foundation awarded **M. Kirilova, PhD** (Department of Molecular Yeasts Genetics) one year scholarship to support her work on the project "The yeasts *Saccharomyces cerevisiae*–a promising model to study brain tumours".
3. **E Boteva, BSc** (Department of Gene Regulation) received the first award in the competition for young scientists of Hasumi International Research Foundation – Bulgaria during the International Meeting "Oncoprogress-Oncovacciness-2010", October 29-30, 2010, Sofia, Bulgaria.
4. **L. Ivanova, MSc** (Department of Biomedical Research) was awarded second prize for a poster presentation at the NATO ASI "Nanotechnological basis for advanced sensors", June, 2010, Sozopol, Bulgaria.

To the institute of Molecular Biology

The Council of the European Scientific and Cultural Society awarded the Institute of Molecular Biology "Roumen Tsanev" the badge "Golden book" for contributions to Bulgarian science.

Projects and achievements

Laboratory of Medical and Biological Research

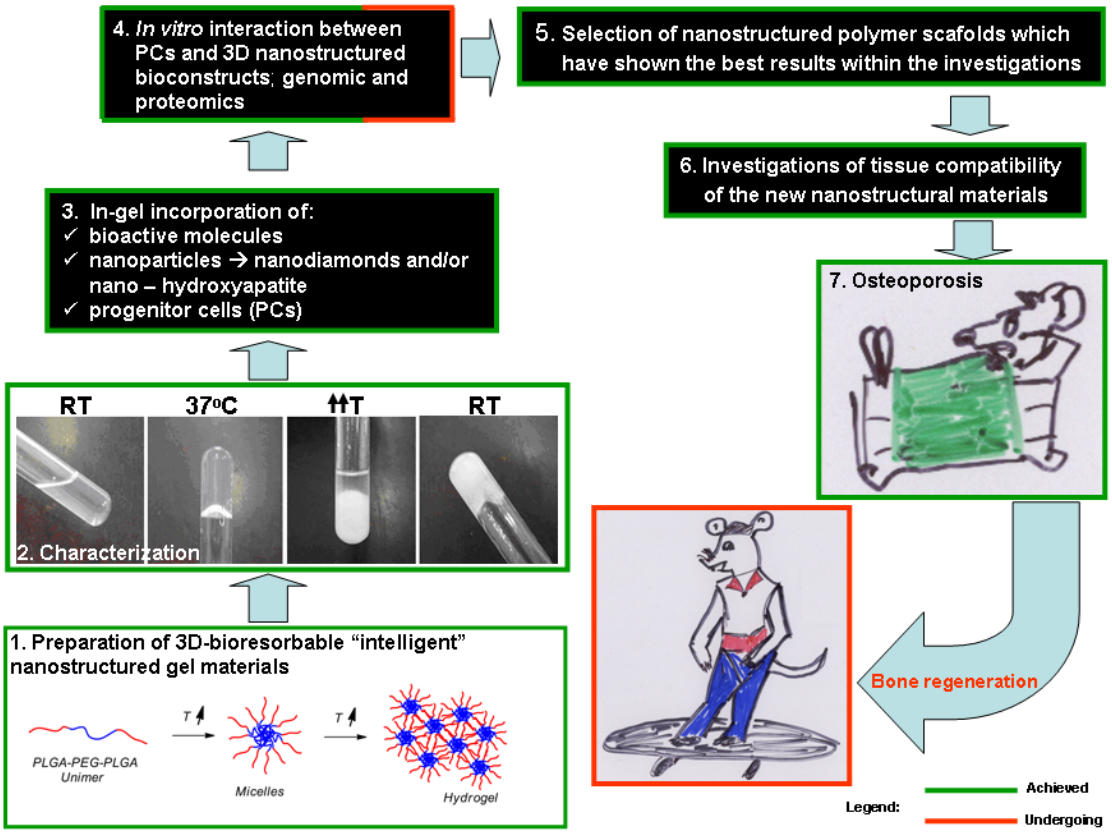
Head: Margarita Apostolova

Novel biodegradable nanostructured materials accelerating osteogenesis

Abstract

Our investigations are aiming at developing of new bioactive injectable highly porous gels, inducing rapid angiogenesis and osteogenesis as a treatment to locally augment the mechanical quality of bone in patients with osteoporosis. The study is combining the application of new 3D biodegradable nanostructural materials which accelerate regeneration of a bone tissue with bioactive substrates delivered locally to reduce the risk of infections in the region of the fracture and to minimize surgical intervention.

Tasks and achievements



Distinctive and novel solutions for possible therapy offered by the results are:

1. The prospect for increasing of cell colonies formation and stimulation of accelerated angiogenesis caused by the 3D morphology of the material, namely by its large specific surface and highly developed porous structure.
2. The capability of the material to be biodegraded at some stage.
3. Options for in gels embedding of low molecular components and nanoparticles for which is believed to enhance mineralization and regeneration of bone tissue.

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Laboratory of Yeast Molecular Genetics

Head: Georgi Miloshev

Study of the role of linker histone Hho1p in chromatin remodelling

Eukaryotic DNA is packed in a complicated nucleoprotein complex called chromatin. The status of chromatin structure and dynamics is essential for the realization of important epigenetic information. Different levels of chromatin compaction exist and govern the expression of genetic information. An interesting phenomenon still raising questions about chromatin structure is the second level of chromatin organization, represented by the so-called "30 nm fiber". Its formation and stability are poorly understood but what is known for sure is that linker histones play important role in its organization.

It has long been believed that the yeast *Saccharomyces cerevisiae* does not have a linker histone. Interestingly, after the sequencing of its genome the yeast linker histone was identified. Therefore, the claimed by some authors absence of "30 nm fiber" in the yeast *Saccharomyces cerevisiae* is quite surprising.

In order to probe the presence of "30 nm" fiber in yeast and the involvement of yeast linker histone, Hho1p, in its organization we have deleted its gene, *HHO1*. Then we have isolated chromatin fragments from the wild type and the linker histoneless mutant, which further have been subjected to AFM (Atomic Force Microscope) analysis.

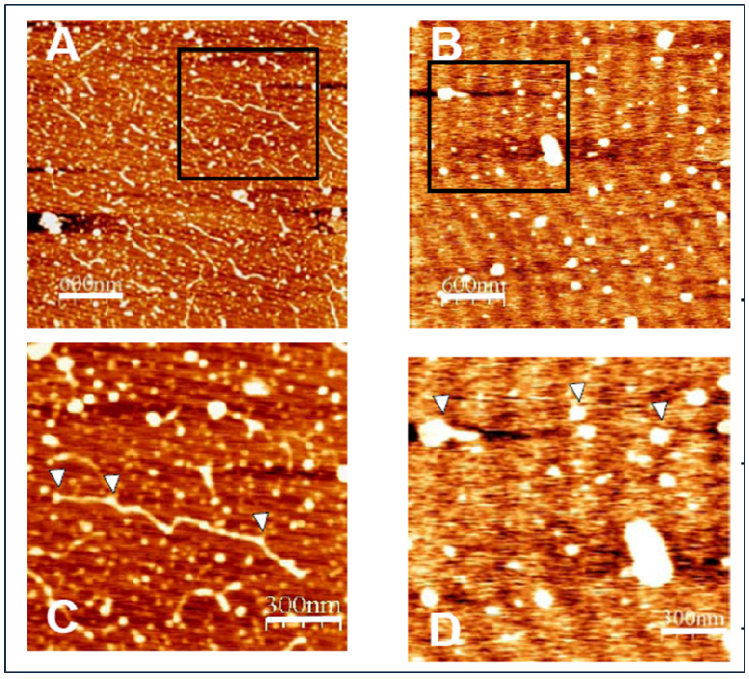


Figure 1.
AFM micrographs of chromatin fragments from wild type and hho1 yeast mutants: **A:** Wild type; **B:** *Δhho1* mutant; **C** and **D** higher magnification of squared areas in **A** and **B**, respectively.

Our results from AFM observations confirmed that there is a well-defined 30 nm chromatin organization in the yeast nucleus (Fig. 1A). Interestingly, these fibers are not detected in the Hho1p null mutant (Fig. 1B), proving that the yeast linker histone is an important player in the "30 nm" fiber organization.

Taken together these results suggest that the higher-order chromatin structure, namely the "30 nm" fiber, has been preserved through almost a billion years of evolution. The single fact of this evolutionary conservation suggests its inevitability for the inheritance of certain important epigenetic information.

Analyses of DNA damage level in indicator plant species from polluted regions

Environmental pollution is an important issue in contemporary times. Huge efforts are engaged in monitoring and preventing it, but results are unsatisfied and disappointing. The most significant tasks in these measures are to correctly determine the sources of environmental pollution, to identify the pollutants and more importantly to determine the level of their effects on organisms.

We are developing a project aiming at toward strict monitoring of environmental pollution with heavy metals and the effects it has on plants. The model plant is *Taraxacum officinale*.

For the purposes of the current research a significant number of soil and plant samples were collected from clean regions and from areas polluted with heavy metals. The samples consisted of soil and seeds and fresh plant leaves. All of them were subjected to chemical analyses, while plant species were additionally investigated with a set of biochemical and genetic methods for characterization of plant genome rearrangements as a result from the pollution with heavy metals.

Results from chemical analyses clearly evidenced increased content of heavy metals like Cd, Mn, Cu, Zn and Pb in all soil and plant samples collected from the polluted areas.

Genetic analyses of all *T. officinale* samples were accomplished by combination of several methods:

A) Surprisingly, the method of Comet assay demonstrated that there is a very slight increase of DNA breaks in plants grown on contaminated soil.
B) PCR-RFLP analyses of the genetic structure of plant samples harvested from clean and polluted areas revealed some fine genetic rearrangements in the polluted plants.
C) Strikingly, karyological analysis of *Taraxacum officinale* plants showed that only in plants collected from the polluted areas an extra chromosomal material was observed.

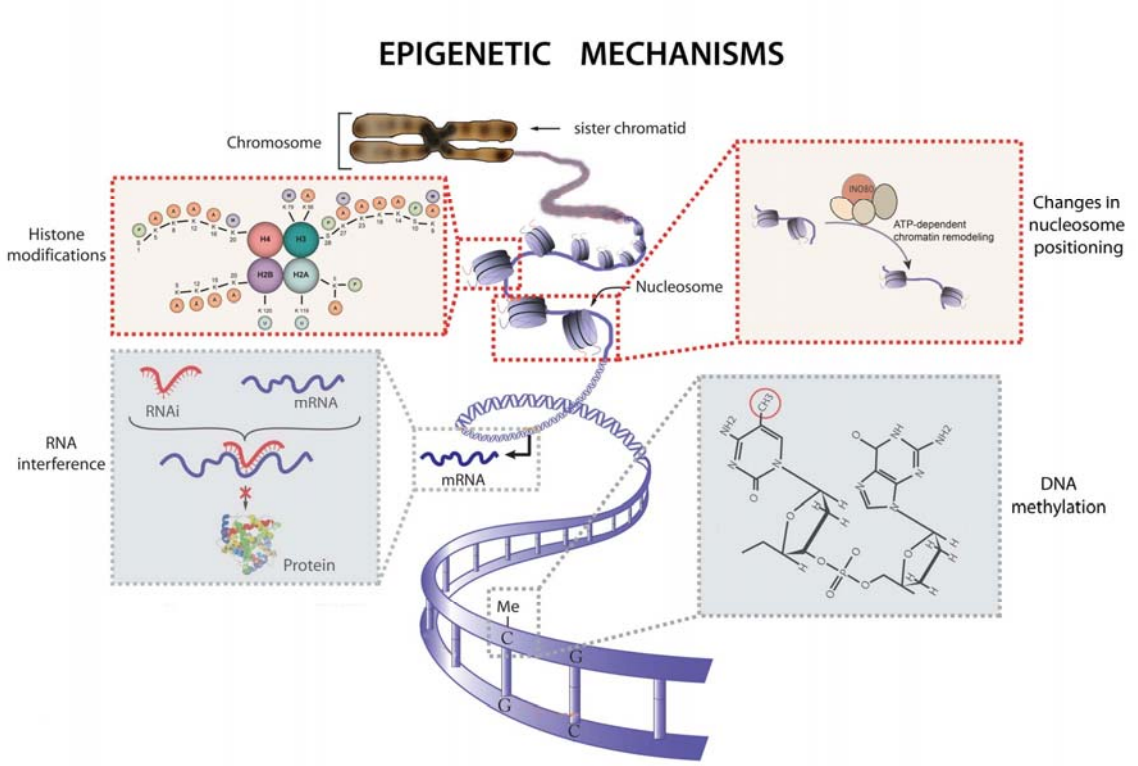
Our results showed very few damages in DNA of plants of the polluted regions at the moment of study, however there are a convincingly large number of chromosomal rearrangements. Therefore, we suggest that the DNA repair systems in such plants are up-regulated in order to cope with heavy metal pollution.

These results state the significance of the very precise chemical determination of heavy metals in the monitored soil and plant samples, and the subsequent study of all impairments in the genome of these organisms. Revealing these complex interactions among the chemical pollutants, their quantity in soil and plant samples and the genome reorganizations, would be very positive and helpful in all further environmental biomonitoring. This is quite prerequisite for the prevention of all problems in human health that could be a consequence of environmental pollution by heavy metals.

Department of Molecular Biology of the Cell Cycle

Head: Boyka Anachkova

The Department of Molecular Biology of the Cell Cycle carries out research in the field of cell cycle regulatory mechanisms. Cell cycle checkpoints activation and abrogation are studied as well as the related processes of DNA damage and repair. Special emphasis is paid on the epigenetic modifications of histones and nucleosomes that determine chromatin structure and hence the progress and fidelity of the basic DNA transactions such as replication, transcription, repair, and recombination. During the last years the aim of our research was to find ways to increase the sensitivity of cancer cells towards the action of anticancer drugs as well as to find means of specific delivery of anticancer drugs to the target cancer cells, which would decrease the toxicity and increase the efficacy of anticancer therapy.



During 2010 the main goal of our laboratory was to study the molecular mechanisms underlying the increased sensibility of cancer cells towards the anticancer drug cisplatin when administered simultaneously with inhibitors of histone deacetylases. It was found that treatment with cisplatin and sodium butyrate lead to decrease of the survival rates of HeLa cells and increase of the survival of mice inoculated with Ehrlich ascites tumour. We have presented evidence that the short term effect of sodium butyirate is hyperacetylation of histones in the euchromatin regions responsible for initiation of DNA replication. Thus butyrate abrogates the cisplatin-induced G1/S phase arrest of the cells as a result of which the cells enter S phase with unrepaired DNA and die of apoptosis. The long term effect of sodium butyirate is hyperacetylation of both eu- and heterochromatin, inhibition of DNA synthesis and apoptosis. These results show that epigenetic therapy with inhibitors of histone deacetylases enhances the cytotoxic effect of cisplatin, allows decrease of its therapeutic doses, and decrease the side effects of the drug.

We have presented evidence that the chromatin modification/remodelling complex TIP60 can modulate the repair process by acetylation of nucleosomes surrounding the double-strand breaks induced by ionizing radiation. By depletion of the cells of subunits of the complex with the siRNA technology we have shown that repair by homologous recombination was reduced. These results indicate that the chromatin modification/remodelling complexes that provide accessibility of the repair factors to the sites of DNA damage are potential targets of epigenetic therapy.

Fluorescence spectroscopy and circular dichroism were used to study the interaction of human galectin-3 (hGal-3) with two anticancer agents: bohemine and Zn porphyrin (ZnTPPS(4)). We have shown that the two compounds with anticancer activity have high affinity for hGal-3 at a site that is distinct from its carbohydrate site. Since hGal-3 binds to several carbohydrate cancer antigens, the results suggest that it may be used in the targeted delivery of anticancer drugs.

Publications:

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Department of Molecular Design and Biochemical Pharmacology

Head: Tamara Pajpanova

Design, synthesis and analysis of biologically active compounds

Synthesis of:

- Non-protein amino acids;
- Peptide mimetics and polypeptides]
- Peptide analogues of nucleic acids;
- Metal-containing cytostatics – complexes of platinum with sulphonamide ligands;
- Waste-free convenient method for obtaining of the parent compound for the synthesis of anticancer drug Cisplatin and other platinum complexes.

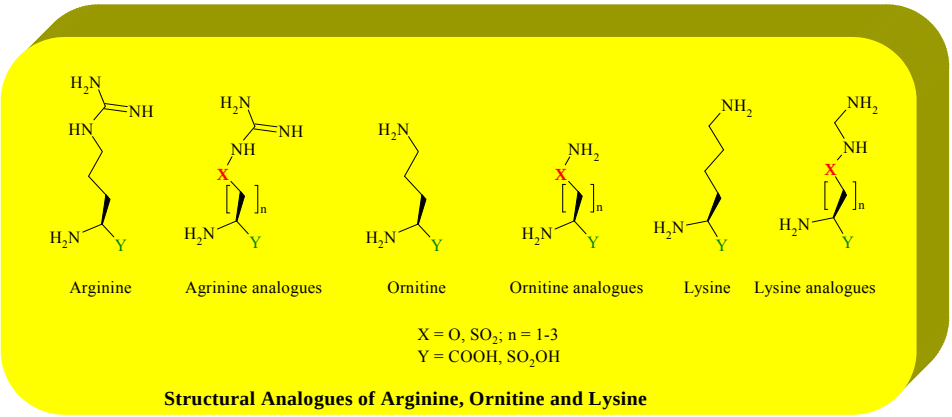
Determination of:

- Opioid activity;
- Cytotoxic and apoptogenic activity.

Characterization of the compounds

Unnatural amino acids, the non-genetically-coded amino acids that either occur naturally or are chemically synthesized, are becoming very important tools for modern drug discovery research. Due to their structural diversity and functional versatility, they are widely used as chiral building blocks and molecular scaffolds in constructing combinatorial libraries. Many of these unnatural amino acids are also critical components in pharmaceuticals and developmental drugs.

In this context, we have focused on the design and synthesis of unnatural amino acids and their derivatives, containing a basic functionality (oxy- and sulfo-amino and guanidino), as structural analogues of, ornithine, lysine and arginine.



Structural Analogues of Arginine, Ornitine and Lysine

Their effects on the growth of microorganisms, cultured tumour cells and their antitumor activity *in vivo* have been evaluated. All tested compounds showed cytotoxic activity against F4N cells.

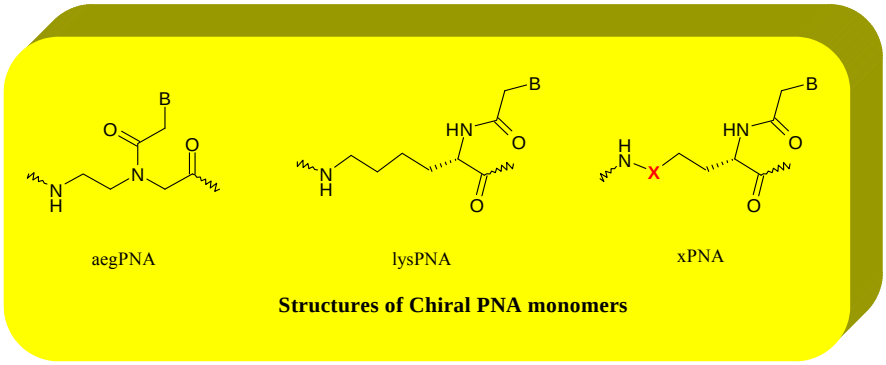
In addition to their interesting biological properties, these unnatural amino acids were incorporated into biologically active peptidomimetics.

Peptidomimetics are compounds whose essential elements (pharmacophore) mimic a natural peptide or protein in 3D space and which retain the ability to interact with the biological target and produce the same biological effect. Peptidomimetics are designed to circumvent some of the problems associated with a natural peptide: for example stability against proteolysis (duration of activity) and poor bioavailability. Certain other properties, such as receptor selectivity or potency, often can be substantially improved.

Our efforts in the area of peptide synthesis encompass a wide variety of opioid peptides possessing useful physiological activity. We synthesized a series of tri- and tetrapeptide mimetics containing non-protein amino acids canavanine (**Cav**) and **sLys** as analogues of analgesic peptides MIF-1 and Tyr-MIF-1. The purpose of this study, therefore was to investigate the analgesic effects of newly synthesized analogues during acute pain. The results suggest that substitution with **Cav** in MIF-1 enhances analgesic activity.

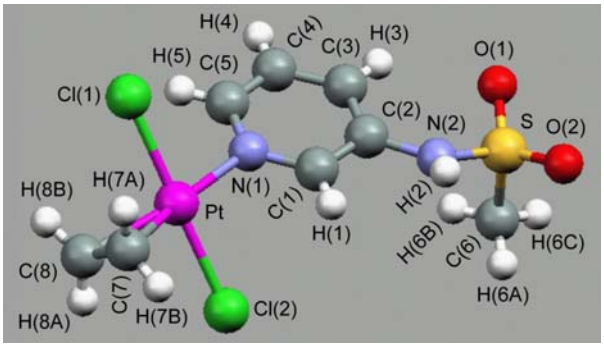
The slow progress in the efficacy of the treatment of severe diseases, e.g., cancer, with low-molecular-weight drugs has revealed a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. The development of new biopolymer materials for applications such as gene carriers and drug delivery systems is of great interest to biomedical sciences. In this field, a number of types of biopolymers have been developed. Along these lines, oligoamino acids, PNA oligomers and dendrimers represent a novel type of polymeric material that has generated much interest in many diverse areas due to their unique structure and properties.

At the present we have initiated a program of research to develop the synthesis of novel polypeptides and PNA analogues.



Another major area of interest of our research group is the development of metal-containing cytostatics – complexes of platinum with sulphonamide ligands. The complexes were characterized by one- and two-dimensional NMR spectroscopy of ^{1}H, ^{13}C and ^{195}Pt, as well as single-crystal X-ray diffraction analysis.

The structure of the newly synthesized complex trans-dichloro(η^2-ethylene)(N-3-pyridinylmethanesulfonamide)platinum(II) has been demonstrated with single-crystal X-ray diffraction analysis.



Molecular and environmental microbiology

- The biodiversity of Bacteria, Archaea and metabolitic genes in waters, soils and sediments from three extreme habitats were studied.
- A large bacterial diversity and adaptation of bacteria to uranium contamination was found in uranium mine Senocos, Southwest Bulgaria. which was studied as a model for biomonitoring of the uranium mining impacted areas.
- The dominance of Archaea was demonstrated in the sediments from Black Sea coast near the town of Sozopol.
- Bacterial diversity in hot springs in the region of the town of Velingrad as sources of thermostable enzymes with potentially importance in the biotechnology was studied. It was demonstrated that bacterial communities, inhabiting those sites were complex, high abundant, location-specific and influenced by the temperature.

References

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Department of Gene Regulation

Head: Ivan Ivanov

Cause of Human Interferon-β Immunogenicity

Background

- Treatment of patients suffering from **multiple sclerosis** (MS) with **human IFNβ** results in anti-drug antibody development in up to 45% of patients.
- In some patients antibodies neutralize drug activity and cause adverse reactions including **urticaria**[1,2] and **anaphylaxis**[3].
- To date, **the reasons for the immunogenicity** of hIFNβ therapeutics are not fully understood.
- In previous studies we have shown that during manufacturing therapeutic proteins accumulate **advanced glycation end products** (AGEs)[4-6].

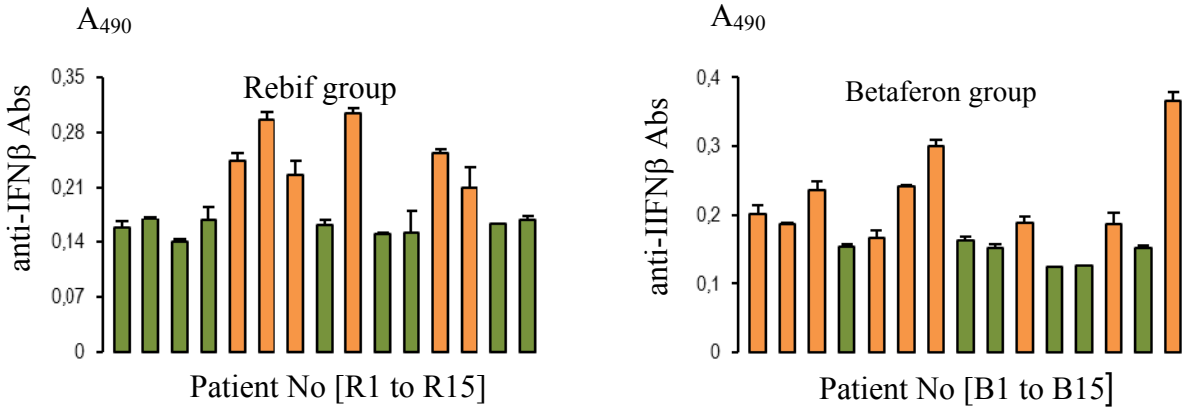
Aim

The present study was designed to test the hypothesis that AGEs form immunogenic epitopes in hIFNβ.

Results

MS patients develop anti-IFNβ antibodies

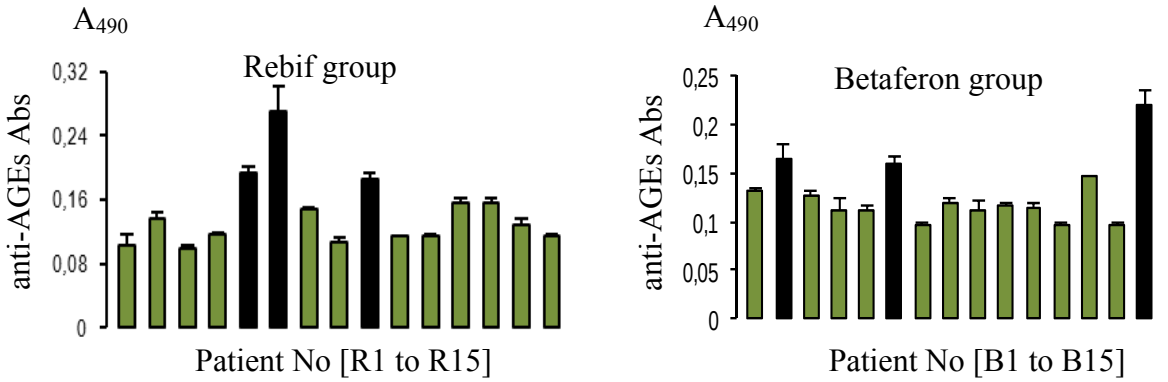
Forty percent (6/15) of patients treated with Rebif® (44 μg Original Formulation) and 60% (9/15) of those treated with Betaferon® develop anti-IFNβ Abs.



Sera were tested for the titer of anti-IFNβ Abs by direct ELISA using one plate assay including control sera (15 anonymous blood donors). Data are means ± SD (n=2). Sera with average titers above the mean + 3SD of the control group were considered positive (orange bars on the graphs). Data in all panels are the representative of three independent experiments.

MS patients develop anti-AGEs antibodies

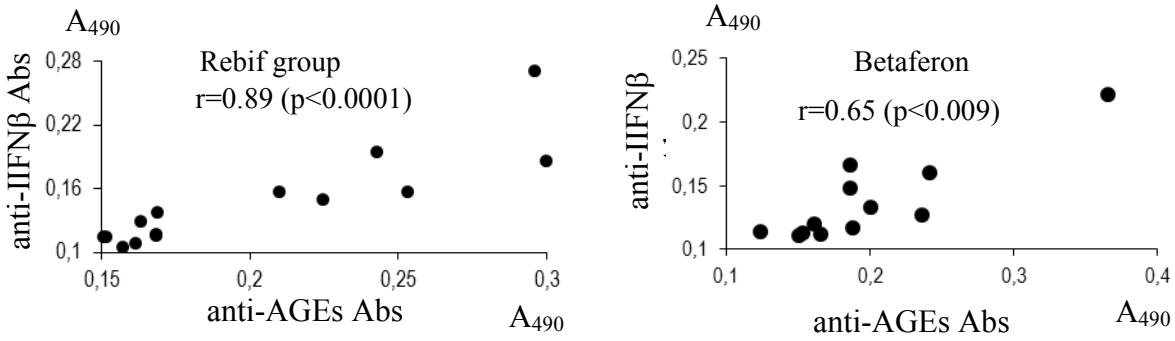
Twenty per cent of the sera in either group (3/15 and 3/15) develop also anti-AGEs Abs.



Sera were tested for the titer of anti-AGEs Abs by direct ELISA using one plate assay including control sera (15 anonymous blood donors). Data are means ± SD (n=2). Sera with average titers above the mean + 3SD of the control group were considered positive (black bars on the graphs). Data in both panels are the representative of three independent experiments

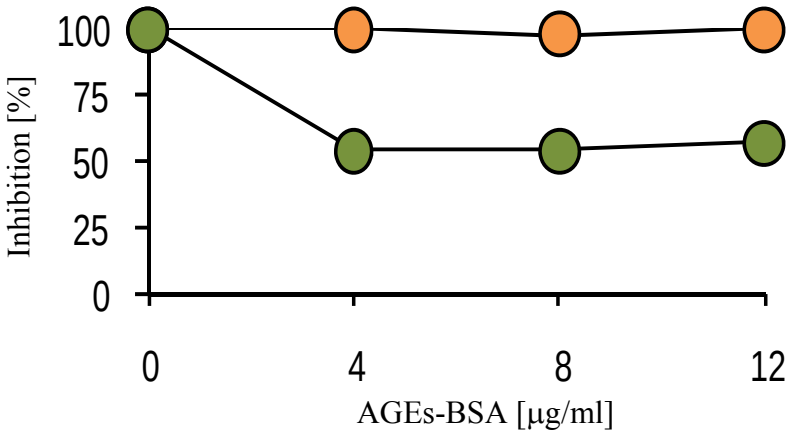
The anti-IFNβ Abs correlate with the anti-AGEs Abs

The serum levels of anti-IFNβ Abs correlate positively with the levels of anti-AGEs Abs, especially in the Rebif group (r=0.89; p<0.0001).

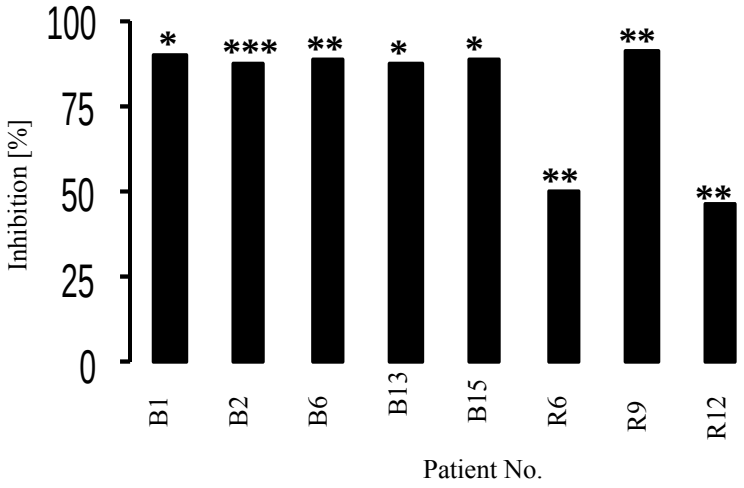


Pearson correlation coefficients (r) were calculated with the "CORREL" function of Microsoft Office Excel 2003, and their statistical significance was evaluated using the software at http://faculty.vassar.edu/lowry/rsig.html

The anti-IFNβ Abs cross-react with AGEs



AGEs-BSA was included as a competitor in the reaction between serum IgGs and IFNβ and competitive ELISA was performed with two sera, one anti-IFNβ Abs^{+} and anti-AGEs Abs^{-} (●), and another positive for both types of Abs (●). Increasing concentrations of AGEs-BSA progressively inhibited the reactivity to IFNβ of the anti-AGEs Abs^{+} serum but not the reactivity of the anti-AGEs Abs^{-} serum.



All anti-IFNβ^{+} sera were tested by competitive ELISA at a fixed concentration of the competitor (10 μg/ml). More than a half of the anti-IFNβ Abs^{+} sera in the Betaferon group (5/9) and half of the Rebif group sera (3/6) responded to AGEs-BSA inhibition with a response range between 9.2% and 54.0% (*p≤0.05, **p<0.001, ***p<0.0001).

Conclusions

- Correlation of anti-IFNβ Abs with anti-AGEs Abs and inhibition of sera reactivity to IFNβ by AGEs-BSA indicate that AGEs form immunogenic epitopes in IFNβ.
- Relevant approaches should be adopted by pharmaceutical companies for manufacturing of AGEs-free IFNβ with reduced immunogenicity.
- Large scale clinical studies with patients treated with IFNβ and other protein therapeutics are required to further evaluate the impact of AGEs on the immuno reactive properties of protein rugs.

References

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Department of Structure and function of chromatin

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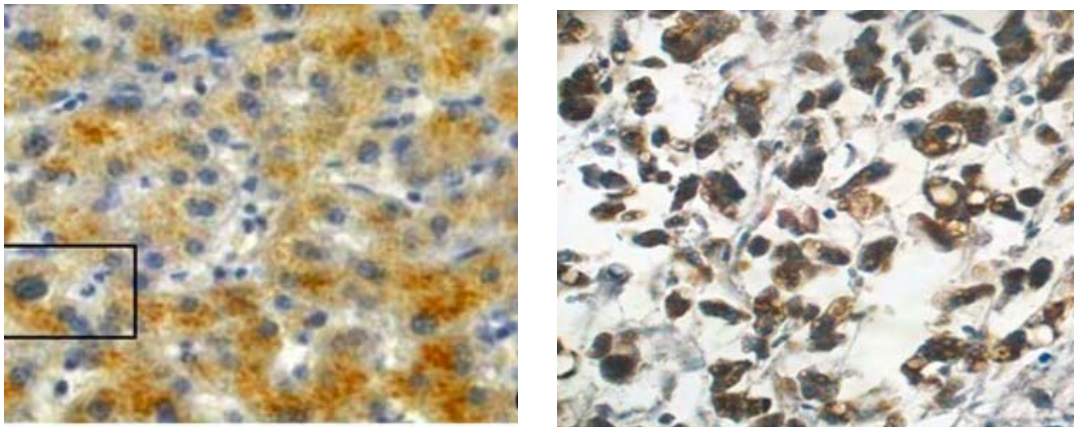
Level of expression of the nonhistone protein *HMGB1* and its receptor *RAGE* in human tumour cell lines and tumours with different histological grade - a putative prognostic marker

Although there are data for the production of HMGB1 in different tumours there is no clear hypothesis for the exact role of the protein in the development of carcinomas and formation of metastasis. Variations in HMGB1 expression at the RNA level have been reported in cases of breast, gastric and hepatocellular carcinomas while no HMGB1 is detected in adrenal gland carcinoma. Very scarce is the information regarding the interaction of HMGB 1 and its receptor RAGE and the correlation with the tumour growth and cell invasion. The data concerning the expression of HMGB1 protein and its receptor RAGE in different tumours reflect mainly the overall production of the proteins but do not refer to their cellular localization and histological grade.

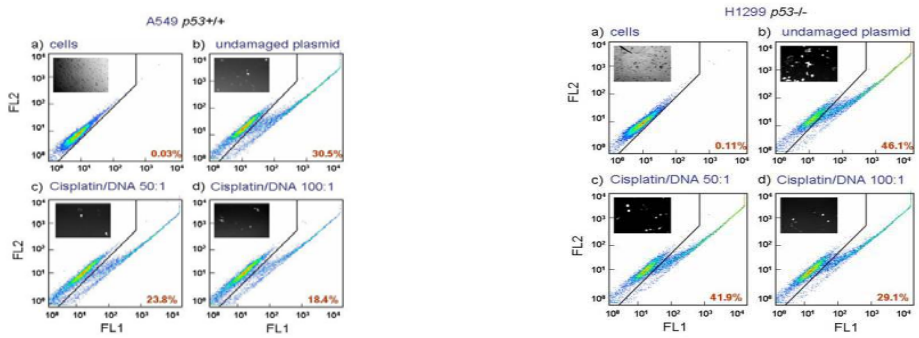
1. High level of expression of the High Mobility Group Box (HMGB) 1 non-histone protein and its specific receptor RAGE in human malignant tumours with different histological grade was detected. The correlation between HMGB1 and RAGE production and their specific localization, and the state of tumour differentiation could have a prognostic meaning in carcinogenesis.

A B



Different cellular localization of RAGE: panel A, in primary hepatocellular carcinomas (cytosolic) and panel B, in liver metastasis (membranious).

2. Two non-small lung cancer cell lines expressing (A549 p53 +/+) and non-expressing (H1299 p53-/-) the tumour suppressor factor p53 were tested for their potential to repair DNA treated with the anti-tumour drug cis-platinum. The tumour cells that are deficient of p53 showed greater repair capacity in this respect.



The repair potential of non-small lung cancer cell lines expressing (A549 p53 +/+) and non-expressing (H1299 p53-/-) the tumour suppressor factor p53 was tested by Host-Cell reactivation assay and analyzed by FACS

The non-histone protein *HMGB1* as a modulator of the anti-tumour effect of the drug cis-platinum: the role of the post-synthetic acetylation and phosphorylation

It is known that the over expression of HMGB1 protein sensitizes the cancer cells in breast carcinomas to the action of the antitumor agent. A putative hypothesis for this effect is the role of HMGB1 protein in the DNA repair synthesis. We reported for the first time that HMGB1 inhibits the repair of cis-platinated DNA at nucleosomal level. The effect is accomplished through the acidic C-tail and modulated by the post-synthetic acetylation. We were motivated to study the correlation between the repair potential of different human tumour cell lines under the conditions of over expression of exogenous HMGB1 as well as HMGB1 mutated at Lys2 in order to analyse the role of the post-synthetic acetylation.

1. Three new antitumor drugs, complexes of Pt(III) were studied for their antineoplastic activity in a set of human cancer cell lines. A direct correlation between the antitumor activity of the complexes and the level of DNA platination was determined.

2. The role of HMGB1 protein as a “chaperone” in the nucleosome formation was studied. HMGB1 stimulates the formation of middle- and end-positioned nucleosomes and the effect is accomplished through interacting with DNA but not with the histone octamer. The post-synthetic acetylation of the protein inhibits its “chaperone” function.

Publications

1. In vitro pharmacological study of monomeric platinum(III) hematoporphyrin IX complexes. (2010), Momekov G, Karaivanova M, Ugrinova I, Pasheva E, Gencheva G, Tsekova D, Arpadjan S, Bontchev PR., *Invest New Drugs*, PMID: 20225009

2. The expression of HMGB1 protein and its receptor RAGE in human malignant tumours. (2010), Kostova N, Zlateva S, Ugrinova I, Pasheva E., *Mol Cell Biochem*. 337, 251-8, PMID: 19876719

3. Repair of DNA damaged by the anticancer drug cis-platinum in human lung cancer cell lines (2010), Yusein-Myashkova S. and Pasheva E., *Comp.Acad. Bulg. Sci*. (in press)

Study of the influence of DNA/DNA and RNA/DNA duplexes' thermodynamic stability on the transcription of RNA in human.

We demonstrated that the 5'-untranslated regions of DNA/DNA and RNA/DNA duplexes in *Homo sapiens* have higher thermodynamic stability compared to the 3'-untranslated regions and coding sequences. The thermodynamic stability of DNA/DNA and RNA/DNA duplexes from the genome of *Homo sapiens* correlates with the speed of RNA polymerase II. The thermodynamic stability of DNA/DNA and RNA/DNA duplexes of entire *Homo sapiens* transcripts correlates with high levels of mRNA in the cell.

Study of the Interactions and Cellular Localisation of Tof1 Mrc1 and Csm3 Subunits of the Triple S-phase Checkpoint Complex Mrc1/Tof1/Csm3 by Means of Fluorescent Microscopy in the Living Cell

A protocol for visualization via fluorescent microscopy of chromatin bound proteins in a whole cell was developed. It was used to study yeast strains, possessing GFP-tagged proteins of the triple S-phase checkpoint complex Mrc1/Tof1/Csm3, with or without deletions of the every single subunit's genes. The application of that protocol helped us to define meaning of every protein of the complex for its DNA binding.