
Session 1. Structure and function of genes and genomes

Lectures

L1.1

The mitochondrial genome

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The sequence of the human mitochondrial genome was established almost 25 years ago and has been used in analysis of human population migrations, forensic medicine, longevity studies and in investigation of the causes of mitochondrial diseases. It is a small compact genome of 16.5 kb containing information about 13 proteins, 2 rRNAs and 22 tRNAs. There are many variants (haplogroups) of the human mitochondrial genome and some of them have been associated — for some populations — with longevity or certain mitochondrial disease. Many of these associations were only detected for certain populations. I will discuss the mitochondrial DNA haplogroups in the Polish population and the association of certain mitochondrial haplogroups with a mitochondrial blindness, Leber Hereditary Optic Neuropathy.

L1.2

The steroid biotransformation genes in genomes of mycobacteria

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The genus of *Mycobacterium* consists of a wide variety of bacteria occupying many ecological niches and displaying very diverse phenotypes. There are saprophytes and dangerous pathogens in this genus including causative agent of tuberculosis (*M. tuberculosis*). One major distinction among members of *Mycobacterium* is growth rate. Saprophytes like *M. smegmatis* or *M. vaccae* can divide every 2–3 hrs. In contrast the slow growing species divide every 12 (*M. avium*), 24 (*M. tuberculosis*) or 168 hours (*M. leprae*). The other distinction, identified more recently, is huge difference in the size of mycobacterial genomes. The genome of *M. smegmatis* (about 6.9 Mb) is two times bigger than genome of *M. leprae* (3.3 Mb). Moreover, *M. leprae* contains a huge number of pseudogenes which

makes this difference even bigger. The common feature of all *Mycobacterium* species is huge metabolism of lipids. At least 8% of *M. tuberculosis* genome is dedicated to this activity. The cell envelope contains a remarkable array of lipids, glycolipids, lipoglycans and polyketides with number of genes encoding enzymes required for their production. Moreover there is estimated from the genome sequence of mycobacteria that lipids and sterols which are more abundant in host tissues than carbohydrates are potential sources of carbon and energy. Both pathogens and saprophytes of mycobacteria contain huge number of genes with putative role in metabolism of lipids. The details analysis of these genes, including heterologous expression, overproduction and gene knock-out could clarify their importance for mycobacteria growing in so distinct ecological niches. The different organization and role of genes, encoding 3-ketosteroid D¹ dehydrogenases and cholesterol oxidases in *M. tuberculosis* and *M. smegmatis*, investigating by construction of unmarked deletion mutant strains, is the keynote of this presentation.

L1.3

Initiation of replication and segregation of bacterial chromosome

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Chromosome replication and segregation are the two major steps of chromosome maintenance in growing cells. In eubacteria and eukaryotes, and very likely in archaea as well, replication is controlled at the initiation stage. Recent studies suggest that the mechanism of initiation of DNA replication is similar across all domains of life. The initiation of DNA replication starts with the binding of specific initiator protein(s) to DNA sites, termed origins, and results in the localized unwinding of the DNA duplex and the establishment of replication forks. DnaA protein plays an essential role in the initiation of bacterial chromosome replication. In contrast to eukaryotic cells, the process of bacterial chromosome segregation is poorly understood. Bacteria have no mitotic apparatus, however, the principles of chromosome segregation in eubacteria, eukaryotes share substantial similarities. A centromere analogue — a locus at which force is applied to separate replicated chromosomes — has been found in bacterial chromosomes. Recent studies have revealed that there is spatial control of chromosome replication and segregation, and that segregation starts concomitantly with replication initiation and does not wait till comple-

tion of replication as is the norm in eukaryotes. We review a recent progress in studies on molecular mechanisms of initiation of replication and chromosome segregation in bacterial cells. Both processes are most extensively studied in the rod-shaped bacteria dividing by binary fission. In our studies we focus on *Actinomycelates*, particularly on slow-growing *Mycobacterium* and mycelial *Streptomyces*, in which chromosome segregation is not closely coupled to DNA replication and occurs only during sporulation.

L1.4

New functions of the plant cap-binding protein complex

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Nuclear cap-binding protein complex (CBC) is involved in several aspects of RNA metabolism. The CBC binds to the cap structure of all polymerase II transcripts and promotes efficient splicing of pre-mRNA, nuclear export of U-rich small nuclear RNAs, mRNA 3'-end formation and also plays a role in nonsense-mediated mRNA decay (NMD). The cap-binding protein complex consists of two subunits called cap-binding proteins (CBP): CBP20 and CBP80. In our laboratory we have characterized CBC from *Arabidopsis thaliana* (AtCBC), which is composed of AtCBP20 (29,9 kDa) and AtCBP80 (96,5 kDa). AtCBP20 is highly conserved and shows 68% identity and 82% similarity with human CBP20. The most unique feature of AtCBP20 is a long C-terminal extension containing two nuclear localization signals (NLS) within it. The second subunit of AtCBC, AtCBP80, is less conserved and, unlike other CBP80 homologues, does not have any NLS. Due to the presence of two NLSs, AtCBP20 is actively transported into the nucleus, while AtCBP80 can reach the nucleus only in a complex with AtCBP20. Site-directed mutagenesis was performed in order to inactivate NLS1, NLS2 or both nuclear localization signals of AtCBP20. The GFP-AtCBP20 constructs were introduced into tobacco protoplasts and subjected to transient expression assay. Mutation in one of the two NLSs did not inhibit transport of AtCBP20 from the cytoplasm to the nucleus. These results indicate that NLS1 and NLS2 act independently and one of these two signals is sufficient for accumulation of AtCBP20 in the nucleus. Using co-transfection of tobacco protoplasts with GFP-AtCBP80 and AtCBP20 Δ NLS1, AtCBP20 Δ NLS2 or AtCBP20 Δ NLS1 Δ NLS2 mutants, we have showed that each NLS can function independently in transport of the AtCBP20/AtCBP80 complex from the cytoplasm to the nucleus. Employing RNAi technology, we obtained *Arabidopsis thaliana* mutants with very low level of AtCBP20 or AtCBP80. These two mutants show identical phenotypes: slightly slowed growth and serrated leaves. Moreover, both mutants are abscisic acid (ABA) hypersensitive suggesting that CBC in plants is involved in ABA signaling.

L1.5

Non-B DNA conformation as an intracellular mutagen

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Repeated sequences are common in animal and plant genomes although our knowledge about their biological role is still limited. For instance, 30% of human genome consists of repeated sequences, which are particularly frequent in Y chromosome as large palindromes located in so called ampliconic sequences. Intensive investigations performed in the last 10 years proved that the genetic instability of different repeated sequences, especially microsatellites, lies at the base of several human genetic disorders. It has been shown that well defined *Escherichia coli* mutants as well as diverse plasmid and phage vectors were very useful models for analysis of molecular mechanisms responsible for instability of trinucleotide repeat sequences (TRS) and other human repeat sequences which are able to adapt *in vivo* several types of non-B-DNA structures. During this lecture, the experimental work will be presented including own results, which point the role of replication, transcription, recombination and DNA repair systems in the stimulation of frequent dynamic mutations within microsatellite tracts. This type of mutations is based on the fact of non-B-DNA structures formation in living cells which act as a spatial obstacle for replication and transcription and are recognized by DNA repair systems. Moreover, as these sequences become longer in cells, they are subject to homologous recombination processes which can destabilize them further. Models of instability of human microsatellite sequences based on the results obtained using bacterial cells were recently confirmed on the eucaryotic models such as yeasts, mice and human cell lines.

L1.6

Repeated motifs within AT-rich region of replication origin

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The replication origins of bacterial chromosomes, bacteriophages, and plasmids as well as certain eukaryotic replicons including DNA viruses and *Saccharomyces cerevisiae* possess characteristic functional elements, including specific binding sites for the appropriate initiation protein and regions with a high content of adenine and thymine

residues. The AT-rich part of a replication origin is probably the most universally conserved structural element identified in both prokaryotic and eukaryotic replicons. This is the region where the double helix is destabilized during process of replication initiation and initial, helicase dependent, unwinding of the double helix takes place. In many cases this regions contain repeated sequences of various lengths. In bacterial chromosome, plasmid and bacteriophage origins these repeated motifs usually are 13-nucleotide long. To investigate how the structure of AT-rich part of origin contributes to the mechanism of DNA replication initiation, mutations in origin of broad host range plasmid RK2 (*oriV*) were introduced which altered the sequence or/and spacing of 13-mer repeats. Twenty six mutants with altered sequence and/or spacing of 13-mer motifs have been constructed and analyzed for replication activity *in vivo* and *in vitro*. The data shows that the replacement of *oriV* 13-mers by similar but not identical 13-mer sequences from *Escherichia coli oriC*, inactivates the origin. In addition, interchanging the positions of the *oriV* 13-mers results in greatly reduced activity. Mutants with T/A substitutions are also inactive. Furthermore, introduction of single nucleotide substitutions demonstrates very restricted sequence requirements depending on the 13-mer position. Only two of the mutants are host specific; functional in *Pseudomonas aeruginosa* but not in *E. coli*. Our experiments demonstrate considerable complexity in the plasmid AT-rich region architecture required for functionality. It is evident that low internal stability of this region is not the only feature contributing to origin activity. Our studies suggest a requirement for sequence specific protein interactions within the 13-mers during assembly of replication complexes at the plasmid origin.

L1.7

Genes and proteins of the Hsp70 chaperone system of Bacteria and Archaea

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Under conditions of stress all organisms rapidly accelerate synthesis of a group of proteins, called heat shock proteins (Hsps), whose functions are various and not yet clearly understood. Many of them are indispensable at all temperatures and extremely highly conserved in evolution. Generally, Hsps are either molecular chaperones, protecting proteins against denaturation and actively renaturing the proteins which have lost their structure, or proteases. The chaperoning systems occur in all three phylogenetic domains: Bacteria, Archaea, and Eukarya. One such system, the molecular chaperone machine, is constituted by the proteins Hsp70(DnaK), Hsp40(DnaJ) and, in bacteria, GrpE (henceforth referred to as DnaK or K, DnaJ or J, and GrpE or E). This KJE machine has been extensively studied in bacteria and in eukaryotes.

In contrast, very little is known about the KJE machine in archaea. Archaea are prokaryotes but some of their chaperoning systems resemble those of eukaryotes. Also, not all archaea possess the stress protein Hsp70(DnaK), in contrast with bacteria and eukaryotes, which possess it without any known exception. Further, the primary structure of the archaeal DnaK resembles more the bacterial than the eukaryotic homologues. Our work addressed two questions: Is the archaeal Hsp70 protein a chaperone, like its homologues in the other two phylogenetic domains? And, if so, is the chaperoning mechanism of bacterial or eukaryotic type? The data have shown that the DnaK protein of the archaeon *Methanosarcina mazei* functions efficiently as a chaperone and that the *M. mazei* DnaK-dependent chaperoning pathway *in vitro* is similar to that of the bacteria *Escherichia coli* used for comparison. This finding is in agreement with the hypothesis of the lateral transfer of the bacterial *hsp* genes from bacteria to archaea. However, *in vivo* results indicate that, intracellularly, the chaperones from the two species differ. Further work gave more detailed information about structural and functional differences between the archaeal and bacterial DnaK proteins, which might be responsible for the species specificity observed *in vivo*.

L1.8

Influence of replication of DNA on organization and evolution of bacterial genomes

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Specific mode of replication of DNA strands — leading and lagging — result in different types of nucleotide substitutions occurring in these strands with different frequencies. It leads in consequence to the biased nucleotide composition of these strands which is called DNA asymmetry. Such asymmetry is a common feature of bacterial chromosomes which have one region of origin and one region of terminus of replication. The bias is high enough to indicate the potential region of origin of replication. The region includes often the *dnaA* gene and clusters of sequences binding the DnaA protein. Asymmetric organization of bacterial chromosomes influences significantly evolution of genes. It is supported by genomic analyses and computer simulations of gene evolution. Genes which were translocated to differently replicating strands and are still subjected to the opposite mutational pressure show a higher probability of elimination than genes which did not change the strand. Interestingly, the genes which change with some frequency the DNA strand are less frequently eliminated and accept more substitutions. Such 'survival strategy' is in agreement with the observed higher divergence of homologs lying on differently replicating strands and is supported by observed frequent rearrangements between DNA strands. It is not true for

genes coding ribosomal proteins which are preferentially located on the leading strand because the location lowers the probability of collisions between replication and transcriptional complexes. Analyses of homologs in closely related genomes show that translocations of genes from the lagging to the leading strand are accepted more often than in the opposite direction.

L1.9

The export system of exopolysaccharide in *Rhizobium leguminosarum* bv. *trifolii*

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Bacteria employ several types of multicomponent systems responsible for polymerization and export of polysaccharides. *E. coli* Group 1 and 4 K-antigens (CPS) and related extracellular polysaccharides in other bacteria follow a Wzx/Wxy-dependent polymerization pathway (Whitfield & Paiment, 2003). The identified in *R. leguminosarum* bv. *trifolii* TA1 (RtTA1) exopolysaccharide (EPS) transport system comprise PssT, PssN, PssP and PssL proteins. Octasaccharide, lipid carrier linked EPS structural subunits are probably flipped across the inner membrane by the action of PssL membrane translocase — a Wzx-like protein. Wzx protein belongs to the PST (polysaccharide specific transport) family and usually acts in cooperation with Wzy polysaccharide polymerase (Whitfield & Paiment, 2003). PssT was recognized as integral inner membrane protein with 12 transmembrane segments (TMS) with conserved motif typical for Wzy proteins (Mazur *et al.*, 2003). PssL/PssT could be responsible for Wzx/Wzy-like-dependent EPS polymerization and translocation in RtTA1. Additional proteins of this multicomponent system are PssP and PssN. The membrane-periplasmic auxiliary (MPA-1 family) PssP protein, equipped with ABC cassette, controls the degree of EPS polymerization (Mazur *et al.*, 2002). PssN protein is a member of the outer membrane auxiliary (OMA) family of proteins, probably acting in the last step of EPS assembly — translocation through the outer membrane. The PssN is likely to form oligomeric structures composed of more than three associated monomers.

References

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L1.10

Sulfur metabolism in fungi: metabolic pathways and their genetic regulation

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Fungi can utilize inorganic sulfur for synthesis of sulfur-containing organic compounds. While having the same sulfate assimilation pathway, generating sulfide — a direct precursor of cysteine and homocysteine, they differ in the organization of sulfur amino acid metabolism. All fungi studied so far can synthesize methionine from cysteine but only some can metabolize methionine to cysteine. We have found that *Aspergillus nidulans* possesses alternative pathways for cysteine synthesis representing the richest repertoire of sulfur amino acid metabolic routes. A number of regulatory systems controlling the expression of sulfur related genes have been found. The most intensively studied is cysteine-dependent sulfur metabolite repression (SMR) system which turns off transcription of several genes, particularly encoding the sulfate assimilation enzymes. This system consists of the negative acting ubiquitin ligase complex of the SCF type and the positive acting transcription factor METR, a member of bZIP family of DNA-binding proteins, need for transcription of several, but not all “sulfur” genes. The SCF complex inactivates METR when cysteine level goes high. Mutations in the *metR* gene lead to auxotrophy, however we have isolated dominant mutations, which relieve METR protein from SCF control. The expression of genes encoding homocysteine metabolizing enzymes is up-regulated by this amino acid. Particularly interesting is regulation of the methionine synthase gene since it is also negatively regulated by choline — the main consumer of methionine methyl groups. The enzyme makes the last step in methionine synthesis but may be also regarded as a first enzyme of choline synthesizing pathway being feedback regulated by the final product. Finally, at least one gene, encoding homoserine acetyltransferase, was found to be negatively regulated by methionine.

L1.11

The use of plastid and mitochondria genome organisation and sequence in the studies of organellar inheritance in bryophytes

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In the case of higher plants the nucleotide substitution rate in plastid genomes is three times lower than in nuclear

genomes and in the case of mitochondrial genomes it is even five times lower than in plastid genomes. Frequent genome rearrangements (deletions, insertions, inversions) that are probably the result of dynamic recombination are observed in the case of higher plant mitochondrial genomes. Our results strongly suggest that the same rules are valid in the case of nuclear, plastid and mitochondrial genomes of bryophyta. In bryophytes a number of allopolyploid species has been reported, with well established parental species. To learn which parental species was the donor of organellar genomes to allopolyploid species we analysed several loci of plastid and mitochondrial genomes. We expected that in the case of allopolyploid species analysed sequences should be identical or nearly identical with the sequences from one parental species and different with the sequences from the other parental species. The results of analysis of several allopolyploid complexes in bryophytes will be presented during the lecture. Generally we conclude that organellar inheritance in bryophyta is uniparental.

L1.12

Cyclic AMP receptor protein-RNA polymerase interactions in *Escherichia coli* as a simplest model of transcription activation

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The *Escherichia coli* cyclic AMP receptor protein (CRP) activates transcription of more than hundred promoters. CRP, in the presence of allosteric effector-cAMP, can bind to the different promoters and interact with the RNA polymerase, activating gene transcription. In the simplest CRP-dependent promoters the transcription activation requires only three macromolecular components such as CRP, RNA polymerase (RNAP), and DNA. This transcriptionally active molecular machinery can serve as a model of the protein-protein and protein-DNA interactions, which occur in more complicated complexes in bacteria or in the much more complicated complexes in eukaryotic cells, requiring tens of the interacting proteins as well as several DNA sequences on the promoter. In this scope, the results of recent structural and functional studies of the CRP-RNAP interactions with different CRP dependent promoters sequences will be presented. The thermodynamic parameters of the CRP-RNAP-DNA interactions as well as distance measurements, using FRET method, between the macromolecular components give more information about this transcriptional complex assembly. The time resolved anisotropy studies of the fluorescently labeled alpha as well as sigma subunits of RNAP indicate a dynamic behavior of this macromolecular complex. These structural studies enabled us to get more information about the simplest transcription activation macromolecular complex in solution.

Oral Presentations

OP1.1

The ecology of bacteria and isoelectric point of their proteomes

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Recent genomic analyses have shown that whole proteomes possess some universal properties, like bimodal distributions of isoelectric points of proteins. In all analysed proteomes, proteins with pI close to 7.4 are the least represented. It could be explained by the fact that pH 7.4 is close to the pH of most of intracellular compartments, and selection eliminates proteins with pI close to this value because they are the least stable, soluble and reactive. The number of the acidic and basic proteins is connected with ecological environment in which an organism is living. The halophiles have more acidic proteins than nonhalophiles. The acidophiles have more basic proteins than alkaliphiles. Some environmental parameters like oxygen or temperature seem to have no influence on the proportion of acidic proteins to basic proteins. There is a relation between the size of a genome and isoelectric point of proteins and between the size of the genome and environment. The biggest proteomes that belong to free living bacteria are acidic while the smallest which belong to endocellular parasites or symbionts are basic. The small genomes in endocellular organisms have a low content of G+C pairs. A connection between a length of polypeptide chain and isoelectric point is visible. The acidic proteins are longer than basic proteins. The extreme pI values are represented by the short proteins, the pI near 7 is represented by long proteins. It is connected with the ability to buffer changes and specific properties of amino acids.

OP1.2

Rapid and systematic approach for sequential analysis of plasmids isolated from hospital environment

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Progress in the efficient analysis of genomic DNA depends not only on the development of new technologies for high-speed DNA sequencing but also on the improvement of existing methods. The overall goal of approaches used today is to make DNA sequencing faster, more accurate, and

less expensive. Here, we present a new method for DNA analysis that allows a systematic sequence determination of genomic DNA by the means of indexer walking. This approach has been tested in our laboratory by sequential analysis of several plasmids isolated from enteropathogenic strains of *Escherichia coli* (EPEC). In first step, an antibiotic resistance cassette (cat) containing primer binding sites has been cloned randomly into plasmid of interest. The random distribution of aforementioned cassette was ensured by digestion of the template with CviJI endonuclease which recognizes a specific sequence 5'-RG→CY-3'. This specificity can be compromised even more to 5'G→C-3' in the presence of DMSO. Obtained recombinants have been introduced into *E. coli* DH5 α . Random insertion of antibiotic resistance cassette enables determination of the total nucleotide sequence of a particular plasmid by an analysis of a few overlapping sequences. On the other hand, the primer binding sites present on introduced cassette (cat) can be used for amplification of DNA of interest. The obtained fragment can be then analyzed by indexer walking technology which was developed in our laboratory. The protocol of DNA sequencing by indexer walking incorporates efficient ligation of double-stranded synthetic oligonucleotides (indexers) to DNA fragments, produced by type IIS restriction endonucleases generating ambiguous four-nucleotide 5' overhangs, and their subsequent amplification (30 thermal cycles) which provides enough template for automated DNA sequencing with Big DyeTM fluorescent terminators. The products of DNA sequencing are then analyzed by capillary electrophoresis. The data gathered in the first sequencing reaction permits further movement into the unknown nucleotide sequence by digestion of analyzed DNA with type IIS restriction endonuclease followed by ligation of the next indexer. A library of presynthesized indexers consisting of 256 oligonucleotides enables bi-directional analysis of any DNA molecule and provides universal primers for sequencing. Based on the results obtained to date, it seems that DNA sequencing by indexer walking can be used as a low-cost procedure for nucleotide sequence determination of DNA molecules, such as natural plasmids, cDNA clones, as well as longer DNA fragments. It can also serve as an alternative method for gap-filling at the final stage of genome sequencing projects.

OP1.3

In vitro transcription from *rpoH* and *ibpB* σ^{54} - controlled promoters

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The heat-shock response controlled by σ^{54} involves two transcriptional units: the known *pspA-E* operon and a

gene coding for a small heat shock protein IbpB, which regulation remains unknown. Moreover, the σ^{54} promoter consensus sequence was found in the regulatory region of the *rpoH* gene coding for the main heat shock factor σ^{32} . Initiation of transcription at σ^{54} promoters is tightly regulated at the step of promoter melting. Isomerisation to the open complex depends on interaction of RNAP σ^{54} with an enhancer-bound activator protein in an ATP dependent manner. This interaction requires looping-out of the intervening DNA, which can be facilitated by the DNA bending IHF protein. Proteins: σ^{54} , IHF and potential activators- NtrC and PspF were purified and used for *in vitro* studies. EMSA tests showed that RNAP σ^{54} and IHF bind in the *rpoH* regulatory region. DNase I footprinting and electron microscopy confirmed RNAP σ^{54} binding exactly to the predicted promoter sequence, and IHF binding to the predicted site, about 350 bp upstream of the *rpoH* gene. Primer extension and transcription *in vitro* experiments show that σ^{54} promoter of the *rpoH* gene is active. However, there is no certainty concerning the activator identity. Despite positive results of PspF binding in EMSA tests, this interaction may be unspecific, since DNase I footprinting did not confirm binding of this activator in the *rpoH* regulatory region. The σ^{54} promoter of the *ibpB* gene was also confirmed to be active in transcription *in vitro*. Preliminary results of DNase I footprinting show that NtrC binds upstream of the *ibpB* σ^{54} promoter, however the question of its influence on *ibpB* expression remains unresolved.

OP1.4

Nonrandom inactivation of the X-chromosome in a girl with Hunter disease

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Hunter syndrome (mucopolysaccharidosis II) is an X-linked recessive disorder, therefore affecting primarily males. Females can be carriers of the disease. In extremely rare cases, Hunter syndrome has been diagnosed in females with abnormal X-chromosome inactivation or chromosomal rearrangements. Hunter syndrome is caused by deficiency of activity of iduronate-2-sulphatase (IDS), an enzyme involved in heparan and dermatan sulphate degradation. Deficiency of IDS results in the lysosomal accumulation and storage of these glycosaminoglycans (GAGs). We report a case of a karyotypically normal girl, diagnosed enzymatically for Hunter disease. Genomic

DNA from the patient and its parents was analysed by automated sequencing for mutations of the *IDS* gene. We revealed a previously described missense mutation A1568G resulting in the substitution of cysteine for tyrosine at position 523 (Y523C). Both mother and patient are heterozygous for the mutant allele and father is a wild-type hemizygote at this locus. To examine the X-chromosome inactivation pattern we determined the methylation status of the X-linked androgen-receptor (*AR*) gene using the methylation-sensitive restriction enzyme *HpaII*. We identified a completely skewed pattern of X-inactivation in the girl-patient. Namely, the paternally derived X-chromosome was inactive and the maternally derived 1568G allele was active. We found no *XIST* promoter mutation that is reported in *Nature Genetics* (17:353-356, 1997 Nov.) to be one of the hypothetical candidates responsible for X-inactivation skewing. We conclude that a skewed X-chromosome inactivation of the paternal gene and a point mutation in the maternal gene were responsible for the Hunter disease in the girl-patient.

OP1.5

Mapping of the *Rhizobium leguminosarum* bv. *trifolii* TA1 genome

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Improved understanding of the rhizobia-legume symbiosis has implications for sustainable agriculture and ecosystem function. For this reason a lot of effort has been put into analyzing both micro- and makro-symbionts genomes. In our study the genomic approach was applied to identify genes involved in polysaccharides syntheses in *Rhizobium leguminosarum* bv. *trifolii* TA1 (RtTA1). Using PFGE electrophoresis we estimated the size of four RtTA1 plasmids. Restriction analysis with rare cutting restriction enzymes allowed us to estimate the size of whole genome (7,3 Mb). Then, physical map of RtTA1 genome was constructed by means of the 2D-PFGE of genomic restriction fragments. To combine genetic and physical maps of the RtTA1 genome, the BAC library of total DNA was constructed (2591 clones). To estimate the insert size distribution in the BAC library, randomly sampled BACs were digested with HindIII. The average insert size was estimated to be 40 kb, with a range of 10-108 kb. DNA sequences derived from random analysis of BAC library and several previously described genes involved in biosynthesis of surface polysaccharides, vitamins, 16S rDNA and nodulation genes were used as a

molecular probes and hybridized to the macro-restriction fragments. Almost all probes derived from the genes involved in polysaccharide biosyntheses hybridized to the restriction fragments assigned to be chromosomal. These data showed that genes involved in the polysaccharide biosynthesis in the strain RtTA1 are localized on the chromosome on contrary to other rhizobial species where they are localized on plasmids.

OP1.6

Mutations of the *NANOS3* gene are associated with testis atrophy and a pure sterility phenotype in men

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The Nanos proteins are highly conserved family of RNA-binding proteins and play critical role in germ cells development in many organisms. There are three homologous proteins in human testes: NANOS1, NANOS2 and NANOS3, and they are encoded by autosomal genes. We previously demonstrated that NANOS1 is crucial for generation and/or maintenance of spermatogonia stem cells in man. The role of NANOS2 and NANOS3 in human germ cell development is still unknown. To approach this issue we screened 124 infertile males with various histological phenotypes of seminiferous tubules (Sertoli Cell Only Syndrome, maturation arrest, hypospermatogenesis or testicular atrophy) for mutations in *NANOS2* and *NANOS3* genes. We found two types of point mutations of the *NANOS3* in two patients and no mutation in *NANOS2* gene. The first mutation was insertion of single amino acid codon, while the second one single nucleotide deletion at +25 position of the intron donor site. These mutations were present only in one allele (the second one being the wild-type) and were absent in 400 fertile men having at least two offspring. The men carrying *NANOS3* mutations were azoospermic (lack of germ cells in semen), had partial testicular atrophy, manifesting with thinning of seminiferous tubules and lack of germ cells except spermatogonia. The testes histological phenotype of the patients carrying *NANOS3* mutations suggests a potential role of this gene in self renewal of spermatogonia. However, further experiments are needed to find out whether mutations we identified cause male infertility.

OP1.7

DNA methylation-dependent expression of the calcyclin gene

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Calcyclin (S100A6) is a small (10 kDa) calcium binding protein expressed in a cell specific manner. High level of calcyclin is observed in fibroblasts and epithelial cells. The gene is located in a tight cluster with other S100 genes on human chromosome 1q21. Calcyclin gene expression can be induced in non-expressing cells by treatment with azacytidine, DNA methyltransferase inhibitor, but not by trichostatin A, a histone deacetylase inhibitor, suggesting that DNA methylation is responsible for suppression of the calcyclin gene ¹. A 500 bp long calcyclin gene fragment encompassing the proximal promoter and the first exon has all features typical for a CpG island. Analysis of cytosine methylation within this region by means of bisulfate modification revealed that cytosine residues are unmethylated in DNA derived from both calcyclin-expressing and non-expressing cells indicating that this region is not involved in cell specific regulation of calcyclin expression. The 3' region of the calcyclin gene is close to a non-coding DNA characterized by the presence of multiple Alu repeats. Bisulfate analysis of DNA fragments within a ~1300 bp region immediately downstream of the calcyclin gene showed differences in methylation pattern with denser cytosine methylation in lymphocytes (no calcyclin expression) as compared to Hep-2 epithelial cells (calcyclin positive). It is thus possible that methylation of DNA proximal to 3' end of the calcyclin gene is responsible for cell specific occurrence of this protein.

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OP1.8

Two GATC sites are necessary and sufficient for stimulation of bacteriophage lambda pR promoter by SeqA protein

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SeqA protein of *Escherichia coli* is a major negative regulator of chromosomal replication initiation. This protein binds GATC sequences in hemimethylated, and under some conditions fully methylated states, but not in an unmethyl-

ated state. However, we found recently that this protein is also a specific transcription factor. Bacteriophage lambda pR promoter is one of promoters positively regulated by SeqA, though mechanism of this transcription activation remains largely unknown. Here we asked which SeqA-binding sites are necessary and sufficient for this activation. There are two GATC sequences located within about 100 bp downstream of the promoter. We found that scrambling one of them (either proximal or distal relative to the promoter) as well as both, results in abolition of the SeqA-mediated stimulation as assessed by measurement of activity of the pR-lacZ fusions. Very similar results were obtained in *in vitro* transcription assays, in which various templates were used. Therefore, we conclude that two GATC sequences, located downstream of pR, are both necessary and sufficient for SeqA-mediated stimulation of this promoter. Therefore, SeqA appears to be one of very few known prokaryotic transcription activators, which bind downstream of the stimulated promoter.

OP1.9

Regulation of the HSPA2 gene expression in testis and cancer cells

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The human *HSPA2* gene is a counterpart of rodents' spermatocyte-specific *Hsp70.2/Hst70* genes. They code for molecular chaperone protein essential for spermatogenesis. However human *HSPA2* gene is expressed also in somatic cells. The function of the *HSPA2* gene in somatic cells is still unknown. Here we present data on the expression of the *HSPA2* gene in testis as well as in selected tumor cell lines. We determined the structure of the *HSPA2* transcripts. RLM-RACE analysis revealed that in testis transcription of the gene is initiated at main transcription start site localized around 120 bp upstream of ATG codon. Next we analyzed expression of the *HSPA2* gene in selected tumor cell lines. By RT-PCR we found that the structure of mRNA transcribed in extra-testicular cells is the same as *HSPA2* transcript synthesized in testis. By Western blot we found that the *HSPA2* protein is expressed only in some cell lines of those which transcribe the *HSPA2* gene. We also analyzed the promoter region of the *HSPA2* gene sufficient for activity of *HSPA2*-CAT constructs. Using transient transfection assay we indicated that the length of the minimal promoter fragment sufficient for CAT activity is cell-type dependent. 243 bp fragment upstream of ATG codon is required for activity in NCI-H1299 and A549 cells (lung carcinomas) but not in HepG2 (hepatoma) and HCT116 (colon cancer). Results of our analysis indicate that the mechanisms regulating activity of the *HSPA2* gene may be cell-type specific. Moreover, it seems that in extra-testicular cells expression of the *HSPA2* gene is regulated on the posttranscriptional level.

OP1.10

Analizis of the factors influencing organization of supercoil domains using *gd* site-specific resolution system in *Salmonella* chromosome

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A typical bacterial chromosome is organized in many independent, dynamic domains of supercoiling which is very important for compaction of DNA in a cell and for the proper course of many genetic processes such as replication, transcription or recombination. Therefore it seems crucial to examine the factors which take part in organization of supercoil domains. A genetic assay utilizing *gd* resolution system was used to study which studies supercoil movement in a large segment of *Salmonella typhimurium*. Process of recombination requires formation of the synaptic complex between the homologous *res* sequences, thus only those sites located within one chromosomal domain may interact. This gives an opportunity to measure the size of the domains *in vivo*, and allows searching for the factors influencing organization of bacterial chromosomes. We used that system to analyze participation of two factors: histonelike protein FIS and the process of transcription as the potential factors involved in supercoiled domains organization. In our studies FIS protein of *Salmonella typhimurium* plays the role of a global regulator for homeostatic control of global supercoil density in DNA but it rather does not organize supercoil domains. The results of these studies point to the role of transcription in formation of superhelical domains in the analyzed region of *Salmonella typhimurium* chromosome. Probably, the knots made on DNA during transcription form a barrier for the motion of interwound supercoils. Consequently, rising transcription levels increases the frequency of barriers formation that define chromosomal domains. This increase is manifested by diminished frequency of site-specific recombination.

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OP1.11

Identification of proteins binding to the 3'UTR of *nad9* gene transcript in cauliflower mitochondria

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The cauliflower (*Brassica oleracea* var. botrytis) *nad9* ORF that code for subunits of the NADH dehydrogenase, the complex I of the mitochondrial respiratory chain, is 573 bp long. Within the 3'UTR (56 bp long) of its transcript, mapped by circularized RNA RT-PCR (CR-RT-PCR), there are small, 4-5 bp repeated sequences that may form secondary structures. Such stem loops are probably recognized by proteins involved in different RNA processing events, including 3' end maturation and transcript stabilization or degradation. Electrophoretical mobility shift assay (EMSA), UV-induced crosslinking and North-Western were used to identify proteins that can bind to 3'UTR of the *nad9* transcript. The specificity of these complexes were checked by using specific and nonspecific competitors. The molecular weight of the proteins interacting with RNA were estimated to 20 kDa, 30-40 kDa, 50-60 kDa and 90 kDa. To purify these proteins, the *nad9* 3'UTR was synthesized *in vitro*, biotinylated on its 5' end and immobilized on Streptavidine-Sepharose column. Cauliflower mitochondrial proteins were purified by affinity to the RNA transcript and the eluted proteins were then analysed by SDS-PAGE and identified by MS. MS analyses indicated the presence of several proteins which belong to metabolic pathways, especially dehydrogenases. Among the proteins we also found the mitochondrial RNA binding proteins — mRBP1a and mRBP2b, which presence was then confirmed by immunodetection. Interestingly, two proteins containing KH RNA-binding and RING finger domain were also indentified. The question whether all these proteins interact with stem loop structure and became factors stabilizing plant mitochondrial transcript are still open.

OP1.12

Regulation of GPVI promoter activation in endothelial cells after collagen and cytokine stimulation

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Collagen is one of the most abundant compounds of extracellular matrix, and serves not only as a structural component of the vessel wall, but also as a substrate for platelet adhesion and activation. In platelets it interacts with several receptors, including two well-characterized membrane glycoproteins, $\alpha 2\beta 1$ integrin and GPVI. The latter is a 62 kD type I transmembrane receptor classified into the immunoglobulin superfamily. GPVI is non-covalently associated with the signal-transducing FcR γ -chain and has now been recognized as a principal platelet re-

ceptor for collagen. Both, genetic damage and pharmacologic blockade of GPVI leads to a loss of collagen signaling in platelets. Until recently, GPVI expression has been restricted to platelets and their precursor cells, megakaryocytes. In 2003, the presence of GPVI expression in cultured human umbilical vein endothelial cells (HUVEC) has been reported (Sun & Platelets, 2003). However, its function in endothelial cells is still not understood. In the present study we attempted to characterize biological agents that may influence GPVI expression in endothelial cells. Changes in GPVI expression were analyzed in HUVECs and immortalized endothelial cell line EA.hy926 at the level of mRNA measured by a real time PCR and at the GPVI promoter activity. The latter was measured in EA.hy926 cells transfected with pGL3-basic containing subcloned the core promoter of GPVI conjugated with a firefly luciferase used as a reporter. Our data show that binding of collagen to endothelial cells significantly up-regulates expression of GPVI. Interestingly, both phorbol 12-myristate 13-acetate and TNF α downregulated GPVI expression. Since the same direction of changes was observed at the level of promoter activity we conclude that modulations of GPVI expression occur at the transcriptional levels.

OP1.13

The role of Hsp70 chaperone system in the Hsp70/Hsp100 dependent disaggregation reaction

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Hsp70 chaperone system co-operates with Hsp100 protein in rescuing proteins aggregated by heat shock or chemical denaturants. However, mechanism of that co-operation, and precise role of each component of that bi-chaperone system in resolubilization and refolding of denatured proteins is still not clear. Using thermally denatured green fluorescent protein GFP we developed an experimental assay suitable for examining renaturation reaction in both real-time fluorescence kinetics measurements and biochemical approaches. We found that ATP-dependent Hsp70 action is a rate — limiting step of renaturation. During this step not only complex formation, but also significant aggregate remodelling takes place, as indicated by gel filtration, gradient centrifugation and light scattering. By experiments with GroEL-trap mutant chaperonin it can be shown that observed change in aggregate size distribution occurs via monomeric protein release. Based on above observations, a model of aggregate renaturation reaction is proposed. In our model Hsp70 acts as monomerizing factor while Hsp100 prevents monomer re-aggregation and allows for a proper refolding.

Posters

P1.1

Association between paraoxonase-1 gene polymorphism and coronary artery disease

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Background — Oxidation of LDL plays an important role in endothelial dysfunction and pathogenesis of atherosclerosis. Paraoxonase-1, an enzyme which is a component of HDL, metabolizes lipid peroxides and protects its accumulation on LDL. Thus activity of PON-1 may influence the risk of atherosclerosis and coronary artery disease (CAD). The PON-1 activity is partially determined by common polymorphism Gln192Arg in the PON-1 gene. The product of Gln (Q) allele has low paraoxonase activity, while Arg (R) — has high activity. Aim — The aim of our study was to estimate possible relationship between a PON-1 gene polymorphism and coronary artery disease (CAD) in the Silesian patients. Materials and Methods — We analyzed 319 individuals including: 139 patients with angiographically documented premature CAD (age 43.6±6.1), and 180 healthy blood donors without familial history of CAD (age 35±10.4). The Q192R polymorphism was genotyped using PCR-RFLP method. Specific primers generate a fragment of 199 bp. Digestion the PCR product by enzyme AlwI gave fragments: 135 and 64 bp. Products were separated on a 8% polyacrylamide gele and visualized by AgNO₃ staining. Results — The R allele frequency is significantly lower in cases than in controls (21.2% vs 32.5%, p=0.002). We also observed statistically lower frequency of R allele carriers in CAD group than in controls (40% vs 52.2%, p=0.02). Especially significant is the difference in the frequency of RR homozygotes (12.8% in controls vs 2.9% in CAD group, p=0.002). Conclusions — Our results suggest that there is an association between Q192R polymorphism in PON-1 gene and coronary artery disease in Silesian patients.

P1.2

Brome mosaic virus (BMV) and barley stripe mosaic virus (BSMV) based vectors: application in gene silencing in plants

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Virus Induced Gene Silencing (VIGS) is currently one of essential methods, successfully applied for functional genomics in plants. So far it has been applied mostly for dicotyledonous plants, like *A. thaliana*, *N. benthamiana*, utilizing potato virus X (PVX), tobacco mosaic virus (TMV) or tobacco rattle virus (TRV). We have prepared a new viral vector, derived from brome mosaic virus (BMV), that can be applied for studies of mono- and dicotyledonous plants' genomes. The vector was successfully used for *gfp* and *pds* (phytoene desaturase) silencing in transgenic *N. benthamiana* and *H. vulgare*, respectively. The experiments showed that only endogene- or transgene-derived insert introduced into BMV RNA3 cDNA cassette in antisense orientation is capable of triggering gene silencing. We conclude that our results are strictly correlated with BMV replication, occurring in spherules that are budded into ER. A successful VIGS application in monocots was also achieved using barley stripe mosaic virus (BSMV)-based vectors. For testing their efficiency barley *pds* silencing was performed. The results showed significant silencing efficiency for BSMV vectors carrying sense, antisense and especially direct inverted-repeats silencing inducing sequences. Therefore BSMV VIGS system has been applied for the functional characterization of barley genes associated with the *Mla13*-mediated resistance towards fungal pathogen *Blumeria graminis* f. sp. *hordei*. The VIGS approach targeted *Rar1*, *Sgt1* and *Hsp90* genes in barley. Their silencing was detected at RNA and protein levels in leaves of main stem and axillary shoots. Additionally, we have observed a resistance breaking phenotype on the BSMV-silenced plants when treated with an incompatible powdery mildew strain carrying *Avr13* gene.

P1.3

DNA damage induced by complexes of ethoxyquin and flavonoids in human lymphocytes

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Ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline, EQ) is widely used in food products and animal feeds because of its powerful antioxidant activity. This compound was recently found to cause many unfavourable side effects (weight loss, increase in the mass of liver and kidney) in animals fed with feeds containing it; also adverse effects in people exposed to EQ at work were

observed [1]. It was also found that EQ is an inducer of pre-neoplastic lesions in the urinary bladder and kidney [2]. Because of that the new compounds, including EQ derivatives, are searched to replace EQ [3]; there are also attempts to synthesize complexes of two biologically active molecules [4]. In the present work we studied the properties of two complexes synthesized from EQ and flavonoids: guercetin and rutin using comet assay; the obtained results were compared with the results obtained earlier for EQ which showed that this compound effectively induced DNA damage in human lymphocytes. The experiments were performed using human lymphocytes obtained from peripheral blood of healthy female donors. The cells were treated with the tested compounds (1–25 µM) for 1 h at 37°C. The results suggest that the complexes of EQ with flavonoids similarly as EQ can induce DNA damage.

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P1.4

Homologous recombination and NHEJ in the double strand breaks repair of *Mycobacterium smegmatis* chromosomal DNA

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In all organisms, genome stability is maintained by a variety of different pathways that they repair the DNA damage. The double strand breaks (DSBs) can appear within DNA due to endogenous or exogenous factors and can be the most lethal form of DNA damage. The unrepaired or misrepaired DNA can initiate processes leading to mutagenesis, tumorigenesis or cell death. The basic mechanisms repairing DSBs are homologous recombination (HR) and non-homologous end-joining (NHEJ). NHEJ is the major pathway for repairing DSBs in mammalian cells. Despite the presence of NHEJ components in budding yeast, *Saccharomyces cerevisiae* repairs DSBs primarily by homologous recombination. Recently, NHEJ system (ligase D and Ku) was identified in several bacterial proteomes (e.g., *Mycobacterium tuberculosis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*). By analogy to the eukaryotic NHEJ, these bacterial Ku and LigD proteins were suggested to participate in a novel prokaryotic NHEJ pathway. The activity of this system was also confirmed by *in vitro* study. The bacterial LigD proteins are multifunctional DNA modifying enzymes composed of an ATP-dependent DNA ligase catalytic domain fused to

putative nuclease and polymerase domains. In our study the unmarked gene deletions were performed to obtain *M. smegmatis* mutants defective in production of LigD; Ku; LigD and Ku; RecA or ligD, Ku and RecA proteins. These mutants were used to observe the participation of LigD and Ku proteins in the repair of DSBs within chromosomal DNA of *M. smegmatis*. However, we have found that the major pathway for repairing DSBs in mycobacterial cells is homologous recombination.

P1.5

The unmarked mutation of the gene encoding for the 3-keto D1 dehydrogenase in *Mycobacterium smegmatis*

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The fast growing mycobacteria degrade natural sterols and use them as source of carbon and energy. The intermediates such as 4-androstene-3,17-dione (AD) and 1,4-androstadiene-3,17-dione (ADD) may be used as precursors for the production of steroid drugs and hormones. The enzyme 3-ketosteroid D¹-dehydrogenase performs the D¹-dehydrogenation of the steroid ring structure and its inactivation may lead to the accumulation of 9a-hydroxy-4-androstene-3,17-dione (9OHAD) from the steroid compounds. The two-step homologues recombination protocol was used to obtain the unmarked deletion of *ksdD-1* and *ksdD-2* genes from *M. smegmatis* chromosome. The resulted mutants were verified by PCR and Southern hybridization. The cholesterol and AD degradation activities of resulted mutants were verified by gas chromatography (GC) analysis. The *Mycobacterium tuberculosis* genome contains two (MT0809; MT3641) and *M. smegmatis* up to six (MSMEG2871; 2873; 4850; 4855; 5801; 5898) ORFs identified as putative 3-ketosteroid D¹ dehydrogenases genes. We found that unmarked gene disruption of MSMEG5898 (*ksdD-1*) inhibiting cholesterol degradation process what results in accumulation of AD. The mutation of *ksdD-1* was complimented by overproduction of MSMEG5898, MSMEG4855 or MT3641 but not MT0809. On the other hand, the gene knock out of MSMEG4855 (*ksdD-2*) did not significantly influence for cholesterol degradation ability of *M. smegmatis* mutant. The double mutant lacking *ksdD-1* and *ksdD-2* genes accumulated AD in cholesterol degradation process but was still able to grow in mineral medium with cholesterol or AD as a soul source of carbon and energy. We concluded the main 3-ketosteroid D¹ dehydrogenase of *M. smegmatis* is MSMEG5898.

P1.6

Identification and computer analysis of a new LEM domain proteins from *Drosophila melanogaster*

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We decided to identify and clone all new LEM domain proteins from *D. melanogaster*. We have designed consensus sequence of LEM motif on the basis of sequences of known proteins containing this region. Designed consensus protein sequence (DDVWNLSDNELRNELNRYGN-NPGPIVGTTRKLYEKKLNLKLRNN) was used as a bait for searching new LEM domain proteins in FlyBase. We have found 5 genes in *D. melanogaster* genome coding for LEM domain proteins. Two of them were identified previously — otefin (CG5581) and bocksbeutel (CG9424). Three other genes: CG3167, CG3748 and CG8679 code for new LEM domain proteins. Identified ORFs were analysed for a presence of structural motifs. We found that gene CG3167 codes for *D. melanogaster* dMAN1 protein, with high homology and structure similarity to human and *Xenopus* protein MAN1. Analysis of a CG3748 gene product, named dLAP2 suggests that this gene may be a *D. melanogaster* ortholog of human protein LAP2 but this needs to be further confirmed. This is because protein homology is not very high and predicted domains are not very similar to LAP2 proteins from vertebrates. Putative product of gene CG8679 possesses also ankyrin repeat therefore we name this protein ARLP (ankyrin repeat LEM protein). Analysis of gene CG8679 led us to conclusion that it can not be a product of alternative splicing of previously reported gene coding for *D. melanogaster* protein MSP300 (an ortholog of human protein nesprin). Analysis of protein sequences of discovered genes indicated that their biological activity may be regulated by cell cycle dependent phosphorylation. Experiments on expression of proteins in bacteria and transfection studies are currently being conducted in our laboratory.

P1.7

Molecular cloning, characterization and protein expression analysis of dMAN1 protein — *Drosophila melanogaster* ortholog of human MAN1 protein

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Using previously designed consensus sequence of Eucaryotic LEM motif we have identified gene (CG3167) coding for *Drosophila melanogaster* protein with high ho-

mology and structure similarity to human and *Xenopus* protein MAN1. The analysis of the open reading frame translation product revealed that protein has molecular mass about 80 kDa, contains LEM domain, two transmembrane regions and RNA recognition motif. Presence of similar domains and sequence similarity to human and *Xenopus* MAN1 allowed us to name this protein as dMAN1. Comparison between sequence of dMAN1 and sequences of known ortholog of protein MAN1 from human and *Xenopus laevis* indicated high level of homology in N-terminal tail (~31% identity, ~68% similarity in fragment between amino acids 1–60) and fragment containing transmembrane regions (~16% identity, ~56% similarity between amino acids 230–430) (in the first transmembrane region ~11.54% identity, 34.5% similarity, in the second one 11.11% identity, 22% similarity). Moreover, analysis of dMAN1 sequence suggested that its biological activity and subcellular localization may be regulated by the cell cycle dependent phosphorylation. In our lab we have generated a series of rabbit antibodies against N – terminal region (containing LEM domain) of *Xenopus* protein XLAP2. After immunoaffinity depletion of serum from anti XLAP2 specific IgGs (on covalently bound antigen), the remaining serum specifically recognized polypeptide with molecular mass about 84kDa, which appears to be dMAN1 protein. dMAN1 protein is present in embryos and larvae but not in adult flies. Western blot analysis suggested that dMAN1 is a nuclear, transmembrane protein. Preliminary experiments using confocal microscopy demonstrated that dMAN1 co-localized with lamin Dm at the nuclear envelope at least during embryogenesis.

P1.8

Polymorphism of DNA repair genes, relation to the repair process and cancer risk

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The level of DNA damage after exposure to genotoxic factors, the rate of its repair, and the efficiency of repair vary considerably in cells of the same type from different individuals. The genetic background is likely to be one determinant of this diversity of responses, and in particular polymorphism in the coding or regulatory regions of genes which encode enzymes for DNA repair, a factor which also affects the risk of cancer. The aims of the present study were: To estimate the changes in the risk of cancer that correlate with the presence of polymorphic variants of the genes *NBS1* (Gln185Glu), *APE1* (Asp145Glu), *XRCC3* (Thr241Met), *XPB* (Asp312Asn and Lys751Gln), *XRCC1* (Arg399Glu). To study the influence of these polymorphic forms on the process of DNA repair.

Polymorphisms were detected by PCR-RFLP using DNA isolated from frozen blood by standard SDS-proteinase K and RNase digestion and phenol-chloroform extraction. Functional tests were performed on lymphocytes isolated from fresh peripheral blood on gradients of Ficoll-Histopaque and exposed *in vitro* to ionizing radiation. Comet and micronucleus tests were used for assessment of DNA damage and repair. The experimental results for samples obtained from 46 head and neck, 33 lung, 30 cervix and 45 colon cancers patients and 84 healthy donors will be discussed.

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P1.9

The role of proteins RI and RIII in control bacteriophage T4 development

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Control of bacteriophage development in the host cells is key process determining its ability to survive in environment. T4 as representative of T-even phages virulent group is not able to lysogenise the host cell. However, T4 is able to adapt its development to growth rate of host cells and, in some conditions, it can stop its development establishing pseudolysogens. Another factor which influences development of T4 phage is superinfection by T-even phage. During this process bacteriophage T4wt prolong lysis time, even by several hours, and this phenomenon is called lysis inhibition (LIN). It is known, that for LIN RI and RIII proteins are responsible, but the mechanism of this process is unknown. Bacteria in natural environment grow with various growth rates. Usually they are much lower than those obtained in standard laboratory conditions. We could set different growth rates of bacteria in minimal medium containing glucose using chemostats in experiments. Cultures of *Escherichia coli* MG 1655 were grown in dilution rates of 0.3 h⁻¹, 0.2 h⁻¹, 0.1 h⁻¹ and 0.05 h⁻¹ what corresponds to generation times of 2.31, 3.47, 6.93 and 13.86 hours respectively. Cultures were infected by phage T4 (wild type), T4rI (defective in the function of the holin inhibitor) and T4rIII. One-step growth experiments were performed and phage development was characterized. Results show increase in latent period length and decrease in phage yield (from 40 to 5) during decrease of bacterial generation time in case of T4wt infection. T4rI and T4rIII mutants developed more rapidly in comparison with wild type phage. Both: latent period and lysis times were different in T4wt and in studied mutant phages.

P1.10

Developmental stage-specific assembly of ParA and ParB complexes in *Streptomyces coelicolor* hyphae

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Bacterial chromosome and plasmid segregation often involve proteins of the ParA (ATPase) and ParB (DNA binding) families. In the mycelial bacterium *Streptomyces coelicolor*, septation-associated chromosome segregation occurs only infrequently during vegetative growth, but it is necessary on a large scale during the formation of chains of unigenomic spores from multigenomic aerial hyphae. Disruption of the *parAB* operon in *S. coelicolor* leads to disturbed chromosome segregation into spores, but has no other obvious effects on colony growth. ParB binds to about 20 *parS* sites clustered around *oriC* region, forming large nucleoprotein complexes that behave differently during vegetative growth and in sporulating aerial hyphae. In vegetative hyphae, foci are small and irregularly spaced but often located about close to hyphal tips. In aerial hyphae in contrast, regularly spaced large foci associated with chromosome separation form immediately before sporulation septation, and they disappear after septation has been completed. Efficient formation of ParB partitioning complexes also depends on ParA, which has a distinctive pattern of localisation; in vegetative hyphae it is found closer to the tips than the ParB foci while in aerial hyphae it forms extended spirals along hyphal tip compartments. Localisation of ParA was independent of ParB. Our results suggest that ParB acts through spatial organisation of the *oriC* proximal part of the chromosome.

P1.11

Purification of proteins binding to the mitochondrial promoter of cauliflower *atp9* gene

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Data on plant mitochondrial DNA-binding proteins are very scarce, despite the fact they are involved in expression and maintenance of mitochondrial genome. We were interested in purification and characterization of proteins showing binding activity to the promoter of the cauliflower *atp9* gene. Purified mitochondria were prepared from

cauliflower (*Brassica oleracea* var. *botrytis*). To identify the location of the 5' termini of the gene, primer extension and CR-RT-PCR experiments were performed. The results revealed the presence of two different termini of the *atp9* gene. The first one resembled promoter consensus motif located at 273 bp from the start of the gene and the second one, without a typical motif, was mapped 115 bp from the ATG codon. In protein purification experiments the *atp9* promoter was represented by three short (50 bp) ds oligonucleotides reflecting different parts of the *atp9* promoter region. The total extract of mitochondrial proteins was partially purified on the Heparine — Sepharose column. The DNA binding activities of the eluted protein fractions were analyzed by EMSA and the molecular weight of individual proteins (complexes) was estimated by Southwestern and UV-induced cross-linking assays. Several candidate proteins of m.w. 25 kDa, 52 kDa and ca. 67 kDa were detected. The results of Southwestern analysis and cross-linking experiments were comparable especially in the case of large protein complexes with m.w. ca. 67 kDa. Our experiments indicate putative protein candidates that could bind to the promoter sequence of plant mitochondrial gene.

P1.12

Transcriptional regulator RosR *Rhizobium leguminosarum* bv. *trifolii* is a prokaryotic zinc finger protein that regulates expression of the *pssA* and *pssB* genes

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The soil bacterium *Rhizobium leguminosarum* bv. *trifolii* synthesizes large amounts of acidic exopolysaccharide (EPS) which plays an important role in symbiotic interaction with clover. Mutants of *R. leguminosarum* bv. *trifolii* deficient in EPS production form empty or partially infected nodules that do not fix nitrogen. The EPS synthesis is complexed and controlled by environmental signals. Recently identified chromosomal *rosR* gene of *R. leguminosarum* bv. *trifolii* encodes a protein of 143 aa (15,7 kDa) that reveals high homology to the family of transcriptional regulators containing DNA-binding motifs of C₂H₂ type zinc fingers. Upstream of the *rosR* -10 and -35 sequences homologous to the *E. coli* Δ₇₀ promoter were identified. A level of *rosR* transcription was determined by deletion analysis of putative promoter cloned in front of the promoterless *lacZ* gene of the broad host range plasmid pMP220. Transcriptional activity of *rosR* was slightly increased at the presence of flavonoids, nitrogen and carbon starvation but significantly reduced in low concentrations of phosphate. In *rosR* promoter, the 44-bp long sequence with 9-bp inverted repeats called

Ros-*box* was found that might be responsible for RosR binding. The *rosR* was required for expression of the *pssA* that encodes glucosyl-IP-transferase involved in the first step of exopolysaccharide units assembly. RosR negatively controlled the expression of *pssB*, that encodes inositol monophosphatase. To study the function and biochemical properties of RosR, we overexpressed and affinity-purified RosR as an N-terminally His₆-tagged protein. A DNA binding ability of RosR protein will be determined using gel retardation method.

P1.13

Effect of Procainamide on transcription of selected signal molecule genes in Jurkat T cells

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Procainamide (PCA) is used to regulate irregular heartbeats to a normal rhythm and to slow an overactive heart. PCA may induce a lupus-like disease in man, but how this drug effect on immune system requires further investigation. It has been suggested that T cells defects can be considered as a primary event in pathogenesis of systemic lupus erythematosus (SLE). The disorders in stimulation of CD4⁺ T lymphocytes can be responsible for alternation in immune system of SLE patients. The direct interaction of the T cell receptor/CD3 complex (TCR/CD3) with an antigen coupled to the major histocompatible complex plays the key role in stimulation of CD4⁺ T lymphocytes. However, the final effect of signal transmission to nuclei depends on contribution of various signal proteins. These proteins mainly include CD3- ζ , FcRIgama, ZAP-70, Syk, LAT and SLP-76 molecules. We determined level of transcripts of ZAP-70, Syk, LAT, SLP-76, signal molecules in Jurkat T cells. Jurkat T leukemia cells were suspended at a concentration 0.5x10⁶ cells/ml in culture medium and incubated without or in the presence of PCA (15, 50, 150 μ M) for 72 hours. After incubation the cells were immediately collected to isolate RNA, which was reverse-transcribed and investigated by Quantitative Real Time-PCR analysis. Jurkat T cells treated with PCA exhibited dose dependent decrease of ZAP-70, Syk, LAT and SLP-76 transcripts level, but PCA did not influence on CD3- ζ transcript level in Jurkat T cells. CONCLUSION: The decrease of ZAP-70, Syk, LAT and SLP-76 transcripts level may contributed in dysfunction of T CD4⁺ cells and changes in the immune system observed in patients with PCA induced SLE.

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P1.14

The frequency of genotypes of the growth hormone (GH) gene in a herd of the Zotnicka Spotted pigs

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Intensive progress in molecular techniques observed in recent years facilitated their wide application both in research and animal breeding. Search for genetic markers for quantitative traits resulted among other things in the detection of point mutations in gene sequences. These mutations are detected using various restriction enzymes. Point mutations were identified within the GH gene both in cattle and pigs (Kirkpatrick, 1992; Nielsen *et al.*, 1995). The effect of the identification in point mutation in the hormone sequence was the development of simple animal genotyping methods with the use of various restriction enzymes e.g. *HaeII* and *MspI* (Kirkpatrick, 1992). The aim of this study was to determine the frequency of genotypes for the GH gene in the Złotnicka Spotted pigs. Experimental material consisted of DNA isolated from blood of 53 Złotnicka Spotted sows. In order to characterize the analyzed pig populations the frequency of GH genotypes were assessed using the *HaeII* and *MspI* enzymes. The identification of genotypes was based on the PCR – RFLP analysis. The frequencies of *HaeII* genotypes: AA, AB and BB in Złotnicka Spotted sows were 18.37, 26.53 and 55.10%, respectively. In turn the frequencies of *MspI* genotypes: CC, CD and DD were 39.02, 29.27 and 31.71%, respectively. In the study by Zwierzchowski *et al.* (1997) conducted on 33 sows coming from matings of Złotnicka Spotted boars with White Large Landrace sows in the course of the determination of the frequency of *MspI* genotypes: AA, AB and BB the following results were obtained: 33.30%, 51.6% and 15.1%, respectively. Studies conducted so far have shown considerable variation between pig breeds in the frequency of individual genotypes (Knorr *et al.*, 1997).

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P1.15

The frequency of genotypes and alleles of ryanodine receptor gene (RYR1) in the Zlotnicka Spotted pigs

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Susceptibility to stress is a trait closely related to the breed of animals (O'Brien, 1993; Houde *et al.*, 1993; Kamyczek *et al.*, 1997; Żurkowski *et al.*, 1995). The frequency of animals with the RYR1 gene within breeds has been verified recently in connection with the implementation into breeding practice of a test facilitating precise definition of animals' breed. The aim of the study was to determine the frequency of RYR1 genotypes and alleles of gene RYR1 in a herd of the Zlotnicka Spotted pigs. Material for investigations consisted of DNA isolated from blood of 53 Zlotnicka Spotted sows kept in the conservative breeding system. In order to characterize the analyzed pig populations frequencies of RYR1 genotypes (RYR^CRYR^C, RYR^TRYR^C, RYR^TRYR^T) and alleles of gene RYR1 were estimated. The identification of genotypes was based on the PCR-RFLP analysis (C1843T) using the *HhaI* restriction enzyme. It was shown that among 53 sows a total of 48 animals (90.57%) were dominant homozygotes (RYR^CRYR^C), while 9.43% (5 head) were heterozygotes (RYR^TRYR^C). In the group of Zlotnicka Spotted sows there were no recessive homozygotes (RYR^TRYR^T). The frequency of alleles of gene RYR1 is as follows: allele C (95.28%) and allele T (4.71%). Literature data assessing the frequency of stress susceptibility genotypes in purebred Zlotnicka Spotted pigs give the following figures: dominant homozygotes 61% and heterozygotes 39%. In turn the frequency of alleles of gene RYR1 reported in literature is as follows: allele C — 82% and allele T — 18% (Żurkowski *et al.*, 1995). Summing up, it needs to be stated that the frequency of stress susceptibility genotypes in pigs and the frequency of alleles of gene RYR1 in the analyzed herd of pigs are low, which makes it possible to eliminate it relatively easily from the analyzed population of sows.

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P1.16

Influence of expression of genes located in *exo-xis* region on phage lambda infection

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The function of majority of phage λ genes is already well known. However, the function of some genes remains unknown. Among them are many "inessential" genes. One of groups of such genes is located between *exo* and *xis* genes of phage λ pL operon. This includes genes *ea22*, *ea8.5* and three ORFs: *orf60a*, *orf61* and *orf63*. These genes were cloned to pGAW3775 plasmid. We noticed that their overexpression in host change phage λ plaque morphology, increasing its turbidity. The effect was most pronounced at 43°C. Altered plaques phenotype was caused by one of genes located on pGAW3775 plasmid, which we identified as *ea8.5*. Increase in turbidity caused by this gene was not caused by increased lysogenisation, as plaques generated by phages λ clb2 and λ cl857 were also turbid. In fact, lysogenisation efficiency was decreased in host bearing plasmid overexpressing investigated genes. The most probable explanation of increased plaque turbidity is high survival ratio of host cells infected with phage. We also observed decreased efficiency of plating of various phage strains at 43°C. We used also single copy *lacZ* fusions to investigate influence of overexpressed genes on phage lambda pL, pI, paQ, and pE promoters activities. Overexpression of genes from *int-xis* region in lysogens also caused increase in both: spontaneous and induced prophage induction. The mechanism of this increase seems to be RecA-independent as it works also in case of λ cl857s7 (ind⁻) lysogen.

P1.17

Efficient preparation of the plasmid vectors for angiogenic gene therapy

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One of the gene therapy strategy are the manipulations on a process of formation of new vessels, commonly defined as angiogenesis. Angiogenic gene therapy is a new therapeutic approach to the treatment of cardiovas-

cular patients. So far, preclinical and clinical studies are successfully focused mainly on the treatment of coronary artery and peripheral artery diseases. Plasmid vectors are often used preparations in angiogenic gene therapy trials. The naked plasmid DNA effectively transfects skeletal muscles or heart and successfully express angiogenic genes which are due to new vessels formation and the improvement of the clinical state of patients. The clinical preliminary data, although very encouraging, need to be well discussed and surely continued. One of the limitations of the gene therapy and non-viral method is the production of pure vectors for a large scale. The main aim of this study was the preparation of the efficient method of plasmid production for the clinic. We propose very effective procedure of the plasmid production from one clone to a large scale. The prepared plasmids have the clinical grade quality. In our opinion the efficient production of the genetic drugs seems to be the resultant of the many factors as time, temperature of the incubation of transformed bacteria, although the procedure of plasmid isolation is also pivotal.

P1.18

PssN is an outer membrane protein, component of polysaccharide export system in *Rhizobium leguminosarum* bv. *trifolii* TA1

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One of macromolecular signals crucial for symbiotic interaction of *Rhizobium leguminosarum* bv. *trifolii* TA1 with clover plants is exopolysaccharide (EPS). Proteins engaged in EPS assembly are proposed to form a complex that transverse both membranes and act in concert in polymerization and translocation of growing EPS chain outside the cell. PssN protein is proposed to act in the last step of EPS assembly — translocation through the outer membrane. In the N-terminal part of PssN protein a signal sequence common to lipoproteins was recognized. Employing a reporter gene fusion approach we evidenced that this part of the polypeptide functions in protein translocation through the inner membrane. We determined that PssN is a membrane protein. Sucrose gradient centrifugation and differential fractionation with Sarkosyl and Triton X-100 of cell envelopes strongly pointed to its outer membrane localization. Comparative trypsin digestion of spheroplasts versus intact cells showed that PssN might not be exposed at the cell surface, but is membrane-embedded with domain(s) exposed to the periplasm. Assessment of PssN by Western immunoblotting after formaldehyde cross-linking of intact cells showed that its monomers as-

sociate into high molecular weight aggregates, probably forming channels in the outer membrane. Further study is necessary to establish the precise function of PssN in the EPS assembly and to find its interacting partners, thus contributing to the overall analysis of the structure and functioning of the EPS synthesis/polymerization/translocation protein complex.

P1.19

***Rhizobium leguminosarum* bv. *trifolii* *pssY* gene product is homologous to a family of galactosyltransferases involved in the polysaccharide synthesis**

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Exopolysaccharide (EPS) produced by *Rhizobium leguminosarum* plays an important role in symbiotic interactions with legumes. The EPS synthesized by *R. leguminosarum* is a polymer composed of repeating octasaccharide subunits which consist of one galactose, five glucose and two glucuronic acid residues with acetyl, pyruvyl and hydroxybutanoyl modifications. The assembly of the unit for acidic EPS of *R. leguminosarum* initiates by the transfer of glucose-1-phosphate to a C₅₅-isoprenylphosphate (IP) carrier by glucosyl-IP-transferase — PssA. The *pssDE* genes encode glucuronosyl-(β1-4)glucosyltransferase and catalyse the second step in the biosynthesis of repeating unit. The *pssC* was identified as encoding a glucuronosyl-(β1-4)-glucuronosyl transferase involved in the third step of the unit assembly. The next steps in biosynthesis of EPS in *R. leguminosarum* bv. *trifolii* remain to be established. The *pssY* gene was identified in the genomic DNA of *R. leguminosarum* bv. *trifolii* during our sequencing project. The PCR product containing *pssY* was cloned and characterized. An ORF encodes a protein of 261 aa length. The PssY displays significant homology to a family of glycosyltransferases, involved in the transfer of galactosyl residues during the polysaccharide units assembly. The role of *pssY* in polysaccharide synthesis was studied by disruption of *pssY* in wild-type strain. The PCR product was cloned into broad host range vector pBBR1MCS-2 and spectinomycin resistance cassette was inserted into the *pssY* gene. Then, the insert with the *pssY*::Sp^r was cloned into pJQ200SK vector and introduced by conjugation into *R. leg. bv. trifolii*. By homologous recombination the *pssY* mutant was isolated and EPS production and symbiotic activity was tested.

P1.20

Characterization of three genes involved in synthesis of exopolysaccharide in *B. subtilis*

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Bacillus subtilis is Gram-positive, soil bacterium which supports plant growth and protects them against pathogenic infection. Mention properties are connected mainly with common bacterial ability to form highly complex communities called — biofilms. Exopolysaccharide (EPS) is thought to be crucial to develop those elaborated structures. Research performed in our laboratory has revealed that deletion mutants in *sigB*, *rsbU* and *rsbP* genes, belonging to *sigB* stress response operon, led to serious defect in biofilm formation. YxaB — hypothetical exopolysaccharide synthetase is supposed to be also under control of s^B factor. Aminoacid sequence analysis of YxaB protein suggests that this enzyme is localized outside the cell and participate in the last stage of EPS synthesis called as a polymerization. Interestingly, deletion of that gene has no impact on biofilm formation in standard conditions (Belicky medium, 30° C, without shaking), which can be partly explain by the fact that *yxaB* gene is expressed only in the particular stress condition. In *B. subtilis* cell we have found two homologues of YxaB protein (YveS and Yvff) encoded by genes belonging to the *yveL-yvff* operon, which includes genes taking part in EPS synthesis. Quantitative researches revealed that over-expression of *yxaB* and *yveS* genes results in two-fold increase the quantity of EPS. Whereas, inactivation of *yvff* gene led to almost two-fold decrease of exopolysaccharide amount and characteristic structural changes. Participation of YxaB and YveS in biofilm formation was also confirmed by the fact that over-expression of those enzymes in $\Delta sigB$ background caused partially restoration of wild phenotype.

P1.21

Involvement of SeqA in replication of Lambda phage and plasmid DNA

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One of the problem we are interested in is to find factors that influence the switch from one type of DNA replication to the another in the case of Lambda DNA. It is generally assumed that Lambda phage DNA during the first stage of its development replicates according to the circle

(theta) mode. Latter under lytic stage of development the switch from the theta to the rolling circle (sigma) mode of replication occurs. Plasmid derived from Lambda DNA theoretically do not replicate according to the sigma mode, but we are able to examine any change between directionality of plasmid DNA replication. In this communication we present results of our studies on the influence of host-encoded protein SeqA on Lambda DNA replication in the absence of DnaA protein and relA function. The influence of DnaA on Lambda replication we have already been demonstrated. In the case of starved relA mutants, new replication complexes are not formed, thus we are able to observe replication driven only by previously synthesized inherited replication complexes.

P1.22

Expression of human homologs of HtrA gene in ovarian and endometrial cancers

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The HtrA family of serine proteases, whose members have been found in most organisms, takes part in cells' protection against stress conditions. So far, at least four human HtrA homologues have been identified. HtrA1 is a candidate tumor suppressor gene downregulated in ovarian cancer cells. It has been shown that its over-expression decreases melanoma tumor growth in mice while downregulation promotes cell proliferation. An evidence exists which indicates that HtrA1 regulates biological processes by modulating growth factor systems such as insulin-like growth factor (IGF) and transforming growth factor (TGF). It has been shown that HtrA1 inhibits TGF β signaling. Another set of data suggest that TGF β can act both as a tumor suppressor during first stages and as a stimulator during late stages of tumorigenesis. HtrA2 promotes apoptosis by binding and degradation of the Inhibitor of Apoptosis Proteins (IAPs). The role of HtrA3 is not well-characterized but high homology to HtrA1 suggests similar functions. The aim of this study was to analyze changes in HtrA1, HtrA2, HtrA3, TGF- β 1 and TGF β -R1 genes expression in healthy and tumor ovarian and endometrial human tissues. We have examined changes in the transcript levels by semi-quantitative RT-PCR technique using β -actin gene as an internal standard. We have found decreased expression levels of HtrA1 and HtrA3 genes in ovarian and endometrial cancers comparing to normal tissues. Underexpression of HtrA1 and HtrA3 genes in ovarian cancer was correlated with the stage of tumor, with lowest expression in the advanced tumors. We did not find significant differences in HtrA2 expression in the examined tissues. HtrA2 and HtrA3

mRNA levels in ovarian cancers have not been analyzed before. Our results further support hypothesis that HtrA1 may function as a tumor suppressor and suggest a similar role for HtrA3. We have found a slightly decreased TGF- β 1 and TGF- β -R1 mRNA levels in the primary tumors and increased levels in advanced ovarian cancers. These results are in agreement with the previously published data. Possible correlation between the HtrA1/3 and TGF- β transcription levels is presently analyzed.

P1.23

Association between angiotensinogen gene M268T polymorphism and coronary artery disease.

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INTRODUCTION — Angiotensinogen (AGT) is a component of renin angiotensin system, and this is peptide precursor of angiotensin II. Many actions mediated by angiotensin II, like vasoconstriction, proliferation and hypertrophy promotion, are potentially proatherosclerotic. AGT gene is located on chromosome 1q42-3. Several AGT gene variants were discovered, and M268T was found to be associated with an increase in plasma AGT concentration and hypertension. **AIM:** The aim of our study was to estimate possible association of AGT gene polymorphism with CAD in cases from Upper Silesia region. **MATERIALS AND METHODS:** We examined AGT genotypes in 163 patients with angiographically documented premature CAD (age 44±6.48) and 157 healthy individuals (age 34±10.62) with negative familial history of CAD. All subjects were white Polish Caucasians from Upper Silesia region (Poland). M268T polymorphism was determined by PCR-RFLP method (BstUI). **RESULTS:** T allele carriers (MT + TT genotypes) are more frequent in cases group, than in controls (79% vs 66%, OR=1.92, p=0.0099). Also the frequency of T allele is significantly higher in CAD group (52% vs 41%, OR=1.56, p=0.0051). There are greater differences in sex-matched subgroups. Men with TT genotype are much more often in cases than in controls (29% vs 16%, OR=2.14, p=0.0237). Carriers of T allele are more frequent in men with CAD than in healthy men (82% vs 63%, OR=2.71, p=0.0018), and than in entire control group (82% vs 66%, OR=2.40, p=0.0034). There are no statistical differences between women subgroups. **CONCLUSION:** Our study indicate, that M268T polymorphism is associated with premature CAD in Upper Silesia region cases, especially in male individuals.

P1.24

Preliminary study on molecular detection and differentiation of plant-parasiting nematodes from genus *Globodera* occurring in Poland

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Potato cyst nematodes (PCN): *Globodera rostochiensis* and *Globodera pallida* are semi-endoparasites feeding on the roots of plants — mainly potato. They cause worse growth, yellow leaves, wilting and loses in yield. PCN are very difficult to control and quite impossible to eradicate. Therefore they have quarantine status, which is the only way to prevent wide spreading of those species. Very important are also establishing of their distribution, removal and also detection. There are some detection methods — mainly morphological or immunological discrimination and identification. However it is relatively easy to differentiate them by observing adult female PCN, juveniles or males discrimination seems unlikely. Additionally those methods are laborious and require strong theoretical background and experience. Therefore molecular diagnostic techniques became recently more frequently applied. In our study we have begun preliminary work on populations belonging to different species of *Globodera*, like *G. rostochiensis*, *G. pallida* and *G. artemisiae* ranging in Poland. We have undertaken their discrimination by using RAPD method. Various primers were tested, that can be utilized to differentiate the *Globodera* species. Because of high variability level in DNA segments used predominantly to detection like ITS1 /2 in rDNA it was impossible to apply methods elaborated in other countries. Thus we have begun to sequence this fragments in genomes of Polish population of *Globodera*. On the basis of that information we are going to prepare appropriate primers that will be used for PCR or PCR/RFLP identification and differentiation of *Globodera* populations occurring in Poland.

P1.25

Organellar transmission in bryophytes

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Both chloroplasts and mitochondria are inherited in plants in a non-Mendelian manner. The mode of cytoplasmic trans-

mission of these organelles has been determined for about 98 families of flowering plants, with about 80% of the species examined displaying uniparental, maternal inheritance and remainder showing biparental inheritance. To our knowledge there is almost no data about organellar inheritance in Bryophytes. This group of plants comprises: mosses, peat-mosses and liverworts. Using molecular markers representing organellar intron sequences we have investigated organellar transmission in the following allopolyploid species of: mosses — *Plagiomnium curvatulum*, *Plagiomnium medium*, *Rhizomnium pseudopunctatum*; peatmosses — *Sphagnum russowii* and liverworts — *Pellia borealis*, *Porella baueri*. We found that in liverworts as well as in mosses both plastids and mitochondria were transmitted uniparentally from one parental species. The following species were the organellar donors for the allopolyploids studied: *Plagiomnium ellipticum* for *P. curvatulum*, *Plagiomnium insigne* for *P. medium*, *Rhizomnium magnifolium* for *R. pseudopunctatum*; *Pellia epiphylla*-species *N* for *P. borealis* and *Porella cordaeana* for *P. baueri*. In the case of *S. russowii* we found that mitochondria and chloroplasts are transmitted probably from different parental species. These data show that there is a general uniparental mode of organellar transmission in Bryophytes, with some exceptions, as in the case of flowering plants. Further studies are necessary to determine whether the transmission in Bryophytes is maternal or paternal.

P1.26

Characteristics of AcpP/AcpM (MSMEG4331/Rv2244) enzyme involved in mycolic acids biosynthesis in *Mycobacterium*

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Mycobacterium tuberculosis is resistant to many antibiotics due to the highly hydrophobic cell envelope consisting of lipophilic molecules among which the most characteristic are mycolic acids. Two types of enzyme systems are involved in their biosynthesis: FAS (Fatty Acid Synthase) I and FAS II. FAS I is encoded by a single gene and it possess capability of *the novo* mycolic acids synthesis. FAS II consists of separable enzymatic proteins responsible for elongation of short acyl chains — products of FAS I system, to long mero chains. In fatty acid biosynthesis the growing acyl is shuttled between the dissociated component enzymes as a thioester of acyl carrier protein (Acp). We have analyzed genes that are a part of operon FAS II: *acpM* (Rv2244) and *acpP* (MSMEG4331), encoding mero-mycolate extension acyl carrier protein. The components of FAS II are putative targets for antituberculosis drug — isoniazid. Expression of *acpP/acpM* is increased in the presence of this antibiotic. The gene has been also classified as essential for growth. We have observed that the amount of mycolic acids in the cell wall of *M. smegmatis*

treated with INH decreased. We have tried to determine whether AcpM activity may influence for pathogenesis of tubercle bacillus. We have used homologous recombination to inactivate *acpP* gene in *M. smegmatis*. We have also purified recombinant AcpM protein in *E. coli* system. The presence of the purified AcpM protein was confirmed by SDS-PAGE, Western-blot and enzymatic tests.

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P1.27

A novel modular polyketide synthase from *Streptomyces coelicolor* A3(2)

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Polyketides are a large and structurally diverse group of natural compounds synthesized in a common mode by repetitive condensations of small carbon units in a manner similar to fatty acid synthesis. Most of the polyketides are produced by bacteria from the genus *Streptomyces*. Their synthesis is carried out by multienzymatic protein complexes — polyketide synthases. There are two main classes of polyketide synthases: type I and II. Type I polyketide synthases are giant multienzymatic proteins in opposition to type II synthases which are complexes of separate enzymes. Here we describe a gene cluster coding for a new type I polyketide synthase, that we named as Kas synthase from *Streptomyces coelicolor* A3(2), a model organism for the genus *Streptomyces*. No polyketide compound is known to be produced by the presented synthase. The synthase is composed of three main subunits, which all together contain 5 extension modules and a loading module. We demonstrate by immunoblotting that the Kas synthase is expressed during transition phase of *S. coelicolor* growth. We also present that Kas synthase is not involved in house-keeping processes. Using sequence comparison to known modular synthases and primary structure analysis of the main synthase proteins we attempt to describe features of the first possible intermediate in the process of biosynthesis of *S. coelicolor* unknown polyketide.

P1.28

Mycobacterium tuberculosis 85B and sigma factor genes expression after exposure to streptomycin.

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The aim of this work was to determine *Mycobacterium tuberculosis* sigE, and H, as well as the reference 85B gene

expression after exposure to streptomycin (STR). Genes expression was analyzed in two *M. tuberculosis* strains cultures: ATCC 25618 strain sensitive to STR, and ATCC 35823 strain resistant to STR. The cells were preincubated, transferred to the Middlebrook 7H9 medium, standardized, and exposed to STR concentrations of 2.0. 4.0. 6.0. 8.0 and 10.0 µg/ml for 1, 12, 24, 48 and 72 hours. Parallel control cultures were provided. Total RNA was extracted by a modified guanidine-phenol-chloroform method. A one-step QRT-PCR reaction was done with ABI-PRISM 7700 sequence detection system (TaqMan). Sequence specific PCR primers and oligonucleotide detector probes labeled with FAM and TAMRA were used. In *M. tuberculosis* ATCC 25618 strain control cultures 85B mRNA was detected from 24h until 72h of incubation. The 85B and *sigma* mRNAs were not detected in 25618 strain cultures exposed to STR. In *M. tuberculosis* ATCC 35823 strain control and exposed cultures, high level of 85B mRNA was detected. *SigE*, and *H* mRNAs were present in all *M. tuberculosis* ATCC 35823 strain cultures. The results of our studies suggest that: 1. quantitative analysis of 85B mRNA enables the determination of STR susceptible and resistant *M. tuberculosis* strains *in vitro*, after 24–72 hours of exposition to all tested STR concentrations; 2. quantitative analysis of *sigE*, and *H* mRNAs may help in the discrimination between STR susceptible and resistant *M. tuberculosis* strains after 48h exposition to the drug.

P1.29

RNA interference-mediated silencing of two *Arabidopsis thaliana* genes encoded nuclear cap-binding proteins

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Nuclear cap-binding protein complex (CBC) consists of two subunits cap-binding proteins (CBP): CBP20 and CBP80 and is involved in several aspects of RNA metabolism. The CBC binds to the cap structure of all RNA polymerase II transcripts and promotes efficient splicing of pre-mRNA, nuclear export of U-rich small nuclear RNAs, mRNA 3' end formation and also plays a role in nonsense-mediated mRNA decay (NMD). In our laboratory we have previously characterized CBC from *Arabidopsis thaliana* (AtCBC), which is also composed of two subunits, AtCBP20 and AtCBP80. An increasing number of genetic mutations that contribute to plant hormones signaling have been characterized. Two of them – T-DNA insertion mutations in *cbp20* and *cbp80* have been described recently that affect response to abscisic acid (ABA). ABA regulates several physiologically important stress and developmental responses throughout the life cycle of plants. During seed development, ABA is respon-

sible for the acquisition of nutritive reserves, desiccation tolerance, maturation and dormancy. During vegetative growth, ABA is a central signal that enables plant responses to various adverse environmental conditions such as drought, salt and cold stresses. *Cbp20* and *cbp80* insertion mutants are recessive and show ABA hypersensitivity in early steps of ABA signaling. We have obtained mutants of *Arabidopsis* with silenced expression of *cbp20* or *cbp80*. using RNAi phenomenon. RT-PCR and Real-time PCR assays showed strongly decreased expression level of both genes studied in silenced plants. The RNAi and knockout mutants of AtCBP20 and AtCBP80 have been compared in the context of phenotype features, drought stress tolerance and reaction on ABA presence. Our observations confirmed high similarity between these plants. Contrary to the wild type, all of them show slightly delayed development and serrated rosette leaf margins, fail to germinate on medium containing 0.3 µM ABA and display an increased tolerance to water deprivation. Our data are consistent with previous observations and strongly suggest that the whole CBC may participate in the transduction of stress signals mediated by ABA.

P1.30

Yeast two hybrid system as a useful tool for testing interactions of mycobacterial cell division proteins.

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The bacterial cell division is effected by a complex macromolecular machinery containing proteins which assemble with a defined dependence hierarchy at the site of division. Many genes and proteins required for division have been defined by classical and molecular genetics especially in *E. coli*, *B. subtilis*. or *C. crescentus*. Very little is known about the molecular aspects of cell division in mycobacteria. Insights into the possible interplay between mycobacterial cell division proteins let us to understand the hierarchy with which they are assembled at the Z ring. We used yeast 2 hybrid assay to study protein-protein interactions. The modular properties of GAL4 and other transcription factors in general fostered this strategy. Indeed many eukaryotic transcription activators have at least two distinct functional domains, one that directs binding to a promoter DNA sequence and one that activates transcription. The DNA binding domain (BD – pAS2-1vector) is on one vector while the activation domain is on the other vector (AD- PGAD10 vector). All tested *M. smegmatis* genes coding FtsZ, FtsW, FtsI, FtsQ proteins are cloned into both vectors, and then these vectors are transformed in to yeast with the reporter gene (β -galactosidase) already present. in contrary to *E. coli* we showed that all tested mycobacterial proteins interact with FtsZ. Generally the yeast two-hybrid assay can iden-

tify novel protein-protein interactions. Secondly, the yeast two-hybrid assay can be used to characterize interactions already known to occur. Characterization could include determining which protein domains are responsible for the interaction, by using truncated proteins.

P1.31

C34T mutation of the *AMPD1* gene affects plasma adenosine and creatinine concentrations in patients with implanted cardioverters-defibrillators

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BACKGROUND: *AMPD1* gene encodes muscle isoenzyme of AMP deaminase. Nonsense mutation C34T has been associated with longer survival in patients with congestive heart failure and ischemic heart disease. This beneficial influence could be caused by increased dephosphorylation of AMP to adenosine. Our aim was to study whether the C34T mutation affects profile of whole blood and plasma purines, lactate, ammonia and creatinine. **MATERIAL:** 112 patients aged 57±12 years with cardioverters-defibrillators (ICD) implanted for secondary prophylaxis of arrhythmia associated with ischemic heart disease or cardiomyopathy. **METHODS:** Venous blood was taken at rest. No "stop-solution" was used to inhibit the metabolism of adenosine in the blood. C34T mutation in exon 2 was detected with the restriction enzyme *Tai* I and C143T mutation in exon 3 was analysed by DHPLC. Concentrations of ATP, ADP, AMP, GTP, GDP, GMP, NAD, NADP, inosine, guanosine, adenosine, uridine, hypoxanthine, xanthine, uric acid and creatinine in heparin-anticoagulated plasma and whole blood were measured with HPLC/UV. Lactate and ammonia concentrations were measured in plasma with spectrophotometric methods. **RESULTS:** C34T and C143T substitutions were in complete linkage disequilibrium. 87 (77.7%) patients were C/C homozygotes, 24 (21.4%) were C/T heterozygotes and 1 (0.9%) was T/T homozygote. 25 carriers of T allele (22.3%) had significantly higher plasma adenosine (median 0.26 vs 0.19 μmol/L, *p*<0.03) and lower creatinine (76.4 vs 83.1 μmol/L, *p*<0.05) concentrations than C/C homozygotes. Whole blood ADP concentrations were slightly higher in T carriers (12.1 vs 11.7 μmol/mmol Hb, *p*<0.04). No other statistically signifi-

cant differences were found. **CONCLUSIONS:** C34T mutation of the *AMPD1* gene does not affect whole blood adenosine, but is associated with higher plasma concentrations. The availability of adenosine for tissues may thus be higher in the carriers of the mutated allele. Lower plasma creatinine concentrations in the carriers suggest some beneficial influence of the mutation on the renal function.

P1.32

Beta2-adrenergic receptor gene polymorphisms and coronary artery disease in Silesian patients

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BACKGROUND: Beta2-adrenergic receptor (*ADRB2*) is cell-surface receptor that activates adenylyl cyclase by coupling to G protein and mediates vasodilation in response to adrenergic agonists in vasculature. Two common polymorphisms: A46>G and C79>G of *ADRB2* gene appear to influence receptor function. *In vitro* studies show that G46 variants demonstrate enhanced downregulation of *ADRB2* while G79 variants seem to be resistant to downregulation. **AIM:** The aim of this study was to investigate association between A46>G and C79>G polymorphisms of *ADRB2* gene and coronary artery disease (CAD) in Silesian patients. **MATERIALS AND METHODS:** We studied 105 patients with angiographically confirmed premature CAD (age 43.4±6.1) and 109 blood donors without familial history of CAD in interview (age 37.5±9.6). All subjects were white Polish Caucasians. The A46>G and C79>G polymorphisms of *ADRB2* gene were assessed by PCR-RFLP method (*Nco*I and *Bse*XI, respectively). **RESULTS:** Genotypes frequencies were in Hardy-Weinberg equilibrium. Frequencies of genotypes and alleles of A46>G polymorphism in CAD group were: AA-34%, AG-31%, GG-35%, A-49% and G-51% and for C79>G polymorphism: CC-37%, CG-49%, GG-14%, C-61% and G-39%. In control group distribution of genotypes and alleles of A46>G polymorphism were: AA-23%, AG-33%, GG-44%, A-39% and G-61% and for C79>G polymorphism: CC-28%, CG-56%, GG-16%, C-56% and G-44%. These differences were not significant. Number of carriers of both polymorphic variants: G46 and G79 also did not differentiate groups. **CONCLUSIONS:** Our results show a tendency to lower frequencies of G allele of A46>G polymorphism and G allele of C79>G polymorphism of *ADRB2* gene in CAD patients but need to be confirm in larger groups.

P1.33

Small interfering RNA as a tool to study CacyBP/SIP function in NB-2a and PC12 cells

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CacyBP/SIP was discovered as a protein that interacted with calcium binding protein, calyculin (S100A6) [1] and Siah-1 [2]. At present, the distribution and structural properties of CacyBP/SIP are well described, but its function remains obscure. For instance, it is known that CacyBP/SIP binds, besides calyculin, some other calcium binding proteins of the S100 family [3] and that CacyBP/SIP is highly expressed in brain tissue, particularly in neuronal cells of rat brain [4]. To elucidate the biological role of CacyBP/SIP we have designed and synthesized siRNA (small interfering RNA) against this protein. This siRNA was used to transfect neuroblastoma NB-2a and PC12 cells, expressing high amount of endogenous CacyBP/SIP. The level of CacyBP/SIP was then monitored in cell extracts by Western blot technique and by immunohistochemistry using anti-CacyBP/SIP antibodies. We found that siRNA against CacyBP/SIP inhibited the expression of this protein, since its level in transfected cells was lower in comparison to control cells. At present, we analyze the effect of diminished expression of CacyBP/SIP on the morphology of NB-2a and PC12 cells and on the activity of ubiquitination pathways.

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P1.34

Genes of different length are non-randomly distributed on prokaryotic chromosomes

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A lot of mechanisms influence non-random location of genes on chromosomes. The most known is organization of genes in operons. For non-random distribution of genes

may be responsible duplication of genes and selection forces placing essential genes on the leading strand and highly-expressed genes close to the origin of replication region. Analyses of 241 prokaryotic chromosomes show that Open Reading Frames (ORFs) of different length are not randomly distributed on chromosomes and they are grouped in clusters containing ORFs of similar length. We have found that in almost all genomes, short ORFs (< 150 codons) have a tendency for clustering which is statistically significant when compared with randomized gene location. These clusters are composed mainly of ribosomal proteins coding genes and ORFs encoding transcriptional regulators and translation initiation factors. Many of clustered short ORFs encode prophage proteins, transposases and insertion element proteins. A great majority of such ORFs are annotated as hypothetical or putative. If an ORF has an assigned function, it is usually clustered with hypothetical ORFs or it is annotated as fragment, truncated or partial. The last group probably represents series of pseudogenes which arose by duplication of fragments of chromosomes. Furthermore, very widespread are clusters of very long ORFs (> 450 codons). These ORFs represent functional genes which were duplicated in tandem and belong to the same protein family (e.g. ABC transporters, polyketide synthases, outer membrane proteins). Identification of clusters of genes and their distribution is helpful in understanding the global organization and evolution of bacterial chromosomes.

P1.35

Phenotypic and genomic diversity of *Robinia pseudoacacia* microsymbionts

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Forty rhizobial strains, rescued from the root nodules of *R. pseudoacacia* grown in Japan and Poland, were characterized for phenotypic properties as well as genomic diversity and compared with reference strains of species and genera of bacteria which form nitrogen fixing symbioses with legumes. They had a moderately slow growth rate, produced acid on yeast-mannitol-agar medium, showed a low tolerance to antibiotics, and moderate resistance to NaCl. Cluster analysis based on the phenotypic features, divided all nodule bacteria included into studies into five phenotypes comprising: (i) *Rhizobium* sp. and *Sinorhizobium* sp. (ii) *Bradyrhizobium* sp. (iii) *R. pseudoacacia* microsymbionts (iv) *Mesorhizobium* sp., and (v) *Rhizobium galegae* strains at similarity coefficient of 78%. *R. pseudoacacia* isolates were placed on single branch together with

Mesorhizobium sp. strains and by this way they were tentatively affiliated into *Mesorhizobium* genus. Such classification of *R. pseudoacacia* microsymbionts was confirmed by analysis of fatty acids methyl esters derived from whole bacterial cells. In Polish isolates 3-hydroxyfatty acids with 14, 15 (*iso*) 16, and 18 carbon atoms characteristics for *Mesorhizobium amorphae* were found, whereas in Japanese black locust microsymbionts 12, 13 (*iso*), and 20 carbon 3-hydroxyfatty acids characteristics for *Mesorhizobium loti* and *Mesorhizobium huakuii* were identified. The composition of saturated and unsaturated fatty acids in all these rhizobia was similar. *R. pseudoacacia* isolates are genomically heterogeneous as indicated by AFLP (Amplified Fragment Length Polymorphism) method. The DNA patterns were found to be highly specific for nearly each strain, although DNA bands specific for most nodule isolates were also noted. These results confirmed the usefulness of AFLP technique for molecular typing of rhizobia and for the rapid screening of their genomic diversity.

P1.36

Increase of neuropeptide Y and decrease of leptin genes expression in hypothalamus of rats with experimental chronic renal failure

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Anorexia is one important causes of malnutrition in the uremic patients. Although the cause of anorexia in uremic patients is not clear, several factors seems to be involved. Neuropeptide Y (NPY) and leptin are involved in the regulation of appetite and energy balance, both in animals and humans. NPY (potent orexigen) mostly produced by the hypothalamus is an important stimulant of appetite and feeding. Leptin is one of an important anorectic hormone which is secreted by adipose tissue. The aim of the present study was to analyse mRNA level of both hormones in hypothalamus of rats with experimental chronic renal failure. Renal failure in rats was induced by two-stage (5/6) subtotal nephrectomy. Using Real-Time PCR reactions with the housekeeping β -actin gene, as internal control, we demonstrated increased NPY mRNA (65%) and decreased leptin mRNA (300%) level, in hypothalamus of rats with experimentally induced chronic renal failure (CRF), as compared to the control rats (fed *ad libitum*). We suggest, that low leptin level due to low leptin mRNA level could be responsible for increase NPY mRNA in hypothalamus of uremic animals.

P1.37

Improved bacteriophage P1 growth on *dksA*-deficient *E. coli* strains.

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DksA, originally identified in *E. coli* as a multicopy suppressor of temperature sensitivity of *dnaKJ* mutations, recently draws plenty of attention as its function in transcription regulation and synergy between DksA and the stringent response alarmon ppGpp action were discovered. *dksA* mutations have pleiotropic defects in cell growth and gene expression. We found that *dksA* deficient strains significantly support growth of bacteriophage P1, commonly used tool in molecular biology. We observed that P1 plaques were much bigger while grown on *dksA* strain comparing to wild type host. Thus, we made an attempt to elucidate mechanism underlying this phenomenon. We studied possible steps in P1 development that could be positively affected by *dksA* mutation. The analysis of P1 growth in time showed minor overall improvement along entire phage yield curve, indicating that growth of the phage might be affected during all steps. This effect was much more pronounced during multi-step growth. The absorption of phage particles on *dksA* cells was moderately more efficient then on wild type cells. The P1 infection did not depend on the growth phase of bacterial culture. The possibility that *dksA* mutation influence the stability of cell membrane resulting in easier phage release has been excluded, indicating that the effect is rather directly on P1 growth. Our studies present evidence that *dksA* influences several steps in P1 development and effects appear to be additive, resulting in improved overall phage yield. Moreover, *dksA*-derived P1 stocks can be used in laboratory work as high-titre, efficient lysates for transduction experiments.

P1.38

Leptin expression in fat tissue and placenta during pregnancy in the rat

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There are many different results among species about leptin expression in placenta and fat tissue. In human, in-

creased leptin level is connected with higher expression of this gene in placenta (Masazuki *et al.*, 1997). In mice and rats, the main source of leptin during pregnancy is fat tissue, whilst in placenta expression was not observed (Malik *et al.*, 2005; Kawai *et al.*, 1997). However, Hoggard *et al.* (2000) reported leptin gene expression in mouse placenta. Also, Garcia *et al.* (2000) using RT-PCR demonstrated leptin expression in rat during pregnancy. Moreover, they found that leptin mRNA level increased during pregnancy. In this experiment, leptin expression at different stages of pregnancy was studied. After fertilization, rats were placed in metabolic cages. Total RNA was used to perform RT-PCR analysis. The cDNA was amplified using multiplex PCR with designed primers for leptin and internal standard HPRT. Result was confirmed by Real-Time PCR. In our study we found that the main source of leptin was fat tissue. However, we found leptin gene expression also in placenta.

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P1.39

The effect of adiposity and leptin on fatty acid synthase gene expression in white adipose tissue of ageing rat

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Ageing in rats is associated with significant reduction of lipogenic enzymes genes expression and the rate of fatty acids synthesis in white adipose tissue (WAT) (1,2,3). Leptin synthesis in WAT is correlated with the mass of this tissue (4). It has been suggested that leptin could be a factor responsible for age-related decrease of fatty acid synthase (FAS) gene expression (5). Ageing in rats is associated with increase of serum leptin concentration and adiposity (6). These two factors (leptin and adiposity) could contribute to age related decrease in FAS gene expression. The aim of this study was to elucidate which factor: leptin or adiposity is more important determinant of FAS activity in WAT of rats. FAS activity in WAT, serum leptin concentration and adiposity index were measured

in 21 male Wistar rats at age from 1 to 27 months. Univariate regression analysis showed strong negative correlation between FAS activity and serum leptin concentration ($r=-0.6$; $p<0.01$) and between FAS activity and adiposity index ($r=-0.7$; $p<0.01$). However, multivariate regression analysis indicated significant negative correlation only between FAS activity and adiposity index ($r=-0.6$; $p<0.05$). Presented data suggest that adiposity is more important determinant of FAS activity than blood leptin concentration.

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P1.40

Age-related changes in the lipogenic enzymes genes expression in brown adipose tissue of rat

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Brown adipose tissue (BAT) is known to be important site for *de novo* fatty acids (FA) synthesis. The controversial findings were reported regarding the effect of age on FA synthesis in BAT. Trayhurn and Wusteman 2 found that in mice lipogenesis reached maximum value at 4–5 weeks of age and there were no major changes thereafter. In contrast, Higgins *et al.* 3 reported that the rate of lipid synthesis was higher in BAT of 32 than 18 weeks old rats. Kochan and Swierczynski (4) found that in rats lipogenesis and fatty acid synthase (FAS) activity reached maximum value between 25 and 35 day of life and then decreases gradually to reach adult level after 2 months. In white adipose tissue (WAT) lipogenesis and lipogenic enzymes gene expression decrease with age (5,6,7,8). The aim of the study was to investigate the effect of age on lipogenic enzymes gene expression in BAT. FAS, ATP-citrate lyase, malic enzyme (ME), glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (6PGDH) activities was measured in BAT obtained from 2 and 9 months old male Wistar rats. FAS and ME protein levels and FAS, ME and 6PGDH mRNA levels were examined in BAT of these rats. Activities, mRNA levels and protein levels of all examined enzymes were higher in BAT of 2 months than 9 months old rats. These results suggest that lipogenic enzymes gene expression in BAT of rats decrease with age.

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P1.41

The promoter fragment with -35 region entirely modified forms open complex with polymerase RNA holoenzyme but is not able to RNA synthesis

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Transcription, the DNA-directed synthesis of RNA, is a major control point of gene expression. The crucial moment for transcription, is the first stage of this process, called initiation. At this stage the recognition and binding of RNA polymerase holoenzyme (RNAP) to the specific sequences of DNA, called promoters, which are composed of two conserved regions -35 and -10, takes place. Our aim was to determine the role of each above mentioned sequences in the binding of RNA polymerase holoenzyme and transcription initiation. A strong A1 promoter of the phage T7 was used in our experiments. This promoter contains -35 hexamer entirely modified, in which consensus sequences TTGACT was substituted by the GAATTC (*EcoRI*). The binding of the RNA polymerase holoenzyme to the promoter fragment was followed by a transcription *in vitro* reaction and a restriction enzyme protection experiments. For these analysis electrophoretic mobility-shift assay was adopted. The results provided evidence that promoter containing completely changed -35 sequence binds RNA polymerase holoenzyme effectively and forms open complex. However, *in vitro* transcription assay showed that its heparin-resistant complex has very low ability to pass to the productive stage of transcription and RNA synthesis. Experiments with digestion by the restriction enzyme *EcoRI* showed that this part of the promoter sequence is in close contact with RNA polymerase holoenzyme and is not accessible for the restriction enzyme. Removal of upstream part of this promoter (digested earlier with restriction enzyme *EcoRI*) interfered significantly with promoter open complex formation.

P1.42

Differential antibacterial activity of genistein arising from global inhibition of DNA, RNA and protein synthesis in some bacterial strains

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Antibacterial activities of various flavonoids have been reported previously, but mechanism(s) of their action on bacterial cells remain(s) largely unknown. Here, we investigated effects of genistein, an isoflavone, and representatives of other flavonoids: daidzein (another isoflavone), apigenin (a flavone), naringenin (a flavanone) and kaempferol (a flavonol), on commonly used laboratory strains of model bacterial species: *Escherichia coli*, *Vibrio harveyi* and *Bacillus subtilis*. We found that *E. coli* was resistant to all tested flavonoids at concentrations up to 0.1 mM, while high sensitivity of *V. harveyi* to most of them (except daidzein, which exhibited significantly less pronounced effect) was observed. Effects of the flavonoids on *B. subtilis* were intermediate relative to the two extremes, i.e. *E. coli* and *V. harveyi*. Action of genistein on bacterial cells was investigated in more detail to indicate changed cell morphology (formation of filamentous cells) of *V. harveyi* and drastic inhibition of global synthesis of DNA and RNA as shortly as 15 min after addition of this isoflavone to a bacterial culture to final concentration of 0.1 mM. Protein synthesis inhibition was also apparent, but delayed. Both cell morphology and synthesis of nucleic acids and proteins was unaffected in *E. coli* cultures under analogous conditions. Studies on cell survival suggest that genistein is a bacteriostatic agent rather than as a bactericidal compound.

P1.43

False positive results of molecular analysis suggesting mtDNA depletion/deletion due to additional PvuII restriction site

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Human mitochondrial diseases are usually caused by dysfunction of mitochondrial DNA (mtDNA), particularly by point mutations, deletions or reduction of mtDNA copy number (called mtDNA depletion). In most procedures

for molecular diagnostics of mitochondrial dysfunction, one of the first steps is linearization of circular mtDNA molecules. The *PvuII* restriction endonuclease is a commonly used enzyme in this step as there is a unique site for this restrictase in human mtDNA (position 2650). We describe a case of false positive results of a Polish patient's mtDNA analysis, which suggested mtDNA depletion or large deletion. More detailed analysis (mtDNA sequencing) revealed that these false positive results were caused by the presence of A12753G substitution in the gene coding for NADH dehydrogenase subunit 5 (ND5). This substitution results in no change in amino acid sequence of the gene product (a silent mutation) but creates an additional *PvuII* site. Two cases of the A12753G substitution were reported previously in the Japanese population, but those patients were not tested for mtDNA deletion or depletion. Therefore, we present a cautionary report indicating that this mtDNA polymorphism (not reported to date in the MITOMAP) exists in human populations as different as Polish and Japanese, suggesting that it may be pandemic and may potentially cause false positive results of standard molecular analyses of human mtDNA. We discovered in the same patient another common polymorphism C12705T in the gene *ND5* (a silent polymorphism) during sequencing analysis.

P1.44

Ghrelin is expressed in ovarian steroidogenic cells and increase progesterone secretion in culture of the preovulatory porcine granulosa cells

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Ghrelin, a 28 amino-acid peptide acylated with *n*-octanoyl group at the N-terminal Ser³, is an endogenous ligand for growth hormone secretagogue receptors (GHS-R). Ghrelin, originally isolated from neuroendocrine cells of the rat stomach, is also expressed in hypothalamus, pituitary, lung, placenta, ovary and testis. The binding of ghrelin to the GHS-R cause its activation and lead to the GH release. Growth hormone secretagogue receptors are mainly expressed in the pituitary, hypothalamus and hippocampus. The aim of this study was to analyze the expression of ghrelin in granulosa and theca cells during the estrous cycle and to verify if ghrelin can exert any effects on steroidogenesis in granulosa cells in *in vitro* culture. Expression of ghrelin was verified by semiquantitative RT-PCR

assay. Data obtain in that study confirm expression of ghrelin in granulosa and theca cells during all stages of the estrous cycle without changes in expression profile. In second experiment, granulosa cells isolated from preovulatory follicles of the porcine ovaries, were stimulated with ghrelin in *in vitro* culture and the medium was collected after 2, 12, 24 and 48 hours for progesterone ELISA assay. The results obtained in this experiment indicate that ghrelin increase progesterone secretion by granulosa cells cultured *in vitro*.

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P1.45

Characteristics of plasmids isolated from clinical strain of *E. coli* CZD1527

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Bacterial plasmids encode an enormous variety of functions including antibiotic resistance, virulence and gene transfer. Therefore, their presence in cell influence properties of bacteria. The clinical strain of *E. coli* that has been analysed is a multi-plasmid strain. With use of two techniques (direct transformation and transformation preceded by insertion of kanamycin transposon), five plasmids from this strain have been introduced into laboratory strain *E. coli* NM522. Two of them, pIGRW12³ and p12¹ do not contain any antibiotic resistance genes. Complete nucleotide sequence of the plasmid pIGRW12³ has been obtained. It is 4995 bp long and encodes mobilization genes *mobA-D*. It shows high homology to plasmid from *Enterobacter cloacae* pEC01 (NCBI Acc. Nr 42733453). Plasmid p12¹ is a bit bigger. Sequencing of this plasmid is in progress, but partial homology to various fragments of plasmid DNA from *Salmonella* and *E. coli* has been proved. Plasmid pK-4, about 3 kb long, carrying resistance to kanamycin and a big plasmid pA-1 with gene of β -lactamase also have been derived from the strain *E. coli* CZD1527. The fifth plasmid p-T15 is about 120 kb long and carries resistance to tetracycline and kanamycin as well as gene encoding β -lactamase of ES β L type. The sequence of this plasmid is to be determined. Already obtained sequence indicate that resistance to kanamycin and β -lactamase is bound up with integron present in this plasmid. The gene of resistance to tetracycline is located in structure of transposone T1721.