BULGARIAN ACADEMY OF SCIENCES

INSTITUTE OF MOLECULAR BIOLOGY

"ROUMEN TSANEV"

50 YEARS
INSTITUTE OF MOLECULAR BIOLOGY
ROUMEN TSANEV

ANNIVERSARY MOLECULAR BIOLOGY CONFERENCE

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The Aanniversary Molecular Biology Conference "50 years Institute of Molecular Biology "Roumen Tsanev" is organized by the Institute of Molecular Biology "Roumen Tsanev" at the Bulgarian Academy of Sciences - the leading scientific institution in the field of molecular biology in Bulgaria.

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ДВОЙНАТА СПИРАЛА[1]

Най-голямото откритие в биологията
през 20 век е изясняване структурата
на наследственото вещество – ДНК,
спиралата на живота

Природата луда, както си играла,
взела, че изплела двойната спирала!
Две сплетени нишки с водородни връзки
във себе си скрили проекти най-дръзки,
проекти велики, проекти безумни,
създали в миг страшен бацилите чумни
или пък родили в някой миг върховен
мозъка на Айнщайн, слуха на Бетховен!

Грозно и красиво в себе си събрала
шества по Земята двойната спирала!
Чрез хиляди гени и с триплетен код
тук създава гений, там пък идиот.
Тук нещо се ражда, там нещо умира –
двойната спирала се редуплицира.
И проекти нови тръгват с двете нишки,
тръгват със живота двете тайни книжки...

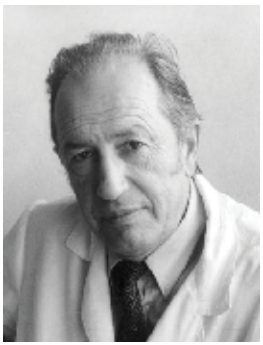
Сякаш чука морзът в двойната спирала:
„Това съм ти дала, туй не съм ти дала...”
И въртят се нишките, сякаш ни орисали,
с тройките кодони всичко в нас записали.
И това, че аз съм АЗ, а пък ти си ТИ,
в ДНК-спиралата някъде седи.
И дори си мисля, дали тез куплети
не са закодирани в нейните триплети!

И си мисля още – в хаоса космичен
как ли е възникнал тоз живот химичен?
На пук на ентропия как ли е израствал,
сякаш е работил демонът на Максвел,
и редил е „буквите” векове милиони,
за да стане хаосът жизнена хармония,
за да станем АЗ и ТИ, за да стане мисъл,
и накрая-редовете, дето съм написал.

Румен Цанев
Из стихосбирката „Живот”

* Double helix, Roumen Tsanev, the poetry “Life”, 2003

THE MEMORY OF GENERATIONS



Roumen Georgiev Tsanev
5.10.1922 – 23.07.2007

TIME GOES ON AND WE WONDER WHICH TRACE IS DEEPER, THE ONE THAT PROFESSOR TSANEV LEFT IN SCIENCE, OR THE ONE DEEP IN OUR SOULS.

Dear Colleagues, today we celebrate 50 years of the Institute of Molecular Biology "Roumen Tsanev". Today, it feels impossible to accept that we shall never meet him again along the corridors of the institute, that we shall never again attend his seminars, discuss his latest scientific ideas and papers, or enjoy his sense of humour.

Prof. R. Tsanev was a founder and for many years head of the Institute of Molecular Biology at BAS. He also established the school of Molecular Biology in Bulgaria and was a member of Academia Europaea (London), the Bulgarian Academy of Sciences (BAS) and of many other international scientific organizations.

His scientific contributions concern the structure and function of chromatin (the genetic apparatus) and the molecular mechanisms of cell proliferation and differentiation. He published more than 200 papers mostly in international journals.

Dear Colleagues, here we present two interviews with acad. Tsanev. His last one **"Was Darwin right in adopting the motto "Natura non facit saltus"*?** appeared in *BIOTECHNOL. & BIOTECHNOL. EQ. VOLUME 21/3, AUGUST 2007.*

Akad. Prof. R. Tsanev and Akad. Bl. Sendov pioneered the idea of a histone code playing the role of epigenetic information for the control of gene activity. They also used the law of mass action and showed the existence of non-linear relations in the genome, which can naturally explain the various aspects of the evolution of species on Earth.

The theme of "Life" continued to excite Prof. Tsanev as a scientist all his life.

Q. 1. Darwin's theory about the evolution of species is not accepted by some people today. Why?

A. 1. This is true, especially in the USA, as seen by a recently published book (19). Two kinds of arguments are used by its critics: either religious considerations or some facts that are not in line with his theory. Leaving aside the first argument, I will discuss the second one.

According to the Darwinian theory of evolution the enormous diversity of living species (between 10 and 50 million (5), not counting those already extinct), are descendents of a common primordial ancestor. Their evolution was based on two processes: 1. Small individual variations, and 2. Natural selection of the modified individuals leading to the survival of the fittest. Thus, gradually new species emerged. This Darwinian phyletic gradualism is in disagreement with some well known facts:

1. Gradual transitions supporting the Darwinian gradualism are observed in some species only;
2. In contrast, the paleontological finds show the existence of large morphological gaps among different species. This fact could not be explained either by incomplete discoveries, or by soft-bodied species that cannot be preserved as fossils;
3. Long geological periods of stasis exist with species "frozen" in evolution before the appearance of new forms;
4. There are geological periods of rapid emergence of new species, such as the well known Cambrian explosion, when many new forms appeared during a relatively short period of 10-20 million years;
5. During some periods mass extinction of species took place (e.g. the extinction of the dinosaurs), that has not yet found a satisfactory explanation;
6. Life on Earth appeared 1.700 billion years after the planet's birth, a period which seems too short for the different living forms to emerge if the pace of evolution proceeded by small gradual changes. For this reason some scientists (e.g. Francis Crick) believed that life emerged somewhere else in space and was later transferred to Earth.

All these facts caused serious discussions and led to various ideas, as for example theories of "quantum jumps" (11), "punctuated equilibrium" (3) etc. forming two opposite camps of thought which became known as the "gradualist-saltationist" schism (18).

* Nature does not make a leap (Webster's dictionary).

To explain the saltatory events observed in evolution, it was logical to see non-linear models being proposed. However, this was done by introducing some variables, such as non-linear fields (1), genome operators (2), stochastic models (4), artificially constructed non-linear genetic networks etc. In fact, all these approaches contributed little to understanding the molecular mechanisms that underlie the process of new pattern formation. Such formal approaches substitute one unknown factor for another. "For the time being – it was concluded – there is no theory at the molecular level which could provide a model for long-term evolution based on non-stationary recursive mechanisms" (2).

Q. 2. How can new discoveries of molecular biology help to solve the contradictory aspects of evolution?

A. 2. Studies of the organization of the genome and of the mechanisms controlling gene activity help in two ways to elucidate the problems of evolution.

1. First of all they show that the basic molecular processes of life are essentially similar, if not identical, in all living species. This may be well illustrated by the processes of information transfer (DNA → RNA → Proteins, known as the dogma of molecular biology), the generation and use of energy, the synthesis of proteins on the ribosomes, the active and passive permeability of membranes, the metabolic pathways from membrane receptors to the nucleus etc. These processes are so complicated that the probability of their emerging at many places and at different times is practically zero. They represent strong evidence in support of the evolution of species from a common ancestor.
2. Secondly, the mechanism of gene control by protein-DNA interactions may explain naturally the saltatory events in evolution. This point needs a more detailed explanation. All experimental data show that the phenotype does not depend on individual genes alone, as indicated by the following facts:
   a. Genes for many basic life processes display a high degree of evolutionary conservation. Such are the genes controlling the cell cycle, genes for embryonic development, histone genes, house-keeping genes etc.
   b. Heterologous transfer of individual genes does not change the phenotypic features of the host. Human genes, for example, have been transferred to mice, to yeast, to bacterial cells where they were perfectly active without affecting the morphological pattern of the host.
   c. Closely related species although displaying different phenotype, have very similar informational content. In this respect humans, for example, are almost identical with chimpanzees, the difference in informational content being 1.3% only.

Thus, it is clear that additional information is needed to translate the linear one-dimensional information in DNA sequences into a three-dimensional phenotype. This new information comes from the fact that genes do not work as separate units but many of them are interconnected in regulatory circuits – **genetic networks**. This is due to the synthesis by some genes of proteins (*trans* factors) which bind to specific DNA sequences (*cis* elements) to control – activate, repress or modulate - gene activity. These *trans-cis* interactions integrate genes into genetic networks.

Q. 3. Could you describe in more detail how the genetic networks help to understand evolution?

A. 3. It is very important to note that most DNA-protein interactions are reversible and obey the Law of Mass Action established by Guldberg and Waage in 1867. I would like to stress especially the importance of this law which, as we shall see, helps to establish the diversity of living forms on Earth. Based on the law of mass action, equations governing the synthesis of mRNA and proteins can be derived (13, 14). A reversible *trans-cis* interaction leads to the formation of a complex (TC) of *trans* factors (T) with *cis* elements (C):

$$T + C \rightleftarrows TC \qquad (1)$$

The fraction of time θ the *cis* element is free of repressor or is bound to an activator can be expressed as follows:

$$\theta = \frac{1}{1 + (\sigma.T)^n} \qquad (2)$$

where σ is the equilibrium constant of the given *trans-cis* interaction and n = 1 if T is a repressor (Tr), or n = -1 if T is an activator (Ta) (13, 14).

The synthesis of mRNA (*m*) should be proportional to the fraction of time the *cis* element is free of repressor (n = 1) or is bound to an activator (n = -1). Thus, the synthesis of mRNA will be described by the equation:

$$\frac{dm}{dt} = a_1.\varepsilon\theta - b_1 m\,, \qquad (3)$$

where a_1 and b_1 are constants and ε is a binary variable which is 0 or 1 depending on whether the concentration of a repressor is lower or higher than its threshold value.

The formation of mRNA-loaded ribosomes r and the synthesis of trans protein T is described by the equations:

$$\frac{dr}{dt} = a_2.m - b_2 r \qquad (4)$$

$$\frac{dT}{dt} = a_3 r - b_3.T \qquad (5)$$

Due to the expression (2) equation (3) is non-linear and the whole system acquires the features of a non-linear dynamics.

We have used these equations to create mathematical models simulating the behavior of different cellular systems: a cell culture (14), regenerating rat liver (9), cancer of the liver (15, 16), mouse epidermis (10), a worm-like creature (called by us "kylindros") (17). It was found that in all cases the computer experiments simulated very well the behavior of the real systems.

In the model of cell culture cell proliferation was induced by decreasing the number of cells and it was observed that "proliferation" stopped when this number was restored.

Similarly, the liver model reacted with cell proliferation after "partial hepatectomy" (simulated by reducing the number of cells in the model) and stopped growing after its mass was restored. The restoration of the "liver" depended strongly on the parameters of the system (**Fig. 1**).

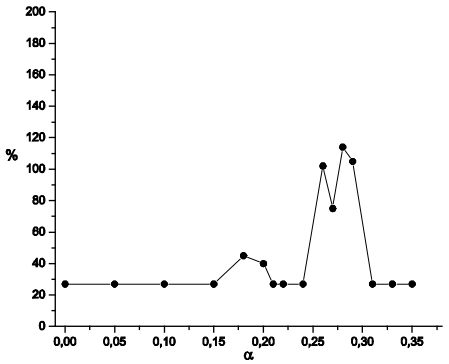


Fig. 1. Effect of the parameter α (which determines the distribution of a diffusable repressor between the cells and the intercellular space) on the extent of restoration of the lever model (ordinates) as simulated by the computer after 66% "hepatectomy" (9)

After damaging some functional genes interrelated with the mitotic genes (D_M-damaged cells) alternating saltatory transitions were obtained between resting liver cells and unlimited cell proliferation upon changing some parameters (**Fig. 2**). The behavior of this system simulated well the properties of cancer cells.

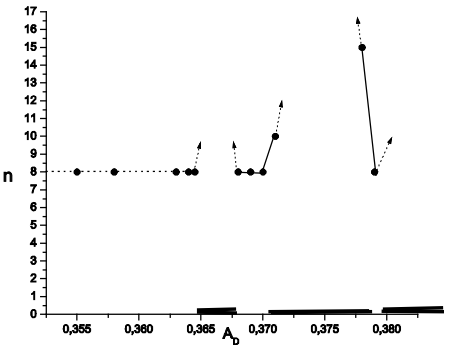


Fig. 2. Effect of the parameter A_D (threshold value of a repressor) on cell proliferation in the liver model (ordinates – number of cells). Regions of stability (white) and of lability (black) of the model containing D_M-damaged cells (15)

The computer simulation gave very conclusive results with the model of epidermis representing a two-layer formation of cells [see details in (10)]. The resting epidermis model showed a random distribution of mitotic cells as in the real case. Damage was simulated by annulling mRNA values for differentiated functions. Our experiments on real epidermis have shown that mechanical injury induces the degradation of cellular RNA [see (12)]. The model reacted to such cell damage with mitotic activity as observed in real experiments (**Fig. 3**).

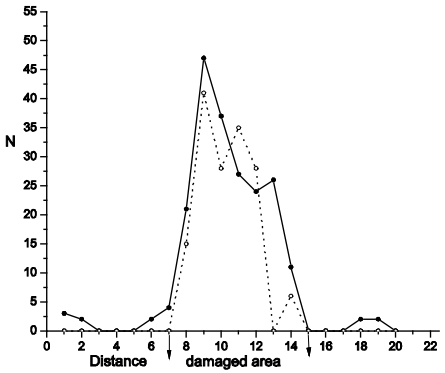


Fig. 3. Increased mitotic cells in the damaged area of the epidermis. Ordinates – relative number of mitoses per unit area. Solid line – in a real experiment; dotted line - computer simulation in the model. According to data from (10)

It is interesting that the computer experiments showed the induction of several fading mitotic waves (2-3 or more depending on some parameters) (**Fig. 4**), as found in real experiments [e.g. (8)]. This disproved the argument that several mitotic waves were not compatible with a model of genes that are functionally interrelated on the basis of a mutual repression. After injury the model showed a wave of cell proliferation limited to the damaged area as observed also in real experiments (**Fig. 4**).

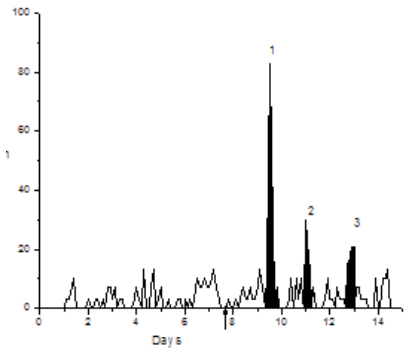


Fig. 4. Three fading mitotic waves after "injury" of the epidermis (in the model simulated by annulling in 60 cells the value X_D of mRNA programming the ribosomes for differentiated function (10). Ordinates – number of mitotic cells per unit area. Abscissae – "days". Arrow – time of "injury"

Q. 4. Are these results a strict proof that the model corresponds to reality?

A. 4. It is true that simulation of the real behavior of a system by a model only shows that this model is possible but it is not considered a rigorous proof of its validity. However, since our model simulated very well the properties of several different cellular systems, this makes highly probable its two main characteristics:

1) Two sets of genes (for cell differentiation and for cell proliferation) interrelated by mutual repression, and
2) Non-linear behavior of the system due to reversible DNA/protein interactions governed by the Law of Mass Action.

In summary, the model predicts the proliferative behavior of the real mouse epidermis: a random distribution of mitotic cells (due to the fluctuation of the repressors around their threshold values); a diurnal variation of the mitotic activity (if a repressor binds an effector like adrenaline); a mitotic activity after injury strictly limited to the damaged area; the appearance of several fading mitotic waves after injury. May I say that the computer mouse predicted very well the epidermal proliferative reactions of the laboratory mouse.

Q. 5. How does this all relate to evolution?

A. 5.With the model "Kylindros" we revealed saltatory phenotypic changes upon changing the parameters of the system. An interesting finding was that the final phenotype depended also on the half-life of the mRNA stored in the egg.

The non-linear relations in the genetic networks give a simple explanation of different aspects of evolution:

1. Within the zone of linearity small changes of the inner and outer parameters of the cellular systems will cause small gradual changes as observed with some species.
2. Outside the linearity zone the gaps in the phenotypic transitions among species are a natural fact due to the saltatory course of the evolution.
3. The existence of different geological periods of "frozen" evolution, of explosion of many new forms or of mass extinction of species is also a consequence of the non-linearity.
4. The saltatory evolution should accelerate the pace of evolution. In the prebiotic period the interactions among the first biopolymers may have also established non-linear relations. In this case the time period for life to appear on our planet may have been sufficient.

Thus, many biological phenomena can be explained on the basis of the Law of Mass Action. This fulfills "one of the objectives of science – to explain more and more with less and less" (7).

Answering the question asked in the title of this interview, we may safely say that Darwin was not right to adopt the motto "Natura non facit saltus". He could not know what molecular biology discovered during the next century. On the other hand, many arguments given today by the critics of his theory [as in (19)] no longer stand in view of these discoveries, especially the non-linearity of the genome.

The most important conclusion is that the biodiversity on Earth, including our own appearance as humans, is due to the physical Law of Mass Action. I want to repeat what the late editor of "Nature" John Maddox wrote in 1992: "So should molecular biologists mend their ways, resurrecting the Law of Mass Action (now conspicuous by its absence from what they publish)" (6)**.** I am glad that we have answered his plea long before it was made by using the law of mass action in our mathematical models of cell proliferation (9, 10, 14, 17).

Q. 6. Could you briefly summarize the main steps in the evolution of life on Earth?

A. 6. The evolution of life comprises two crucial periods: 1. A prebiotic period of transition from inorganic matter to the first biopolymers and 2. Emergence of biological systems – from biopolymers to Man. These main steps are presented in the following scheme:

EVOLUTION OF LIFE ON EARTH

1. Inorganic matter
↓
2. Synthesis of organic compounds
↓ **?**
3. Synthesis of the first biopolymers
↓ **→ Emergence of Life**
4. Reversible interactions among biopolymers
↓ **→ Law of Mass Action comes into force**
5. Non-linear dynamics of biopolymers reproduction
↓
6. Saltatory emergence of new forms
↓ **→ Saltatory evolution**
7. Natural selection – survival of the fittest
↓
8. Biodiversity on Earth

Q. 7. You have one question mark in this scheme. Could you comment on it?

A. 7. It concerns the crucial transition from organic compounds to biopolymers. This process is fundamental for the emergence of life. But we know very little how it was achieved. I would not like to go into the many existing speculations. A step forward has been made by the discovery of ribozymes leading to the idea that the first biopolymers were not DNA but RNA molecules which contain both the information and the enzymatic properties to replicate.

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The inerview was taken by S. Pavlova,

with respect and inspiration, the rest is silence.

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From *Chemical Embryology* to Nucleosome Patterning

An interview with Roumen G. Tsanev

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ABSTRACT Roumen Tsanev was a prominent Bulgarian scientist whose pioneering ideas about the role for chromatin in cell differentiation and development led him to propose the first hypothesis for epigenetic information based on a histone code. To test experimentally his ideas, Dr.Tsanev explored nucleosome structure and heterogeneity and generated seminal data on nucleosome segregation in replicating chromatin. Roumen Tsanev made significant contributions to the understanding of chromatin changes that underlie zygotic gene activation. He identified sperm specific chromatin components tightly bound to DNA and demonstrated that the histone complement of the male pronucleus appears before the onset of DNA synthesis in the mouse zygote. In this interview, Roumen Tsanev talks about his passion for science and literature, reminisces about surmounting the harsh realities in post-war communist Bulgaria through creativity and determination, and explains what led him to propose that histones were carriers of epigenetic information. Dr. Tsanev discusses mathematical models of gene regulation and recalls computer simulations that reveal the non-linearity of genetic networks. He explains how this non-linearity could affect cell proliferation, differentiation, development and evolution.

KEY WORDS: *chromatin, histone code, epigenetics, early development, mathematical modelling, evolution*

Professor Roumen Tsanev left us suddenly on July 23rd 2007 at the age of 84. A few months earlier we talked about chromatin, developmental biology and his life in science for this Special Issue of *The International Journal of Developmental Biology (IJDB)*. To his last days Tsanev was a man of extraordinary energy, passion and burning commitment to research; witty and provocative in a debate, a poet, scholar and philosopher, he imprinted a style of thinking on generations of young Bulgarian biologists. His whole scientific career is marked by innovation and originality - one of his early achievements was the development of RNA fractionation by gel electrophoresis. But above all, ideas – which he generated in abundance - had to be put to rigorous experimental testing. Dr Tsanev's lifelong interests lay in understanding the complex mechanisms of gene regulation that lead to cell differentiation. He was among the pioneers who tackled the structure and function of chromatin back in the sixties, as he was convinced that chromatin and particularly the histones, held the key to understanding cell differentiation. Well ahead of his time, Dr. Tsanev formulated some fundamental ideas about epigenetics based on a "histone code". He proposed a model of cell differentiation, which introduced a level of gene expression control based on long-term association of specific proteins to particular regions of DNA. In effect, Tsanev's model suggested ahead of his time the heritable influence of chromatin silencing at developmentally regulated loci. Aficionado of mathematical methods he tested his ideas about gene regulation in multicellular organisms and epigenetics using mathematical modelling and computer simulations. The model systems included epidermal proliferation, liver regeneration and tumour malignancies.

To search for experimental evidence for his epigenetic ideas Tsanev and his group embarked on a research programme starting with analysis of histone variants in the early seventies, and later, nucleosome heterogeneity and segregation during replication. In the late eighties, Tsanev developed experimental approaches to investigate zygote genome activation in the early mammalian embryo and obtained seminal data about tightly bound proteins on DNA and ultrastructures in ram sperm nuclei. Using pronuclear microsurgery and electron microscopy, Tsanev studied the dynamics of protamine to histone replacement, DNA replication and

Abbreviations used in this paper: BAS, Bulgarian Academy of Sciences.

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nucleosome assembly in the freshly fertilised mouse egg.

He was deeply convinced that the nucleosomes are "patterned" by histone variants and dedicated a tremendous amount of work to investigate nucleosome heterogeneity and analyse histone variants in heterochromatin of mouse and midge (*Glyptotendipes barbipes*), polytene chromosomes in different developmental contexts. More recently Dr. Tsanev focused on the role of genetic networks in the process of evolution, highlighting the sophisticated concept of genomic non-linearity.

Roumen Tsanev was a man of moral integrity. He started his career under the political oppression of Lyssenko and spent the greater part of his professional life in the stifling world of "realsocialism". Through all this time he fostered and protected talented people at odds with the official ideology. The memory of these years is now fading away, but those who were there will not forget how Roumen Tsanev endorsed and stood by the values of intellectual freedom refusing to be intimidated by political pressure. He firmly believed that molecular biology held a key to improving human health and understanding *die Welt im Innersten Zusammenhält* [1]. Roumen Tsanev had a magical personality, and was able to capture and galvanize your imagination with few words and gestures. He was a fascinating speaker with the charm of a Hidalgo convinced in the rational certainty of his dreams.

Fig. 1. Between Science and Art. *"The charm of a Hidalgo convinced in the rational certainty of his dreams". After authoring more that 200 scientific papers, Dr. Tsanev finished the Bulgarian translation of Faust by Goethe in 2005.*

As a classical warming up introduction, would you try to describe the sources of your inspiration to get involved in Developmental Biology?

Between Science and Art

Since my childhood I was fascinated by the wonderful world of living creatures. I enjoyed collecting insects and spending hours in observing the life of spiders. This was in spite of having been born in a family of litterateurs - my father was a well-known literary critic, my mother was a poetess and our home was a place continuously visited by Bulgarian writers and people of art. I deviated from this world of art and with the years became more and more interested in the secrets of Nature, wanting to understand one day what *die Welt im Innersten zusammenhält?*[1] (Goethe, *Faust*). Nevertheless, a vivid interest in literature and especially in poetry remained - for better or for worse - as an inherited burden in my life. This tragedy of Goethe affected me so deeply that its translation into Bulgarian became my hobby since my youth. My translation of "Faust" in Bulgarian was published only last year to my great satisfaction from the point of view of the translation but not from that of my understanding "what the universe engirds"!

Along with my constant involvement in scientific research I was deeply sensitive to poetry and even tried to write verses, which I dared to publish in two booklets only recently. One of my verses was dedicated to DNA whose structural beauty inspired me to write the verse "The Double Helix" which begins:

Our Mother Nature, frenziedly witted,
had the double helix so nicely knitted.

The scientist

Very early I realized that knowledge in chemistry, physics and mathematics would be needed to understand life. In 1941 I graduated from a Gymnasium in Sofia with a special prize for mathematics. At that time the Medical Faculty in Sofia University offered the best opportunity for studying life sciences and I graduated in Medicine, but never used my medical knowledge as a GP except on my two daughters - a practice which they fortunately survived.

Soon after I obtained my degree in Medicine I met the famous Bulgarian geneticist Doncho Kostov who recruited me as his research assistant in the newly founded Institute for Applied Biology and Development of Organisms - an Institute at the Bulgarian Academy of Sciences, which opened up in 1946 with no rooms, no staff and no equipment. Long years followed in the worst working conditions and under the pressure of the officially imposed pseudoscience of Lyssenko. Such was the situation more than a half-century ago when I started my scientific carrier. Unfortunately D. Kostov, who had actively opposed Lyssenko during his work in the USSR, soon died of heart failure under the pressure to recognize the teaching of Lyssenko.

After the death of D. Kostov, his institute merged with the institute of Biology led by another famous Bulgarian biologist – Methodi Popov. There I founded a small laboratory, which later became an independent unit at the Bulgarian Academy of Sciences (BAS) under the name of "Central Biochemical Laboratory", where in 1954 I was appointed as Director.

With a group of young enthusiasts I started research on the structure and function of nucleic acids under the worst post-war conditions. Moreover, nucleic acids were a dangerous field under the situation created by the total submission of our authorities to the pseudoscientific ideas imposed by Lyssenko. To this should be

[1] "What the universe engirds" Faust by J.W. von Goethe. p.I, Penguin Books, 1949. English translation by Ph.Wayne.

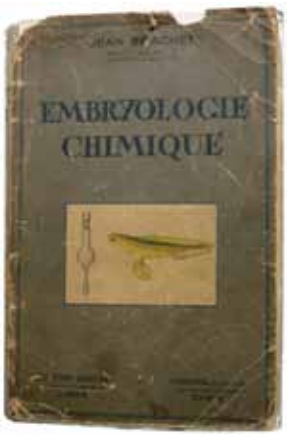

Fig. 2. "The Bible" of the newly founded "Central Laboratory of Biochemistry" (1954).

added the difficulties, sometimes the impossibility, to publish in western journals.

Very early my imagination to start studies on nucleic acids was excited by two books: the first was a volume of the English Society for Experimental Biology containing the papers read at the first Symposium on nucleic acids held in Cambridge in 1947 brought to me by D. Kostov. The second was the wonderful book «Embryologie chimique» (Fig. 2) by Jean Brachet (Brachet, 1945) which at that time became our Bible in the laboratory. It was a gift in 1949 by the young Swedish student Lars Ehrenberg whose enthusiasm to help post-war Bulgaria brought him as a volunteer in our youth work brigades.

Without any supply of special reagents and equipment I started work. Influenced by the book of Brachet, I isolated RNase from my own saliva (later from calf pancreas) and used it to carry out cytochemical studies on nucleic acids during mechanical injury of tissues. Thus, I obtained my first experimental results indicating the role of RNA degradation products in the wound process.

Despite the bad working conditions, an enthusiastic group of talented young people formed the nucleus of our future Institute of Molecular Biology. The lack of an ultracentrifuge led us to discover that electrophoretic separation of RNA in agar gels is a much better method for nucleic acid fractionation than ultracentrifugation (Tsanev, 1965). This was my first achievement and soon this technique and its many variations became a preferred procedure world- wide.

In 1969-71 you proposed an original mathematical model of cell proliferation and differentiation. How does this model account for the networks of gene regulation controlling development and differentiation?

In 1961 I read the paper of Jacob and Monod who proposed a model explaining the regulation of gene expression in prokaryotes (Jacob and Monod, 1961). It was a fascinating idea and I was tempted to apply this model to eukaryotes, not realizing yet the important consequences of this idea.

At that time I used to spend our summer vacation with my family on the Black Sea coast in the International House of Scientists "Joliot Curie" near Varna. There I met on the beach the Bulgarian mathematician Blagovest Sendov (now member of our Academy and Bulgarian Ambassador to Japan in Tokyo). Burning on the sand under the hot August sun we discussed science and I started to explain a model of cellular activity based on interrelated genes and the equations that may express the control of their activity. Sendov was very much interested in this idea. In these early days of informatics he was involved in work with computers that were already installed in the Institute of Mathematics at the BAS. Finally we decided to use a computer to study a model of cellular activity based on a network of genes interrelated on the basis of equations describing the synthesis of mRNA, controlled by DNA-protein interactions and programming the ribosomes for the synthesis of proteins.

After the vacation we started computer simulations of our model using the Russian computer "Minsk 22" that occupied a whole room in the Institute of Mathematics and worked for days to solve some of our simulation experiments. With today's modern computers, such problems take only seconds to be solved.

We adapted to eukaryotic cells the concept of gene regulation based on a circuit of two sets of genes – one controlling cell differentiation and another mitotic activity - interrelated on the basis of mutual repression. The main process which affected our model was the reversible interaction of proteins with DNA. We introduced an equation describing the synthesis of some proteins (trans factors) that controlled the activity of genes by binding to their specific DNA sequences (cis elements). It was clear that such reversible trans/cis interactions should obey the Law of Mass Action, a law formulated in 1867 by Guldberg and Waage.

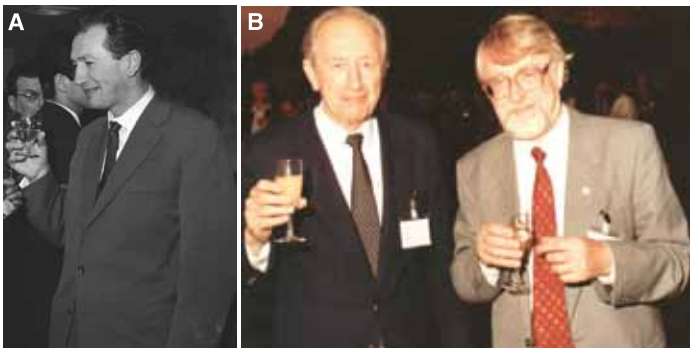

Fig. 3. Fellowships abroad in the 1960s and 1970s. (A) *At a reception at the International Atomic Agency, Sacley, France (1963).* **(B)** *With Vladimir Skulachev (right) in Moscow (1979).*

Today we know that some of these trans-acting proteins are repressors and others are activators of gene activity but our approach would still apply. Based on the law of mass action, the fraction of time θ a trans-cis complex is free of repressor or is bound to an activator can be expressed as: $\theta = 1/1 + (\sigma.T)^n$ where n is 1 if T is a repressor or -1 if it is an activator and σ is the equilibrium constant of a given trans-cis complex involving the trans factor T. Thus, the synthesis of mRNA should be proportional to the fraction of time θ that the cis element is free of repressor (n = 1) or is associated with an activator (n = -1).

The use of θ in the equation of mRNA synthesis imposes features of non-linearity to the whole system. The properties of such a system depend on its parameters. There is a definite space of parameters when small changes in their values cause small changes in the properties of the system. In a non-linear system when the parameters reach the boundaries of this space, small changes of their values lead to drastic, saltatory changes in the property of the system. These "jumps" were clearly revealed when we carried out computer experiments with models of different cellular systems: a proliferating cell culture, a regenerating mammalian liver (Sendov and Tsanev, 1968), liver cancer, wounded skin epidermis, as well as a model of a worm-like creature called by us "Kylindros" (Tsanev and Sendov, 1971b).

Fig. 4 shows the abrupt changes of liver "regeneration" after 2/3 "hepatectomy" (simulated by decreasing the number of cells) upon changing one of the parameters of the system. It is seen that there are regions of stability where changes in the concentration of a critical repressor synthesized by differentiation genes do not affect the reaction of the system. However, extremely small deviations out of these regions of stability, change drastically the behaviour of the system and an unlimited growth is obtained.

With the model "Kylindros" we obtained sudden morphological changes when the half-life of the stored mRNA in the oocyte changed (Fig. 5). Thus, changes in the stability of mRNA in the oocyte will lead to phenotypic changes of a species.

The validity of our model was confirmed with computer experiments on the model of epidermis. It simulated well the random distribution of mitotic cells, their diurnal variations and increased mitotic activity after injury, simulated by decreasing the number of cells in a segment of the model. It is interesting that in these experiments, our model predicted several fading mitotic waves after injury - 2, 3 or more, depending on various parameters as found in some real experiments where two maxima of mitotic rate were observed on days 1-2 and 7 (Rohrbach *et al.*, 1977). Our real experiments have shown that injury leads to degradation of RNA and this was used to simulate injury in the model.

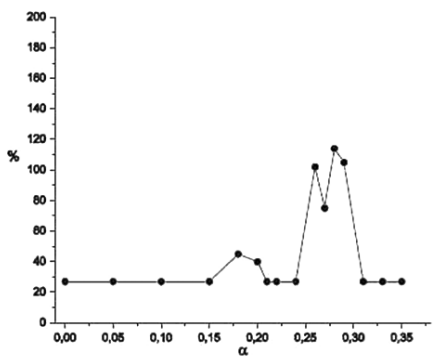

Fig. 4. A non-linear genome. *Effect of the parameter α (which determines the distribution of a diffusible repressor between the cells and the intercellular space) on the extent of restoration of the lever model as simulated by the computer after 66% "hepatectomy" (Sendov and Tsanev, 1968).*

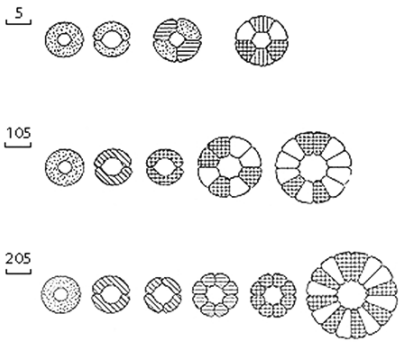

Fig. 5. The Kylindros model. *Cross sections of the model Kylindros showing different phenotypes depending on the half-life time (expressed as computer steps) of the mRNA stored in the egg (Tsanev and Sendov, 1971b).*

Some authors have argued that the presence of several mitotic waves following injury speaks against a model of functionally interconnected operons. As seen, our model experiments show that such a conclusion is not correct; the model did predict such results. May I say that in this case the computer mouse predicted very well some properties of the laboratory mouse!

In all cases the computer experiments with our models have shown that the behaviour of these systems is a non-linear function of their parameters. When the latter cross some space boundary, abrupt changes take place leading either to a chaotic unstable state, or to a new steady state, characterized by a different set of active genes. The idea that non-linear relationships should take place in the genome has been suggested by other authors. To explain saltatory phenomena in evolution, non-linear models have been proposed by introducing variables, as for example non-linear fields, genome operators, stochastic models (Karev *et al.*, 2003), artificially constructed non-linear genetic networks etc. In fact all these approaches did not contribute to understanding the molecular mechanisms underlying the process of new pattern formation. Such formal approaches substitute one unknown factor for another. In our studies we reached such non-linear relations on the basis of a natural molecular process – interactions between specific DNA sequences (cis elements) and regulatory factors (trans proteins) as governed by the Law of Mass Action. This answers one of the objectives of science to explain more and more with less and less.

Being among the first to use the Law of Mass Action in a mathematical model of cell proliferation, it was gratifying to read a

Fig. 6. Talking about Evolution. *With Alexander Oparin and his wife Nina Petrovna in the Rila mountains (1962).*

while ago what the former editor of "Nature" John Maddox wrote in 1992: *So should molecular biologists mend their ways, resurrecting the Law of Mass Action (now conspicuous by its absence from what they publish)* (Maddox, 1992).

Development and Evolution were two recurrent themes in the main projects of your laboratory. Early in the sixties you talked with Alexander Oparin (Fig. 6), who provided the first and principal modern appreciation of the origin of life. You were particularly interested in the evolution of histones and its implications for histone binding and role on DNA. In the light of the resurgent interest in Darwin's theory, what could be the impact of genetic network non-linearity on the evolution of organic forms?

I wonder if Darwin was right in adopting the motto "*Natura non facit saltus*"[2]? Some contradictory features of the evolution of species have caused many debates and disagreements concerning the pace of evolution. The Darwinian phyletic gradualism was opposed to the "un-Darwinian" abrupt changes ("quantum jumps"). Long geological periods of "frozen" evolution were found in contrast to the periods of rapid emergence of new biological forms (e.g. the Cambrian explosion). The mass extinction of species during some geological periods also remains still unexplained. There was no theory at the molecular level that could provide a natural model explaining these varying aspects of evolution. Attempts to explain these peculiar features have caused the division of opinions referred to as "gradualist-saltationists" schism.

As I already explained, gene regulation based on interactions between trans-acting proteins and cis-DNA sequences leads to non-linear differential equations describing gene activity. Thus, the genome becomes a non-linear system as a function of its parameters. The parameter space is divided into zones of stability (gradual changes of the phenotype) and zones of unstable "chaotic" behaviour - abrupt changes ("jumps") leading either to lethality for a living system (extinction of species) or to a new stable state representing the emergence of a new biological form for the natural selection. Our data have also shown the importance of some parameters for the stability of a cellular system. Such are the equilibrium constants of the trans/cis interactions, the threshold values of the concentrations of trans proteins, membrane permeability, the half-life of different mRNAs etc. Of special interest is the organization of DNA in separate domains by some tightly bound proteins that seem to be evolutionary conserved (Avramova *et al.*, 1989).

The conclusion that the genome is a non-linear system has important implications explaining in a natural way the different aspects of the biological evolution. A genetic drift caused by small Darwinian changes, even without any adaptive values, may be in fact the driving force of evolution by shifting the molecular parameters of a population outside the boundary of the stability zone. The results of such small changes may be species variations - within the zone of stability, extinction of species (within the zone of unstable behaviour), emergence of new species (transition into a new zone of stability) and long periods of stasis separating these events. This is a new approach to the problem of evolution which will be very helpful in reconciling different view points.

Life on Earth appeared more than 3,700 billion years ago, i.e. very soon after our planet was formed. Such a short period for life to emerge has led some authors (e.g. Francis Crick) to believe that life was created somewhere else in the Universe and then transferred to the Earth. The period of about 1,300 billion years for life to appear on Earth is really short if evolution proceeded according to Darwinian phyletic gradualism. However, the presence of non-linear relations in the genome will strongly accelerate the pace of evolution. It cannot be excluded that in the prebiotic time, non-linear relations were established among the new biopolymers, leading rapidly to new forms. Thus the famous motto of ancient philosophers "*Natura non facit saltus*" [2] is far from being true. Darwin was right to insist on the role of small variations, but he could not know what molecular biology was going to discover a century later.

Back in 1971, when nucleosomes had not yet been described, you proposed an epigenetic mechanism of differentiation and carcinogenesis based on the assumption that regulatory units could be based on "specific arrangement of histone and non-histone proteins". What were your first steps in the field of chromatin structure and function and how did you come so early in time to the idea of epigenetic information?

My first studies on the role of nucleic acids in wound healing induced my interest in the molecular mechanisms of cell proliferation and differentiation. This led me to study the molecular organization of chromatin, a problem that became a central objective of our team. On the basis of electron-microscopic observations I reported in 1975 details on the substructure of the nucleosome showing the possibility of its splitting into two half-nucleosomes (Tsanev, 1978). The behaviour of nucleosomes during DNA replication and transcription then became the focus of our further studies. I was especially interested in the problem whether stable DNA-protein complexes may be inherited playing the role of a new type of biological information – epigenetic information (Tsanev and Sendov, 1971a).

As far as the histone code is concerned, first of all I would like to clarify what I think we should understand by epigenetics, because there is some confusion in the literature concerning this notion. Epi- comes from the Greek, meaning "upon", "after", and

[2] Nature does not make a leap (Webster's dictionary).

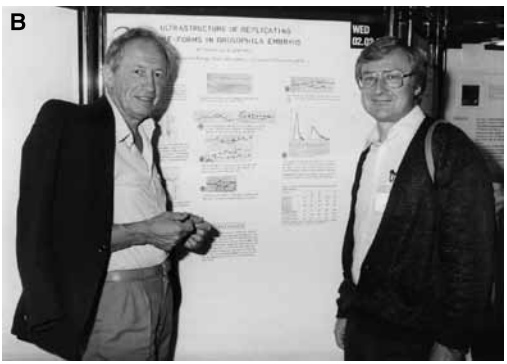

Fig. 7. Chromatin Meetings. (A) *At the Gordon Conference (1986), Tsanev in row 5, 4th from the left to right (arrow).* **(B)** *FEBBS meeting 1988, with Tibor Igo-Kemenes (right).* **(C)** *"Witty and provocative in a debate….he imprinted a style of thinking on generations of young biologists". Talking to students and post-doc fellows, Nucleolar workshop, Suzdal, Russia (1989).*

"following". Thus, epigenetic information is a stable factor with the following characteristics: It affects gene expression, it is independent of DNA sequences and it is transferred to the progeny (it is stably inherited).

Histones attracted our attention due to their peculiar properties: 1) They bind firmly to DNA without recognizing DNA sequences; 2) The genes that code for histones are repeated hundreds of times; 3) Histones are highly conserved in evolution, showing very few mutations. For example, while many proteins undergo tens to hundreds of mutations per one million years per 100 amino acids, the most conservative histone H4 has undergone only 1 mutation in such a period. This shows that even one mutated gene in the histone repeat will produce "false" histones which would not be tolerated, even though the bulk of normal genes in the repeated cluster will supply normal histones. This unusual and intriguing fact especially aroused my interest. One of the roles of histones is packaging and protecting DNA. However, when the problem is limited to such a role only, various other proteins can be used, as is the case of the proteins associated with sperm DNA. This suggested another important role of histones, which may be informational. Then, in 1969-1971 we put forward the idea that different arrangements of histones may play the role of an epigenetic histone code for the recognition sites of different genes, thus controlling gene expression. Concerning this model in 1971 we wrote in the Journal of Theoretical Biology: *"Such a model does not require molecules able to recognize DNA base sequences but introduces a new information contained in the histone arrangements where the histone molecules play the role of elements of a new, epigenetic code"* (Tsanev and Sendov, 1971b). Activation of genes *"is realized by non-histone proteins which can specifically and irreversibly bind to different histone arrangements"* at the initiation sites of genetic units. During the period of 1974 -1978 it was largely recognized that non-histone proteins are involved in the control of gene expression (Elgin and Weintraub, 1975), (Bekhor, 1978). In agreement with such a mechanism we found in different cellular systems metabolically stable non-histone proteins, a property needed to preserve the cellular type during DNA replication (Koleva and Tsanev, 1978; Srebreva *et al.*, 1979).

At that time (1970-71) nucleosomes and their histone complement had not yet been discovered. When later it was found that DNA is organized in nucleosomes which have the same histone complement, our attention was focused on the non-allelic histone variants and their chemical post-translational modifications, which became well known in 1974-75. It could be calculated that with two variants of H2A and three of H2B, 18 different nucleosomes can be assembled, without changing the overall design of the particle. If the two halves of the nucleosome contained different variants, then this number becomes 324. On the other hand it was also calculated that if all possible chemical modifications of only H4 took place, 240 different nucleosomes could arise (DeLange and Smith, 1975).

Nucleosome heterogeneity became the main object of our further research. We found differences between nucleosomes containing different histone variants. Thus, the accessibility to iodination of nucleosomes containing H2A1 or H2A2 variants was different and nucleosomes reconstituted with one or the other of these variants showed differences in the rate of DNAse I digestion (Venkov *et al.*, 1985). Upon dissociation of nucleosomes with increasing salt concentration, a multiphase dissociation curve was obtained indicating the presence of different nucleosomes with respect to their stability (Vassilev *et al.*, 1981). The transcriptionally active macronucleus of *Tetrahymena* was found to contain differ-

ent histone variants from those of the inactive micronucleus (Allis *et al.*, 1980). Similar differences we observed in the mouse centromeric chromatin (Russanova *et al.*, 1989). A chromatin fraction containing rapidly labelled RNA also showed some differences in histone variants (Gabrielli *et al.*, 1981).

Concerning the post-translational modifications of histones, we found that the most highly acetylated forms of H3 and H4 as well as the acetylated subspecies of H2B were localized almost entirely in the active chromatin fraction (Georgieva *et al.*, 1982).

In 1979/80 we reached the following conclusion specifying our model: Histone variants could be involved in gene regulation by making the nucleosomes structurally and functionally heterogeneous. I also drew attention to the fact that on the basis of the structures of nucleosomes, the H2A-H2B pair has an inner site for interaction with the H3-H4 tetramer and an external site for interaction with some nonhistone proteins and that evolutionary conservation of the protein-binding domains in H2A-H2B shows the vital importance for the cell of some specific protein-protein interactions.

The idea that histones play a role in the control of gene expression met a serious difficulty in the accepted view that in the sperm nucleus of some species histones are lost completely and replaced by protamines. However, more detailed studies showed the presence of tightly bound proteins in mature sperm nuclei (O'Brien and Bellve, 1980a; O'Brien and Bellve, 1980b). Moreover, electron-microscopic studies of such nuclei revealed DNA fibres with nucleosome-like beads resistant to guanidine chloride, high salt and urea (Tsanev and Avramova, 1981). These data suggested that in the course of spermatogenesis somatic histones may have been preserved at some important sites of the genome. The complex protein structure of the sperm nucleus may be important for the reconstitution of a somatic nucleus preserving the sites with informational combinations of histones and their modifications. This specific structure may explain the need to replace the protamines with histones before the first replication takes place after fertilization (Nonchev and Tsanev, 1990).

We had previously shown that histones could be incorporated into chromatin in the absence of DNA replication, but the problem arises how a specific histone pattern could be transmitted to the progeny during DNA replication, what is the behavior of histones and nucleosomes during this process. The idea of a histone code supposed that during DNA replication old and new histones should not be distributed randomly between the two DNA strands. This question was first addressed experimentally by our group and we found that old histones remained associated with the old DNA strand and the new one – with the newly synthesized strand (Russev *et al.*, 1980). We also found bilateral dispersive nucleosome segregation in groups of several adjacent nucleosomes distributed between the two daughter DNA molecules (Pospelov *et al.*, 1982) The distribution of old and new histones between the two DNA strands could be explained by differences in the interaction of histones with the two complementary DNA strands. We were then able to demonstrate that the nucleosomal histone core, when in the chromatin, is slightly asymmetrically placed in the region where the H2A-H2B pairs have been shown to interact with DNA (Chipev *et al.*, 1987). Such an asymmetry was confirmed by later publications and by the fact that an alternating asymmetrical protection of DNA in nucleosomes was seen inside the chromatin higher-order structure (Staynov, 2000). An asymmetry in the relative position of these two dimers has also been detected by X-ray crystallography of core particles (Richmond *et al.*, 1984). Thus, histones interact more strongly with one of the DNA strands and this may explain why the old histones remains attached to it during replication. I have suggested that a strong binding site may be due to arginine residues. The need for a subtle balance of binding forces may explain the extraordinary fact that mutations substituting lysine for arginine have not been tolerated in evolution. *"This asymmetric interaction of histones with the two DNA strands may have important implications for the segregation mode of the old histone octamers during replication and for the cooperative alignment of the new nucleosomes"* (Tsanev and Russev, 1974).

After our publications suggesting epigenetic information realized by histones and their interactions with non-histone proteins, there was a period of silence in the literature concerning epigenetics, which lasted about 20 years. Then, especially after 1990 there was an explosion of papers discussing epigenetic information. Although authors do not necessarily look back to see that the idea of an epigenetic information played by histones has been expressed 20-30 years earlier, the model of a code, based on non-allelic histone variants and histone tail modifications, read by regulatory proteins and governing downstream events, is now a central dogma of modern epigenetics (Strahl and Allis, 2000; Jenuwein and Allis, 2001). Still many aspects of the nature of the histone code remain elusive and I hope that among the different "omics" of today, epigenomics will soon find its place.

Fig. 8. A Man of the Bench. (A) *In the hot room with Georgy Dessev (left).* **(B)** *What does the sequence say? (with Ivan Ivanov, right).*

Based on the experimental results in your work, can you describe your view on the complexity of gene interactions during embryogenesis and morphogenesis and shortly summarize the essential points of your concept?

All data show that in the complex process of development at least three sources of information for gene expression and morphogenesis are used: genetic - available as DNA sequences, epigenetic - expressed as combinations of histone modifications ("histone

code") and genetic networks - based on trans-cis interactions inducing non-linear dynamics in the genome.

Thus, not only in the evolution of species, but also during the individual development of a living organism, saltatory changes may take place that would explain some aspects of morphogenesis. Due to non-linear relations, changes in the parameters of a living system could drastically lead to cellular death, cellular transdetermination, to the appearance of a new forms and also to cancer. A good example is the transformation of the caterpillar into a butterfly – a saltatory transition from a creeping creature to a flying beauty. Nothing could be more different than these two forms. Another example is the unicellular organism *Naegleria* which in the absence of water is an amoeba and in water is transformed into a flagellate. For some time these two forms were considered to be two different species.

As a summary I would like to suggest tentatively the following four interrelated informational systems controlling the development of living organisms:

1. Genetic information expressed as DNA sequences coding for proteins and different RNAs.

2. Epigenetic information represented by histone variants and their chemical modifications involved in the control of gene expression.

3. Genetic networks - information for phenotypic expression, translation of the linear information in DNA into a three-dimensional phenotype – a new type of information realized by the genetic networks making the genome a non-linear system.

4. Audio-visual informational signals transmitted from parents to descents, forming by learning the behaviour of a species. I don't dare to discuss this aspect, but with the development of a neural system, this is likely to play a more and more important role.

It should be added that the parameters involved in all these informational steps play a crucial role both in the function of a living system and in the course of evolution. Speciation was not only due to genes *per se*, but to changing interrelations among genes – the structure of the genetic networks and their parameters. Let me finish with the probably striking conclusion that development, like the whole biodiversity on Earth, including our own human presence, owes a great deal to the Law of Mass Action.

Fig. 9. Enhancers and Nucleosomes. *R.G. Tsanev (right) with Walter Shaffner (left) and Weyma Lübbe (middle) at the DKFZ, September (1991) Heidelberg.*

Fig. 10. Science and Politics. *R.G. Tsanev (left) in London (1986), with the Russian geneticist and famous Soviet dissident Dr. Jores Medvedev (right).*

What type of new technologies will help to decipher the relationship between ontogenesis and evolution?

Studies on cloning and stem cells would be my first suggestion. Cloning may help to elucidate the factors that impose differences on organisms with identical DNA, which seems important for the role of ontogenesis in evolution. Such differences have been observed in cloned mammals as nicely illustrated by the "parents" of Dolly (Wilmut *et al.*, 1997; Wilmut and Campbell, 1998). Cloning may also help to understand how a somatic cell nucleus is reprogrammed to start the development of a new organism. The development of a whole organism from a somatic cell - i.e. somatic embryogenesis - where the nucleus should be "cleaned" from some proteins to become able for reprogramming and stem cells may help to understand the mechanisms and factors controlling their differentiation into various cellular types, an important factor for ontogenesis.

How did creativity and original thinking help you in studying the role of chromatin in development and could you venture to say in what way imagination operates in art, literature and science?

I think that in addition to intellectual capacity, the study of such a complex structure as chromatin and such a multiphase process as development, requires a lot of creative imagination when observing how powerful life is in creating forms and functions. If I have made some contributions to Science it is due to the persistence and will to solve a problem and to overcome the difficulties always accompanying our activities and of course, the help of my enthusiastic and clever colleagues. The lack of appropriate working conditions was a stimulating factor to find new procedures. I enjoy the intellectual play to develop a hypothesis and to design experiments which could prove or disprove it. If I had some success in my work, it is connected with the constant and continuous occupation of my mind and imagination with possible solutions of a problem and sharing my efforts with my co-workers.

In all spiritual areas of human, creativity imagination plays an important role. This is evident in Art and Literature, which cannot

be realized without imagination. In Science, imagination also plays a role that is not less important than intellect and knowledge. Having imagination to formulate an idea is an important step for the progress of Science. For me it is a real pleasure in seeing a theoretical idea experimentally proven, or even disproved, the latter being also a step forward. This demonstrates the power of human intellect and imagination. We have to recognize two types of scientists: the strict, pedantic researcher without imagination, who does not deviate from the bare facts (Wagner in *Faust*) and the creative scientist with an imagination looking ahead of the facts (Faust in *Faust*). Of course, both types of scientists are important and necessary for progress in Science; some are more like Wagner and others – like Faust!

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Dynamic alterations of linker histone variants during development
James S. Godde and Kiyoe Ura
Int. J. Dev. Biol. (2009) doi: 10.1387/ijdb.082644jg

A histone H1 variant is required for erythrocyte maturation in medaka
Osamu Matsuoka, Norihisa Shindo, Daisuke Arai and Toru Higashinakagawa
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Dynamic distribution of the replacement histone variant H3.3 in the mouse oocyte and preimplantation embryos
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Histone methylation defines epigenetic asymmetry in the mouse zygote.
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Idealism and romantic patriotism for science - an interview with José Francisco David-Ferreira
Maria Carmo-Fonseca and José Francisco David-Ferreira
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Marek Maleszewski and Andrzej K. Tarkowski
Int. J. Dev. Biol. (2008) 52: 163-169

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Putting evo-devo into focus. An interview with Scott F. Gilbert
Alexander T. Mikhailov
Int. J. Dev. Biol. (2005) 49: 9-16

A life in research on lens regeneration and transdifferentiation. An interview with Goro Eguchi
Kunio Yasuda
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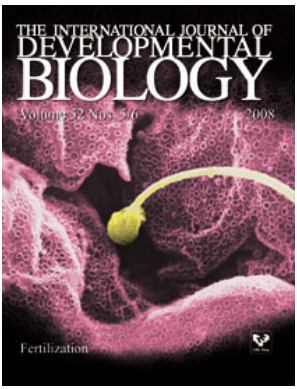

PROGRAM

THURSDAY OCTOBER 6, 2011

8:00 – 9:00

REGISTRATION

9:00 – 9:30

OPENING CEREMONY

9:30 – 10:30

OPENING PLENARY LECTURE (IN BULGARIAN)

THE INSTITUTE OF MOLECULAR BIOLOGY AND THE TIME OF ITS FOUNDERS

George Markov & Evgeny Golovinsky

ИНСТИТУТЪТ ПО МОЛЕКУЛЯРНА БИОЛОГИЯ И ВРЕМЕТО НА НЕГОВИТЕ СЪЗДАТЕЛИ

Георги Марков & Евгени Головински

SESSION: EPIGENETICS AND GENOME INTEGRITY

Chairpersons: Iliya Pashev, Boyka Anachkova

10:30 - 11:00

THE CHROMATIN OF ACTIVE GENES IS NOT IN A PERMANENTLY OPEN CONFORMATION AND IS HEAVILY LOADED WITH LINKER HISTONES

Colyn Crane-Robinson

11:00 - 11:30

COFFEE BREAK/POSTER SESSION A

11:30 - 12:00

HOW DO THE SMART CHROMATIN REMODELING NANOMACHINES WORK?

Stefan Dimitrov

12:00 - 12:20

THE ROLE OF HMGB1 PROTEIN IN CHROMATIN REMODELING AND REPAIR OF CISPLATIN-DAMAGED DNA AT NUCLEOSOME LEVEL
Evdokia Pasheva

12:20 - 12:40

THE INFLUENCE OF THE REMODELING COMPLEX INO80 ON THE HIGHER-ORDER CHROMATIN STRUCTURES OF YEAST *SACCHAROMYCES CEREVISIAE*
George Miloshev

12:40 -13:00

DIFFERENT ROLES OF RUVBLS AND INO80 PROTEINS IN MAMMALIAN DOUBLE STRAND BREAK REPAIR
Anastas Gospodinov

13:00 - 14:00

LUNCH

SESSION: EPIGENETICS AND GENOME INTEGRITY
Chairpersons George Russev, Stefan Dimitrov

14:00 - 14:30

OXIDATIVELY GENERATED DAMAGE TO CELLULAR DNA: FORMATION AND REPAIR
Jean Cadet

14:30 - 15:00

BASE EXCISION REPAIR WITHIN NUCLEOSOMAL SUBSTRATES
Dimitar Angelov

15:00 - 15:20

DYNAMICS AND INTERDEPENDENCE OF THE SUBUNITS OF THE S-PHASE CHECKPOINT COMPLEX MRC1/TOF1/CSM3
Marina Nedelcheva-Veleva

15:20 - 15:40

SODIUM BUTYRATE INCREASES HELA CELLS RADIOSENSITIVITY BY SUPPRESSING DNA REPAIR
Miglena Koprinarova

15:40 - 16:10

COFFEE BREAK/POSTER SESSION B

SESSION: STEM CELLS AND BIOTECHNOLOGY

16:10 - 16:40

HAIR FOLLICLE STEM CELLS NICHE DISRUPTION IN THE MOUSE MUTANTS "BALD MILL HILL"
Stefan Nonchev

16:40 - 17:00

HOW BACTERIA EAT WASTE GASES AND PRODUCE FUEL AND CHEMICALS?
Svetlana Boycheva

18:00 - 20:00

COCKTAIL

FRIDAY OCTOBER 7, 2011

SESSION: BIOMEDICINE
Chairpersons: Stefan Nonchev, Margarita Apostolova

9:30 - 10:00

INDOXYL SULFATE, A TRYPTOPHAN METABOLITE, INDUCES NEPHRO-VASCULAR CELL SENESCENCE
Toshimitsu Niwa

10:00 - 10:30

SCHIZOPHRENIA GENOME

Draga Toncheva

10:30 - 11:00

COFFEE BREAK/POSTER SESSION C

11:00 - 11:20

POLYMORPHISMS OF MATRIX METALLOPROTEINASES (*MMP*) IN COPD

Tatyana Vlaykova

11:20 - 12:00

NOVEL GENERATION SEQUENCING FROM ILLUMINA: THE PRESENCE AND THE FUTURE

ELTA'90 M – General Sponsor of the Conference

12:00 - 13:30

LUNCH

SESSION: BIOMEDICINE

Chairpersons: Draga Toncheva, Ivan Ivanov

13:30 - 14:00

IS THERE ROOM FOR EXPERIMENTAL PHYSICAL MODELS IN MOLECULAR BIOLOGY?

Zdravko Lalchev

14:00 – 14:20

QUANTITATIVE PROTEOMIC IDENTIFICATION OF HOST FACTORS INVOLVED IN THE *TYPHIMURIUM* INFECTION CYCLE

Dora Kaloyanova

14:20 - 14:40

INTERACTION OF THE TUMOR SPECIFIC PROTEIN HGAL-3 WITH ANTICANCER AGENTS

Vanya Bogoeva

14:40 - 15:00

PROTEOMICS FOR CARDIOVASCULAR DISEASE BIOMARKERS DISCOVERY

Margarita Apostolova

15:00 – 15:30

TOUCH THE FUTURE OF PCR – FASTER, SMARTER, EASIER PCR WITH 1000–SERIES THERMAL CYCLERS

A&A Medical – Conference Sponsor and Official Representative of Bio-Rad for Bulgaria

15:30 - 16:00

COFFEE BREAK/POSTER SESSION D

16:00 - 16:20

STABILITY OF MUTANT HUMAN INTERFERON-GAMMA DERIVATIVES A COMPLEX APPROACH STUDY

Genoveva Nacheva & Elena Lilkova

16:20 – 16:40

QUANTITATIVE EXPRESSION ANALYSIS OF CBF GENES IN BULGARIAN WINTER WHEAT CULTIVARS

Elena Todorovska

16:40 - 17:00

GLYCATION AND IMMUNOGENICITY OF PROTEIN THERAPEUTICS

Roumyana Mironova

17:00 - 17:10

CLOSING CEREMONY

POSTER SESSIONS

THURSDAY OCTOBER 6, 2011

10:00 - 13:00

SESSION A: PROTEOMICS, MEMBRANES, BIOINFORMATICS AND BIOTECHNOLOGY

PA1

EFFECT OF HISTONE DEACETYLASE INHIBITOR SODIUM BUTYRATE ON INTERSTRAND CROSSLINK REPAIR

Stanislava Popova

PA2

ADAPTIVE RESPONSE TO DNA-DAMAGING AGENTS – A NONSPECIFIC PHENOMENON

Stephka Chankova

PA3

INVESTIGATION OF THE LEVELS OF PROTEIN FACTORS INVOLVED IN THE PROCESS OF INITIATION OF DNA REPLICATION IN UV DAMAGED HUMAN CELLS

Vera Djeliova

PA4

MAMMALIAN INO80 IN RECOVERY FROM REPLICATION STRESS

Kalina Goranova

PA5

MICROBIAL COMMUNITY DIVERSITY IN THE RHIZOSPHERE OF *ZOSTERA SP.* BEDS IN SOZOPOL BAY, SW BLACK SEA REGION

Nadezhda Todorova

PA6

POLYMORPHISMS IN CYTOKINE GENES IN BRONCHIAL ASTHMA

Dimo Dimov

PA7

POLYMORPHIC CHARACTERISTICS AND INITIAL PCR-BASED POLYMORPHISM ANALYSES OF PLANTS FROM FIVE BULGARIAN POPULATIONS OF *HABERLEARHODOPENSIS* FRIV

Slaveya Doncheva

PA8

INITIAL DETERMINATION OF POLYMORPHISM AND *IN VITRO* CONSERVATION OF SOME *RAMONDA SERBICA* POPULATIONS FROM ALBANIA AND BULGARIA

Slaveya Doncheva

PA9

GENE-SPECIFIC PCR AMPLIFICATION OF TECHNOLOGICALLY IMPORTANT LACTOCOCCAL GENES

Zoltan Urshev

PA10

THE FTO RS9939609, ADIPOQ RS1501299, RS822391 AND ADIPOR2 RS16928662 POLYMORPHISMS RELATIONSHIP TO OBESITY AND METABOLIC SYNDROME IN BULGARIAN SAMPLE

Deyana Vankova

PA11

METHOD FOR DEVELOPMENT OF STRAIN-SPECIFIC MARKERS FOR BIFIDOBACTERIA

Zhechko Dimitrov

PA12

STRUCTURAL ORGANIZATION OF IGS AND LEVEL OF METHYLATION OF RIBOSOMAL RNA GENES IN RECONSTRUCTED BARLEY KARYOTYPES (*HORDEUM VULGARE* L.)

Anna Dimitrova

PA13

GENOMIC DISTRIBUTION OF AC-LIKE TRANSPOSABLE SEQUENCES IN *SPHAEROCOCCUM* TYPE MUTANT FORMS OF *TRITICIM AESTIVUM* L. AND *TRITICALE*

Georgi Bonchev

PA14

DNA ANALYSIS OF YEAST ECOSYSTEM OF SELECTED BULGARIAN FOOD PRODUCTS

Dilnora Gouliamova

PA15

COMMUNITY STRUCTURE OF YEASTS AND ACTINOMYCETES IN SELECTED ANIMAL FEESES

Dilnora Gouliamova

PA16

GENOTYPING OF ENDEMIC THE RHODOPE MOUNTAINS SHORTHORN RHODOPEAN COW BREED

Peter Hristov

PA17
S-MOTIFS AS A NEW APPROACH TO SECONDARY STRUCTURE PREDICTION: COMPARISON WITH STATE-OF-THE-ART METHODS
Elena Todorovska

PA18
***IN SILICO* SOLUTION FOR MERGING OF PHENOTYPE ONTOLOGIES**
Elena Todorovska

THURSDAY OCTOBER 6, 2011

14:00 - 17:00

SESSION B: EPIGENETICS AND GENOME INTEGRITY

PB1
STUDY OF THE ANTICORROSION EFFECT OF EXOPOLYSACCHARIDES PRODUCED BY *LACTOBACILLUS DELBRUECKII B5* CULTIVATED ON DIFFERENT CARBOHYDRATES
Tsveteslava Ignatova-Ivanova

PB2
***IN SILICO* AND BIOCHEMICAL ANALYSES OF SUPEROXIDE DISMUTASE AND CATALASE ENZYMES IN CD - ACCUMULATING YEASTS**
Ventsislava Petrova

PB3
COMPUTATIONAL RNA/PROTEIN STRUCTURE PREDICTION AND FOLDING
Roumen Dimitrov

PB4

BIOLOGICAL SEQUENCE COMPARISON, MOLECULAR EVOLUTION AND PHYLOGENETICS

Roumen Dimitrov

PB5

VESICLES WITH TUBULAR PROTRUSIONS IN SYMMETRICAL AND NON-SYMMETRICAL CONDITIONS

Julia Genova

PB6

GLYCOLIPID CONTENT OF THE NUCLEAR MEMBRANE IN HYPOXIC RAT BRAIN

Emilia Petrova

PB7

EFFECT OF LINSEED DIETARY SUPPLEMENTATION ON FREE FATTY ACID CONTENT OF RAT BRAIN SYNAPTOSOMAL MEMBRANE

Emilia Petrova

PB8

SURFACE ELECTRICAL EFFECTS OF THE PHOSPHOLIPASE A_2 SUBCOMPONENT OF THE NEUROTOXIN VIPOXIN ON HUMAN ERYTHROCYTES

Virjinia Doltchinkova

PB9

NATIVE AND RECOMBINANT FATTY ACID BINDING PROTEIN 3 FROM *FASCIOLA HEPATICA* AS A POTENTIAL ANTIGEN

Denitsa Teofanova

PB10

COBALT(II)-INDUCED CHANGES IN HEMOGLOBIN CONTENT AND IRON CONCENTRATION IN MICE FROM DIFFERENT AGE GROUPS

Yordanka Gluhcheva

PB11

AUTOANTIGENICITY OF HUMAN C1Q IS BASED ON CONFORMATIONAL TRANSITION LEADING TO INCREASED HYDROPHOBICITY OF ITS GLOBULAR HEAD FRAGMENT

Vishnya Stoyanova

PB12

IMPACT OF GLYCATION INHIBITORS ON THE BIOLOGIC ACTIVITY OF RECOMBINANT HUMAN INTERFERON-GAMMA

Rositsa Tsekovska

PB13

HMGB1 PROTEIN AS A CHAPERONE: THE ROLE OF THE POSTSYNTHETIC ACETYLATION

Taner Osmanov

PB14

FIRST REPORTED NATURAL HISTIDINE TAILED PROTEIN FAMILY (PCHTP FAMILY)

Aneliya Yoveva

PB15

MASS-SPECTROMETRIC IDENTIFICATION OF STRESS-RELATED PROTEINS IN GROWTH MEDIUM OF SALT-TREATED SUSPENSION CULTURES OF *DACTYLIS GLOMERATA* L.

Lyuben Zagorchev

FRIDAY OCTOBER 7, 2011

9:30 - 12:30

SESSION A: BIOMEDICINE AND DRUG DESIGN

PC1

SUBCELLULAR LOCALIZATION OF HMGB1 AND ITS RECEPRTOR RAGE IN NORMAL AND TUMOUR TISSUE

Jordana Todorova

PC2

CORRELATION OF THE HMGB1 PROTEIN ENDOGENOUS LEVEL IN HUMAN CANCER CELL LINES WITH THEIR ABILITY TO REPAIR DNA DAMAGED WITH THE ANTICANCER DRUG *CISPLATIN*

Shazie Yusein-Myashkova

PC3

EXPRESSION OF IGF-1R $_{M}$RNA IN COLORECTAL CARCINOMA PATIENTS

Iliya Karakolev

PC4

ANTIGENIC CHARACTERISTICS OF RECOMBINANT NUCLEOCAPSID PROTEINS OF THE LASSA AND MARBURG VIRUSES

Anatoli Krasko

PC5

STUDIES ON THE SEROPREVALENCE OF FIVE HPV GENOTYPES IN BULGARIAN HIGH-RISK GROUP BY USING NEWLY DEVELOPED RECOMBINANT HPV VLPS

Evelina Shikova

PC6

THE USE OF RECOMBINANT VIRUS-LIKE PARTICLES HARBOURING INSERTED TARGET ANTIGEN TO GENERATE ANTIBODIES AGAINST CELLULAR MARKER $_{p}16^{INK4A}$

Evelina Shikova

PC7

INCLUSION BODIES OBTAINED FROM *E.COLI* CELLS EXPRESSING HUMAN INTERFERON-GAMMA CONTAIN NUCLEIC ACIDS

Elena Krachmarova

PC8

STABILIZING EFFECT OF SUCROSE, DEXTRAN AND HYDROXYETHYL STARCH ON RECOMBINANT HUMAN INTERFERON-GAMMA

Milena Tileva

PC9

INHIBITION OF GLYCATION BY SELECTED NATURAL AND PHARMACEUTICAL SUBSTANCES

Albena Stratieva

PC10

HIGHER TNF-ALPHA PRODUCTION DETECTED IN COLORECTAL CANCER PATIENTS MONOCYTES

Spaska Stanilova

PC11

INVESTIGATION OF IL-6 EFFECTS ON SP-A EXPRESSION IN A549 LUNG CELL LINE

Jordan Doumanov

PC12

MOLECULAR CLONING, EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF EXTRACELLULAR α-AMYLASE FROM EMBRYOGENIC SUSPENSION CULTURES OF *DACTYLIS GLOMERATA* L.

Goritsa Rakleova

PC13

OPPOSITE MODELS OF EXPRESSION OF ANDROGEN RECEPTOR (AR) AND RETINOIC ACID RECEPTOR α (RAR-α) IN THE ONSET OF MALE GERM CELL DEVELOPMENT IN HORMONALLY MANIPULATED RATS

Ekaterina Pavlova

PC14

INTEGRATION OF LANGERHANS-LIKE CELLS INTO A HUMAN SKIN EQUIVALENT

Vesselina Laubach

PC15

SYNTHESIS OF PLGA-PEG POLYMER MICELLES-CARRIERS OF COMBRETASTATIN-LIKE ANTITUMOR AGENTS

Iliyan Kolev

FRIDAY OCTOBER 7, 2011

13:00 - 16:00

SESSION A: BIOMEDICINE AND DRUG DESIGN

PD1

AN APPLICATION OF LOGISTIC REGRESSION AND MULTIFACTOR DIMENSIONALITY REDUCTION FOR DETECTING GENOTYPE-PHENOTYPE INTERACTIONS ON RISK OF ATHEROSCLEROTIC PLAQUE PROGRESSION

Nadya Ivanova

PD2

GENETIC MAPPING OF EMS-INDUCED MUTATIONS WHICH INTERACT WITH *DROSOPHILA FRAGILE X MENTAL RETARDATION 1 (DFMR1)*

Dimitrina Georgieva

PD3

ROLE OF +1245 A/G MT1A AND -209 A/G MT2 POLYMORPHISMS IN THE PATHOGENESIS OF CORONARY ARTERY DISEASE AND DIABETES MELLITUS

Rahila Kozarova

PD4

DROSOPHILA DFMR1 **INTERACTS WITH GENES CONTROLLING ACTIN FILAMENT ORGANIZATION IN NEURONAL DEVELOPMENT**

Mariya Petrova

PD5

DRUG DESIGN BY REGRESSION ANALYSES OF NEWLY SYNTHESISED DERIVATIVES OF 8-QUINOLINOL

Nedyalka Georgieva

PD6

A NEW ANTIOXIDANT WITH NATURAL ORIGIN CHARACTERIZED BY ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY METHODS

Antoaneta Zheleva

PD7

RADICAL SCAVENGING CAPACITY OF SEEDS AND LEAVES ETHANOL EXTRACTS OF *CYNARA SCOLYMUS* L -A COMPARATIVE STUDY

Ekaterina Georgieva

PD8

COMPARATIVE INVESTIGATION ON RADICAL SCAVENGING ACTIVITY AND PROTECTIVE PROPERTIES OF NATURAL ISOLATED AND SYNTHETIC ANTIOXIDANTS

Yanka Karamalakova

PD9

STUDY OF THE PHYTOTOXIC EFFECTS OF ATRANORIN, GYROPHORIC ACID AND PARIETIN ON CULTURES OF APOSYMBIOTICALLY GROWN PHOTOBIONT *TREBOUXIA ERICI*

Veneta Ivanova

PD10

β-CARBOLINE ALKALOID CONSTITUENTS FROM A *THERMOACTINOMYCES SP.* STRAIN, ISOLATED FROM LIVINGSTON ISLAND, ANTARCTICA

Veneta Ivanova

PD11

METHANOL: CLOROFORM EXTRACTS FROM *LAMIUM ALBUM* L. AFFECT CELL PROPERTIES OF A549 CANCER LUNG CELL LINE

Veseslina Moskova-Doumanova

PD12

AN ORGANOPLATINUM (II) COMPLEX OF *N*-3-PYRIDINYLMETHANESULFONAMIDE WITH EXPECTED CYTOSTATIC ACTIVITY. STRUCTURAL, SPECTROSCOPIC AND THERMOANALYTICAL STUDY

Nikolay Dodoff

PD13

PREPARATION OF CHITOSAN GEL BEADS FOR TRYPSIN IMMOBILIZATION

Michail Kamburov

PD14

NOVEL SYNTHETIC COMBRETASTATIN A-4 ANALOGUES WITH POTENTIAL ANTITUMOR ACTIVITY

Lyudmila Ivanova

PD15

MOLECULAR MODELING OF THE KYOTORPHIN MIMETICS

Tatyana Dzimbova

PD16

IN VITRO **ASSESSMENT OF THE CYTOTOXIC EFFECTS OF SULFOARGININE ANALOGUES AND THEIR HYDRAZIDE DERIVATIVES IN 3T3 AND HEPG2 CELLS**

Tamara Pajpanova

PD17

DEGLYCATING ENZYMES FROM EMBRYOGENIC SUSPENSION CULTURES OF DACTYLIS GLOMERATA L.

Eva Popova

ABSTRACTS OF LECTURES

L1-L21

L.1.

THE CHROMATIN OF ACTIVE GENES IS NOT IN A PERMANENTLY OPEN CONFORMATION AND IS HEAVILY LOADED WITH LINKER HISTONES

Colyn Crane-Robinson[1], Nelly Sapojnikova[2], Alexandra Trollope[1],

Alan Thorne[1], Fiona Myers[1], Dontcho Staynov[3]

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Andronikashvili Institute of Physics, Tibilisi, Georgia[2]

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A widespread view is that whereas heterochromatin, both constitutive and facultative, adopts a condensed 30 nm fibre structure, active genes are fully open in the beads-on-a-string conformation. To give the lie to this rather unrealistic concept, we used DNaseI digestion coupled to Taqman PCR to define local accessibility, i.e. with a resolution of <100 bp. At the beta globin locus in 15-day chicken embryo erythrocytes, accessibility along the active genes is the same as that within the adjacent 16 kb of constitutive heterochromatin. Only the 'traditional' hypersites (DHS) exhibited enhanced accessibility. The concept of active genes as permanently in highly extended structures must therefore be abandoned. The basis of 'General DNaseI Sensitivity' at active genes will be discussed. To define the role of linker histones in chromatin structures, antibodies to the multiple chicken subtypes were generated and used in ChIPs. At the same beta globin locus, a remarkably uniform distribution of all the subtypes was observed, broken only by gaps at the DHS. Linker histones are not therefore generalized gene repressors and a wide variety of chromatin transactions can take place in their presence.

L.2.

HOW DO THE SMART CHROMATIN REMODELING NANOMACHINES WORK?

Stefan Dimitrov[1], Manu Shubhdarshan Shukla[1], Sajad Syed[1], Fabien Montel[2], Cendrine Faivre-Moskalenko[2], Jan Bednar[3], Andrew Travers[4], Dimitar Angelov[5]

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Chromatin remodelers are sophisticated nano-machines. They are high molecular multiprotein complexes, which are able to alter histone-DNA interactions, to evict histones and to mobilize nucleosomes. Despite the considerable efforts, both the mechanism of their action and the conformation of the remodeled nucleosomes remain still elusive. We have studied the mechanism of RSC and SWI/SNF nucleosome mobilization by using high resolution microscopy and novel biochemistry techniques. Atomic Force Microscopy (AFM) analysis shows that two distinct products are generated as a result of the RSC remodeling reaction: (i) nucleosome-like non-mobilized particles, termed remosomes, which contain 180-190 bp of DNA associated with the histone octamer and, (ii) mobilized particles located at the end of DNA. Electron-Cryo Microscopy visualization reveals that individual remosomes exhibit distinct and highly irregular DNA shape. The use of the novel "In gel one pot assay" for studying in a single reaction the accessibility of nucleosomal DNA towards restriction enzymes all along its length and the DNase I footprinting demonstrate that the histone-DNA interactions within the remosomes are strongly perturbed, in particular in the vicinity of the nucleosome dyad. The data suggest a two-step mechanism of RSC nucleosome remodeling. During the first step RSC pumps 15-20 bp DNA from each one of the free DNA linkers without repositioning of the histone octamer, which results in the formation of remosomes, a multitude of stable nucleosome-like particles with highly altered histone-DNA interactions. During the second step of the reaction, RSC functions as a true translocase, by pumping and releasing DNA.

We speculate that the major *in vivo* function of RSC is the generation of remosomes.

In agreement with this we will show that base excision repair (BER) is greatly facilitated by the generation of the remosomes.

L.3.

THE ROLE OF HMGB1 PROTEIN IN CHROMATIN REMODELING AND REPAIR OF CISPLATIN-DAMAGED DNA AT NUCLEOSOME LEVEL

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The high mobility group box (HMGB) proteins are the most abundant nuclear non-histone proteins, known for their ability to recognize non typical DNA structures and to bend DNA. Those properties define HMGB-1 and -2 as architectural factors of chromatin and implicate them in numerous nuclear processes such as repair, remodeling, replication, etc. We report the first results that link HMGB1 to repair of cisplatin-treated DNA at nucleosome level. The experiments were carried out with three types of reconstituted nucleosomes strongly positioned on the damaged DNA: linker DNA containing nucleosomes (centrally and end-positioned) and core particles. The highest repair synthesis was registered with end-positioned nucleosomes, two and three times more efficient than that with centrally positioned nucleosomes and core particles, respectively. HMGB1 inhibited repair of linker DNA containing nucleosomes more efficiently than that of core particles. Just the opposite was the effect of the *in vivo* acetylated HMGB1: stronger repair inhibition was obtained with core particles. No inhibition was observed with HMGB1 lacking the acidic tail. Binding of HMGB1 proteins to different nucleosomes was also analysed. HMGB1 bound preferentially to damage nucleosomes containing linker DNA, while the binding of the acetylated protein was linker independent. We show that both the repair of cisplatin-damaged nucleosomes and its inhibition by HMGB1 are nucleosome position-dependent events which are accomplished via the acidic tail and modulated by acetylation.

The participation of HMGB-1 and -2 proteins in chromatin remodelling is also investigated. The ability of these proteins and their posttranslationally acetylated forms to affect SWI/SNF and RSC dependent nucleosome mobilization was studied. Both proteins assisted nucleosome sliding induced by the two remodelers. Following acetylation, these proteins acquire the ability to bind to core particles, a property that has not yet been documented with parental proteins. We report that compared to the non-modified proteins, acetylated HMGB-1 and -2 exhibited both stronger binding to linker DNA-containing nucleosomes and a higher co-remodeling activity. Acetylation of HMGB-1 and -2 proteins enhanced binding of SWI/SNF to the nucleosome but did not affect its ATPase activity.

L.4.

THE INFLUENCE OF THE REMODELING COMPLEX INO80 ON THE HIGHER-ORDER CHROMATIN STRUCTURES OF YEAST *SACCHAROMYCES CEREVISIAE*

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Certain evidence has been collected about the imperative role of chromatin remodeling complexes (CRCs) in the fine tuning of genome activity. One of the most abundant CRCs is INO80 which is evolutionary conserved from yeast to human. INO80 consists of several subunits, all of them playing important roles in its functioning. The actin-related protein -Arp4p, is one of these subunits. Although *ARP4* gene is essential for the yeast cells certain *arp4* mutants do exist, thus providing good opportunities for studying of INO80 roles in the higher-order building of chromatin.

Using the advantages of the yeast *S. cerevisiae* as a model system we have shown that there is a physical contact between Arp4p and the yeast linker histone Hho1p. Furthermore, we were able to observe successive number of phenotypic characteristics of the constructed double *arp4 deltahho1* cells. These include changes in chromatin loop organization and abundance, severe disruption of the "30 nm" chromatin fibre, alterations in the size and the appearance of the nucleus. Moreover, certain changes in the cellular morphology have been also detected. Strikingly, the deletion of the linker histone in *arp4* mutant genotype led to partial restoration of the wild type phenotype.

The detected straightforward connection between chromatin remodelling complexes, in particular INO80 and linker histones could be envisaged by the involvement of both factors in gene activity regulation mainly by building up and maintaining of the higher-order chromatin structures.

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L.5.

DIFFERENT ROLES OF RUVBLS AND INO80 PROTEINS IN MAMMALIAN DOUBLE STRAND BREAK REPAIR

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Chromatin modifications/remodeling are important mechanisms by which cells regulate various functions through providing accessibility to chromatin DNA. However, the underlying mechanisms by which chromatin complexes mediate repair in mammalian cells remains largely enigmatic. We studied the ways by which RUVBL proteins and Ino80 ATPase affected double strand break repair in mammalian cells. RUVBLs form a dodecameric ring structure and are present in mammalian Trrap-TIP60, INO80 and SRCAP complexes. Ino80 ATPase is the catalytic core component of mammalian INO80 chromatin remodeler. Depletion of either RUBVLs or Ino80 protein by RNA interference resulted in reduction of homologous recombination repair but the defects where due to deficiencies at different stages of the process.

RUVBL proteins reduced RAD51 recruitment to chromatin and nuclear foci formation to about 50% of that of the control, but did not affect DNA damage signalling as judged by the normal histone H2AX phosphorylation and cell cycle distribution. Treatment with the histone deacetylase inhibitor restored RAD51 foci formation in the RUVBL1-depleted cells, suggesting that RUVBL facilitate access of the repair machinery to sites of DNA damage via their participation in TRRAP-TIP60 histone acetyltransferase.

On the other hand, silencing of Ino80 protein did not impair Rad51 recruitment but impeded chromatin association of the early stage repair protein 53BP1. Further analysis by using BrdU-labelled single-stranded DNA and RPA immunofluorescent staining showed that INO80 mediates 5'-3' resection of double strand break ends.

Our data suggest that different mammalian chromatin modification and remodelling complexes are required at different stages of DSB repair.

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L.6.

OXIDATIVELY GENERATED DAMAGE TO CELLULAR DNA: FORMATION AND REPAIR

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Emphasis has been placed in the last decade on the elucidation of the main degradation pathways of DNA model compounds mediated by •OH radical and one-electron oxidation reactions as the result of indirect and direct effects of ionizing radiation, respectively. This has led to the isolation and characterization of almost 100 oxidized purine and pyrimidine nucleosides if hydroperoxide precursors and diastereomers are considered. However, far less information is currently available on the mechanisms of radiation-induced degradation of bases in cellular DNA mostly due partly to analytical difficulties. It may be reminded that the measurement of oxidized nucleosides and bases in nuclear DNA is still a challenging issue which until recently has been hampered by the use of inappropriate methods such as the GC-MS that have led to overestimated values of the lesions by factors varying between two and three orders of magnitude. At the present, when using the accurate and sensitive HPLC/MS/MS assay, 11 single modified nucleosides and bases were found to be generated in cellular DNA upon exposure to gamma rays and heavy ions. This validated several of the •OH-mediated oxidation pathways of thymine, guanine and adenine that were previously inferred from model studies. The concomitant decrease in the yields of oxidized bases with the increase in the LET of heavy ions is accounted for by the preponderance of indirect effects in the damaging action of ionizing radiation on DNA. Further evidence for the major role played by •OH radical was provided by the results of irradiation of cells with high intensity 266 nm laser pulses. Under these conditions 8-oxo-7,8-dihydroguanine is mostly produced as the result of bi-photonic ionization of DNA nucleobases and subsequent hole migration to guanine bases. It is likely that some of the oxidized bases that have been isolated as single lesions are in fact involved in clustered damage. Interestingly it was recently shown that a single oxidation hit is capable of generating complex lesions in DNA. Thus •OH-mediated abstraction at C4 of the 2-deoxyribose moiety gives rise to DNA strand cleavage together with the formation of a highly reactive aldehyde that undergoes an addition reaction to the amino group of a proximate cytosine, leading to 4 diastereomeric cycloadducts as components of likely interstrand cross-links. These adducts are slowly repaired in cellular DNA. It was also shown that the (5'*R*)-5',8-cyclo-2'deoxyadenosine, a tandem lesion, that arises from intramolecular addition of the •OH-mediated C5' radical to the C8 position of the adenine moiety is generated in DNA, however, in a low yield upon exposure of cells to gamma radiation.

L.7.

BASE EXCISION REPAIR WITHIN NUCLEOSOMAL SUBSTRATES

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Eukaryotic cells are constantly subjected to oxidative stress leading to a tremendous number of insults, which have to be efficiently repaired. In the DNA, 8-oxo-7,8-dihydroguanine (8-oxoG) is the major lesion induced upon oxidative stress, which is repaired by the Base Excision Repair (BER) pathway. BER uses a limited number of enzymes and how BER functions on naked DNA template is well characterized. 8-oxoG is recognized and removed by the 8-oxoguanine DNA glycosylase (OGG1), which exhibits both a glycosylase and an apurinic/apyrimidinic (AP) lyase activity. How BER operates on chromatinized templates is far from being clear. In general, the presence of nucleosomes interferes with BER but the DNA interacting proteins involved appeared to be differently affected by the nucleosome structure.

As a model system we have used precisely positioned dinucleosomes assembled with linker histone H1. A single 8-oxoG was inserted either in the linker or the core particle DNA within the dinucleosomal template. We found that in absence of H1 the removal of 8-oxoG from the linker DNA by OGG1 proceeds with identical efficiency as in the naked DNA. In contrast, the presence of histone H1 resulted in close to ten-fold decrease in the OGG1 efficiency in linker DNA independently of linker DNA length. At the same time, the very strongly inhibited removal of 8-oxoG within the core particle DNA remains independent on H1. Chaperone-induced uptake of H1 restored the efficiency of the glycosylase from linker DNA, but not from the octamer wrapped DNA. Finally, we show that chaperone-assisted removal of histone H1 and ATP-dependent nucleosome remodeling are both necessary and sufficient for an efficient initiation of 8-oxoG repair within nucleosomal DNA. A model for a quasi-stochastic mechanism of BER within chromatin will be discussed.

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L.8.

DYNAMICS AND INTERDEPENDENCE OF THE SUBUNITS OF THE S-PHASE CHECKPOINT COMPLEX MRC1/TOF1/ CSM3

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The basic function of the S-phase checkpoint is to prevent the cell from large single-stranded DNA generation that is easily broken and causes genome instability. The *S. cerevisiae* heterotrimeric complex Tof1/Csm3/Mrc1 is a key player in the S-phase checkpoint. When DNA synthesis is prohibited (as a result of spontaneous replication block or induced arrest), the activated complex stops the progression of DNA unwinding and stabilizes the MCM helicase to give time to the repair machinery to cope with the problem. The triple complex is also related to the subsequent restart of replication process, as well as the so called "adaptation" to a specific blocking factor action. Our study aims to evaluate the individual impact of every subunit of the complex in regard to those processes.

We examined the cellular localization and consequent DNA binding of GFP-tagged subunits of Tof1/Csm3/Mrc1, when the complex is intact and when one of the subunits is missing. By fluorescent microscopy analysis of all combinations, conclusions about the assembly, cellular localization and changes in the complex, necessary for its activation and regulation in response to replication defects can be made. We demonstrate the independence of each subunit from the other two with regard to their nuclear localization. It is also shown the necessity of Tof1 for Csm3 chromatin binding and vice versa. Our results demonstrate that Mrc1 is Tof1 and Csm3 dependent for its chromatin association, but in contrast, Mrc1 is not required for chromatin binding of Tof1 and Csm3. We also studied the nuclear behaviour of Mrc1 subunit in the process of adaptation to hydroxyurea. The results indicate that for the yeast cell to proceed further into the cell cycle, still in the presence of a blocking agent, Mrc1 dissociation from DNA is required.

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L.9.

SODIUM BUTYRATE INCREASES HELA CELLS RADIOSENSITIVITY BY SUPPRESSING DNA REPAIR

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Histone deacetylase inhibitors (HDACi) are a new class of anticancer agents targeting the epigenome. The combined administration of HDACi and DNA-damaging agents increases the effect of the latter. This allows the application of lower doses of genotoxic agents and reduces the toxic effects, which substantially ameliorates the survival and quality of life of some patients. The challenge now is to improve our understanding of the underlying mechanisms. We treated HeLa cells with HDACi sodium butyrate (NaBu) and γ-radiation, to investigate the mechanisms by which the epigenetic agent increases the effect of the genotoxic agent. The γ-radiation induces double strand breaks (DSB) in the genomic DNA. HeLa cells activate G2-phase checkpoint, which provides time to repair the damages before cell division is continued. The epigenetic agent NaBu could increase the effect of γ-radiation by two possible mechanisms: by abrogation of activated G2-phase checkpoint, allowing the cells to enter mitosis with damaged DNA or by inhibition of the DSBs repair. Using FACS analysis we determined that NaBu does not abrogate the activated G2 checkpoint. By immunofluorescence we demonstrated that in the addition of NaBu, γ-H2AX-foci indicating DSBs were present for a longer period of time. This showed that NaBu inhibits repair of DSBs. We investigated which of the two possible ways of DSB repair-homologous recombination (HR) or non-homologous end joining (NHEJ), is inhibited by NaBu. HeLa cells in G2-phase were synchronized and after γ-radiation released synchronously in G1-phase, where NHEJ was the only possible mechanism for repair of DSB. We found out retention of the repair in the presence of NaBu. To study the repair by HR, the formation and disappearance of the Rad51 foci (typical of this type of repair) was followed. These foci in the addition of NaBu were present for a longer time period in the nucleus. By Host cell reactivating assays with two different constructs containing a single DSB repairable with HR or NHEJ we confirmed the above results that NaBu inhibits the HR as well as the NHEJ. We reached the conclusion that the increased acetylation under HDACi treatment prevents the correct mechanisms of DSB repair by both NHEJ and HR. The initial histone deacetylation in the vicinity of the damage is probably necessary to hold the broken ends in close proximity to each other, facilitating the upcoming repair.

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L.10.

HAIR FOLLICLE STEM CELLS NICHE DISRUPTION IN THE MOUSE MUTANTS "BALD MILL HILL"

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The mouse *hairless* gene encodes a nuclear receptor co-repressor implicated in the control of in the epidermal differentiation and hair follicle (HF) cycling. The integrity of hairless protein (HR) is required for proliferation, migration and differentiation of keratinocyte precursors in the stratum basale and the outer root sheath (ORS) of the HF. This nuclear protein interacts with HDACs and seems to harbour motifs required for chromatin remodelling activities. HR likely affects the fate and identity of precursor cells by interfering in the WNT and VDR signalling pathways. Spontaneous and targeted mutations at the *Hr* locus are instrumental in deciphering interaction networks underlying the correct HF development and skin morphogenesis. In the mouse mutants "bald Mill Hill" (bmh), the *Hr* gene product displays abnormal cellular localisation in the epidermis and hair follicle during post-natal development. The main goal of our investigation was to identify stem cells in bmh mice and follow their fate in mutant skin and hair follicle development.

We have used these mutant mice to address gene expression and morphology in the putative stem cell reservoirs of the epidermis and HF. Our data show that disintegration of the lower part of the HF in early catagen is associated to defects in their adhesion properties and in particular to a specific loss of E-cadherin expression. In these mutants the HF is gradually transformed in an abnormal skin structure termed utricle. The utricle's epithelium appears to be gradually converted to epidermal identity. Gene gun transfection of bmh skin explants indicates that specific markers for the granular layer of the epidermis are expressed in epidermal pattern in upper and lower utricles in bmh mice. Using a panel of stem cell markers to delineate the HF bulge area, we observed a marked disruption of this putative stem cell niche in first catagen of the hair cycle.

We discuss the relevance of the hairless protein mis-localization to the specific skin phenotype and stress the impact of these data with respect to the molecular basis of the pathology in mouse hairless mutants. Our findings reveal a complex molecular network that potentially links several signalling pathways underlying hair follicle formation. These results suggest that *Hr* might control key events in the first steps of precursors proliferation and differentiation, rather than be responsible for the maintenance of stem cell identity.

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L.11.

HOW BACTERIA EAT WASTE GASES AND PRODUCE FUEL AND CHEMICALS?

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Our research interest is focused on understanding the genetics and biochemistry of microorganisms which are capable of using industrial waste gases as its sole source of carbon and energy for production of fuels and high value chemicals for industry. Thus the gas fermentation process not only results in reduction of the polluting industrial gas emissions but also enables the conversion of the waste into products with industrial importance. To date, most gas fermenting microorganisms have exhibited the ability to form acetate, formate and butyrate; ethanol and butanol were also reported as products. In this article we demonstrate for the first time that three non-pathogenic Clostridium species: *C. autoethanogenum*, *C. ljungdahlii* and *C. ragsdalei* are able to produce 2,3 butanediol utilising waste gases as the sole carbon and energy source. 2,3-butanediol is a highly valuable chemical, as it can be readily converted to key building blocks that are used in the production of hydrocarbon fuels, plastics, synthetic rubbers, polymers and textiles. The examination of the results from the high-performance liquid chromatography (HPLC) showed that a very small amount of 2,3-butanediol (1.4-2 mM) was produced during the stationary phase by all three species. This result was independently confirmed by gas chromatography-mass spectrometry (GC-MS) analysis. We also showed that all genes involved in the 2,3-butanediol pathway were highly upregulated during the stationary growth phase, when 2,3-butanediol was detected. These findings reveal that gas fermenting microorganisms possess advantages for fuel and chemical production and hold significant potential for future metabolic and genetic engineering efforts.

L.12.

INDOXYL SULFATE, A TRYPTOPHAN METABOLITE, INDUCES NEPHRO-VASCULAR CELL SENESCENCE

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Indoxyl sulfate (IS) is markedly accumulated in the serum of chronic kidney disease (CKD) patients. IS is metabolized by the liver from indole, which is generated from tryptophan in dietary proteins by intestinal flora such as *E. coli*. The oral sorbent AST-120 reduces serum levels of IS in CKD patients by adsorbing indole, a precursor of IS, in the intestine. IS is taken up by proximal tubular cells through organic anion transporters (OAT1, OAT3), and induces free radicals with impairment of cellular anti-oxidative system. IS stimulates progression of CKD by increasing renal expressions of TGF-β1. Further, IS promotes the expression of p53 by ROS-induced activation of NF-κB and p53 itself, thus accelerating senescence of proximal tubular cells with progression of CRF.

Administration of IS to hypertensive rats reduced renal expression of Klotho, and promoted cell senescence with expression of senescence-associated β-galactosidase, p16, p21, p53 and Rb accompanied by renal fibrosis.

IS induces free radicals and cell senescence in vascular smooth muscle cells and endothelial cells. IS stimulates proliferation and osteoblastic transdifferentiation of vascular smooth muscle cells, and inhibits viability and NO production of vascular endothelial cells. IS promoted aortic calcification and aortic wall thickening in hypertensive rats with expressions of osteoblast-specific proteins. IS promoted cell senescence with expression of senescence-associated β-galactosidase, p16, p21, p53 and Rb in the aorta of hypertensive rats.

Thus, IS accelerates the progression of not only CKD but also cardiovascular disease (CVD) by inducing nephro-vascular cell senescence.

L.13.

SCHIZOPHRENIA GENOME

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Schizophrenia is a severe mental disorder marked by hallucinations, delusions, cognitive deficits and apathy, with a heritability estimated at up to 80%. Copy number variants (CNVs) have been identified in individual patients with schizophrenia, but large-scale genome-wide surveys have not been done.

We performed a genome-wide association survey of CNVs in patients with schizophrenia and matched controls, using high-density microarrays.

Associations with schizophrenia were found for rare and common CNVs. Large deletions on chromosome 15q13.3 and 1q21.1 were associated with schizophrenia. CNVs in at least three loci act as strong risk factors for schizophrenia in a minority of individuals. Many common variants of small effect were also identified. We also established the contribution of the variants in the major histocompatibility complex for the molecular pathogenesis of the disorder.

In conclusion, our results provide a strong proof of different models of schizophrenia (monogenic/polygenic component) that include the effects of multiple rare structural variants across the genome or at specific loci and the role of thousands of common alleles of very small effect. Our data could explain at least one-third of the total variation in liability to schizophrenia.

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L.14.

POLYMORPHISMS OF MATRIX METALLOPROTEINASES (*MMP*) IN COPD

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COPD is a chronic disease of the lung that is characterized by decreased air flow and is associated with abnormal chronic inflammation in the airways and development of extensive tissue remodelling. Matrix metalloproteinases (MMP) comprise a family of proteolytic enzymes capable to degrade practically all components of exstracellular matrix (ECM). MMP play a key role in normal physiological processes of development, tissue remodelling and repair, as well as in various pathological conditions. Based on their substrate specificity and structural organization they are subgrouped into collagenases, stromelysins, gelatinases, matrilysins, and membrane-type matrix metalloproteinases.

The MMP activity is very strictly controlled at the level of gene transcription, latent zymogene activation, and inhibition by endogenous inhibitors. Most of MMP genes are highly polymorphic with allele-specific effects on transcriptional activity of the corresponding gene or on the enzyme activity.

In the current report we attempt to summarize the information about the role of polymorpisms of MMPs and their inhibitors in development and progression of COPD.

In addition we present our own data concerning the effect of two promoter polymorpisms: *MMP1 -1607insG* (*1G>2G*, rs1799750) and *MMP3 -1171insA (5A>6A*, rs3025058) on the risk of COPD.

The results suggest that -1607 2G allele of *MMP1* gene and -1171 6A allele of *MMP3* gene do not represent risk factors for development of COPD, however the homozygous 2G/2G genotype of *MMP1* seems to affect the lung function, especially in smokers, possibly by enhancing *MMP1* gene expression.

L.15.

QUANTITATIVE PROTEOMIC IDENTIFICATION OF HOST FACTORS INVOLVED IN THE *SALMONELLA TYPHIMURIUM* INFECTION CYCLE

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In order to identify host factors involved in *Salmonella* replication, stable isotope labelling by amino acids in cell culture (SILAC)-based quantitative proteomics was used to investigate the interactions of *Salmonella typhimurium* with the secretory pathway in human epithelial cells. Protein profiles of Golgi-enriched fractions isolated from *Salmonella typhimurium*-infected cells were compared with those of mock-infected cells, revealing significant depletion or enrichment of 105 proteins. Proteins annotated to play a role in membrane traffic were overrepresented among the depleted proteins whereas proteins annotated to the cytoskeleton showed a diverse behaviour with some proteins being enriched, others being depleted from the Golgi fraction upon *Salmonella* infection. To study the functional relevance of identified proteins in the *Salmonella* infection cycle, small interfering RNA (siRNA) experiments were performed. siRNA-mediated depletion of a selection of affected proteins identified 5 host factors involved in *Salmonella* infection. Depletion of peroxiredoxin-6 (PRDX6), isoform beta-4c of integrin beta-4 (ITGB4), isoform 1 of protein lap2 (erbin interacting protein; ERBB2IP), stomatin (STOM) or TBC domain containing protein 10b (TBC1D10B) resulted in increased *Salmonella* replication. Surprisingly, in addition to the effect on *Salmonella* replication, depletion of STOM or ITGB4 resulted in a dispersal of intracellular *Salmonella* microcolonies. It can be concluded that by using SILAC-based quantitative proteomics we were able to identify novel host cell proteins involved in the complex interplay between *Salmonella* and epithelial cells.

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L.16.

IS THERE A ROOM FOR EXPERIMENTAL PHYSICAL MODELS IN MOLECULAR BIOLOGY?

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The capabilities of the simplest model of biomembranes, Langmuir monolayers, to experimentally study in quantitative term the molecular interactions at membrane interface between membrane lipids and variety of membrane-active agents are illustrated. Successful combination of the monolayer model with surface potential measurements, axisymmetric drop shape analysis (ADSA) and Brewster Angle Microscopy (BAM) is demonstrated by studying of molecular interactions in two cases: (i) between enkephalins and membrane phospholipids and (ii) between benzalkonium chloride (BAC) and human tears, meibum, and rabbit corneal epithelium lipid extracts.

The enkephalins are pentapeptides (Tyr-Gly-Gly-Phe-Met/Leu) with a proven antinociceptive action. It is believed that the interaction between them and the lipids composing the membranes is important for conversion of the peptides into a "bioactive" conformation. The interaction of the synthetic Methionine-enkephalin (Met-enk) and its amidated derivative (Met-enk-NH_2) with monolayers of zwitterionic dimyristoylphosphatidylcholine (DMPC) and negatively charged dimyristoylphosphatidylglycerol (DMPG) were evaluated by the Langmuir's monolayer technique. The surface tension (γ, mN/m) of pure DMPC and DMPG monolayers and that after injection of the peptides under the interface as a function of time were detected. The decrease in γ values showed that there was a strong penetration effect of both types of Met-enk molecules into the lipid monolayers, being significantly stronger for the amidated derivate, Met-enk-NH_2. We suggested that the interaction between the neuropeptides and DMPC was predominantly determined by peptides amphiphilicity while the electrostatic forces played significant role for the insertion of the cationic Met-enk-NH_2 in DMPG monolayers. Our results demonstrate the potential of Langmuir monolayers as an elegant and simple membrane model to study quantitatively lipid-peptide interactions.

The interactions between BAC, a common preservative used in ophthalmic formulations, and human tears, meibum, and rabbit corneal epithelium lipid extracts at the air/water interface were examined *in vitro* during an artificial blink (compression/expansion of film area) by Langmuir monolayers, surface potential measurements and ADSA. Surface pressure-area isotherms and isocycles were used to assess the sample's lateral elasticity and capability to compress and spread during dynamic area changes. The lipid films morphology was monitored by BAM.

In the Langmuir-monolayer and ADSA experiments, the interactions between BAC and lipids or tears result in: a) impaired lipid spread and formation of discontinuous non-uniform surface layers; b) increased surface pressure-area hysteresis during compression/expansion; and c) displacement of the lipids by BAC from the surface. The effects occurred within seconds after BAC exposure and their magnitude increased with BAC concentration (kept within the clinical range of 0.005-0.02%). The above model *in vitro* approach provides molecular scale insights of the detrimental effect of BAC on tear film, which explains well the film instability and corneal epithelial barrier dysfunction after exposure to BAC in the human eye *in vivo*.

L.17.

INTERACTION OF THE TUMOR SPECIFIC PROTEIN HGAL-3 WITH ANTICANCER AGENTS

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Human galectin-3 (hGal-3) is a mammalian lectin involved in regulation of RNA splicing, apoptosis, cell differentiation, and proliferation. Multimerized extracellular hGal-3 is thought to crosslink cells by binding to glycoproteins and glycosylated cancer antigens on the cell surface or extracellular matrix.

Fluorescence spectroscopy and circular dichroism (CD) were used to study the interaction of hGal-3 with two anticancer agents: bohemine and Zn porphyrin ($ZnTPPS_4$). The dissociation constant for binding of bohemine with hGal-3 was k_D 0.23±0.05 μM. The hyperbolic titration curve indicated the presence of a single bohemine binding site.

The binding of $ZnTPPS_4$ to hGal-3 is of high affinity having k_D= 0.18-0.20 μM and is not inhibited by lactose, indicating that $ZnTPPS_4$ and carbohydrate bind different sites. CD spectra of the hGal-3 complex suggested that the binding of the hydrophobic compounds changed the hGal-3 secondary structure.

In summary, we show that two compounds with anticancer activity, bohemine and $ZnTPPS_4$, 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 have utility in the targeted delivery of drugs for cancer.

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L.18.

PROTEOMICS FOR CARDIOVASCULAR DISEASE BIOMARKERS DISCOVERY

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Cardiovascular diseases (CVD) are the number one cause of death and are projected to remain so. Mechanisms to prevent and reduce the effects of CVD have been the focus of intense research for many years. The discovery of novel proteins, diagnostic biomarkers, and potential drug targets is crucial in the development of new therapeutic agents and strategies for treatment.

The aim of this study was to investigate the participation of proteins in the progression of the CVD and to clarify the molecular mechanisms of action in sera from patients with coronary artery disease and heart failure.

Proteome analysis was conducted for sera from 293 patients with the following diagnoses: Stable Angina Pectoris- SAP (n=59), Unstable Angina Pectoris UAP (n=120), diabetes type I (n=114), hearth failure (n=20, HYHA class 2 and 3) and for sera from 131 healthy controls.

The identified proteins changed over the threshold in patients with UAP, SAP and diabetes type I participate in 6 different signal transduction pathways. Ingenuity Pathway Analysis identified 6 specific potential biomarkers for patients with UAP, 6 for patients with diabetes and 5 for SAP.

The changes of expression of the chosen biomarkers can probably determine the differences in the characteristic and progression of the disease in the studied patients.

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L.19.

STABILITY OF MUTANT HUMAN INTERFERON-GAMMA DERIVATIVES – A COMPLEX APPROACH STUDY

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The autoimmune diseases result in an overactive inappropriate response of the immune system against self-components. There is substantial evidence during the last two decades that the pathogenesis of certain autoimmune diseases such as multiple sclerosis, alopecia areata, autoimmune uveitis and myasthenia gravis is related to the abnormal production of human interferon-gamma (hIFNγ). The treatment includes application of hIFNγ antagonists (such as IFNβ) or neutralization of hIFNγ activity by anti-IFNγ antibodies. However, none of these have proven to be effective. A new approach for counteracting hIFNγ could be based on the competitive inhibition by hIFNγ analogs incapable to trigger the hIFNγ signal transduction pathway but active towards the hIFNγ receptor. To this aim the hIFNγ analogs were designed on the basis of the three-dimensional structures of IFNγ and its receptor as well as on the hIFNγ functional map. An important region for the intracellular functioning of the cytokine - the putative upstream NLS (amino acids 84-94 in α-helix E) - was selected for mutagenesis where Lys 86-88 were randomly mutated using PCR. The mutant hIFNγ genes were cloned and expressed in *E. coli*. The potential conformational changes in the structure of the obtained mutant proteins were investigated employing molecular dynamics simulations. The free energy surface of Lys 86 backbone torsion angles space in hIFNγ wild type and derivative proteins was analyzed using metadynamic model. Based on the obtained results 12 out of 100 mutants were singled out as preserving the structure of α-helix E and stability similar to that of the wild type protein. The selected recombinant proteins were investigated for biological activity and affinity to the IFNγ receptor on WISH cell culture by their capacity to compete with the native hIFNγ. High correlation between results of the molecular dynamics simulations and biological data was obtained. These hIFNγ analogs are potential candidates for treatment of autoimmune diseases based on competitive inhibition of the endogenous hIFNγ.

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L.20.

QUANTITATIVE EXPRESSION ANALYSIS OF CBF GENES IN BULGARIAN WINTER WHEAT CULTIVARS

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Cold acclimation induces the expression of cold-regulated genes needed to protect plants against freezing stress. This induction is mediated, in part by *CBF* (*C-repeat Binding Factors*) transcription factor family. Natural differences in frost tolerance in wheat have been mapped to the *FR2* (*Frost Resistance 2*) locus on 5 chromosome group and are associated with differences in threshold induction temperatures and/or transcript levels of several CBF genes.

This study compared the relative expression patterns of four *Triticum aestivum CBF* genes (*CBFA19, CBF15.2, CBFA2* and *CBFD21*) in crown tissues of two Bulgarian hexaploid winter wheat cultivars (*vrnA1/vrnB1/vrnD1/vrnB3*) with different LT tolerance - the high tolerant Milena and low tolerant Roussalka, as well as the spring cultivar (*Ch. spring*) by Real-Time qRT-PCR. The objective of the work is to expand our understanding of the molecular mechanisms underlying low temperature (LT) acclimation in Bulgarian winter wheat and to detach candidate functional markers with application in breeding programs for abiotic stress tolerance.

The transcription profiles of *TaCBF* genes showed that they are induced by cold treatment at 2°C, attained max level between the 1st and 4th hour and then declined to basal levels after 7 hours of treatment. The transcript abundance comparison revealed that *CBFA2, CBFD21,* and *CBF15.2* are expressed to higher levels in the frost tolerant cultivar with the highest level for *CBF2.* This suggests that the higher *TaCBF* expression is associated with the winter cultivar's superior FT development capacity.

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L.21.

GLYCATION AND IMMUNOGENICITY OF PROTEIN THERAPEUTICS

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The development of anti-drug antibodies in patients treated with protein therapeutics appears to be a rule rather than an exception even when therapeutics are identical to the original human proteins. The clinical manifestation of protein drug immunogenicity ranges from lack of complications to aggravated conditions, including severe anaphylaxis and autoimmune responses. To date, the reasons for the immunogenicity of protein therapeutics are not fully understood. The aim of the present study was to test the hypothesis that advanced glycation end products (AGEs) contribute to the immunogenicity of interferon-β (IFNβ) pharmaceuticals.

We have found that the level of antibodies (Abs) against human IFNβ in sera of multiple sclerosis patients treated with either IFNβ-1a (Rebif® or IFNβ-1b (Betaferon®) correlates positively with the level of Abs against AGEs. Over 50% of the anti-IFNβ Abs^+ sera have demonstrated cross-reactivity with AGEs, which implies that AGEs form immunogenic epitopes on IFNβ. Consistent with these results, we have shown that IFNβ-1a and IFNβ-1b contain the products of advanced glycation N^{ε}-(carboxymethyl) lysine (CML) and imidazolone. Immunoassay performed with anti-CML and anti-imidazolone Abs provides further evidence for the immunogenic nature of AGEs in IFNβ. Finally, using *Escherichia coli*-derived human interferon-γ as a model, we propose strategies for prevention of AGEs formation in therapeutic proteins.

Data provided in this study demonstrates that IFNβ pharmaceuticals contain advanced glycation end products that form immunogenic epitopes on the therapeutic molecule. Relevant strategies should be adopted by pharmaceutical companies for the production of AGEs-deprived IFNβ with reduced immunogenicity.

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ABSTRACTS OF POSTERS
PA1-PD16

PA1

EFFECT OF HISTONE DEACETYLASE INHIBITOR SODIUM BUTYRATE ON INTERSTRAND CROSSLINK REPAIR

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Histone acetylation has been implicated in a number of DNA damage response processes such as nucleotide excision repair (NER), DNA double-strand break repair and cell cycle checkpoint control. Modulation of these processes by histone acetylation is the mainstream approach of epigenetic cancer therapy by histone deacetylase (HDAC) inhibitors. Repair of interstrand crosslinks requires participation of NER, homologous recombination repair, Fanconi anemia proteins network and translesion synthesis. Crosslinks are highly cytotoxic, slowly repairable lesions and agents that cause them are efficient chemotherapeutics. To understand whether combination of crosslinking damage and modulation of histone acetylation may prove more efficient in cell killing than either treatment alone, we studied the effects of HDAC inhibitor sodium butyrate on DNA damage responses after treatment with the crosslinker mytomicin C. Our results showed that combined treatment reduced cell survival. The effect was not due to changes of repair rates by NER, homologous recombination or changes in the way each of the agents affected cell cycle distribution. Both mytomicin C and sodium butyrate induced phosphorylation of gamma-H2AX and apoptosis. Taken together our data indicate that the increased cytotoxicity of MMC in the presence of sodium butyrate is likely due to the strong apoptotic induction caused by the HDAC inhibitor.

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PA2

ADAPTIVE RESPONSE TO DNA-DAMAGING AGENTS - A NONSPECIFIC PHENOMENON

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Any exposure to minimal stress that causes a very low level of DNA damage can trigger a nonspecific phenomenon - adaptive response (AR) resulting in an increase of cell's/organism's resistance to higher levels of the same or other type of stress.

Here we discuss how AR depends on the genetic constitution and experimental designs. As a model different test-systems *(Chlamydomonas reinhardtii*, *Hordeum vulgare* and human lymphocytes) and chemical agents with specific mode of action have been used.

The induction of DNA damage and the magnitude of AR have been measured by CFGE and A/N comet assay.

Our data show that AR strongly depends on the level of "priming" dose and the duration of "intertreatment" time given between "priming" and following "test" dose. Optimal inter-treatment time for AR induction was found to be 2 and/or 4 h. We have defined also the level of "small" dose that can trigger an AR. There was no induction of AR when "priming" treatment did not induce at least 1.5-2 folds higher level of DNA damage compared to the control samples.

Our experiments also demonstrate that the magnitude of AR depends on the genetic constitution of cells. Surprisingly genotype resistance did not abrogate the cells' ability to further "adapt", but the magnitute of AR was measured to be smaller than that of WT or sensitive genotypes.

Acceleration of DSB rejoining was confirmed as an underlying mechanism involved in adaptive response.

Our results contribute for the enlargement of the present state of knowledge concerning the response of cells/organisms to low doses of environmental pollutants as well as to the mechanisms involved in the formation of the genetic elite of populations.

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PA3

INVESTIGATION OF THE LEVELS OF PROTEIN FACTORS INVOLVED IN THE PROCESS OF INITIATION OF DNA REPLICATION IN UV DAMAGED HUMAN CELLS

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Cells are constantly exposed to both endogenous and exogenous agents that damage DNA and threaten their genomic stability. Ultraviolet radiation (UV) as a DNA damaging agent induces cyclobutane pyrimidine dimers or pyrimidine pyrimidone (6-4) DNA lesions. These alterations elicit cellular responses that maintain the integrity of the genome by both DNA repair and by preventing cell cycle transitions. Recently it was suggested that the protein factors involved in process of the initiation of DNA replication in addition to regulating origin licensing play an important role in DNA damage response. The origin recognition complex (ORC) plays a central role in the process of organization of pre-replicative complexes and functions as a platform on which the additional initiation factors such as the cell division control protein (Cdc6) and hexameric minichromosome maintenance (Mcm) helicase complex are loaded. The Cdc6 protein is down-regulated in response to DNA damage, probably to prevent further activation of replication origins. However, there is no detailed analysis of the interrelationship between the multiple degradation pathways of Cdc6 and to the best of our knowledge there are no data about the stability of ORC proteins under conditions of DNA damage. In this study we investigated the protein levels of the initiation factors Cdc6 and Orc2, Orc3, and Orc4 in the human cell lines HeLa and HEK 293 after UV light damage. We found that UV light exposure caused reduction of the cellular levels of the initiation proteins Cdc6, Orc2 and Orc3 in HeLa cells but not in HEK 293 and triggered apoptosis in HeLa cells, while UV damage was repaired in HEK293 cells without any changes in the distribution of the cells in the cell cycle. The results suggest that the induction of the apoptotic process in HeLa cells lead to the process of degradation and cleavage of some of the investigated protein factors to ensure the inhibition of the process of initiation of DNA replication in the presence of DNA damage. As degradation of Cdc6 was inhibited by the kinase inhibitor caffeine we conclude that its degradation is regulated by the DNA damage response pathway.

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PA4

MAMMALIAN INO80 IN RECOVERY FROM REPLICATION STRESS

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Stalled replication forks arise when nucleotide levels are low, when the replication machinery encounters DNA lesions or replication slow zones.

The data about the role of the yeast INO80 complex in the recovery from replication stress is controversial: on one hand it was found necessary for the resumption of DNA synthesis following fork stall by hydroxyurea, while on the other INO80 was found dispensable for fork resumption after HU. In higher eukaryotes the role of INO80 in recovery from replication stress has not been studied at all.

We investigated the effects of Ino80 depletion in human cells on the rate of replicative DNA synthesis after replication stress induced by hydroxyurea. Our data indicate that replication recovery in Ino80 knock-down cells is strongly inhibited as judged by incorporation of labelled nucleotide precursors and decreased ssDNA formation indicated by impaired RPA focal recruitment. This effect is accompanied by increased gamma-H2AX foci formation in silenced cells showing induction of double-strand DNA breaks caused by replication fork collapse. These observations are consistent with a model in which mammalian INO80 chromatin remodeler is required to maintain integrity of stalled replication forks.

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PA5

MICROBIAL COMMUNITY DIVERSITY IN THE RHIZOSPHERE OF *ZOSTERA SP.* BEDS IN SOZOPOL BAY, SW BLACK SEA REGION

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Seagrass communities (g. *Zosteretae*) are used as valuable indicators for assessment of the ecological state of marine nearshore zones. They play an important role in the sediment's structure and function development as well. The present study aims to assess the bacterial and archaeal diversity in microbial communities in *Zostera* sp. beds and in bare sediments by using Amplified rDNA Restriction Analysis (ARDRA) and 16S rRNA gene retrieval.

Undisturbed sediment cores from *Zostera sp.* beds and bare sediments were collected along a gradient of an anthropogenic impact from a point source of municipal waste waters. Station 1, as the most remote from the source was used as a reference one, Station 2 was the one exposed to intensive wave actions. Station 3 was the closest one to the point source, and Station 4 was situated at the outer part of *Zostera* sp. meadow.

ARDRA approach was used for a rapid assessment of dominant members of bacterial communities. Significant bacterial diversity was detected in *Zostera* sp beds, as well as in bare sediments. Clear differences were observed in the restriction patterns of Station 2 samples, probably reflecting the effect of the specific matrix and environmental influences. The analyzed archaeal 16S rDNA clone libraries from the sediments revealed high diversity. Phylogenetic analysis showed that all of the retrieved 16S rDNA sequences were affiliated with the phyla *Crenarchaeota* and with environmental sequences with marine origin.

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PA6

POLYMORPHISMS IN CYTOKINE GENES IN BRONCHIAL ASTHMA

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Bronchial asthma is a common chronic lung disease that is driven by abnormal inflammatory reactions in the airways in response to the complex interaction between genetics and environmental factors. The underlying inflammation in asthma is manifested by prominent eosinophil infiltration of bronchial wall with an increased number of Th2 ($CD4^+$) lymphocytes and active mast cells. The profile of inflammatory mediators in asthma is composed mainly of bronchoconstrictor mediators, such as histamine, cysteinyl leukotrienes, kinins, and prostaglandin D_2, as well as cytokines derived from Th2 cells (IL-5 and IL-13). Key regulators also are the eosinophilic chemotactic cytokine eotaxin, IL-4 and NO, however the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 have also been found at increased amount in the sputum and BAL fluid in individuals with bronchial asthma, especially in cases with more severe state. Therefore, inflammatory mediators are considered to be of pivotal importance in pathogenesis of this condition as the protein levels of the cytokines are finely regulated by variety of factors, including genetics.

In this respect we aimed to summarize the available information about the role of polymorphisms of several cytokine genes for development and progression of Bronchial asthma.

We also present our results from the study focused on the role of promoter polymorphisms in IL-6, TNF-α and IL-1β as possible predisposing factors in development and progression of Bronchial asthma.

The results of our analyses showed no significant difference in -174G>C *IL6* and 308G>A *TNFA* genotypes and the allele distribution between controls and patients with asthma, suggesting that these polymorphisms have no effect as risk factors for Bronchial asthma. Similarly, we did not observe statistically significant differences in genotype frequencies of *IL1B* -511C>T and *IL1B* +3953C>T between controls and patients with asthma. However the minor T allele of *IL1B* -511C>T was less frequently found in controls (0.305) than in the asthmatic patients (0.415, p=0.0002), determining 1.7 fold higher risk for Bronchial asthma.

PA7

POLYMORPHIC CHARACTERISTICS AND INITIAL PCR-BASED POLYMORPHISM ANALYSES OF PLANTS FROM FIVE BULGARIAN POPULATIONS OF *HABERLEA RHODOPENSIS* FRIV.

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Haberlea rhodopensis Friv. is a Balkan endemic plant and tertiary relic with highly fragmented habitat. Its populations are isolated and/or grow at very different environmental conditions. In this respect, it is interesting from evolutionary, genomic and ecological point of view, to inspect the level of DNA polymorphism between some of these populations.

Recent studies of our research group reveal significant variations in many morphological and phenological traits between and even within *H. rhodopensis* localities. In order to detect whether and to what extent the observed variations are fixed in the *Haberlea* genomes, we performed initial molecular analyses. As a donor of plant material we used plants from the *in vitro* gene bank established at the Department of Plant physiology and Molecular biology. *In vitro* plants from five populations with significant morphology and phenology variations were used for DNA extraction and PCR-based analysis of polymorphisms in three types of sequences: (1) internal transcribed sequences (ITS); (2) partial sequences of stress-related genes, and (3) uncharacterized transcribed sequences. Initial results show various levels of polymorphism between some of the populations.

Our results show that the genomic systems of *Haberlea rhodopensis* Friv. have adaptive potential and the genome of this ancient plant is still evolves.

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PA8

INITIAL DETERMINATION OF POLYMORPHISM AND *IN VITRO* CONSERVATION OF SOME *RAMONDA SERBICA* POPULATIONS FROM ALBANIA AND BULGARIA

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The "resurrection plant" *Ramonda serbica* is included in the Albanian and Bulgarian list of endangered plants as rare, Balkanic-endemic and tertiary relic species. Our research groups have established national *in vitro* collections of *R. serbica* in Albania and Bulgaria. The aim of this joint study is (1) the choice of successful micropropagation methods as the basis for *in vitro* collections and (2) the evaluation of polymorphism of some natural populations.

In both collections, seeds were used as a convenient starting point for micropropagation in the nutrient medium JG-B. In the Bulgarian *in vitro* collection, a dry sterilization of seeds was applied for a first time for *Gesneriaceae* family. The micropropagation and conservation of *Ramonda* seeds and plantlets was similar in Albanian and Bulgarian collections. The plantlets *in vitro* as an explant material were developed in JG-B medium with different phytohormones. The method of conservation *in vitro* with minimal growth method (modification of nutrient medium) was used.

Meiosis and mitosis examinations of the natural populations and plantlets cultivated *in vitro* were carried out by standard Squash method. Cytogenetical study of natural populations showed polyploid forms as a mixture (2n=72) and (2n=96). Mitosis preparations in plantlets *in vitro* showed a predominance of the most frequent form (2n=96).

The biodiversity in interspecific level of the natural populations of *Ramonda serbica* of Central Albania and cultivated plantlets *in vitro* from Albania and Bulgaria was evaluated via molecular markers (RAPDs and ITSs). Estimation of genetic relationships was conducted using Jaccard's similarity coefficient.

The micropropagation and *in vitro* conservation method is a convenient way for the international exchange of germplasm of rare and endemic plants. From the data obtained in this study it can be concluded that RAPD markers provided a useful technique to study genetic diversity in *Ramonda serbica* populations. This technology allows the identification of different populations as well as the assessment of the genetic similarity among different populations.

PA9

GENE-SPECIFIC PCR AMPLIFICATION OF TECHNOLOGICALLY IMPORTANT LACTOCOCCAL GENES

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The selection of industrial lactococcal cultures in Bulgaria has been performed in the 1970s with many of them still on the market as starters for cheese, butter and cream. These cultures however have not been characterized with contemporary molecular methods, while for the last 30 years there has been a significant development in the studies of *Lactococcus lactis* as a model of lactic acid bacteria. To close this gap we have undertaken a study of the lactococcal cultures in the LBB bacterial collection using gene-specific PCR.

As a first step we have confirmed the species identification of lactococci by targeting the glutamate decarboxylase gene (*gadB*) obtaining the *L. lactis*-specific amplification products of 600 bp for *L. lactis* ssp. *lactis* and 560 bp for all *L. lactis* ssp. *cremoris* strains. PCR amplification of the genes for membrane proteinase (*prtP*), citrate permease (*citP*) and nisin structural genes (*nisA* and *nisZ*) was used to select strains which grow rapidly in milk, ferment citrate or produce nisin, respectively. For all strains for which amplification products were obtained, the *prtP* $^{+}$, *citP* $^{+}$ *nisA/Z* $^{+}$ phenotype was confirmed using differentiating microbiological media. Additionally the *citP* $^{+}$ genotype was always associated with strains previously identified as *L. lactis* ssp. *lactis* biovar. *diacetylactis* - an important aroma forming variety of dairy lactococci. Hybridization experiments determined the localization of the *citP* gene on a 8.2 kbp plasmid in biovar. *diacetylactis* strains. Furthermore potentially lysogenic lactococcal cultures which carry in their genome the gene of prophage integrase (*int*) were outlined within the group of tested strains. Nearly one third of the strains tested positively for integrase, suggesting a wide distribution of potentially lysogenic lactococci. The thermal induction of prophage activity was demonstrated for the *int* $^{+}$ strain *L. lactis* ssp. *lactis* C1/6.

The gene-specific PCR for the detection of the presence of technologically important lactococcal genes permits a rapid and cost effective selection process for strains with high potential for industrial application.

PA10

THE FTO RS9939609, ADIPOQ RS1501299, RS822391 AND ADIPOR2 RS16928662 POLYMORPHISMS RELATIONSHIP TO OBESITY AND METABOLIC SYNDROME IN BULGARIAN SAMPLES

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Obesity is an established risk factor for the development of metabolic syndrome (MetS). In recent years genome-wide association (GWA) studies have generated evidence that associates genetic variations at multiple regions of the genome with quantitative traits reflecting obesity. Variations in the fat-mass and obesity-associated gene (FTO) are associated with the obese phenotype in many Caucasian populations. There are controversial data for adiponectin (ADIPOQ) and adiponectin receptor (ADIPOR2) genes and their contribution to the risk of high BMI and metabolic syndrome.

The present study aims to investigate allele frequencies of four SNPs and the relationship between certain SNPs in the FTO, ADIPOQ and ADIPOR2 genes in relation to obesity in a Bulgarian population.

The study included 164 Bulgarian volunteers at or older than18 years of age. Patients with secondary obesity and obesity related hereditary disorders were not included, as well as patients with medication-induced obesity. Obesity-associated indices (BMI, levels of plasma glucose, triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL), blood pressure (systolic/diastolic) and waist and hip circumferences) were measured. Genomic DNA isolated from whole blood was genotyped for four different SNPs (rs9939609 in FTO; rs822391, rs 1501299 in ADIPOQ and rs16928662 in ADIPOR2 genes) by 5'-exonuclease assay using Real-time PCR 7500.

This is the first study of a Bulgarian population for genotype distribution and allele frequencies of SNPs in the FTO, ADIPOQ and ADIPOR2 genes. Significant association between the risk allele SNP carriers for the FTO and ADIPOQ genes and obesity and increased risk of MetS was not established for the Bulgarian cohort. The only TT carrier for ADIPOR2 gene had significantly higher BMI, waist/hip ratio, TAG and total cholesterol, as compared to GG and GT carriers - a distribution corresponding to data from other (GWA) studies.

A direct linkage between obesity and the studied SNP genotypes in Bulgarian population has not been established at present, possibly due to the small sample size. Studying interactions with environment (metabolic flexibility) could be more predictive of obesity-related diseases than the causative relationship between gene variations and MetS.

PA11

METHOD FOR DEVELOPMENT OF STRAIN-SPECIFIC MARKERS FOR BIFIDOBACTERIA

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Several health-promoting effects are attributed to *Bifidobacteria* as a major part of the normal intestinal microbiota. Because the probiotic properties are strain-specific, the use of reliable and discriminative molecular methods is very important.

The goal of the present work is to present a new method for development of strain-specific DNA markers, as well as AFLP genotyping for *Bifidobacteria* with satisfactory discriminative power and reproducibility.

AFLP genotyping is based on restriction cleavage of DNA with enzyme couple *Xho I* and *Taq I*, specially designed adapters, preselective and selective PCR primers. AFLP derived ampliphicants were successfully used as a source of strain-specific markers towards one probiotic *Bifidobacterium longum* strain, and subsequently, based on their specific sequences - for design of strain-specific probe. Its specificity was confirmed upon 70 *Bifidobacteria* strains. The strain specific markers and primers were successfully applied for quantitative detection of the same strain by help of real-time PCR in faeces of volunteers after consumption of probiotic products.

The developed AFLP for Bifidobacteria proved to be at least as discriminative as PFGE. The approach for design of strain-specific markers would be used to confirm the presence and quantify certain strains in complex bacterial communities.

PA12

STRUCTURAL ORGANIZATION OF IGS AND LEVEL OF METHYLATION OF RIBOSOMAL RNA GENES IN RECONSTRUCTED BARLEY KARYOTYPES (*HORDEUM VULGARE* L.)

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The main objective of this study was to analyze the molecular structure of the rDNA intergenic spacer (IGS), and to investigate the methylation pattern of rRNA genes in reconstructed barley karyotypes (*Hordeum vulgare* L.) with altered position or structure of the two nucleolus organizers (NORs).

The analysis was carried out using different NOR-reconstructed karyotypes T-1586 (control) (Gecheff, 1978), T-35 (NOR6H-deletion karyotype) (Gecheff et al., 1994), T-21 (NOR6H split into two approximately equal parts, the terminal of which is transferred to the short arm of the chromosome 5H) (Gecheff, 1989). Restriction enzyme analysis was conducted using two restriction enzymes (Eco RI and Eco RV), followed by molecular hybridization with different ^{32}P-labelled rDNA probes (plasmids R10, R1.8, R3.2 and R3.8). The structure of IGS was determined based on the database sequences of the 9.8 kb (GenBank HQ825319) (L. Karagyozov, personal communication) and 8.8 kb (GenBank AF1475501) rDNA repeat units in barley. Results showed that the 5' end of IGS of rRNA genes in both NOR5H and NOR6H consists of 7 complete or partial copies of 79 bp-lenght. In downstream direction a set of complete or partial subrepeats of 116 bp-length was found, (20 in NOR6H and 6 in NOR5H). The position of the promoter was determined by analogy with the published nucleotide sequence of IGS in wheat (GenBank X07841). The methylation pattern of rRNA genes in reconstructed barley karyotypes was determined after restriction with Eco RI and consistent digestion with methylation sensitive Msp I, Hpa II or Hha I restriction enzymes. The length of the resulting DNA fragments was determined by the method of "indirect end labelling" using 0.7 kb EcoRI-TaqI rDNA fragment as rDNA probe. Hypomethylated sites were found in the 79 bp and 116 bp subrepeats and in the external transcribed spacer (ETS) in the both NORs of the karyotypes analyzed.

Our results clearly showed that the compensatory effect in the expression of rRNA genes in T-35 observed earlier (Dimitrova et al., 2008) was accompanied by a very strong hypomethylation of the rRNA genes in the only remaining NOR5H.

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PA13

GENOMIC DISTRIBUTION OF AC-LIKE TRANSPOSABLE SEQUENCES IN SPHAEROCOCCUM - TYPE MUTANT FORMS OF *TRITICIM AESTIVUM* L. AND TRITICALE

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DNA sequences homologous to the maize Activator (Ac) element are widespread in plant genomes. Nowadays, several reports are available concerning the distribution and characterization of Ac-homologous sequences in natural populations of different cereal species but these elements still remain to be comprehensively characterized. In this respect, there is a particular lack of information about the dynamics of Ac-homologous sequences in mutant germpalsm background.

The present study was aimed at characterization of the distribution and dynamics of Ac-homologous sequences in wheat and Triticale sphaerococcum mutant forms. They display specific characteristics typical for the influence of transposable elements such as continuous variability in the phenotype expression with reversions to the wild aestivum phenotype and high level of chromosomal rearrangements. The pattern of the DNA methylation as a major epigenetic factor responsible for the regulation of Ac transposon activity in plants was also investigated.

EMS-induced sphaerococcum-type mutant forms show genome instability namely revealed by structural and functional dynamics of Ac-like transposable sequences. These sequences share different level of homology to the original maize Ac9 element, which is locus specific and is due to their structural divergence. For example, Ac-like sequences corresponding to the central (exon) part of the Ac transposase show lower level of homology and structural diversity in comparison to the exon-intron boundary regions. In addition, it has been shown that the copy number of Ac-like transposable sequences correlates with the ploidy level and genome size of plant genomes. Such findings support the fact that transposable elements contribute to genome size fluctuation and structural variation within species. The observed alterations in the PCR generated genomic pattern enabled us to clearly distinguish different types of mutant forms which is also in agreement with their origin and phenotypical characteristics. This fact highlights the promising potential of DNA transposons for more thorough fingerprinting in cereals and assessment of the impact of abiotic stress factors on genome stability. In addition, DNA hybridization analysis has revealed modulation in the methylation status of Ac-like transposable sequences between the control and mutant form of Triticale.

The obtained data favor the assumption that initial EMS treatment has modulated the wheat genome stability by affecting the behavior of Ac transposon homologous sequences in *T. aestivum* and Triticale sphaerococcum mutant forms. Insertion polymorphism and enhanced transcriptional activity of the most abundant BARE-1/WIS 2 retrotransposons recently was also attributed to these mutants (Bonchev et al. 2010), which supports the notion that modulation of transposable elements dynamics appears as a distinguishing mark of EMS-provoked genome instability in wheat.

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PA14

DNA ANALYSIS OF YEAST ECOSYSTEM OF SELECTED BULGARIAN FOOD PRODUCTS

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The production and maintenance of good quality food products contribute to the quality of life. Yeasts and food are intimately related since the early days of human civilization. The contribution of yeast to the food products can be either beneficial or detrimental. In recent years, the interest in food yeast ecosystem has increased considerably. Little knowledge is available on yeast ecosystem of various Bulgarian food products. Among the traditional Bulgarian food products are yogurt, boza and cheese.

The aim of the present study was to explore yeast ecosystem of some traditional Bulgarian food products using molecular analysis of ribosomal DNA.

Yeast strains were isolated by spreading of aliquots of food samples on Petri Dishes with YM agar, pH-3.4 . Total genomic DNA was isolated using modified Bolano method. Partial 26S ribosomal DNA was amplified and sequenced using primer pair NL1/NL4. Phylogenetic affiliations of isolated yeast strains were determined through BLAST similarity search analysis.

In total 20 strains of yeast were isolated from various food products. DNA analysis has demonstrated that the yeasts affiliated with such genera as *Saccharomyces, Kazachstania, Kluyveromyces, Rhodotorula, Pichia* and *Candida.*

Our study have demonstrated occurrence of various yeasts in selected Bulgarian food products. Most of yeast strains identified in our analysis considered not to constitute a risk for healthy individuals.

PA15

COMMUNITY STRUCTURE OF YEASTS AND ACTINOMYCETES IN SELECTED ANIMAL FACES

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Yeasts and actinomycetes represent an important source of biologically active compounds. They are used extensively in biotechnology for production of antibiotics and enzymes.

In the present work we studied diversity of yeasts and actnomycetes in the fecal pellets of selected animals. Extracellular enzymatic activities of isolated yeasts and actinomycetes were analyzed. Additionally, the ability to grow at various temperatures of isolated strains was studied.

Ribosomal DNA was amplified using colony PCR using specific primers. Extracellular enzymatic activities of the isolated strains were tested qualitatively on selective agar media.

In total thirty yeasts and ten actinimycete strains were isolated from animal feeses**.** Molecular analysis have shown that majority of yeasts strains belong to ascomycete genera such as *Kluyveromyces, Clavispora, Pichia, Wickerhamomyces, Candida, Zygowilliopsis, Galactomyces, Hanseniaspora,* and *Debaryomyces*. Few yeasts belong to basidiomycete genera such as *Trichosporon, Cryptococcus, Rhodotorula and Guehomyces* All isolated actinimycete strains were identified as *Thermoactinomyces saccharii.* The analysis of extarcellular enzymatic activities has shown that two yeast strains posses amylolitic activity, six strains have proteolitic activity and fourteen strains have lipolitic activity. All yeast strains were able to grow at 37º C. Four starins demonstrated ability to grow at 42º C. Two strains were able to grow at 45º C.

Thermoactinomyces saccharii has shown strong proteolitic activity. The optimal growth temperature of *Thermoactinomyces sacchari* was shown to be 55° C, with maximum and minimum growth temperatures 60° C and 40° C, correspondingly.

Animal feeses represent rich source of thermostable yeasts and actinomycetes with important biotechnological properties.

PA16

GENOTYPING OF ENDEMIC FOR RHODOPY MOUNTAIN SHORTHORN RHODOPEAN COW BREED

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There are only two cow breeds endemic for Bulgaria. One of them is Shorthorn rhodopean cattle. Therefore it is of high significance to reveal the genotype profile of that cow population which represents the aim of the present study.

Thirty eight animals of that breed were genotyped for three lactoprotein genes (kappa-casein, alfaS1-casein and beta-lactoglobulin) through PCR-RFLP analysis. The results for kappa-casein locus show slight superiority of heterozygous AB genotype and prevalence of B allele. With regards to beta-lactoglobulin gene, dominant is AB genotype as well but allelic frequencies are equal. Analysis of polymorphism of alfaS1-casein gene displays that about 53% of the animals are with homozygous BB genotype, about 34% - with heterozygous BC genotype and only approximately 13% (5 representatives) - with homozygous CC genotype. In the last gene case there is about triple prevalence of B allele above the C allele.

It may be concluded that Shorthorn rhodopean cow breed is with specific genotype profile with respect of all investigated genes. That profile is different than the one described for other cattle breeds in Bulgaria.

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PA17

S-MOTIFS AS A NEW APPROACH TO SECONDARY STRUCTURE PREDICTION: COMPARISON WITH STATE OF THE ART METHODS

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The development of protein structure prediction still has not reached the point from which there can be only small improvements in the quality of the results. However, the methods for secondary structure prediction are much closer to this leveling point, than the much more complex field of tertiary (3D) structure prediction. Here we present a novel approach to the simpler of the two problems: the assignment of secondary structure elements to a sequence of amino acid residues.

The average quality of the results from state-of-the-art methods is slowly increasing past 70% correctly predicted residues. In theory the maximal average quality for this particular problem is around 80%. Our new method gives a significant improvement of quality over most known methods, with an average of correct predictions around 75%. The method breaks known protein molecules into pairs of secondary structure elements, called S-motifs, which are used to infer the probable structure of the query sequence. While it does not use multiple sequence alignment directly, and does not include complex mathematical models like the artificial neural networks (ANN) that are otherwise commonly used, it still offers reliable prediction in most cases. This, and the novel S-motif approach it uses, make the method a viable part of any consensus prediction methods that may be developed in the future.

PA18

IN SILICO SOLUTION FOR MERGING OF PHENOTYPIC ONTOLOGIES

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The phenotypic ontologies of various species typically contain thousand of terms and relations between them but often the semantics employed in them is enormous in scale. The major problem with using such ontologies is that they lack tools for: cross-species text (literature) searches as well as for providing conditions to design of new experiments with other species.

This is where the process of merging phenotypic ontologies comes into use. To merge two or more phenotypic (anatomical source) ontologies basically means: 1. to draw cross-species edges between the DAGs representing their ontologies (DAG – directed acyclic graph); 2. to merge nodes and edges from the two source ontologies and to promote them to a more general model (again an ontology) thus generating as a result a new ontology (super-ontology or target ontology). The only requirement for the super-ontology is that it *makes sense* from the points of view of both source (input) species-specific ontologies.

For solving that task an intelligent software system for merging two (or more) source anatomical ontologies into a generic super-ontology has to be designed and implemented. The working title of our software system is *OntoMerger* (abbreviation from "Ontologies Merger") and its core module is based on two novel models designed to bridge the gap between the two source ontologies and an original automation procedure proposed by the authors for merging the source anatomical ontologies into a more general super-ontology.

Additional modules of the software system include but may not be limited to:

• Communications module interrogating (querying) external structured anatomical knowledge sources like UMLS, FMA, WordNet, GO (Gene Ontology).

• Visualization module allowing the user to easily navigate through the super-ontology as well as visualize its links to the two (or more) source ontologies.

• Searching (mining) module providing capabilities for performing intelligent text searches or text mining into various external unstructured (natural language based) knowledge resources (scientific literature, the web) using the richness of the generated super-ontology model. The results of those searches is the main merit brought by *OntoMerger* and aims to help researchers in finding scientific results already published by others which may help them extrapolating the results obtained by their own experiments or help them design new experiments to perform on their own.

PB1

STUDY OF ANTICORROSION EFFECT OF EXOPOLYSACCHARIDES PRODUCED BY *LACTOBACILLUS DELBRUECKII B5* CULTIVATED ON DIFFERENT CARBOHYDRATES

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It has been proven that the strain Lactobacillus delbrueckii B5 synthesizes exopolysaccharides in the presence of different amounts of sucrose (2 and 4%) and mixture 2% sucrose and 4% maltose. The obtained information was used in a study on the anticorrosive properties of exopolysaccharides synthesized by the latter strain. The study on the corrosive stability of steel samples was conducted according to the gravimetrique method. The rate of corrosion, the degree of protection, and coefficient of protection ware calculated. Microscope pictures of the treated steel samples confirmed the corrosive activity.

PB2

IN SILICO AND BIOCHEMICAL ANALYSES OF SUPEROXIDE DISMUTASE AND CATALASE ENZYMES IN CD - ACCUMULATING YEASTS

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A comprehensive analysis of the complete genome sequences coding the key antioxidant enzymes - superoxide dismutase (SOD) and catalase (CAT) of the Cd-accumulating yeasts *Candida glabrata*, *Schizosaccharomyces pombe* and *Pichia pastoris* was performed. This study was conducted in order to reveal different adaptive strategies providing cellular robustness and adaptability during production of Cd-nanoparticles.

Cd-containing nanoparticles may cause toxic effects on the yeast cells via reactive oxygen species (ROS), which mediate oxidative stress. The putative orthologs of *S. cerevisiae* superoxide dismutase and catalase genes, responsible for the detoxification of ROS in the cell, were found and analyzed. In addition, the electrophoretic patterns of the enzymes were also investigated. The obtained bioinformatic results showed that the corresponding genes were conservative (approxiamtely 68% identity for *SOD1*; approximately 53% identity for *SOD2*; and approximately 57% identity for *CAT1*) in all tested Cd - accumulating yeast species. Nevertheless, the biochemical data display some differences. In *S. pombe* the number of the observed electrophoretic enzyme bands coincided with the identified two genes, encoding Cu/Zn and Mn SOD, respectively. In contrast, in *C. glabrata*, although only two SOD genes were established - *SOD1* and *SOD2*, electrophoretically three protein bands, one corresponding to Cu/Zn SOD and two to Mn SOD activity, were observed. A similar phenomenon was found in *P. pastoris*, where besides one Cu/Zn SOD, three Mn SOD isoenzymes were visualized, encoded by a single gene. The comparative analysis of the bioinformatic data and the results of the electrophoretic studies confirmed the existence of only one catalase enzyme in the three studied yeast species, encoded by a single gene in the corresponding genome.

In silico analysis of the intracellular distribution of the antioxidant enzymes was also performed. It was confirmed that Mn SOD enzymes possess typical N-terminal signal sequences for mitochondrial localization with Gavel cleaving sites at positions 31, 30 and 34, for *C. glabrata*, *S. pombe* and *P. pastoris*, respectively. Furthermore, a PTS2 - RAIFKSTQ sequence at position 6 was observed in the Mn SOD of *C. glabrata*, while such was not found in the other studied yeast strains. For Cu/Zn SOD enzymes it was revealed that all of them can be located not only in the cytosol but probably also in mitochondria, since the three protein sequences possess Gavel cleaving sites at position 19. *In silico* data about the intracellular distribution of catalase enzymes in *P. pastoris* and *C. glabrata* showed that both proteins successfully can be targeted to cellular peroxisomes because they have typical PTS1 signals in the C-terminus of their protein sequences. The analysis of the amino acid sequence of *S. pombe* catalase revealed that the enzyme is exclusively localized in the cytoplasm.

The conducted biochemical and *in silico* studies demonstrate a stable and coordinated antioxidant enzymatic defense against oxygen radicals, generated in each cell compartment, preventing them from ROS damage.

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PB3

COMPUTATIONAL RNA/PROTEIN STRUCTURE PREDICTION AND FOLDING

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RNA and protein molecules provide a large variety of biological functions within cells including information transfer, enzymatic catalysis and regulation of cellular processes. Understanding how these molecules fold is important to define their functions in the cellular context. Significant advances in theory and experiments have resulted in a conceptual framework which describes the folding mechanisms of proteins and RNA molecules. In this review, we outline general principles that govern RNA and protein structure prediction, as well as comparison of folding behavior of proteins and RNAs such as - the denatured state, initiation of folding pathway, folding rates and major folding events.

PB4

BIOLOGICAL SEQUENCE COMPARISON, MOLECULAR EVOLUTION AND PHYLOGENETICS

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DNA sequence data unites all organisms into the fold of comparative analyses allowing reconstruction of their evolutionary histories even if they enormously differ in morphology and lifestyle. But while nucleotide sequences are universal their tempo and mode of evolution is not.

For closely related sequences there is a single optimal alignment which provides an accurate measure of similarity, structure, function and evolutionary history. However, with increasing evolutionary distances between nucleotide sequences the single optimal alignment method is replaced by an ensemble of alignments of almost equal quality and ensemble of different self-folded conformations.

Thus, recurring difficulties associated with diverged sequence data include alternative alignment possibilities of insertions and deletions, regions with length variations in which homology assessment is questionable or impossible, occurrence of localized excessive mutations to the point of saturation and loss of phylogenetic signals. Therefore, for diverged sequences optimizing similarity will not necessarily improve structure, function and evolutionary history assessments.

Here our aim is to represent an overview of sequence alignment methods from computational, biological, and statistical perspectives.

Although the search for globally optimal similarity alignment is an ongoing process, the sequence alignment method diverged in its alignment objectives in a few major directions: 1) structure predictions; 2) database searching; 3) sequence comparison; and 4) phylogenetics.

The goal of structure prediction is to deduce the 2D and 3D structure of the gene product from a given gene sequence. The goal of alignment for database searching is to maximize the distinction between the homologous and non-homologous sequences. The major role of alignment for sequence comparison is to find out conserved sequence features (for example functional sites). Finally, the goal of alignment for phylogeny is to align residues only if they have descended from common ancestral residue.

Here we examine the exhaustive schemes, which are classically formulated as dynamic programming algorithms. They consist either of optimization schemes which find the best alignment for a given model, or of probabilistic schemes based on partition functions - in which all alignments, with their respective weights, are evaluated.

It is now evident that DNA sequence evolution is far more complex than previously supposed. It cannot be treated as an arbitrary string of characters, rather than as a macromolecule with specific biological constraints. The molecular structure and function may influence sequence evolution by generating sequence conservation or mutational hot spots of both nucleotide substitution and insertion/deletion events. Therefore, sequence alignment should model molecular processes that have led to the observed sequence variation rather than similarity-based patterns.

PB5

VESICLES WITH TUBULAR PROTRUSIONS IN SYMMETRICAL AND NON SYMMETRICAL CONDITIONS

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Experimental study of the morphology of StearoylOleoylPhosphoCholine (SOPC) vesicles with long tubular protrusions (tethers) connected to them in symmetrical and nonsymmetrical isoosmolar and non isoosmolar environment was performed. It is shown that in case of adding a solution with different osmolarity to the outer for the membrane medium the vesicle with tether changes its shape to a vesicle with chain structure of small spherical formations. This shape transformation is reversible with time. After around 40 minutes equilibrium is achieved and the object of interest acquires its initial form. It was experimentally shown that such morphological changes can also be observed with the change of the temperature. The bending elastic modulus of the lipid membrane of vesicles, containing tethers is measured using the method of thermally induced shape fluctuations and is compared to that of vesicles with the same lipid composition without tubular protrusions.

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PB6

GLYCOLIPID CONTENT OF THE NUCLEAR MEMBRANE IN HYPOXIC RAT BRAIN

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The present investigation was undertaken to evaluate the level of glycolipids in rat brain nuclear subcellular fraction following hypoxia.

Male Wistar rats at the age of three months were subjected to sodium nitrite-induced hypoxia. Sodium nitrite was administered intravenously at 20 mg/kg body weight.

In the nuclear subcellular fraction of hypoxic brains we found increased levels of total glycolipids (5.2-fold, $p<0.001$), gangliosides (4.1-fold, $p<0.001$), and cerebrosides (5.9-fold, $p<0.001$). Gangliosides and cerebrosides accounted for 32% (0.892±0.08 mg/g dry lipid residue/ml) and for 68% (1.903±0.04 mg/g/ml) of the total glycolipids, respectively. The high content of glycolipids and especially gangliosides can apparently be explained by their neuroprotective effect. It is supposed that gangliosides can acutely reduce the extent of central nervous system injury by protection of membrane structure and function. Considering gangliosides as neuroprotectors, these changes may be interpreted as a defensive and compensatory mechanism against hypoxic damage.

Probably the high content of cerebrosides makes the membrane steadier because cerebrosides contribute to a dense network of H-bonding between three hydroxy groups of cholesterol, the hydroxy group of the sphingosine, the hydroxy groups of the acyl chains and the amide bond of the sphingolipids.

Our data provide evidence that sodium nitrite-induced hypoxia influences glycolipid metabolism in rat brain nuclear subcellular fraction. They also show that nuclei respond to hypoxia by synthesizing a high amount of cerebrosides and gangliosides and this is probably involved in the cell survival pathways.

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PB7

EFFECT OF LINSEED DIETARY SUPPLEMENTATION ON FREE FATTY ACID CONTENT OF RAT BRAIN SYNAPTOSOMAL MEMBRANE

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The aim of the present investigation was to establish the changes of free fatty acids (FFA) in rat brain synaptosomal subcellular fraction in linseed-supplemented diet.

Male Wistar rats at the age of three months were used in the experiment. Animals were fed a standard chow diet supplemented with linseed at a dose of 3g/day for three weeks.

Feeding linseed resulted in significant increase of total FFA (2.7-fold, from 6.449±0.1 to 17.621±0.07 mg/g dry lipid residue/ml, $p<0.001$) and all individual FFA in the rat brain synaptosomes. The most notable effect was observed for linoleic acid, whose concentration increased 2.8-fold ($p<0.001$). Linoleic acid, linolenic acid, arachidonic acid and docosahexaenoic acid had the largest percentage of the total FFA and their estimated concentrations were 2.241±0.02mg/g/ml, 3.173±0.03 mg/g/ml, 6.236±0.01 mg/g/ml, and 3.339±0.02 mg/g/ml, respectively. Studies in the literature consider concentrations of arachidonic acid in animal tissues as a parameter of linoleic acid desaturation. Besides the FFA pool size, the composition of the FFA pool was also modified by linseed supplementation. The latter was comprised of mono- and polyunsaturated FFA, some of which were absent in controls ($C_{16:1}$, $C_{18:1}$, $C_{18:3}$, $C_{20:2}$, $C_{22:6}$). These findings indicate that the FFA pattern resembles those of linseed regarding the high content of linoleic and linolenic acids.

Alterations in the FFA pool composition of the synaptosomal fraction were observed in response to linseed dietary supplementation. There was a tendency to synthesize high amounts of long-chain PUFA which indicates that both ratios n-3 to n-6 PUFA and PUFA to saturated FFA can be modulated by dietary intake. This would be beneficial for further nutritional implications.

PB8

SURFACE ELECTRICAL EFFECTS OF THE PHOSPHOLIPASE A_2 SUBCOMPONENT OF THE NEUROTOXIN VIPOXIN ON HUMAN ERYTHROCYTES

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Snake venoms are one of the most complex mixtures of proteins exhibiting a variety of pharmacological effects such as neurotoxicity, myotoxicity, cardiotoxicity, platelet aggregation, hemolysis, anticoagulation and others. The main and most toxic component isolated from the venom of Bulgarian *Vipera amm. Meriodionalis* is the neurotoxin Vipoxin - a heterodimeric postsynaptic ionic complex composed of two protein subunits - a basic and strongly toxic His48 $sPLA_2$ enzyme (secretory phosphatide sn-2 acylhydrolase, Phospholipase A2, EC 3.1.1.4) and an acidic, enzymatically inactive and nontoxic component. Both subunits have the same polypeptide length (122 amino acids) and are closely related sharing 62% sequence identity.

$sPLA_2$ superfamily of enzymes catalyze specifically the hydrolysis of the 2-acylester bond of 1,2-diacyl-3-sn-phosohoglycerides in a calcium-dependent manner, releasing fatty acids and lisophospholipids. We have demonstrated previously that PLA_2 subcomponent of the neurotoxin vipoxin exhibits also hemolytic and anticoagulant properties.

Erythrocytes are an intermediate between artificial membranes and biological systems. The blood cells bear a stable electric charge determined by their surface structure and by the chemical composition of their environment ($I=0.21945\ M^{-1}$). Here, we have presented data about the influence of $sPLA_2$ on the surface electric charge on the human erythrocytes, which decreases compared to that of the native membranes. The mechanism of the topography modification of the surface charge upon the incorporation of Phospholipase A_2 involves as well structural dislocations in the cytoskeleton that influence the distribution of the surface residues on the membrane.

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PB9

NATIVE AND RECOMBINANT FATTY ACID BINDING PROTEIN 3 FROM *FASCIOLA HEPATICA* AS A POTENTIAL ANTIGEN

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Lipid-binding proteins are members of widely distributed protein family. They take part in metabolism of different lipophilic ligands and also have a role in physiological activity, metabolism and disposition of essential hydrophobic compounds (fatty acids, phospholipids, eicosanoids, retinoids, etc.) which are important molecules involved in several cellular processes including gene transcription, immune responses, etc.

Fatty acid binding proteins (FABPs) are mostly cytosolic but some members are excretory-secretory (E/S) proteins. There is a lot of data that helminthic FABPs from E/S products cause antigenic and allergenic reactions.

The aim of present study is to make a detailed investigation of native and preformed recombinant FABP3 as a potential antigen for vaccine creation.

The native FABP3 from *Fasciola hepatica* was purified and the recombinant one was expressed in *E. coli*. Their fatty acid binding activity has been determined using the fluorescent fatty acid analogue DAUDA and [^{14}C]-palmitic acid. Polyclonal antibody was produced against recombinant FABP3 and it was tested on the native one.

The obtained results give the opportunity to investigate the effectiveness of the native and recombinant FABP3 as an immunotherapeutic agent and if it could be used for immunoprophylaxis.

PB10

COBALT(II)-INDUCED CHANGES IN HEMOGLOBIN CONTENT AND IRON CONCENTRATION IN MICE FROM DIFFERENT AGE GROUPS

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Cobalt (Co) and its compounds are shown to improve haematological parameters. Data suggest a relationship between plasma cobalt and iron concentrations. The aim of the present work is to study the effect of water-soluble cobalt(II) compounds ($CoCl_2$ and Co-EDTA) on haemoglobin content and iron concentration in mice from different age groups.

Pregnant balb/c mice in late gestation were subjected to cobalt chloride ($CoCl_2$.6H2O) or Co-EDTA treatment at daily doses of 75 mg/kg or 125 mg/kg until day 90 of the newborn pups. Cobalt(II) compounds were obtained from drinking tap water. The newborn pups were sacrificed on days 18, 25, 30, 45, 60 and 90 which correspond to different stages of development. Plasma samples were used for measuring cobalt(II), haemoglobin (Hb) and iron (Fe) concentration.

Long-term treatment with Co(II) increased haemoglobin content in a dose- and time-dependent manner in mature mice (day 45 to day 90) while it was reduced in immature mice (day 18 to day 30). Higher Hb was measured in samples treated with $CoCl_2$ compared to those treated with Co-EDTA. Plasma Fe concentration was significantly higher in samples treated with Co-EDTA compared to those exposed to $CoCl_2$. Lower concentrations were measured only in mature animals. Co(II) concentration increased in a dose-dependent manner. More Co(II) was measured in samples treated with $CoCl_2$ except for day 60 mice possibly due to the stability of the complex Co-EDTA. Surprisingly, mature mice had less Co(II) in their plasma compared to day 30 mice. Strong correlation between plasma Co(II) and iron concentration was found in samples of mice treated with Co-EDTA. Co(II) concentration showed inverse correlation with haemoglobin in mice treated with low dose Co-EDTA. Such relationship was found for day 45 and day 60 mice exposed to high dose $CoCl_2$.

The effect of chronic exposure to cobalt(II) depends on the type of compound used, dose, time duration as well as on the age of the experimental animals. Immature mice are more sensitive to Co(II) treatment and show signs of anaemia. Cobalt(II) has a significant impact on haemoglobin biosynthesis possibly due to its effect on iron metabolism.

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PB11

AUTOANTIGENICITY OF HUMAN C1Q IS BASED ON CONFORMATIONAL TRANSITION LEADING TO INCREASED HYDROPHOBICITY OF ITS GLOBULAR HEAD FRAGMENT

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Human C1q as the first subcomponent of the classical complement pathway takes part in maintenance of homeostasis and normal function of the immune system. Apart from its physiological role C1q is involved in pathological conditions which are associated with repetitive generation of anti-C1q autoantibodies. Such autoantibodies have been found in patients with systemic lupus erythematosus (SLE), mixed connective tissue disease, Felty's syndrome, rheumatoid vasculitis, polyarteitis nodosa, polychondritis, Sjögren's syndrome, different types of glomerulonephritis, HIV, as well as in healthy individuals. The timing and the events that turn a protective molecule like C1q into a target for the self immune response are unknown. C1q is thought to undergo a conformational transition exposing neo-epitopes that renders the molecule antigenic. This conformational transition is supposed to be due to immobilization of C1q on a target surface. This assumption is supported by the fact that anti-C1q autoantibodies do not bind fluid phase C1q.

We addressed the issue of C1q autoantigenicity by analysing its structural features and functional activity affected by the amphiphilic polyzwitterion (PZ) poly(ethylene oxide-b-*N*,*N*-dimethyl(methacryloyloxyethyl) ammonium propanesulfonate).

We analysed the intrinsic protein fluorescence of C1q interacting with PZ by fluorescence spectroscopy. Fluorescence spectrum of C1q was dominated by fluorophores in a polar microenvironment (em. max at 345 nm). Upon binding of increasing amounts of PZ to C1q the emission maximum position of Trp fluorescence indicated that the fluorophores tended to burry in the hydrophobic core of the protein. Non-linear regression analysis of the experimental data revealed one class of hydrophobic binding site in C1q. The observed changes in fluorescence intensity and emission maximum positions suggested that the interaction with ZP induced a series of conformational transitions in C1q. The fluorescence spectra of the recombinant analogues of the globular fragments of A, B and C chains of C1q molecule (ghA, ghB and ghC, respectively) revealed one class of hydrophobic binding site and conformational transitions in ghB.

We analysed by ELISA the effect of PZ, present during the immobilization of C1q, ghA, ghB and ghC, on their recognition by anti-C1q autoantibodies, purified from patients' sera with Lupus Nephritis (LN). The results showed that the conformational changes in C1q, induced by 25 mM and 50 mM PZ, resulted in increased recognition of C1q and ghB by the anti-C1q autoantibodies.

In conclusion our data suggests that the increased hydrophobicity of C1q and ghB due the conformational changes induced by PZ underlies the increased binding of the LN anti-C1q autoantibodies.

PB12

IMPACT OF GLYCATION INHIBITORS ON THE BIOLOGIC ACTIVITY OF RECOMBINANT HUMAN INTERFERON-GAMMA

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The process of glycation includes spontaneous attachment of reducing sugars to proteins and DNA. The final result of this interaction is the formation of advanced glycation end products (AGEs), which can affect the structure and function of the target molecules. We have recently demonstrated that glycation occurs in bacteria and affects both bacterial and recombinant proteins including recombinant human interferon-gamma (rhIFN-γ). As a result of glycation, rhIFN-γ undergoes chemical and conformational alterations affecting the therapeutic properties of the protein. The clinical use of rhIFN-γ is limited by the fact that the structural alterations cause reduction of rhIFN-γ biologic activity. The aim of this study was to find approaches for overcoming rhIFN-γ glycation in order to obtain a stable protein with sustained biologic activity.

In our study, we included seven compounds which affect different steps of the glycation process. The inhibitors were added as supplements to the fermentation media, where recombinant bacteria were grown. The effect of the glycation inhibitors was evaluated by monitoring the change in the antiviral activity of rhIFN-γ over time. We have examined the following glycation inhibitors - aspirin, vitamin B_1, aminoguanidine, arginine, pyridoxine, pyridoxal 5'-phosphate and pyridoxamine. Aspirin proved to be the best inhibitor of rhIFN-γ glycation. When rhIFN-γ was isolated from cells grown in 0.1 mM aspirin it demonstrated high biologic acitivity (~$2x10^6$ IU/mg) over three months of storage in solution at 20°C. Noteworthy, control rhIFN-γ isolated from cells grown in the absence of glycation inhibitors showed two orders of magnitude lower biological activity.

The use of glycation inhibitors appears a promising tool for the prevention of glycation of recombinant proteins in host bacteria. In turn, this is expected to result in the production of therapeutic proteins with improved quality in terms of efficacy and safety.

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PB13

HMGB1 PROTEIN AS A CHAPERONE: THE ROLE OF THE POSTSYNTHETIC ACETYLATION

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The High Mobility Group Box (HMGB) chromosomal proteins are well known for their properties as architectural factors: they facilitate the assembly of site-specific DNA binding proteins to their cognate binding sites within chromatin. HMGB1 binds to the minor groove of the DNA, causing a local bending and untwisting of the double helix. It has binding preference for altered DNA structures, like bends, bulges caused by UV, the anticancer drug cis-Pt and four-way junctions. Although HMGB1 binds naked DNA with low affinity and no sequence specificity, it interacts specifically with DNA at the entry into the nucleosome. Those findings suggest the involvement of HMGB1 in the formation of the nucleosome particle and motivated us to study its "chaperone" function. The essential role of the post-synthetic acetylation of HMGB1 for its properties was shown in our laboratory. This modification increases the binding affinity of the protein to some distorted DNA structures and stimulates DNA end-joining. In this study acetylated and parental forms of HMGB1 were compared relative to their influence into a process of nucleosome reconstitution. For the core histones, several chaperones have been identified so far, with nucleoplasmin being the first one. Almost any molecule that could shield the basic charge of histone proteins from DNA, including pectin, RNA, polyglutamic acid and even salt, was found to function as a histone chaperone *in vitro* with little or no relevance for the assembly of chromatin *in vivo*. We used DNA fragment which contained strong nucleosome positioning wild type sequence at the middle for the nucleosome reconstitution. We applied two methods: stepwise dialysis and direct reconstitution. For both cases a stimulation effect on the reconstitution process with the native form of the protein was observed. The post-synthetic acetylation of HMGB1 strongly reduced the "chaperone" effect. The successive incubation of HMGB1 with the histone core and DNA and vice versa clearly showed that the mechanism of stimulation was based on HMGB1/DNA interaction. This fact suggests that HMGB1 acts as a chaperone on the level of the DNA bending.

PB14

FIRST REPORTED NATURAL HISTIDINE TAILED PROTEIN FAMILY (PCHTP FAMILY)

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Protein poly-histidine regions are often associated with metal binding affinity for different divalent cations. These metal binding sites may consist of either consecutive histidine residues or histidine residues in combination with one to three other amino acids in between. In a variety of poly-histidine region containing proteins this motif is situated in the middle of the polypeptide chain and it is often associated with a metal binding function. On the other hand, polyhistidine-tags are often used for metal affinity purification of chimeric proteins. The most common polyhistidine motifs are composed of six histidine residues which are added at the C-terminal or N-terminal of the protein of interest.

Present investigation reports a novel type of nematode specific family of poly-cysteine and histidine-tailed proteins (PCHTP). Its first member is Ts-PCHTP and it was identified in parasitic nematode *Trichinella spiralis*. Histidine motif located at the C-terminus is useful for Ni-affinity purification of both native and recombinant protein. On the basis of Ts-PCHTP we composed *in silico* full hypothetical protein sequences from different nematode nucleotide database fragments. Alignment of these sequences showed high homology in the poly-cysteine domains and histidine tail. The sequence analysis showed no homology with other proteins. We suggested that the encoded Trichinella and Trichuris proteins constitute a novel PCHTP protein family, specific for the Order Trichocephalida. It may be phylogenetically related to Caenorabdites similar proteins, typical for all nematodes. Members of that family are all putative secreted or extracellular proteins.

Up to date only a few natural histidine tailed proteins were reported. However, all of these proteins are from prokaryotes and described as hypothetical or predicted in the genomic databases. Considering that fact we suggest PCHTP as the first reported family that comprises natural histidine tagged proteins.

PB15

MASS-SPECTROMETRIC IDENTIFICATION OF STRESS-RELATED PROTEINS IN GROWTH MEDIUM OF SALT-TREATED SUSPENSION CULTURES OF *DACTYLIS GLOMERATA* L.

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Proteomics is the large-scale functional analysis of proteins extracted from intact organisms, tissues or cell compartment. Recently plant proteomics has evolved as an important field with a large impact on plant biology, signalling, reproduction and stress physiology. Although proteomic studies are often associated with two-dimensional protein gel electrophoresis the recent development of the methodology in mass-spectrometry allows new, less complex approaches including shotgun proteomics and SDS PAGE-based shotgun proteomics.

The aim of the present study was to identify proteins, potentially related to salt stress response and somatic embryogenesis. Suspension cultures of *Dactylis glomerata* L. of the highly embryogenic genotype Embriogen-P were treated with 0.085 M NaCl. At this salt concentration the formation of somatic embryos was shown to be enhanced. At the end of the cultivation period proteins from the growth medium of control and salt-treated embryoids were separated on SDS PAGE. Selected bands were digested with trypsin and subjected to liquid chromatography - tandem mass spectrometry (LC-MS/MS). The resulting spectra were analysed by SEQUEST software, FASTA algorithm in monocot database as the genome of *Dactylis glomerata* was not sequenced up to date. Furthermore samples were subjected to *de novo* sequencing by mass spectrometry.

According to the LC-MS/MS results similarity with a total of 20 proteins from other monocot species was found. Results were further confirmed and clarified by the *de novo* sequencing. About 15 of them are up or down-regulated in salt-treated compared to control cultures and therefore could be stress-related. As salt stress induces the expression of proteins involved in the process of somatic embryogenesis it could be proposed, that some of the proteins identified are also somatic embryogenesis related.

In conclusion the simplified, SDS PAGE - LC-MS/MS approach proved to be useful for large scale protein identification in complex protein mixtures. Although there are very few database entries for proteins of *Dactylis glomerata* the high similarity with proteins from other monocot species allow identification of putative stress and somatic embryogenesis-related proteins.

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PC1

SUBCELLULAR LOCALIZATION OF HMGB1 AND ITS RECEPTOR RAGE IN NORMAL AND MALIGNANT TUMOUR TISSUE

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High Mobility Box 1 protein (HMGB1) is a chromatin associated nuclear protein found in almost all eukaryotic cells. It is known as an important architectural factor that facilitates the assembly of site-specific DNA binding proteins to their cognate binding sites within chromatin. Thus, it has been implicated in transcriptional regulation, DNA repair and recombination. Beyond this nuclear role, HMGB1 has been shown to be released passively by necrotic cells, and actively by macrophages/monocytes in response to inflammatory stimuli. There are several findings linking HMGB1 protein to cancer progression. Elevated expression of HMGB1 occurred in some primary tumours and in most cases HMGB1 was associated with invasion and metastasis. The main signalling pathway is accomplished through the interaction of HMGB1 with its Receptor for Advanced Glycation End products (RAGE). A few data suggest that not always the elevated expression of RAGE and HMGB1 is a prerequisite for bad prognosis of tumour development but its cellular localization is also important.

We have studied different samples from primary hepatocellular tumour tissues and liver metastases. In primary tumour cells RAGE is located predominantly in cytoplasm while in the metastatic cells it is mainly on the cell membrane. We also compared the level of HMGB1 and RAGE protein synthesis in normal rat organs and tumour tissue. We found out that in normal tissue the proteins are in their soluble form whereas in the tumour tissue they are predominantly in the membrane fractions.

Within all cases we detected increasing amounts of HMGB1 and RAGE in the tumour samples. We suggest that there is a correlation between membrane localization of RAGE and HMGB1 and cell tumour potential.

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PC2

CORRELATION OF THE HMGB1 PROTEIN ENDOGENOUS LEVEL IN HUMAN CANCER CELL LINES WITH THEIR ABILITY TO REPAIR DNA DAMAGED WITH THE ANTICANCER DRUG *CISPLATIN*

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Cisplatin is a well-known anticancer drug with high efficacy against various types of human cancers. However, the efficiency of the drug highly depends on cancer type and on variety of cellular factors that modulate its potential. The cis-Pt exerts its anti-tumour effect by forming DNA adducts and if these are not repaired, cell apoptosis is induced. Therefore the repair capacity of the tumour cell lines is essential for successful chemotherapy. The DNA lesions are target sites for damage recognition proteins such as HMGB1 which makes this protein an important factor that may modulate the activity of the drug.

In order to investigate the link between DNA repair capacity and HMGB1 we studied the ability of several cell lines: breast (MCF-7), prostate (PC-3), hepatocellular (HepG2), cervical (HeLa), and two nonsmall cell lung cancers (A549 and H1299) and human embryonic kidney cells (HEK 293), to repair *cisplatin* damaged DNA using Host-Cell Reactivation assay (HCR). The extent of repairs of the DNA adducts was compared with the endogenous expression of HMGB1 protein. The latter was determined by Western blot analyses using specific fluorophore anti-HMGB1 antibody.

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PC3

EXPRESSION OF IGF-1R $_{M}$RNA IN COLORECTAL CARCINOMA PATIENTS

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The IGF-1R signalling pathway can positively regulate cell-cycle progression and thus, play a critical role in cancer development. Although recent studies provide sufficient evidence supporting the functional importance of IGF-1R in cancer, the prognostic significance of IGF-1R expression levels to colorectal cancer (CRC) remain elusive. Expression of IGF-1R mRNA was examined in paired samples of CRC and adjacent normal mucosa, as well as in CRC patients' venous blood. We also investigated the effect of two monocytes stimulus - C3bgp and LPS on induced mRNA of IGF-1R from normal and colorectal human monocytes. The role of a member of MAPK signal transduction pathways - JNK in *IGF-1R* expression was assessed. The expression of IGF-1R mRNA was measured by relative RT-PCR. The results demonstrated that expression of IGF-1R mRNA in venous blood from CRC was down-regulated compared to venous blood from healthy donors (1.426 vs. 0.645; p=0.024). Mean IGF-1R mRNA level was found to be approximately fivefold higher in tumour tissue compared to adjacent normal mucosa (22 vs. 3.8; p=0.02). Strong IGF-1R mRNA expression was found for early stages of CRC. The results show that both stimuli used strongly up-regulated mRNA expression for IGF-1R in CRC monocytes, then in monocytes from healthy donors. The highest level of *IGF-1R* expression was detected after C3bgp stimulation, regardless of JNK inhibitor presence. The significance for increasing expression of *IGF-1R* was observed for early CRC when patients were divided according to the tumour stage. We conclude that down-regulation of mRNA expression of IGF-1R in venous blood and its up-regulation in tumour tissue and stimulated CRC monocytes from advanced stages reveal the role that IGF-1R plays in tumour progression, and might be used as prognostic factor.

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PC4

ANTIGENIC CHARACTERISTICS OF RECOMBINANT NUCLEOCAPSID PROTEINS OF THE LASSA AND MARBURG VIRUSES

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The Marburg (*Filoviridae*) and Lassa (*Arenaviridae*) viruses belong to especially dangerous group of infectious agents with high case fatality rates. Methods for specific treatment and preventive maintenance of these infections are not developed. Timely diagnosis of disease, isolation of the patient and symptomatic treatment is of crucial importance.

For the purpose of development of diagnostic test systems we studied the antigenic structure of the nucleocapsid proteins (NP) of these viruses. The fragments of 341 bp from Marburg virus, strain Voege, and 1306 bp for Lassa virus, strain Josiach, were cloned into pJC40 plasmid and expressed into *E. Coli* BL21 (DE3) strain. The fragments included the main antigenic sites of the NP of these viruses.

Both of the recombinant polypeptides are able to bind specific antibodies from human serum (Table 1). This presents an opportunity for their usage as antigenic components of immunoassay diagnostic test kits.

TABLE 1

Estimation of the antigenic specificity of the recombinant peptides from Lassa and Marburg viruses

Human serum with antibodies to virus:	**Optical density in ELISA**	
	recombinant peptide from the Lassa virus	recombinant peptide from the Marburg virus
Marburg	0.134±0.015	**1.101±0.0165**
Lassa	**1.018±0.004**	0.137±0.0075
Crimean-Congo hemorrhagic Fever Virus	0.173±0.0065	0.154±0.009
Hemorrhagic Fever with Renal Syndrome Virus	0.162±0.034	0.158±0.011
Dengue virus	0.119±0.0015	0.12±0.007
K-	0.15±0.0105	0.175±0.024

PC5

STUDIES ON THE SEROPREVALENCE OF FIVE HPV GENOTYPES IN BULGARIAN HIGH-RISK GROUP BY USING NEWLY DEVELOPED RECOMBINANT HPV VLPS

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Infection with Human papillomavirus (HPV) causes cervical cancer and premalignant dysplasia. HPV serology may provide an important epidemiological tool for the assay of past and present HPV infections and for prediction of HPV-associated cancers or premalignancies. The current study, used newly developed HPV virus-like particles (VLP) to investigate the seroprevalence of three oncogenic HPV genotypes (HPV-16, HPV-18, HPV-33) and two low-risk HPV genotypes (HPV-6, HPV-11) in healthy individuals and a study group of patients attending the Dermato-Venerological (DV) Centre in Sofia.

Recombinant HPV L1 proteins self-assembled to VLPs were used in an indirect enzyme-linked immunosorbent assay (ELISA) to identify IgG antibodies in serum specimens collected from patients attending the DV center (n=400), the control group of age-matched healthy individuals (n=101) and the control group of healthy children aged 0-13 years (n=200). Recombinant HPV L1 proteins were expressed in yeast *S. cerevisiae* and purified by a density gradient ultracentrifugation. The self-assembly of HPV L1 proteins to VLPs was confirmed by electron microscopy. To increase the specificity of the serologic assay, the VLPs were immobilized on heparin attached to the microtiter plate. The relevance of HPV type-specific ELISA was evaluated by testing serum specimens collected from vaccinated women (n=5). The ELISA test revealed the differences in the levels of HPV type-specific IgG antibodies in the study group of patients attending the DV centre as compared to the control groups.

Recombinant yeast-expressed HPV L1-based VLPs represent a useful tool for serologic investigation of the HPV infection.

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PC6

THE USE OF RECOMBINANT VIRUS-LIKE PARTICLES HARBOURING INSERTED TARGET ANTIGEN TO GENERATE ANTIBODIES AGAINST CELLULAR MARKER $P16^{INK4A}$

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Protein engineering provides an opportunity to generate new immunogens with desired features. Previously, we have demonstrated that major capsid protein VP1-derived virus-like particles (VLPs) from hamster polyomavirus are highly immunogenic. In the current study, we aim to design and characterize mosaic VLPs consisting of an intact VP1 protein and VP2 protein fused with the target antigen (the cellular marker $p16^{INK4A}$) at its C terminus.

Both proteins were co-expressed in yeast *S. cerevisiae* and self-assembled to mosaic VLPs harbouring the inserted target antigen on the surface. The mosaic VLPs were used for immunization of mice and generation of monoclonal antibodies against $p16^{INK4A}$, a cell cycle regulation protein that might be used as a specific marker for human papillomavirus (HPV)-transformed cells. The mosaic VLPs induced a strong immune response against the target antigen bound to VP2 protein in immunized mice. The specificity of polyclonal antisera raised against mosaic VLPs was proven by an immunohistochemistry of cervical tissue specimens. The antisera showed specific immunostaining of malignant cervical tissue. Spleen cells of the immunized mice were used to generate monoclonal antibodies against $p16^{INK4A}$ protein. The specificity of monoclonal antibodies was proven both by Western blot using recombinant full-length $p16^{INK4A}$ protein expressed in *E. coli* and immunostaining of HPV-tranformed cells.

The current study demonstrates the potential of mosaic VLPs with inserted target antigen as a new type of immunogens, for generation of antibodies of high diagnostic value.

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PC7

INCLUSION BODIES OBTAINED FROM *E. COLI* CELLS EXPRESSING HUMAN INTERFERON-GAMMA CONTAIN NUCLEIC ACIDS

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Recombinant human interferon-gamma (hIFNγ) aggregates in *Escherichia coli* cells in the form of insoluble protein particles called *inclusion bodies* (IBs). The latter are typical for many eukaryotic proteins expressed in bacteria. It is known that besides foreign (eukaryotic) proteins, IBs contain also different host proteins. To our knowledge, no literature data are presently available indicating that IBs contain nucleic acids. The aim of this study is to carry out adequate analysis in order to prove whether or not IBs isolated from *E. coli* cells expressing hIFNγ contain nucleic acids that may affect the protein aggregation.

To this end *E. coli* LE392 cells were transformed with the expression plasmid pP1R9-hIFNγ carrying a synthetic hIFNγ gene. Under optimal growth conditions the yield of hIFNγ reached 30% of the total bacterial protein. The cells were disrupted by ultrasonication and IBs were isolated by centrifugation. The latter were purified by sucrose density gradient centrifugation and extensively washed until no living *E. coli* cells were found in the precipitates. Protein content of the final IBs was analysed by polyacrylamide gel electrophoresis and (hypothetical) nucleic acids (DNA and RNA) were isolated by TRI®zol. Agarose gel electrophoresis of the isolates undoubtedly showed the presence of both DNA and RNA. The latter were further identified by UV spectroscopy, specific (DNase and RNase) enzymatic digestion and fluorescent microscopic observation of DAPI stained samples. In model experiments we proved that adding total RNA to samples containing hIFNγ enhanced the aggregation of the protein.

Based on these data we hypothesize that the nucleic acids are active participants in the aggregation of hIFNγ in the form of IBs.

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PC8

STABILIZING EFFECT OF SUCROSE, DEXTRAN AND HYDROXYETHYL STARCH ON RECOMBINANT HUMAN INTERFERON-GAMMA

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Recombinant DNA technology is a common approach for manufacturing of biologically active proteins for medical purposes. A plethora of evidence, however, indicates that recombinant proteins undergo structural changes during purification and storage, which can jeopardize their biologic activity and safety. Therefore, one of the most challenging tasks in the development of protein pharmaceuticals is how to overcome the physical and chemical instability of the recombinant proteins. Human interferon (hIFNγ) is endowed with multiple biological activities, including antiviral, antiproliferative, immunomodulating, etc., thus rendering hIFNγ of great potential for clinical application. A disadvantage of the recombinant hIFN (rhIFNγ) is its instability related with the protein aggregation, covalent multimerization and non-enzymatic glycosylation. Protein aggregates are known to have reduced or no biologic activity.

In this study we investigate the effect of some carbohydrates (sucrose, dextran and hydroxyethyl starch) on rhIFNγ stability. To this end rhIFNγ was purified in two-step chromatography in the presence or absence of sucrose. The purified rhIFNγ was supplemented with either dextran or hydroxyethyl starch, and stored at 20°C. Its structural changes were analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and its biological (antiproliferative) activity was determined by a kynurenine bioassay.

We found that the three carbohydrate compounds substantially reduced the aggregation of rhIFNγ during both isolation and storage conditions. Furthermore, they also inhibited the rhIFNγ glycation, which was explained by favouring its native state thus, preventing the potential glycation sites from modifications.

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PC9

INHIBITION OF GLYCATION BY SELECTED NATURAL AND PHARMACEUTICAL SUBSTANCES

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Non-enzymatic glycosylation (glycation) is a spontaneous process starting with formation of Shiff bases between reducing sugars and reactive amino groups in proteins, nucleic acids, etc. The Shiff bases are further converted to Amadori products and finally transformed into advanced glycated end products (AGEs). The formation of AGEs is an irreversible process, causing structural and functional changes in the proteins and therefore it is related with various complications in diabetes patients like nephropathy, retinopathy, neuropathy and angiopathy. This study aims to find inhibitors of glycation amongst the group of some harmless natural and officially registered pharmaceutical products. The group of natural products included alanine, arginin, lysine, glycine, adenosine, thymidine, thiamine, pyridoxine, glucosamine and resveratrol; and the pharmaceutical substances were represented by isoniazid, aminoguanidine, acyclovir and amiloride.

Inhibitory effect of the selected substances was studied in an *in vitro* system consisting of bovine serum albumin (BSA) or histone H1 (substrates) and glucose-6-phosphate (glycating agent) incubated at neutral pH at 37^{o}C for 1, 3, 12 and 17 days. The content of AGEs in BSA was determined by measuring the fluorescence at λ_{ex}=370 nm and λ_{em}=436 nm.

The amino-compounds thymidine, thiamine, aminoguanidine, isoniazid, amiloride and resveratrol showed a detectable inhibitory effect on glycation, which was better expressed with the resveratrol. The latter demonstrated also a synergistic anti-glycated activity with glucosamine and aminoguanidine. Surprisingly, the two basic amino acids lysine and arginine showed enhancing rather than inhibitory effect on glycation.

The polyphenolic compound and popular antioxidant resveratrol (native of wine) is the most potent inhibitor of glycation amongst the fourteen chemical compounds tested and therefore it is a promising candidate for developing drugs for diseases associated with glycation.

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PC10

HIGHER TNF-ALPHA PRODUCTION DETECTED IN MONOCYTES FROM COLORECTAL CANCER PATIENTS

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Many studies have shown that the progression of colorectal cancer is associated with suppression of host cell-mediated immunity and of cytokines that control this branch of immune defence, including TNF-alpha. Other studies have reported an increase of serum levels of TNF-alpha in patients with colorectal cancer. This study was conducted to elucidate some of these discrepancies. We examined the ability of monocytes from patients with colorectal cancer to produce TNF-alpha after stimulation and the involvement of JNK signal transduction pathway in TNF-alpha synthesis. Purification of monocytes was done by plastic adherence of PBMC. Monocytes were stimulated with LPS or C3bgp. The involvement of JNK MAP kinase in TNF-alpha secretion was evaluated by using selective JNK inhibitor SP600125. The quantity determination of TNF-alpha was performed by ELISA in culture supernatants for 24 h. Monocytes from patients with colorectal cancer produced significantly higher level of TNF-alpha in response to LPS stimulation in comparison with monocytes isolated from healthy donors (1926±690 pg/ml vs. 1465±771 pg/ml; p=0.034). Moreover, we observed that LPS-stimulated TNF-alpha production increased in stage - dependent manner; because monocytes from patients with advanced cancer secreted significantly more TNF-alpha than monocytes from patients in early stage of the disease (2298±127 pg/ml vs. 1687±780 pg/ml; p=0.041). Inhibition of JNK MAPK led to significant suppression of TNF-alpha production in both healthy individuals and patients with colorectal cancer. However, even after inhibition of JNK, monocytes from patients produced a greater quantity of TNF-alpha compared with monocytes from healthy donors. We conclude that monocytes from patients with colorectal cancer are prone to produce higher levels of TNF-alpha after stimulation and this production is disease stage dependent.

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PC11

INVESTIGATION OF IL-6 EFFECTS ON SP-A EXPRESSION IN A549 LUNG CELL LINE

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Pulmonary surfactant reduces surface tension at the alveolar air-liquid interface, thereby preventing lung collapse. Surfactant protein A (SP-A) is the most abundant surfactant protein. It binds to alveolar macrophages. SP-A also stimulates chemotaxis of alveolar macrophages, and serves as an opsonin in the phagocytosis.

Low SP-A levels are associated with increased inflammation in the lung. In the absence of infection SP-A inhibited interleukin-6 (IL-6) production. In macrophages infected with *M. tuberculosis*, SP-A augmented IL-6 production. In humans, SP-A levels are altered by a number of disease states. In some of them there is increased levels of SP-A in the bronchoalveolar lavage fluid.

The aim of this study was to investigate presence/absence of negative feedback between increased levels of IL-6 and production of SP-A from A549 lung cell line. The line originated from pneumocytes type II. These cells are main producers of pulmonary surfactant and show increased phagocytotic activities.

Our preliminary results show that incubation of A549 cells with IL-6 for different time changes the expression levels of SP-A.

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PC12

MOLECULAR CLONING, EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF EXTRACELLULAR A-AMYLASE FROM EMBRYOGENIC SUSPENSION CULTURES OF *DACTYLIS GLOMERATA* L.

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Somatic embryogenesis is the developmental process by which somatic cells, under suitable induction conditions, undergo restructuring through the embryogenic pathway to generate embryogenic cells. These cells then go through a series of morphological and biochemical changes that result in the formation of a somatic embryo and the generation of new plants. The transition of somatic cells into cells that are capable of forming an embryo is the most intriguing and also the part of somatic embryogenesis that is least understood. Somatic embryogenesis in cell suspension cultures is a good model system for investigating early plant development. A complex array of molecules, mainly derived from the cell walls, is found in the conditioned medium which modulate either positively or negatively embryo development.

In search of early markers for somatic embryogenesis an extracellular 48 kDa protein from the primary cell wall and the culture medium of microcluster cells from *D. glomerata* L. was identified as an acidic α-amylase. using MALDI-TOF-MS. 5' RACE/3' RACE methods were employed to clone its full-length cDNA sequence and it showed to contain a 1536-bp open reading frame encoding a 427 aa protein with a signal peptide (24 residues), indicating that this protein is synthesized as a preprotein and secreted outside the cells. The protein was designated *D. glomerata* α-amylase, isoform 1 (*Dg*Amy1). *Dg*Amy1 was cloned into the expression vector pET20b(+) (Novagen) and expressed in *E. coli* BL21(DE3) with C-terminal His-tag. The soluble recombinant protein was purified using an affinity nickel resin column. Activity assays showed that the protein was a functionally active α-amylase.

A monoclonal antibody raised against the recombinant protein labelled the entire surface of microcluster cells as well as the region of cell-to-cell adhesion. It is assumed that the extracellular proteins are indispensable for differentiation and morphogenesis taking part in signal transduction, cell-cell recognition, cell expansion and adhesion. The plant cell wall is largely a dynamic structure and the extracellular α-amylase could modify some of its structural components during development. Research is currently underway to determine the crystal structure of *Dg*Amy1 and to elucidate its possible role for somatic embryo development.

PC13

OPPOSITE MODELS OF EXPRESSION OF ANDROGEN RECEPTOR (AR) AND RETINOIC ACID RECEPTOR A (RAR-A) IN THE ONSET OF MALE GERM CELL DEVELOPMENT IN HORMONALLY MANIPULATED RATS

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Vitamin A together with testosterone (T) and FSH play an essential role in regulating spermatogenesis. Androgens and Vitamin A act through specific nuclear receptors: AR, retinoic acid receptors (RAR-α, β, γ). The present study aimed to investigate the relationship between expression of RARα and AR in the initiation of spermatogenesis during puberty in conditions of neonatal hormonal manipulation.

An experimental model of neonatal treatment with diethylstilbestrol (DES), GnRH-antagonist, and T-therapy was used. Immunostaining for RARα and AR, and 121-point counting were applied. Absolute nuclear volume (ANV) was estimated for Sertoli (SC), germ cells (GC) and their subtypes. At onset of puberty (d18) over expression of RARα was evident in SCs after DES-10 but not GnRHa treatment. Co-administration T+DES restored the normal pattern of weak immunoreactivity. An opposite mode of expression was seen for AR - loss of expression after DES but not GnRHa application and recovery by T-therapy. Similar reduction was found for spermatogonial ANV in both groups, whereas ANV of spermatocytes (Sc) was reduced in greater extent by DES (10x) than GnRHa (6x). Preleptotene (Pl), leptotene (L) and zygotene (Z) stages were also more affected by DES than GnRHa. Sertoli cell support toward spermatocytes (Sc/SC ratio) was more affected by DES (4x decrease) than GnRHa (2x) and the same tendency was found for Pl/SC and L-Z/SC.

In conclusion, data suggest possible interplay between retinoid and androgen signalling in Sertoli cells in the differentiation of male germ cells. The anti-androgenic effect of estrogens on meiotic germ cells is probably mediated by augmentation of RAR α expression.

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PC14

INTEGRATION OF LANGERHANS-LIKE CELLS INTO A HUMAN SKIN EQUIVALENT

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Langerhans cells (LCs) are immature dendritic cells residing suprabasally within the epidermis with intracellular Birbeck granules and cell surface receptor langerin (CD207) as typical markers. Studies regarding cellular interaction between Langerhans cells and other skin cells are somehow hampered by the difficult cultivation of these cells *in vitro*. The human CD34+ MUTZ-3 cell line, derived from an acute myeloid leukaemia, can be differentiated into Langerhans-like cells in the presence of a cytokine cocktail including GM-CSF, TGF-β1 and TNF-α. As differentiation markers served the expression of langerin, CD1a, CCR6, Birbeck granules and the ability to antigen internalization.

The aim of this study was to integrate differentiated MUTZ-3 cells (MUTZ-3-LCs) into a three-dimensional full-thickness skin model consisting of primary human keratinocytes and fibroblasts. On top of fibroblast-containing collagen matrix (Henkel AG & Co. KGaA, Düsseldorf) a mixture of keratinocytes and MUTZ-3-LCs were seeded and cultured for 24 hours. Subsequently, the models were lifted up to the air-liquid interface. Histological evaluation featured a fully stratified epidermis with all characteristic epidermal strata. Langerin-positive cells were detected suprabasally within the epidermis indicating that keratinocytes and/or fibroblasts provide environmental conditions for long-term maintenance of MUTZ-3-LCs.

These skin models provide a relevant research tool to study LC biology *in vitro*. Particularly, the interactions between LCs and other skin cells and their contribution in cutaneous immune responses can be investigated.

PC15

SYNTHESIS OF PLGA-PEG POLYMER MICELLES - CARRIERS OF COMBRETASTATIN-LIKE ANTITUMOR AGENTS

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One of the most important tendencies for development of the modern chemistry is related to the new trends in biomedicine and pharmacy. Today, significant advances have been made in the development of new biodegradable and biocompatible polymer materials and synthesis of corresponding nanoparticles- polymer micelles.

The aim of our research was to synthesize new biodegradable and biocompatible block copolymers based on poly(lactide-co-glycolide) (PLGA) and poly (ethylene glycol) (PEG) with linear (ABA-type) architecture; to investigate their ability to form nanosized particles; and its aptitude to acts as carriers of combretastatin-like antitumor agents. The synthesis of linear PLGA-PEG-PLGA block copolymer was carried out in line with standard procedures. The self-assembly behaviour of the copolymers in aqueous media was studied by UV-VIS and fluorescent spectroscopy. The size and morphology of the obtained micelles were determined by TEM and DLS. The capability of obtained micelles to adsorb and deliver combretastatin-like antitumor agents into living cells was also demonstrated.

We showed that the obtained copolymers formed nanosized micelles (particles with size up to 50 nm), which adsorbed and delivered combretastatin-like antitumor agents into living cells. *In vitro* biocompatibility results denote that all tested polymer particles are devoid of cytotoxic effects and may be used as a non-toxic drug carriers to target cells.

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PD1

AN APPLICATION OF LOGISTIC REGRESSION AND MULTIFACTOR DIMENSIONALITY REDUCTION FOR DETECTING GENOTYPE-PHENOTYPE INTERACTIONS ON RISK OF ATHEROSCLEROTIC PLAQUE PROGRESSION

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Inflammation and genetic predisposition play a critical role in the initiation and progression of atherosclerosis, especially in the process of plaque destabilization. The apolipoprotein A-I (apoA-I) component of High Density Lipoproteins (HDL), Plasminogen activator inhibitor-1(PAI-1) and *C-reactive protein (*CRP) have been implicated in participation of vascular inflammation regulation.

Our aim was to examine interactions among a panel of selected gene polymorphisms with serum CRP level in order to assess the risk for progression of atherosclerosis. Data from 42 patients with stable angina pectoris (SAP), 42 unstable angina pectoris (UAP) and 122 age-matched healthy subjects was used.

Data examination using both conditional logistic regression and multifactor dimensional reduction (MDR) methods showed significant genetic interactions between -390 (C/T/A) CRP, ^{33}Leu/Pro GPIII, IL-1 RA intron 2 polymorphism, 4G/5G PAI-1, ^{55}Leu/Met PON1, C677T MTFHR, -75 (G/A) APOA-I polymorphisms together with serum CRP levels in patients with NAP compared to SAP/Controls.

Using ten-fold cross validation, the best model showed Odds ratio equal to 7.4667 (3.5823, 15.5629), x2=31.6391 at $p<0.0001$ indicating that the combination of presented polymorphisms and elevated CRP levels are a good model for predicting plaque destabilization in patients with NAP.

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PD2

GENETC MAPPING OF EMS-INDUCED MUTATIONS WHICH INTERACT WITH DROSOPHILA FRAGILE X MENTAL RETARDATION 1 (DFMR1)

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Fragile X mental retardation is the most common form of human mental retardation, which is caused by the functional loss of the Fragile X Mental Retardation Protein 1 (FMRP). It is not precisely known how the absence of this protein is related to the disease pathology. One way to further understand FMRP functions is to look for new genes which genetically interact with it in a common biological pathway.

In our work we used the Drosophila Fragile X-model which has a single FMRP ortholog-dFMRP. We performed a forward genetic screen for EMS-induced X-chromosomal modifiers of a mutant "wing phenotype", induced by over-expression of the gene dfmr1 in the wing imaginal discs. We looked for dominant mutations, enhancing or suppressing this phenotype which at the same time had recessive lethal effect on viability.

Altogether we identified 8 enhancers and 11 suppressors of this phenotype.

Using genetic methods we mapped them to specific loci in the X-chromosome. The obtained information from these experiments will serve as a base to select appropriate candidate genes which will be further validated as possible dfmr1-interactors.

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PD3

ROLE OF +1245 A/G MT1A AND -209 A/G MT2A POLYMORPHISMS IN THE PATHOGENESIS OF CORONARY ARTERY DISEASEAND DIABETES MELLITUS

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Metallothioneins are cysteine-rich metal-binding proteins which play important role in zinc and copper metabolisms and are involved in free radicals scavenging. MTs involved in the zinc homeostasis can affect it, and could influence Zn-dependent processes such as the regulation of the blood pressure. MTs have close relation with hypertension which is associated with diabetes mellitus and cardiovascular disorders.

The aim of the present study was to investigate the role of +1245 A/G MT1A and -209 A/G MT2A SNPs in the pathogenesis of diabetes and coronary artery disease (CAD) in the context of Bulgarian cohort.

Molecular analysis was done in 142 patients with CAD, 101 diabetic patients without clinical evidence for cardiovascular disease and 21 aged- and sex-matched controls. Genotyping of both +1245 A/G MT1A and -209 A/G MT2A SNPs was done using PCR-RLFP analysis.

A binary logistic regression following adjustment for the conventional risk factors (hypertension, obesity, smoking, family history, sex, age, cholesterol and triglyceride levels) showed that the carriage of the +1245 A allele (compared to GG-genotype) was independent predictor only for developing of CAD (adjusted OR 6.6, 95% CI=1.6 to 26.8; p=0.008), while the -209 G allele (compared to AA-genotype) was independent predictor only for diabetes (adjusted OR 6.03, 95% CI=0.7 to 53.1; p=0.105).

In conclusion +1245A allele is independently associated with an increased risk of CAD after adjustment for classical risk factors for atherosclerosis in Bulgarian cohort, where the -209A/G MT2A polymorphism is predominantly associated with diabetes.

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PD4

DROSOPHILA DFMR1 INTERACTS WITH GENES CONTROLLING ACTIN FILAMENT ORGANIZATION IN NEURONAL DEVELOPMENT

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Fragile X syndrome is a heritable mental retardation caused by the loss of function of the fragile X mental retardation protein FMRP. It is believed to be a neurodevelopmental disorder and in this context elucidation of the role of FMRP in neuronal development is very important.

In our work we used the *Drosophila* model of Fragile X syndrome to study the genetic interactions of *dfmr1* with genes, controlling actin filament organization in neuronal development and axonal growth.

According to previous data, there are a number of *Drosophila* genes that are important for this process. To study their possible genetic interactions with *dfmr1*, the following procedure was performed. We over-expressed *dfmr1* in the larval imaginal discs and used the induced "notched wing" phenotype as a background to look for suppressor/enhancer interactions. The logic was that if they interacted with *dfmr1*, meaning they performed a common function, they would show dose sensitive effects and would suppress/enhance the "notched wing" phenotype.

In our experiments we found that some genes, controlling actin filament organization, interact with *dfmr1* and modify the background wing phenotype.

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PD5

DRUG DESIGN BY REGRESSION ANALYSES OF NEWLY SYNTHESIZED DERIVATIVES OF 8-QUINOLINOL

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The aim of the present study was to establish a relationship between antibacterial activities of newly synthesized derivatives of 8-Quinolinol and their structure. To accomplish the latter, the minimal inhibitory concentrations (MIC, mg/dm^3) of the investigated compounds against *Staphylococcus aureus* were determined and applied in the mathematical model.
Twenty four substituted derivatives of 8-Quinolinol
were tested through regression analysis and their MIC were determined and compared to reference strain of *Staphylococcus aureus*. A linear model for the relationship between the biological activity expressed as -ln(1.MIC^{-1}) and the structure of the compounds described by descriptors reflecting electronic, steric and thermodynamic parameters was proposed. A selection of descriptors having the greatest impact on biological activity was performed by stepwise regression:

-ln(1.MIC^{-1})=0.503135 + 0.650967ClogP - 0.00991Tot.E - 0.000001Bind - 0.014489MR + 0.001922G + 0.000028E + 0.292877LUMO.

A correlation between the antimicrobial activity of the resulting compounds and some selected descriptors with the coefficient of determination R^2=0.988 was established. Its high value defined the model as adequate and proved its high predictive ability. The resulting regression equation allowed the prediction of the antimicrobial activity of new not synthesized derivatives of 8-Quinolinol.

PD6

A NEW ANTIOXIDANT WITH NATURAL ORIGIN CHARACTERIZED BY ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY METHODS

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Formerly, naturally isolated SQGD (IBG-21) exhibited good *in vitro* radical scavenging capacity towards 1,1-diphenyl-2-picrylhydrazyl (DPPH). By the present research using *in vitro* and *ex vivo* EPR methods we report our further studies on the antioxidant and free radical properties of SQGD. By *in vitro* EPR direct spectroscopy a powdered form and aqueous solution of SQGD were studied before and after 2 h of UV irradiation and a single almost symmetrical EPR signal with a g value of 2.0056±0.0002 was registered for the powdered form and g=2.0044±0.0002 for the solution form. Based on the calculated g values and the strong EPR signal stability we accept that the recorded radical can be safely ascribed to a semiquinone radical anion of SQGD. To study *in vivo* antioxidant properties of SQGD, white laboratory mice were inoculated i.p.: first group with SQGD (20mg/kg), second with anticancer drug N-cyclohexyl-N-(2-chloroethyl)-N-nitrosourea (CCNU, 80 mg/kg), third with SQGD plus CCNU and controls were only inoculated with the solvent. At the 3rd h after treatment mice livers were isolated and homogenates in DMSO solution of the spin trap N-tert-butyl-alpha-phenylnitrone (PBN) were prepared and their EPR spectra were recorded. Statistically significant increased level of ROS production was found in liver homogenates of mice treated by CCNU compared to those of the controls. ROS production in livers of mice treated by SQGD, or by the combination of SQGD plus CCNU was slightly decreased compared to the controls.

In conclusion, obviously SQGD does not cause oxidative stress in the livers of mice for the followed period and behaves *in vivo* as an excellent antioxidant and hepatoprotector.

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PD7

RADICAL SCAVENGING CAPACITY OF SEEDS AND LEAVES ETHANOL EXTRACTS OF *CYNARA SCOLYMUS L.* – A COMPARATIVE STUDY

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Artichoke scientific name *Cynara scolymus L*. is an herbal medicinal plant widely used in traditional European medicine. This medicinal plant possesses many properties, including hepatoprotective ability and antioxidant effect.

The aim of the present study was to evaluate and compare the radical scavenging capacity of ethanol extracts prepared from *Cynara scolymus L.* seeds and leaves towards 1,1-diphenyl-2-picrylhydrazyl (DPPH) by an Electron Paramagnetic Resonance (EPR) method and visible spectrophotometry.

Both methods have demonstrated that at any studied concentration of the seeds' extract the per cent of the scavenged DPPH radicals was considerably higher than that calculated for the leaves extract. We also found that the percentage of DPPH radical scavenging capacity determined by EPR spectroscopy either for Cynara seed or leave extracts was higher than those determined by the spectrophotometry method.

In conclusion, we consider that the results obtained by the EPR method are more reliable compared to those obtained by the spectrophotometry method. It is a well established fact, that plant extracts might give rise to reduction of DPPH free radicals, which results in an increase of the DPPH-H concentration measured by the spectrophotometry method, while by EPR spectroscopy the EPR registered spectra in the studied samples are due only to the presence of DPPH free radicals.

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PD8

COMPARATIVE INVESTIGATION ON RADICAL SCAVENGING ACTIVITY AND PROTECTIVE PROPERTIES OF NATURAL ISOLATED AND SYNTHETIC ANTIOXIDANTS

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The purpose of this study is to evaluate and compare the free radical scavenging activity and protective properties of the isolated natural radioresistant product SQGD-IBG-21 and synthetic nitroxyl-labeled antioxidant SLENU.

A higher reducing power potential was found for SQGD (1.336±0.03357 U_{abs}) compared to that of SLENU (0.196±0.002273U_{abs}); also a higher nitric oxide radical scavenging activity-35.645±1.122% for SQGD in comparison with SLENU (19.964±2.233%). Total antioxidant capacity of SQGD was found to be 75±0.06%, while for SLENU it was only 22±0.03%.

Maximum protection to the liposomes calculated in % inhibitory activity of the two agents was found to be 50.04±0.037% for the natural agent and 27.54±0.33% for nitroxyl-labeled agent.

By direct EPR spectroscopy stable radical structures were recorded in the solutions of both studied antioxidants: an o-semiquinone anion radical structure was recorded in aqueous solution of SQGD and nitroxyl free radical struture in the SLENU solution, respectively.

In conclusion the studied agents exhibit strong radical scavenging and antioxidant activities. It should be mentioned that the natural antioxidant possesses higher protective properties in comparison with the synthetic antioxidant. Further experimental studies with both antioxidants are in progress in our laboratory.

Acknowledgments: This study was supported by a grant of Indo-Bulgarian project (BIn-7/08).

PD9

STUDY OF THE PHYTOTOXIC EFFECTS OF ATRANORIN, GYROPHORIC ACID AND PARIETIN ON CULTURES OF APOSYMBIOTICALLY GROWN LICHEN PHOTOBIONT *TREBOUXIA ERICI*

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The aim of the work was isolation and identification of didepside atranorin, tridepside gyrophoric acid and anthraquinone parietin from common lichens containing photobiont and to evaluate their potential phytotoxic effect on aposymbiotically grown lichen photobiont *Trebouxia erici*.

Atranorin, gyrophoric acid and parietin were isolated from lichens *Pseudevernia furfuracea*, *Umbilicaria hirsuta* and *Xanthoria parietina* in order to compare their phytotoxicity on cultures of aposymbiotically grown lichen photobiont *Trebouxia erici*. Cortical didepside atranorin had a strong phytotoxic effect on the photobiont cells. Presence of atranorin at highest tested dose of 0.1mg/disk decreased the growth of photobion cells and altered composition of assimilation pigments (mostly by decrease of chlorophyll *a* content and increase levels of its phaeophytinization). Atranorin decreased the levels of chlorophyll *a* fluorescence as well. Its presence also induced formation of reactive oxygen species, hydrogen peroxide as well as superoxide. It seems that atranorin which may be in direct contact with photobiont cells forming layer in lichens with stratified thallus, may act as allelochemical, controlling cell division of algal partner inside thallus and so regulating the balance between symbionts.

However, phytotoxicity of tridepside gyrophoric acid and anthraquinone parietin on the photobiont cells was not confirmed at tested concentrations in this study.

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PD10

B-CARBOLINE ALKALOID CONSTITUENTS FROM A *THERMOACTINOMYCES SP*. STRAIN, ISOLATED FROM LIVINGSTON ISLAND, ANTARCTICA

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The aim of the work was isolation, characterization, identification and biological activity of the isolated β-carboline alkaloids from strain *Thermoactinomyces sp*. imbas-14.

Two natural β-carboline alkaloids [1and 2] were isolated from the culture filtrate of *Thermoactinomyces sp.* imbas-14 strain. The organism was isolated from penguin excrements, collected on Antarctic Livingston Island. Based on the effect of temperature on its growth rate, the strain is thermophilic with an optimal growth temperature at 50°C. β-Carbolines belong to the group of indole alkaloids found in significant amounts in many plant families, animals and microorganisms. The plants were used in traditional medicine to treat asthma, jaundice, lumbago and other human ailments. The compounds were purified by solvent extraction, silica gel column chromatography and preparative TLC and HPLC consecutively. The structures of 1 and 2 were elucidated by using high-performance liquid chromatography, using double online detection with a diode array spectrophotometer and a mass spectrometer (HPLC-DAD-ESI-MS), and extensive one- and two-dimensional NMR experiments.

Both substances 1-methyl-β-carboline (harmane) (1) and 1-acetyl-4-methyl-β-carboline (2) are natural products, isolated for first time from the culture filtrate of antarctic strain *Thermoactinomyces sp*. imbas-14. These compounds showed activity against important Gram-positive (*Bacillus mycoides*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria, yeasts (*Candida tropicalis*) and filamentous fungi (*Penicillium notatum*).

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PD11

METHANOL:CHLOROFORM EXTRACTS FROM *LAMIUM ALBUM* L. AFFECT CELL PROPERTIES OF A549 CANCER LUNG CELL LINE

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Lamium album L. (white died nettle) possess astringent, anti-inflammotary, antibiotic, antispasmotic and bacteriostatic properties and is used for bladder, kidney and menstrual problems. In the present study we investigated potential effects of several combinations of methanol and chloroform extracts from *in vivo* and *in vitro* propagated *Lamium album* L., extracted by Soxhlet method on A549 cancer lung cell line.

After 24 h and 48 h of incubation, both *in vivo* and *in vitro* extracts Methanol:Chloroform 1mg/ml:1mg/ml and 2.5mg/ml:2.5mg/ml showed cytotoxic effects on cells, measured by MTT assay. Damage on the adhesion properties and cell cycle were also detected.

We consider that *Lamium album* L. extracts possess potential anti-cancer effects that need to be investigated in grater details.

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PD12

AN ORGANOPLATINUM(II) COMPLEX OF *N*-3-PYRIDINYLMETHANESULFONAMIDE WITH EXPECTED CYTOSTATIC ACTIVITY. STRUCTURAL, SPECTROSCOPIC AND THERMOANALYTICAL STUDY

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In the last years special attention is given to the "non-classical" cytostatic Pt(II) complexes, and in particular, those containing planar *N*-heterocyclic ligands in *trans*-configuration [M. A. Jakupec et al., *Rev. Physiol. Biochem. Pharmacol.*, 1, 146 (2003); I. Kostova, *Rec. Pat. Anti-Cancer Drug Discov.*, 1, 1 (2006)]. In continuation of our previous studies on the coordination chemistry [N. I. Dodoff, *Internet J. Vibr. Spectrosc.*, 4, 3 (2000) 5 (http://www.ijvs.com); *idem, Z. Naturforsch., B*, 56 (2001) 1217; N. I. Dodoff *et al*., *ibid.*, 59 (2004) 1070] and cytotoxic effect [N. I. Dodoff *et al*. *Z. Naturforsch., C*, 64 (2009) 179] of platinum-group metal complexes with *N*-3-pyridinylmethanesulfonamide (PMSA), now we report the synthesis, crystal structure and physico-chemical characterization of *trans*-dichloro(η^2-ethylene)(*N*-3-pyridinylmethanesulfonamide)platinum(II) (1) Crystals of 1 (monoclinic; $P2_1/c$; a=5.1260(1), b=19.1600(4), c=12.7990(3) Å, β=97.242(2); Z=4) contain complex molecules in which the Pt(II) has a planar coordination, the ethylene molecule is perpendicular, and the pyridine ring is inclined to *ca*. 45° with respect to the coordination plane.

Complex 1 was characterized by IR and electronic spectra, as well as by thermoanalytical (TG/DTG-TGA) measurements. A detailed multi-NMR (^{1}H, ^{13}C and ^{195}Pt) spectroscopic study of 1 and the free ligand was performed. Complex 1 along with the starting compounds PMSA and K[Pt(η^2-C_2H_4)Cl_3]×H_2O were tested for cytotoxic activity against human hepatocellular carcinoma HepG2 and human leukaemia K562 cell lines. Marginal activity was registered only for K[Pt(η^2-C_2H_4)Cl_3]×H_2O against K562 cells after 72 h of treatment (cell viability, % of control: 52±4, 87±5 and 88±8 at concentration of 1000, 500 and 250 μmol l^{-1}, respectively).

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PD13

PREPARATION OF CHITOSAN GEL BEADS FOR TRYPSIN IMMOBILIZATION

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Chitosan is a natural polysaccharide obtained by N-deacetylation of chitin and is consequently a copolymer of N-acetyl-D-glucosamine and D-glucosamine. Chitosan possesses distinct chemical and biological properties, in its chains it has reactive amino and hydroxyl groups, amenable to chemical modification. Along with unique biological properties that include biocompatibility, nontoxicity, physiological inertness, affinity to proteins chitosan offers an extraordinary potential of applications. It is available in different forms: powder, gel, fibres and membranes. Different-sized particles can be formed by several methods, e.g. precipitation, emulsion cross-linking, spray drying, ionotropic gelation. Covalent cross-linking leads to the formation of a permanent network with improved mechanical properties of the gel. This is particularly useful for biomedical applications: cell culture support, enzyme immobilization and biomaterial for implants.

Enzyme immobilization is a method to keep enzyme molecules confined or localized in a certain defined region of space with retention of their catalitic activities. Immobilization is achieved by fixing enzymes to or within solid supports, as a result of which heterogeneous immobilized enzyme systems are obtained.

The purpose of this study is to immobilized trypsin by covalent binding onto chitosan gel beads cross-linked and activated with glutaraldehyde; to study the effect of the chitosan molecular weight and the degree of deacetylation on enzyme loading, leaching and activity, and to characterize the particles.

This work describes the preparation of chitosan beads by precipitation and covalent gelation. The effect of preparation conditions was investigated. The gel beads were characterized by Fourier transformed infrared spectroscopy for chemical modification and scanning electron microscopy for morphology.

Immobilized trypsin was characterized for optimum functional range and stability. The effect of substrate concentration was also studied.

PD14

NOVEL SYNTHETIC COMBRETASTATIN A-4 ANALOGUES WITH POTENTIAL ANTITUMOR ACTIVITY

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Combretastatins are a group of organic compounds found in the bark of South African tree *Combretum caffrum*. The most promising of these natural products is combretastatin A-4 (CA-4) because of its simple structure and high cytotoxic activity against a variety of human cancer cell lines. The stilbene CA-4 is a potent anti-cancer drug and represents a new class of therapeutic compounds known as vascular disrupting agents.

The aim of the present study was to synthesize a series of new heterocyclic analogues of CA-4 containing benzoxazolone and benzothiazolone moiety, and to investigate their effect on different human cell lines.

Investigating more than 40 novel synthetic analogues, the results showed potent cytotoxic activity of four studied compounds. Immunofluorescent staining of treated cells demonstrated the formation of abnormal mitotic spindles and alterations in chromatin condensation. Further evaluating the cell cycle distribution by flow cytometry presented the persistent accumulation of treated cells in G2/M transition pathway. The arrest of cell cycle caused mitotic blockade and apoptotic cell death in the studied cell lines. The mechanisms of action will be discussed.

The heterocyclic analogues of CA-4 showed a potent antitumor activity and it is important because new anticancer therapeutics may emerge from these efforts.

PD15

MOLECULAR MODELING OF THE KYOTORPHIN MIMETICS

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The endogenous peptides take part in the regulation of various adaptive reactions of the organism, including pain perception. The dipeptide kyotorphin (Tyr-Arg, Kyo) plays a role in pain modulation in the mammalian central nervous system (CNS), and is one of the most investigated neuropeptides. One of the successful strategies in the design of neuropeptides with enhanced stability and improved delivery to the CNS is the use of non-protein amino acids, like canavanive (Cav), a structural analogue and amtimetabolite of arginine (Arg). In our previous *in vivo* experiments we demonstrated that Tyr-Cav exerted a strong-reversible analgesic effect, more pronounced than that of Kyo. Bearing in mind these and the fact that norsulfoarginine (NsArg) is a structural analogue of arginine and canavanine, we synthesized a series of new peptides with expected analgesic activity, containing NsArg residues in their molecules: NsArg-Tyr, Tyr-NsArg, Tyr-NsArg-NH_2 and Tyr-NsArg-OBzl.

The conformational features of these dipeptides are of particular interest, both from theoretical and pharmacological point of view. Since no single-crystal X-ray diffraction data for the compounds are available until now, we undertook a quantum-chemical modelling of their structure.

Here we present our preliminary computational results for Kyo (1) and NsArg-Tyr (2). Molecular mechanics (MM+ force field) conformational search for the two species was performed, and the global minimum-energy conformations thus obtained, were further optimized at HF *ab initio* (3-21G** basis set) level of theory.

In both cases, specific, scorpion-like conformations are realized, with hydrogen bonds involving the guanidino-group and the phenolic hydroxyl.

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PD16

IN VITRO ASSESSMENT OF THE CYTOTOXIC EFFECTS OF SULFO- ARGININE ANALOGUES AND THEIR HYDRAZIDE DERIVATIVES IN 3T3 AND HEPG2 CELLS

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The significance of the guanidine moiety's importance in many biologically active compounds cannot be disputed. The guanidine moiety often causes significant changes in the biological activity of organic molecules. On the other hand, the intensive development of combinatorial chemical libraries has drawn more attention to the preparation of amino acids with a non-proteinogenic side chain in order to increase the diversity of the resulting compounds. In this context we have been concerned for several years with the preparation and characterization of unnatural amino acids containing a basic functionality (oxy- and sufo-guanidino group) in the side chain. Their effects on the growth of microorganisms, model plant systems and cultured tumour cells have been evaluated as well as their antitumor activity *in vivo*. We reported that modification of Cav at the carboxyl group selectively changed toxicity against bacteria, plants and leukaemia cells.

In this study, the cytotoxic activities of arginine analogues sArg, NsArg, and their hydrazide derivatives sArg-$CONHNH_2$, sArg-$CONHN(CH_2CH_2Cl)_2$, sArg-$CONHNC_6H_5$, NsArg-$CONHNH_2$ and NsArg-$CONHN(CH_2CH_2Cl)_2$, NsArg-$CONHNC_6H_5$ on 3T3 (standard mouse embryonic fibroblast cell line) and HepG2 (*human liver hepatocellular carcinoma cell line)* cells were examined.

The cultivated cells were treated with amino acid analogues in a wide concentration range (0.015-2 mM). MTT assay was performed 24 hours after treatment. PrizmaPlot.4 (ANOVA-test) was used for statistical analysis. sArg analogues exhibited higher cell growth inhibitory effects on both cell lines in comparison with their parent compound. $_S$Arg-$CONHN(CH_2CH_2Cl)_2$ cell growth inhibitory effect was considerably higher in the tumour cells HepG2 in comparison with 3T3 cells.

It may be concluded that the substitution in the carboxylic group of sArg increases the cell growth inhibitory effects of the compounds, especially in the case of the *bis*-(2-chloroethyl) hydrazide substitute. The same correlation was observed in the case of NsArg and its analogues.

The results confirm our previous findings that the cell growth inhibitory effect of the compounds depends mainly on the modification in the carboxylic group.

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PD17

DEGLYCATING ENZYMES FROM EMBRYOGENIC SUSPENSION CULTURES OF DACTYLIS GLOMERATA L.

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Glycation or non-enzymatic glycosylation is a spontaneous chemical reaction of biological amines with reducing sugars (e. g., glucose, fructose, ribose, erythrose) and other carbohydrate derivatives such as ascorbic acid. Glycation includes a series of reactions known as Maillard reactions in which early, intermediate and advanced glycation end products are formed. The formation of early glycation products (Schiff's bases and Amadori products) is a reversible process. To date, three groups of enzymes capable of catalyzing the degradation of the Amadori products (amadoriases) have been discovered - fructosamine oxidases, fructosamine-3-kinases and a fructoselysine-6-phosphate deglycase. These deglycating enzymes were found in animals, many bacterial species and there is also evidence for the presence of amadoriases in plants (*Arabidopsis thaliana* and *Spinacia oleracea*).

The aim of the present study was to investigate the monocot, *Dactylis glomerata* L., for the presence of deglycating enzymes. To this end, we used the phage display technology to produce monoclonal antibodies against the *Escherichia coli* amadoriase. A number of clones were selected against the target antigen and tested for the ability to recognize the *E. coli* amadoriase by ELISA and Western blotting.

To detect the presence of deglycating enzymes in our plant model system, total cell wall protein from embryonic suspension cultures of *D. glomerata* L. was isolated and separated by SDS-PAGE. The Western blotting was performed with a monoclonal antibody against the *E. coli* amadoriase produced as described above. The antibody was shown to recognize a single protein band from the total cell wall protein with a molecular mass of nearly 40 kDa as estimated by SDS-PAGE. This protein fraction was purified and sent for custom sequencing.

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