Mechanism of the multiprotein RNA polymerase II

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RNA polymerase II (pol II) produces all mRNA in eukaryotic cells. Structures of the ten-subunit core of yeast pol II in free form [1, 2], in complex with DNA and RNA [3], and in complex with alpha-amanitin [4] have elucidated the mRNA transcription mechanism [reviewed in 5, 6]. Based on this work, models for the complete 12-subunit pol II were obtained [7, 8]. We have subsequently solved the first structure of pol II in complex with a transcription factor, the elongation factor TFII S [9, 10]. TFII S stimulates a weak intrinsic RNA cleavage activity of pol II and is required for efficient mRNA proofreading and for escape from DNA arrest sites. A detailed model of this 13-polypeptide 536 kDa complex, derived at 3.8 Å resolution, shows a spectacular binding mode of TFII S to the polymerase surface, and reveals that pol II has a
single tunable active site for both RNA polymerization and cleavage. Very recently we did complete the atomic structure of the 12-subunit pol II both in free form and in form of an elongation complex with bound DNA, RNA, and substrate NTP [11, 12]. After a summary of this work, I will present new structural and functional data that provide insights into the coupling of transcription to mRNA processing [13] and into pol II recycling [14].

References
7. PNAS 2002; 100: 6964–6968.

K1-002
The structure and function of Gfh (GreA2), an inhibitor of RNA polymerase from Thermus thermophilus
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In thermophilic bacteria of Thermus genus there are two GreA-like transcription factors, GreA1 and GreA2 (Gfh) that display opposite functional properties. GreA1, which is a functional analog of E. coli GreA, possesses nucleolytic activity and stimulates transcription elongation, whereas Gfh does not display any nucleolytic activity, but inhibits the activity of GreA1. We demonstrated that Gfh functions as a general transcription repressor that inhibits all enzymatic activities of RNA polymerase (RNAP) including RNA synthesis, pyrophosphorolysis and hydrolysis. During transcription initiation, Gfh decreases the rates of abortive synthesis, affecting both the Vmax and Km for NTPs. During elongation, Gfh increases the Km, and its inhibitory activity is stimulated by Mg-ions. Gfh binds to RNAP through its C-terminal domain (CTD), whereas Gfh’s inhibitory activity resides in the lower portion of the N-terminal domain (NTD) containing the “tip” region with four essential Asp residues. In Gfh–RNAP complexes, the “tip” of NTD reaches the RNAP catalytic center. Gfh and GreA1 bind competitively to RNAP with apparent Kd values of 1.0 and 0.2 μM respectively. We determined the crystal structure of Gfh at 2.6 Å resolution. The overall structure is similar to that of E. coli GreA, however, the CTD of Gfh is flipped and rotated around the linker loop by 120° relative to NTD.

Conclusion: Based on our structural, biochemical and genetic data, we proposed a model in which Gfh could assume two conformations in the cell. In the “flipped” conformation, Gfh is unable to bind to RNAP and is functionally inactive. In the alternate, GreA-like conformation, Gfh binds to RNAP and inhibits its catalytic activity. The Gfh action entails a partial occlusion of NTPs to the active site and an alteration of the geometry of Mg-ion coordination in the catalytic center.

K1-003
Transcriptional regulatory complexes
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Transcription of eukaryotic protein-coding genes by RNA polymerase II is a complex process regulated by a large collection of transcription factors and co-regulatory complexes, including the Mediator of RNA polymerase II transcription and chromatin remodelling and modifying enzymes. Mediator is a multiprotein transcriptional coactivator that is expressed ubiquitously in eukaryotes from yeast to mammals and is required for induction of RNA polymerase II (pol II) transcription by DNA binding transcription factors. We exploited multidimensional protein identification technology (MudPIT) to carry out a proteomic analysis of the subunit composition of the mammalian Mediator complex. By comparing MudPIT data sets obtained from multiple independent Mediator preparations immunoaffinity purified through their different subunits, we have identified a set of consensus mammalian Mediator subunits. In addition, we have identified as Mediator-associated proteins the CDK8-like cyclin-dependent kinase CDK11 and the MEDI3-like KIAA1025 protein (MEDI3L), which is mutated in patients with the congenital heart defect transposition of the great arteries (TGA). We have taken a similar approach to analysis of several chromatin modifying and remodelling complexes, including the TPRAP-Tip60 histone acetyltransferase (HAT) and the SRCAP chromatin remodelling complexes.

K1-004
Regulation of transcription complex assembly in chromatin
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We have used model genes and cellular model systems to analyze the general principles underlying the formation of transcription complexes on human genes. Here we compare inducible simple synthetic model genes with more complex endogenous enhancer-driven genes using biochemical methods, chromatin immunoprecipitations and functional assays. One general result is an impressive mechanistic richness. Specifically the class of general transcription initiation transcription cofactors that will be discussed here underlies a significant dynamics in chromatin that correlates well with gene activation.

K1-005
TBP-Like Protein (TLP/TRF2) is a novel checkpoint factor that regulates TATA-less housekeeping genes
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TBP binds to TATA-box to trigger PIC formation. However, no consensus model for TATA-less genes has been provided. Metazoans express a distantly TBP-related protein referred to as TBP-like protein (TLP/TRF2). TLP does not bind to TATA-box and replace by TBP in an in vitro transcription. TLP binds to TFIIA stronger than TBP. TLP behaves as an unconventional transcriptional activator when it is anchored at a core promoter. In an ordinary reporter assay, TLP preferentially enhances TATA-less
promoters. TLP is thus thought to affect the basal transcription machinery. TLP is not essential for cell growth. We investigated the cellular functions of TLP by using TLP-knockout chicken DT40 cells. TLP prolonged the G2 phase and functioned as a negative regulator for cell growth. Ectopic expression of TLP increased the proportion of cells in the G2/M phase and apoptotic state. TLP-null cells showed an insufficient G2 checkpoint when cells were exposed to stresses such as UV light and methyl methanesulfonate, and the population of apoptotic cells decreased after stresses. Moreover, TLP transiently translocated to the nucleus shortly after stress treatment. Various kinds of stresses induced nuclear translocation of TLP. The expression of several stress-response and cell-cycle regulatory genes, which are categorized in TATA-less genes, drifted both in TLP- and stress-dependent manners. TLP is therefore thought to work for checking cell integrity through its transcription regulatory ability.

K1-006
Regulation of transcription by RNA polymerase II by the multi-functional Ccr4-Not complex

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The highly conserved Ccr4-Not complex regulates mRNA expression at multiple levels. The core complex consists of nine subunits, which were first identified in yeast genetic screens for alterations in basal expression of HIS3 and ADH2. Intriguingly, the Ccr4 and Caf1 subunits represent the major cytoplasmic mRNA deadenylase activities in yeast. In addition, Not4p can act as a RING-dependent protein-ubiquitin ligase. By SGA-screening of not4 alleles in yeast we identified synthetic interactors, which are involved in DNA damage repair pathways. Indeed, ccr4-not mutant strains are HU-sensitive and display a reduced mRNA induction of RNR genes. Analysis of the RNR3 locus suggests impaired histone modifications in ccr4-not mutant strains. Functional analysis of human Ccr4-Not orthologues in transient reporter assays shows that promoter recruitment of CNOT2 and CNOT9 results in a strong transcriptional repression. TSA-sensitivity suggested involvement of HDAC activity in CNOT2-mediated repression. Both overexpression and siRNA inhibition experiments indicate that the N-CoR, SMRT, HDAC3 complex could act as a CNOT2-corepressor. In addition, yeast two-hybrid assays showed that (fragments of) the N-CoR protein interact with CNOT2 and, also, with CNOT1. Interestingly, the CNOT1 protein contains several nuclear receptor interacting domains and CNOT1 overexpression inhibits ligand-dependent transcriptional stimulation by the estrogen-receptor. In conclusion, we have found that several subunits of the Ccr4-Not complex can regulate gene transcription directly. This involves well-known corepressors and links Ccr4-Not function to chromatin modification pathways.

K1-007P
Functionally distinct ADA2-containing histone acetyltransferases in Drosophila

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In several metazoan organisms, there are two genes encoding related, but distinct homologues of ADA2-type transcriptional adaptors. Previous protein purification and protein-protein interaction studies suggested that the two Drosophila ADA2 proteins, together with the histone acetyltransferase (HAT) GCN5, and other factors, form multiprotein transcriptional regulatory complexes. Here we present evidences obtained from in vivo studies that the two ADA2 proteins of Drosophila have distinct and essential functions in development. Ada2a or Ada2b null homozygous animals are late-larval and late-pupal lethal, respectively, and overproduction of one ADA2a protein from a transgene cannot rescue the defects resulting from loss of the other, indicating that the two Ada2 genes of Drosophila have different functions. In the absence of ADA2b the acetylation of lysine 14 (K14) in nucleosomal histone 3 (H3) is reduced, and the localization of TAF10 (TATA binding protein associated factor 10) at several sites on the polytene chromosomes is diminished. In contrast, the lack of ADA2a protein does not affect either H3 K14 acetylation or TAF10 localization, but distorts the structure of the polytene chromosomes. The decreased level of histone H3 K14 acetylation in Ada2b mutants does not affect profoundly the localization of RNA polymerase II, as K14 acetylated H3 is observed mostly with bands, while poll II localizes mostly to the interbands. The two ADA2a also differ in their transcriptional co-activator activity. The data presented demonstrate that the two ADA2 proteins of Drosophila are both essential but functionally distinct.

K1-008P
Functional studies of a TATA-binding repressor (TabR) from Xylella fastidiosa and its target genes

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The TabR protein controls the expression of a set of genes of unknown function arranged in a single operon that is conserved in Xylella fastidiosa and Agrobacterium tumefaciens. TabR is similar to ArsR/SmtB transcriptional repressors, which control tolerance of heavy metals in prokaryotes. However, the TabR operon differs remarkably from the ArsR/SmtB operons since it does not have the effector proteins like thioneins and ATPases. Instead, the TabR operon contains, in addition to TabR, three predicted membrane permeases and a putative glyoxalase II (GloB), a member of the beta-lactamase family. To show whether TabR binds to the DNA upstream the GloB gene, the GloB promoter was fused to the GFP as a reporter gene. Escherichia coli cells carrying this construct showed high constitutive GFP fluorescence, whereas in Xylella and Agrobacterium, fluorescence was barely detected. When TabR was expressed in E. coli carrying the reporter gene, GFP fluorescence dropped sharply, showing that TabR is a repressor in vivo. Electrophoretic mobility shift assays combined with DNaseI footprinting revealed that TabR binds to a TATA box-like region comprising a 9-4-9 palindrome in the GloB promoter. Binding of the TabR to its target DNA was diminished in the presence of cadmium, zinc, copper and iron suggesting that the operon might control metal tolerance in Xylella. However, the regulation of the operon by TabR in response to metals could not be demonstrated in vivo since Xylella and Agrobacterium cells carrying the reporter plasmid did not show significant alterations in GFP fluorescence in the presence of metals. Agrobacterium mutants deficient in either TabR or GloB will be used to evaluate the function of these proteins in metal tolerance or detoxification of organic molecules.
The Na\(^+\) iodide symporter (NIS) is a specific thyroid gene located in the plasma membrane and it is responsible for the uptake of iodine into the thyroid cells. Its synthesis is mainly regulated by the thyroid stimulating hormone (TSH) through the transcription factors Pax-8 and CREB. It has been demonstrated that cholesterol as a component of the plasma membrane can regulate the function of different proteins. Moreover, cholesterol and some oxysterols derived from its synthesis can regulate gene expression through the transcription factor SREBP (Sterol Regulatory Element Binding Protein). In the present study we analyze whether changes in the cholesterol cell content can modify the expression of specific thyroid genes such as NIS and Pax-8. We decrease the cholesterol cell content incubating the PCCl3 thyroid cells in lipoprotein deficient serum (LPDS). Under these conditions we find an increase in the levels of NIS expression. We demonstrate that this effect is due to low levels of intracellular cholesterol and not to inhibition of its synthesis since HMG-CoA reductase inhibitors do not increase NIS expression levels. We also find that this effect is specific for NIS and Pax-8 and not for Thyroglobulin. To study whether the lack of cholesterol regulates transcription of the NIS gene we analyzed the activity of the NIS promoter using a luciferase reported DNA construct containing the full-length NIS promoter. We observed that NIS promoter showed a significant stimulation of transcription by LPDS, whereas the addition of exogenous cholesterol reverted this effect. We also demonstrate that TSH can increase SREBP activity and that LPDS stimulates the phosphorylation of CREB, indicating a crosstalk between the signalling cascades stimulated by TSH and the cholesterol levels to increase NIS expression. Our results indicate that cholesterol levels determine the expression and function of NIS through the transcription factors Pax-8, CREB and SREBP.

K1-010P
A Zymomonas mobilis autoregulated transcription unit controlled by the extracellular osmotic pressure

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Studies on transcription regulation in Zymomonas mobilis, an ethanologenic gram-negative bacterium with unusually high tolerance to glucose concentrations up to 30 g/l, have revealed so far only constitutively expressed promoters. We have recently described the isolation of a DNA fragment on the basis of complementation of a glucose defective mutant that contains four putative open reading frames (ORF1-4) apparently controlled by a single promoter element [1]. The putative promoter (Pglc) was verified by stimulating the expression of the gfp reporter gene. The Pglc promoter was subject to osmoregulation as its activity was induced specifically by increasing the extracellular osmotic pressure with various sugars or salt. Interestingly, it was demonstrated that the product of ORF4 interacts with the Pglc promoter, suggesting an auto-regulated expression mechanism. Additionally, reverse transcription - PCR analysis showed that the gene cluster is transcribed into a single mRNA verifying the operon organization of this transcription unit (glc operon). Further transcriptional analysis by semi-quantitative RT-PCR demonstrated that glc operon expression was regulated by the concentration of glucose, hence supporting its direct involvement in the glucose tolerance mechanism of this bacterium. The individual ORFs were subcloned and their products were overexpressed. Two of the products of this operon appear to be involved in the non-mevalonated isoprenoid biosynthesis pathway, implying indirectly their role to osmoadaptation. This is the first indication of a self-regulated transcription unit in Z. mobilis controlled by the extracellular osmotic pressure.

Reference

K1-009P
Regulation of the Na\(^+\) iodide symporter (NIS) by cholesterol

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K1-011P
In vivo and in vitro effects of HIF-1\(\alpha\) inhibitor, RX0047

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HIF-1\(\alpha\) plays such a major role in activating gene transcription important for maintaining homeostasis under hypoxic conditions, so it is an obvious target for the development of novel cancer therapeutics. This study was performed to determine the antitumoral efficacy of a novel HIF-1\(\alpha\) inhibitor, RX0047 (Rexahn corporation, Rockville, MD, USA) by the Bioluminescence imaging system (BLI) at different times after tumor cell implantation. Various cancer cell lines, including pancreatic (Mia and Panc1), breast (MDA-MB-231, HME 50-T), prostate (PC-3), and lung (A549, H1299), were all sensitive to RX0047 treatment, even in the low nanomolar ranges, as shown by the inhibition of cell growth. For in vivo studies, A549 lung cancer cells were transfected with a lentiviral construct containing the luciferase gene (A549-Luc), injected intravenously for lung colonization model and implanted subcutaneously to the flank area of nude mice. Progressive tumor development in control groups was observed and quantified in living animals in real time using BLI in addition to ex vivo imaging and histology. RX0047, a novel Hif-1\(\alpha\) inhibitor, in addition to inhibiting the growth of various cancer cells in vitro, inhibits the formation of A549 lung metastasis in xenograft animal models and shrinks the tumor size in flank models. Noninvasive in vivo luciferase imaging provides easy visualization of the tumor size and location so the imaging results can be used as an indicator of the treatment effectiveness.
K1-012P
Entamoeba histolytica TATA-box binding protein has the ability to bind to different TATA variants in vitro

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Strains of the protozoan parasite Entamoeba histolytica, responsible for human amoebiasis, have distinct capacity to damage cultured cells and human tissues. The expression of many molecules involved in E. histolytica pathogenicity correlates with its virulence. Thus, the variability in virulence exhibited by E. histolytica strains might be controlled in part by transcription of these and other virulence genes. Certain E. histolytica genes are activated or down regulated during liver abscesses production by trophozoites and during epithelial colonization and invasion. However, we ignore which transcription factors modulate these events. The ability of Entamoeba histolytica TATA binding protein (EhTBP) to interact with different TATA boxes in gene promoters may be one of the key factors to perform an efficient transcription. In this work we studied the EhTBP DNA-binding activity for several TATA variants and determined the TATA-EhTBP dissociation constants (Kd). First, we demonstrated the presence of EhTBP in the complexes formed by nuclear extracts (NE) and TATTAAA oligonucleotide (considered as the canonical E. histolytica TATA box) by gel shift assays. In these experiments a single NE-TATTAAA oligonucleotide complex was detected. This complex was super-retarded by anti-EhTBP antibodies, which also recognized the UV cross-linked complex in Western blot assays. Recombinant EhTBP (rEhTBP) formed specific complexes with TATA variants found in E. histolytica gene promoters and other TATA variants generated by mutation of the TATTAAA sequence. The dissociation constants of rEhTBP for TATA variants ranged between (1.04 ± 0.39) × 10−11 and (1.60 ± 0.37) × 10−10 M. It is noteworthy that rEhTBP affinity for TATA variants is stronger than other TBP variants found. In addition, EhTBP is more promiscuous than human and yeast TBPs, probably due to modifications in amino acids involved in TBP-DNA binding.

K1-013P
Transcript processing of the Rtdpoz genes in the rat testis

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The Tdpoz is a recently discovered gene family [I]. The putative Tdpoz protein contains 365 amino acids, including two functional domains, TD and POZ, located in the amino and carboxyl end, respectively. TD and POZ are derived from tumor necrosis factor receptor (TNFR)-associated factor (TRAF) domain and POZ/ BTB, respectively. The first protein reported to contain both TD and POZ is PPOP, a speckle nuclear protein in human and mice. Based on the sequence of mouse Tdpoz1, exhaustive database searches have uncovered other Tdpoz genes in animal and plant species including mice, human, rat, C. elegans, Drosophila, Arabidopsis and rice. Tdpoz is thus an evolutionary conserved gene family. In BLAST searches, using the complete rat genome sequence database that was published in April 2004, we found 193 hits encoding rat Rtdpoz genes; the Rtdpoz sequences are distributed over 3 chromosomes. RT-PCR and Northern blots have established that the major tissue expressing the Rtdpoz gene(s) is in the rat testis; low but detectable expression is also detected in the liver, brain and heart. By way of 5- and 3'-RACE analysis, we show in this work that two or more Rtdpoz homologs are expressed in the testis. Alignment between the genomic and cDNA sequences indicate that in the testis Rtdpoz genes, the coding sequences are uninterrupted and the transcripts share a common 3-end. On the other hand, the 5-termini of the Rtdpoz transcripts are highly heterogeneous and arise from complex alternative splicing. Intriguingly, some exon 1 sequences are separated from the bulk of the transcripts by > 200 kb and may have been derived from trans-splicing.

Reference

K1-014P
Comparative analysis of the Xenopus C/EBP alpha promoter to mammalian homologues

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CCAT/Enhancer binding protein alpha (C/EBP alpha) liver enriched transcriptional activating protein is a member of the C/EBP family of transcription factors and is involved in hepatocyte specific gene expression and in the process of tissue differentiation. We present here the molecular characterization of the promoter of Xenopus C/EBP alpha gene. The sequence comparison of the promoter with mammalian C/EBP alpha promoters showed the sequence of Xenopus C/EBP alpha promoter is quite diverged compared to mammalian counterparts. Comparison of the 5’-UTR shows that the conserved open reading also is also conserved in Xenopus gene. CpG analysis of the promoter revealed that mammalian promoters have a CpG islands whereas this GC rich region is absent in Xenopus promoter. Our functional analysis on Xenopus promoter reveals that another family member, C/EBP delta, is able to autoactivate the Xenopus C/EBP alpha gene in common with C/EBP alpha and LAP genes whereas LIP (Liver inhibitory protein) that is a shorter form of C/EBP alpha cannot transactivate the gene transcription. Our cotransfection experiments with USF and CREB expression plasmids also reveal that Xenopus C/EBP alpha promoter could be activated by USF implicated to play important role in indirect autoregulation of the human C/EBP alpha gene and CREB that is regulator of C/EBP alpha gene.

K1-015P
Identification of cis-regulatory elements controlling two differentially expressed Pit-1 genes in the duplicated genome of Cyprinus carpio

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The pituitary-specific transcription factor Pit-1 is involved in transcriptional regulation of a network of hypothalamo-hypophysial
Abstracts

Factors including prolactin, growth hormone and its own gene. According to our working hypothesis, Pit-1 plays an important role in regulation of the adaptive response to cyclic environmental changes in the ectotherm teleost Cyprinus carpio, a tetraploid vertebrate that is functionally diploidized. In the duplicated genome of the carp, two Pit-1 genes exist and transcripts of both genes were detected by RT-PCR analysis. Mainly Pit-1 gene-I transcripts were observed by in situ hybridization in pituitary gland sections of winter-acclimatized carp, where overall Pit-1 expression is significantly lower as compared to summer-acclimatized fish. Gene copy specific characteristics in the architecture of these naturally evolved promoter sequences comprise the transcription start site and the 5'-UTR region. Electromobility shift assays using carp hypophysal nuclear extract revealed binding of pituitary factors to sites conserved in both promoters (CRE, Pit-1) and to a site unique to the gene-I promoter. We are currently analyzing the in vivo associations by ChIP. Our data suggest that the specific control elements identified in the proximal regulatory region of the two Pit-1 genes are physiologically relevant and may be related to the differential expression of the duplicated Pit-1 genes in carp. Furthermore, in an interspecies comparative context, these studies could shed light on the dynamics of duplicate vertebrate genomes. Acknowledgment: Supported by grant DID-UACH-2004-57, AGCI/CGRI-DRI-10/01068-3.4, FNC1040073.

K1-016P
Characterization and expression studies of three Hypoxia-Inducible Factors from the grass carp
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Hypoxia-inducible factors (HIFs) are heterodimeric transcription factors that upregulate genes involved in erythropoiesis and angiogenesis to increase oxygen delivery, and/or elevated glucose uptake and glycolysis for non-oxidative energy production. Numerous studies have shown that HIF proteins play a key role in oxygen homeostasis in mammals, but the function of HIFs in fish is still poorly known. This report describes the cloning and functional characterization of three HIF-alpha isoforms - gcHIF-1α, gcHIF-2α and gcHIF-4α - from the grass carp. The deduced proteins of gcHIF-1α and gcHIF-2α share 62-72% sequence identity with the corresponding mammalian and fish homologues, and contain various characteristic sequence motifs present in all mammalian HIF-αs including the basic helix-loop-helix dimerization domain, PER-ARNT-SIM domain, oxygen-dependent degradation domain, transactivation domain and nuclear localization signals. Computer analysis indicated that gcHIF-4α is a novel HIF-α isoform, which has not been reported previously in other animals. Northern blot analysis revealed upregulation of gcHIF-1α, gcHIF-2α and gcHIF-4α in various tissues of 4-h hypoxically stressed grass carp. Ecopic expression of the three gcHIF-α cDNAs in CHO Ka13 cells (deficient in HIF-1α) showed that gcHIF-2α significantly upregulated expression of the endogenous vascular endothelial growth factor (VEGF) gene as well as HRE-driven luciferase reporter genes.

K1-017P
Activation of different defence-related genes expression by ergosterol
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Ergosterol is the main sterol of most fungi. Production of reactive oxygen species by NADPH oxidase, alkalization of extracellular space and increase of cytosolic concentration of calcium after the ergosterol treatment on tobacco cells was previously observed as well as the activation of some stress activated mitogen-activated protein kinases on alfalfa cells, even in nano-molar concentrations. Ergosterol activates a signal pathway including phospholipase A2 and protein kinase C. Here, we report about the expression of some defence-related genes after the treatment of tobacco cells and plants with nano-molar concentration of ergosterol. The gene expression of pathogenesis related proteins of families PR1, PR3, PR5 and proteinase inhibitors together with enzymes as phenylalanine ammonia lyase, sesquiterpene cyclase and lipoxygenase was observed by a real-time PCR. In addition, the concentrations of some phenolic compounds and phytoalexins increased in time. On the other hand, ergosterol did not provoke any leaf necrosis or cell death and the accumulation of PR proteins was slower when compared with another well-defined elicitor cryptogein from Phytophthora cryptogea. These data shows that ergosterol is able to activate expression of a number of defence genes and could increase the resistance of plants against the pathogens.

K1-018P
Possible similarities between the subunit of eukaryotic TFIIIC and the bacterial transcription factor
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Eukaryotic genes for small RNAs and short interspersed repetitive elements have internal promoters. They are transcribed by RNA polymerase III (RNAP III). RNAP III requires the multi-subunit protein factor TFIIIC in transcription initiation. TFIIIC contains the B-block binding subunit, which recognizes the internal promoter. B-block binding subunits are diverse in eukaryotes, but there are several domains with conserved sequence similarities. The bacterial IS1 is a small DNA segment, which transposes to numerous sites on chromosomes and plasmids. IS1 appears as a repetitive sequence in the bacterial genome. The internal region of IS1 acts as a cis-element to stimulate RNA synthesis from the upstream promoter. The product of the bacterial artA gene works with the IS1 internal sequence to stimulate transcription. Both of the bacterial ArtA protein and the eukaryotic B-block binding subunits act with the regions downstream from the transcription initiation sites. Thus, I compared the primary structure of ArtA with those of the B-block binding subunits. Interestingly, amino acid sequence of ArtA was partially similar to those of the N- and C-terminal regions conserved in many B-block binding subunits: several aromatic residues are well conserved in the bacterial and eukaryotic sequences. Additionally, the N- and C-terminal regions of the S. cerevisiae B-block binding subunit are tentatively identified as HMG boxes, which is a DNA binding motif. HMG boxes have a loose consensus sequence, and aromatic residues are conserved. I will discuss the functional relationship between the transcription machinery of RNAP III and that with bacterial ArtA.

K1-019P
Evidence for the regulatory role of LMG160 protein on DNA transcription in vitro
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Low mobility group (LMG) proteins are a group of non-histone proteins characterized by their relative mobility through
electrophoresis and are very heterogeneous with a wide spectrum of chromatin proteins. In this study a fraction of these proteins with a molecular weight of 160 KDa, designated as LMG160, was isolated from rat liver and its effect on DNA transcription was studied in cell-free RNA-synthesizing systems. Using in vitro transcription systems in the presence and the absence of LMG160 showed that this protein causes a strong inhibition of the DNA-templated RNA synthesis in a dose dependent manner; as in an LMG160: template DNA ratio of 1:1, a completely inhibition was observed. Concerning our previous research demonstrating the presence of LMG160 in the nuclear matrix extract of the hepatocyte cells of the rat liver tissue, and since the nuclear matrix is known as a network anchoring important activities such as DNA replication, transcription, DNA repair, dNTP synthesis and so on, this finding suggests a regulatory role for LMG160 on DNA transcription in the nuclei.

K1-020P
Role of the RPB4 subunit of yeast RNA polymerase II in transcriptional regulation
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A major role in the regulation of eukaryotic protein-coding genes is played by the gene-specific transcriptional regulators, which recruit the RNA polymerase II holoenzyme to the specific promoter. Recently was shown that Rpb4, a non-essential subunit of RNA polymerase II, is involved in activation of subset of genes in Saccharomyces cerevisiae [1]. Conserved from archaea to humans, Rpb4 forms a sub-complex with Rpb7 that easily dissociate from the polymerase under mild denaturing conditions. rp4 yeast strains grow at moderate temperatures slowly, cannot survive at temperatures above 32 °C or below 15 °C. Recently was found strong genetic interaction of RPB4 and other genes coding components of transcription machinery, RPB9, SPT8 and DST1 [2]. The activity of RNA polymerase II is tightly regulated by a number of factors. The phosphorylation state of its C-terminal domain (CTD) makes it competent either for initiation or elongation of transcription. Although many kinases are known to phosphorylate the CTD, so far only one phosphatase, Fcp1 (TFIIF-dependent CTD phosphatase) has been found to be responsible for the dephosphorylation. We have identified and isolated the gene of the Drosophila melanogaster homologue of Fcp1 and expressed the protein in bacterial and human cells. We found that coexpression of Fcp1 decreased the activity of several promoters in a heterologous HeLa transient transfection system. In order to study the function of Fcp1 in vivo we generated Drosophila lines that carry transgenes for overexpression or silencing of Fcp1. Examination of these lines revealed Fcp1 as a essential component of the transcription machinery as both kinds of lines are lethal. The biochemical experimental possibilities of CTD phosphorylation and the well-developed genetic tools provided by the Drosophila system gives us a unique opportunity to examine the different roles of Fcp1 in transcription regulation.

K1-021P
Functional cooperation between FACT and the MCM helicase complex facilitates chromatin DNA replication
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Chromatin is supressive in nature to cellular enzymes that metabolize DNA, mainly due to the inherent inaccessibility of the DNA template. Despite extensive understanding of the involvement of chromatin-modifying factors in transcription, role of related activities in DNA replication remains largely elusive. Here we show that the transcriptional elongator FACT is functionally linked to DNA synthesis progression. Its involvement in DNA replication is partly mediated by the stable association with the replicative helicase complex, MCM, and further by the coexistence with MCM on replication origin. Furthermore, reliant on its nucleosome-reorganizing activity, FACT possesses the ability to facilitate chromatin unwinding by the MCM complex, which is otherwise inert on the nucleosomal template. As a consequence, FACT positively regulates DNA replication and S phase progression in vivo, and such replicative role is evolutionarily conserved. Together, our findings identify FACT as an integral and conserved component of the endogenous replication machinery, and further outline a model in which the concerted action of helicase and chromatin-modifying activities promotes chromatin replication.

K1-022P
Structure and function of the Drosophila melanogaster Fcp1 phosphatase
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The activity of RNA polymerase II is tightly regulated by a number of factors. The phosphorylation state of its C-terminal domain (CTD) makes it competent either for initiation or elongation of transcription. Although many kinases are known to phosphorylate the CTD, so far only one phosphatase, Fcp1 (TFIIF-dependent CTD phosphatase) has been found to be responsible for the dephosphorylation. We have identified and isolated the gene of the Drosophila melanogaster homologue of Fcp1 and expressed the protein in bacterial and human cells. We found that coexpression of Fcp1 decreased the activity of several promoters in a heterologous HeLa transient transfection system. In order to study the function of Fcp1 in vivo we generated Drosophila lines that carry transgenes for overexpression or silencing of Fcp1. Examination of these lines revealed Fcp1 as a essential component of the transcription machinery as both kinds of lines are lethal. The biochemical experimental possibilities of CTD phosphorylation and the well-developed genetic tools provided by the Drosophila system gives us a unique opportunity to examine the different roles of Fcp1 in transcription regulation.

K1-023P
Expression of the alpha2-macroglobulin gene in rat liver involves participation of hepatocyte nuclear factor-6
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Turpentine-induced acute-phase (AP) response in rats is followed by transcriptional activation of the alpha2-macroglobulin (alpha2-MG) gene mediated by cytokine interleukin-6 and glucocorticoids. It was previously shown that members of two families of transcription factors - CCAAT/enhancer binding protein (C/EBP) and Signal Transducers and Activators of Transcription (STATs), mostly regulate the expression of alpha2-MG gene.

Based on nucleotide sequence analysis of promoter region in the alpha2-MG gene we postulated that binding of hepatocyte nuclear factor-6 (HNF-6), a novel liver enriched transcription factor, also might participate in transcriptional regulation of this gene. HNF-6 represents the prototype of a new class of cut-homeoproteins that contains a bipartite DNA binding region consisting of a single cut domain and a highly distinctive homeodomain. By Western immunoblot analysis the presence of two HNF-6 isoforms in the liver nuclear extracts of untreated and turpentine-treated rats was established. The relative concentration of both HNF-6 isoforms remained unchanged during the turpentine induced AP response. Using the combination of DNA affinity column coupled with the alpha2-MG hormone response element and Western immunoblotting we found same DNA binding ability of HNF-6 under basal and AP conditions. Our results revealed for the first time that HNF-6 could bind alpha2-MG gene and participate to the regulatory network of factors that control primarily basal expression of the alpha2-MG gene.

K2 – Epigenetic Regulation of Transcription

K2-001
Dosage compensation: old complex new facts
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Dosage compensation mechanisms ensure equal dosage of X-linked genes. In *Drosophila* this involves hyper-transcription of the male X chromosome. Genetic studies have identified five proteins and two non-coding RNAs important for regulating this process. However, it is unknown whether additional proteins associate with MSL proteins to regulate the X chromosomal genes. Here we report the purification of the RNA/protein containing dosage compensation complex from *Drosophila* embryos and Schneider cells. Using TAP-tagged MOF as well as Flag-tagged MSL-3 we have purified a functionally active complex that acetylates histone H4 at lysine 16. We find stable association of MSL-1, MSL-2, MSL-3 MOF as well as intact roX1 and roX2 RNAs. Characterization of new associated members is ongoing and the progress will be discussed.

K2-002
Dosage compensation in *Drosophila*: mechanism, models, mystery
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Dosage compensation in fruit flies involves doubling the transcription of genes on the single male X chromosome as a way of adjusting their expression to the levels originating from the two female X chromosomes. Fine-tuning gene expression is vital for fruit flies: Failure leads to male-specific lethality. Dosage compensation is regulated through modification of chromatin. Acetylation of histone H4 at lysine 16 by the acetylase MOF is crucial. MOF is recruited to the X chromosome as part of a dosage compensation complex (DCC) consisting of other male-specific lethal (MSL) proteins and non-coding roX RNA. We are searching for determinants for efficient interaction of DCC with X chromosomal targets. So far the DNA sequences on the X chromosome and the interacting DCC structures have been elusive. Using a Chromatin Immunoprecipitation approach we monitor the interaction of DCC within 2.6 Mbp of the *Drosophila* X chromosome. The representation of MSL protein binding along the chromosome reveals a “patchy” association, hinting at sequence-specific binding determinants, but also identifies direct target genes. Ablation of MSL proteins by RNA interference leads to an approximately twofold drop of target gene expression. We have isolated a series of *in vivo* binding sites for the DCC by Chromatin immunoprecipitation. Characterizing the DCC binding to these sites in various genetic backgrounds reveals a gradient of affinities and suggests ways of how DCC is targeted to and distributed over the male X chromosome. Our ongoing characterization of one novel DCC binding site at high resolution will be presented.

K2-003
Higher-order chromatin and chromosome folding in transcription
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The activity state of a gene can often be correlated with its intranuclear localization to specific sub-regions and/or position relative to constitutive heterochromatin. Active genes are often found toward the interior of the nucleus whereas silent genes are often associated with the nuclear periphery and/or heterochromatic clusters. Thus epigenetic modifications to chromatin correlate with differential nuclear positioning and gene activity states. Nascent RNA production and RNA polymerase II (RNAP II) are compartmentalized into discrete foci or “transcription factories”. Individual transcription factories contain a 1000-fold higher local concentration of RNAP II than the surrounding nucleoplasm. By RNA FISH we find that nearly all transcription of individual genes takes place in these transcription factories. Transcription of most “active” genes is not continuous but oscillatory, with transcription switching on and off in conjunction with gene movements into and out of transcription factories. We have shown that the number of transcription factories per cell nucleus is severely limited compared to the number of active genes and transcription units in several mouse tissue-types. This compels many genes to share the same transcription factory and we have
shown that genes as far as 40 megabases apart co-localize in the same factory at exceptionally high frequencies. Here we extend this analysis for several genes over mouse chromosome 7 as well as other mouse chromosomes. Our results indicate that epigenetically potentiated genes dynamically engage transcription factories in order to be transcribed and thus highly dynamic chromatin movements and higher-order chromatin and chromosomal folding play major roles in regulation of gene expression.

K2-004
The role of chromatin architecture in gene transcription
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Activation of transcription is mediated through the recruitment of chromatin modifying and remodeling factors, which alter the local chromatin structure to generate a permissive environment. Chromatin modification and subsequently remodeling occur in a precise order determined by the local chromatin structure. Here we show that proinflammatory cytokines induce interleukin-8 (IL-8) transcription in epithelial cells but not in B cells, despite the fact that the transcription factors known to regulate IL-8 expression (NF-kB and C/EBP) are present and/or activated in every cell type. Here we show that the lineage-specific transcription of IL-8 is due to the distinct chromatin architecture on the enhancer-promoter of this gene. More specifically, in expressing cells the enhancer/promoter is nucleosome-free whereas in non-expressing B-cells there is a nucleosome masking the entire regulatory region of the gene. The B-cell nucleosome contains the histone variant macroH2A, thus obstructing both transcription factor binding and nucleosome remodeling in vivo. Depletion of macroH2A by siRNA in B-cells leads to activation of IL-8 transcription. Recruitment of the macroH2A nucleosome to the IL-8 enhancer in B-cells requires a nearby AP1 site and siRNA against some of the AP1 proteins rescues IL-8 transcription in B-cells. Thus, AP1 proteins recruit the macroH2A nucleosome leading to lineage-specific silencing of IL-8 expression.

K2-005
Novel small-molecule inhibitors of DNA methylation
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DNA methyltransferase enzymes are promising targets for cancer therapy. In many cancer cells promoters of important genes are hypermethylated, which results in gene inactivation. It has been shown that DNA methyltransferase inhibitors can suppress tumor growth and have significant therapeutic value. However, established inhibitors are limited in their application potential because of their mechanism-dependent enzyme inhibition causing high toxicity. The aim of our work was to find new structures that would be mechanism-independent inhibitors of human DNA methyltransferases enzyme. To discover such compounds we have searched the Diversity Set of the National Cancer Institute database using a previously established three-dimensional model of the human DNA methyltransferase 1 (DNMT1) active site and a novel docking and scoring procedure. The result of our in-silico screen is a set of compounds that have high affinity to the active site and a different structure than original substrate - cytosine, which suggests a mechanism-independent enzyme inhibition. Two of the most promising structures discovered in this screen were tested on a human cancer cell line and were found to be potent inhibitors of genomic DNA methylation. These compounds can provide a new approach to the design of inhibitors for the methylation process and could also be used as a base for further rational drug development.

K2-006
Epigenetic repression in living Drosophila. Stability and dissociation constants of Polycomb group Protein-chromatin complexes
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PcG genes products act in multimeric complexes that associate with specific sequences in the genome called Polycomb Repression Elements (PREs) and inhibit transcription of the associated genes throughout development. We have addressed fundamental questions about the stability and lifetime of PcG repression complexes in living organisms on their target sites to understand the mechanism by which long-term silencing of chromatin over many generations is also compatible with the ability to modulate the transcriptional state. Green fluorescent protein (GFP) fusion proteins of two PcG proteins, polycomb (PC) and polyhomeotic (PH), have been constructed and introduced into transgenic flies. We performed fluorescence bleaching and recovery studies (FRAP and a new technique, 3D- inverse FRAP) in living embryos and live larval tissues of these transgenic stocks. Using multicomponent data analysis and simulations we have determined diffusion rates and dissociation constants for the proteins during Drosophila development. These are the first FRAP experiments that we know of on whole living animals. In embryos we found dissociation rates of koff = 0.047/s for PHGFP and koff = 29/s for PCGFP. In larval tissue 85% of the complexes had these same rates whereas 15% of the loci were slower but fully exchangeable in less than 6 minutes. By multicomponent analysis, we were able to analyze complexes on individual polytene chromosome bands in intact glands. Reproducible individual dissociation rates presumably reflect the specific mixture of PcG and non-PcG auxiliary proteins at individual PREs. Our data demonstrate that the mechanism of long-term silencing maintained over 14 cell generations is achieved by mass action equilibria of freely dissociable complexes undergoing continuous exchange and thus available for epigenetic reprogramming by changes in the local concentration of proteins that compete for the same binding sites.

K2-007P
Identification of a DNA methylation-dependent activator sequence in the pseudoxanthoma elasticum gene, ABC6
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ABC6 encodes MRP6, a member of the ABC-protein family with unknown physiological role. The human ABC6 and its two pseudogenes share 99% identical DNA sequence. Loss-of-function mutations of ABC6 are associated with the development of pseudoxanthoma elasticum (PXE), a recessive hereditary disorder.
affecting the elastic tissues. Various disease-causing mutations were found in the coding region, however the mutation detection rate in the ABC6 coding region of bona fide PXE patients is only approximately 80%. This suggests that polymorphisms or mutations in the regulatory regions may contribute to the development of the disease. Here, we report the first characterization of the ABC6 gene promoter. Phylogenetic in silico analysis of the 5′ regulatory regions revealed the presence of two evolutionarily conserved sequence elements embedded in CpG islands. The study of DNA methylation of ABC6 and the pseudogenes identified a correlation between the methylation of the CpG island in the proximal promoter and the ABC6 expression level in cell lines. Both activator and repressor sequences were uncovered in the proximal promoter by reporter gene assays. The most potent activator sequence was one of the conserved elements protected by DNA methylation on the endogenous gene in non-expressing cells. Finally, in vitro methylation of this sequence inhibits the transcriptional activity of the luciferase promoter constructs. Altogether these results identify a DNA methylation-dependent activator sequence in the ABC6 promoter.

K2-008P
Studie on the position of non-histone protein, LMG160 in chromatin
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Purpose: Cadherins are cell-cell adhesion glycoproteins, which present distinct adhesive specificity and distribution in human tissues. P-cadherin, expressed in normal breast myoepithelial cells, has been reported in breast carcinomas, where it is associated with high-grade histological tumours and proliferation. This study aimed to analyse the expression of classical cadherins in invasive breast cancer and to correlate them with breast tumour biological markers, pathological features and patient survival.

Additionally, other purpose of this study was to evaluate the methylation pattern of P-cadherin promoter as a potential molecular mechanism underlying the regulation of this gene.

Experimental Design: Using a series of invasive breast carcinomas, P-cadherin expression was evaluated and correlated with histological grade, oestrogen receptor expression, p53 and c-erbB-2. In order to assess whether P-cadherin expression in breast cancer was associated with changes in CDH3 promoter methylation status, we studied the methylation status of a 5′-flanking region of this gene in the same invasive breast carcinomas.

Results: P-cadherin expression in breast carcinomas showed a strong correlation with high histological grade, high rates of proliferation, c-erbB-2 and p53 expression, lack of oestrogen receptor, and poor patient survival in a short-term follow-up. Additionally, we found that 71% of P-cadherin negative cases showed gene promoter methylation, while 65% of positive ones, were unmethylated.

Conclusions: P-cadherin expression was highly associated with tumour aggressiveness, being a good indicator of clinical outcome. Moreover, we demonstrate that aberrant expression of P-cadherin in breast cancer cells might be controlled by gene promoter methylation.

K2-010P
S-Gal-Elastin receptor triggering by matrix-derived peptides induces a potential up-regulation loop through IL-1b expression and NF-kB activation in melanoma cells.
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Tumour progression implies cell-cell and cell-matrix interactions. In a previous work, we showed that elastin fragmentation at site of melanoma invasion was associated with matrix metalloproteinase-2 (MMP-2) up-regulation generating an auto-stimulation loop. Besides matrix components, other substrates such as proIL-1b are cleaved by activated MMP-2. We here investigated whether elastin-derived peptides (EDPs) could generate another autostimulation loop by inducing IL-1b expression. Our results first evidenced that EDPs treatment of melanoma cells led to an increased expression of IL-1b. This effect was mediated by the S-Gal-Elastin receptor (or EBP for Elastin Binding protein) occupancy as being suppressed by lactose and reproduced upon cell stimulation with the active peptide VGVAPG. Transduction pathways mediating this effect were then studied using specific chemical inhibitors. Although both Erk1/2 and p38 signalling cascades were activated in melanoma cell treated with EDPs, neither U0126 nor SB203580 pre-treatment could prevent their effect on IL-1b expression. Thus, we focused on another critical element involved in IL-1b up-regulation and showed that EDPs activated NF-kB pathway. The involvement of NF-kB cascade in EDPs-mediated IL-1b stimulation was further assessed using either adenovirus IkB or a specific chemical inhibitor of NF-kB nuclear translocation (SN-50). Finally, the role of NF-kB over AP-1 DNA-binding in the IL-1b promoter activation was assessed by gel-shift competition analysis with specific IL-1b promoter sequences. Hence, we demonstrated the activation of NF-kB pathway in EDPs-stimulated melanoma cells. Processing of the proIL-1b form to its active form upon MMP-2 activation could generate a second potential auto-stimulation loop. These data further illustrate that elastic fibers are important sources of matrixkines in pathologic conditions such as melanoma invasion.
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K2-011P
Inhibition of lipopolysaccharide-induced nitric oxide formation by simvastatin in RAW264.7 macrophages involves heme oxygenase-1 induction
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Statins, inhibitors of cholesterol biosynthesis, exert various beneficial effects including anti-inflammatory activity that are incompletely explained by the cholesterol-lowering effect alone. Heme oxygenases (HO) are rate-limiting enzymes that catalyze the conversion of heme into CO and biliverdin. Recent studies have suggested protective roles of HO-1, an inducible form of HO, in various inflammatory conditions. Overproduction of nitric oxide (NO) and PGE2 derived from inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), respectively, has been implicated in the pathogenesis of inflammatory diseases. This study was to investigate whether the inhibition of lipopolysaccharide (LPS)-induced NO and PGE2 formation by simvastatin is associated with the induction of HO-1 in RAW264.7 macrophages. Our results showed that simvastatin significantly inhibited LPS-induced nitrite and PGE2 formation in RAW264.7 macrophages accompanied by attenuation of the expression and activity of iNOS and COX-2. Interestingly, simvastatin also dose-dependently enhanced the HO-1 expression and activity in LPS-treated macrophages. Pretreatment of zinc protoporphyrin (Znpp), a HO-1 inhibitor, reduced the inhibitory effect of simvastatin on LPS-induced nitrite formation but no effect on LPS-induced PGE2 formation. Furthermore, the inhibitory activity of simvastatin on LPS-induced activation in nuclear NF-κB and the degradation of cytosolic IκB-α, was decreased by addition of Znpp. These results provide a novel mechanism to explain the anti-inflammatory activity of simvastatin through enhancing HO-1 induction, which may play an important role in the attenuation of LPS-induced NO production but no significant effect in COX-2/PGE2 pathway.

K2-012P
Ets and the maintenance of spatial homeostasis of cranial sutures
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The Ets family of transcription factors is essential in normal and abnormal development. Some Ets target genes have been known to modulate bone formation and degradation. Mammalian cranial morphogenesis is a complex, coordinated, interplay between intrinsic genetic controls and extrinsic environmental factors. Among the mechanisms that affect Ets coupling to target gene expression is the Ca2+-dependent phosphorylation of Ets. We propose that the intracellular Ca2+ change is the immediate result of perturbation in the mechanical environment forming a self-stabilizing mechano-morphological control circuit. In the current study, we are testing the hypothesis that Ets-2 is an important intermediate in mechanotransduction linking stretch-induced changes in intracellular Ca2+ to key alterations in gene expression at the bone/cranial suture interface to maintain suture homeostasis. We showed that an immediate increase in intracellular Ca2+ occurs in response to suture stretch. We performed immunohistochemistry on 1-day-old rat calvaria with anti-Ets antibodies and showed localization of Ets in the nuclei of cells of the immature cranial sutures and bone. Furthermore, we showed that bacterially expressed GI-70 Ets-2 protein was phosphorylated in vitro by CaM kinase II. To further test the hypothesis in vivo, we have taken a peptide array approach to develop phosphorylation-specific antibodies against Ets-2. Potential CaM kinase II phosphorylation sites in Ets-2 were identified using NetPhos 2.0. A total of 22 nanopeptides containing putative serine/threonine phosphorylation sites (p > 0.5) were synthesized using Fmoc solid phase technology, and assayed for CaM kinase II phosphorylation. Our preliminary data suggest that phosphorylation results from the peptide arrays are in general agreement with published studies by conventional methodologies.

K2-013P
Evaluation of the combined activity of PCI and Iressa against the EGF/ErbB pathway
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New molecular mechanisms related with proliferation and survival of tumour cells have been elucidated in the last years. This knowledge has allowed to search and design new agents in a more specific way and with less secondary effects. One of the most important and studied pathways is the polypeptide growth factor family of epidermal growth factor (EGF) and their receptors (ErbB3). We have studied the combined effect of a tyrosine kinase inhibitor (Iressa) with a natural molecule from the potato, which has previously been seen that acts as an EGF antagonist, Potato Carboxypeptidase Inhibitor (PCI). These studies have been done with a breast cancer cell line, the MDA-MB 231 one. This cell line is a metastasic one which overexpresses the EGF receptor (EGFR) and has low levels of erbB2. Proliferation assays were carried out combining a pre-treatment of 6 days with PCI followed by a treatment of 3 days with Iressa. After this, protein levels of the receptors and ligands of the ErbB family were analysed using a commercial ELISA kit. Finally, expression levels of either receptors or ligands were analysed using semiquantitative...
RT-PCR. The results showed a deregulation in the expression of almost all ligand when cells were treated with PCI. These levels were always upregulated, indicating that the pre-treatment favoured an autocrine loop. This could explain why these cells do not respond to Iressa and also why they grew even more than control ones. This study could help to explain the resistance of cells to chemotherapy treatments. Our aim is to carry on with this study and to enlarge either the cell lines or the kind of tumours analysed.

K2-014P
Mutator-like transposable element of cassava (Manihot esculenta Crantz) is highly methylated
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DNA methylation has been linked with gene silencing. The suppression of repeated genes is found in several eukaryotes. One possible role of DNA methylation and inactivation of repeated sequences is suppression of transposons within the genome. Transposons induce detrimental changes in the host genes for example by the disruption of genes through insertion mutations. Mutator (Mu) is a highly mutagenic transposable element system. It transposes preferentially into low copy number DNA sequence resulting in an extremely high rate of mutations. Epigenetic silencing has been a widely discussed means of Mu element regulation. Mu is inactivated when 5′-methylation of cytosine occurs at CG and CNG sequences within terminal inverted repeats (TIRs) lacking transposase binding. Our study with cassava (Manihot esculenta Crantz) revealed highly methylated Mutator-like transposable element (MULE) sequences as shown by the hybridization pattern of the DNA digested by the isoschizomers, Hpa II and Msp I, known to have differential sensitivity to DNA methylation. Northern analysis also showed low levels of MULE transcript in this important food crop.

K2-015P
Role of nuclear IκB kinase (IKK) in tumorigenesis: phosphorylation and functional regulation of CREB-binding protein (CBP)
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The IKK/NF-κB plays a critical role in the regulation of gene expressions involved in inflammation, immune responses, cell growth, differentiation, apoptosis, and transformation. NF-κB-mediated transcription has been shown to require the transcriptional coactivators, CBP and p300, to link NF-κB and basal transcriptional apparatus. It has been known that CBP and p300 has been known as phosphoproteins, however, only a few studies could correlate CBP/p300 phosphorylation with the functional effect. In this study, our data showed that IKK translocated to the nucleus, and phosphorylated GST-CBP (669-1673) in vitro in response to TNF-α. Base on a database search, we further demonstrated the consensus residues of Ser1382/1386 in CBP are the consensus residues of Ser1382/1386 in CBP. This phosphorylation induces recruitment of CBP to the NF-κB-responsive promoters, leading to the acetylation of histones in chromatin. Furthermore, we also found that inhibition of IKK activity attenuated the TNF-α-induced recruitment of CBP to the NF-κB-responsive promoters, suggesting that the Ser1382/1386 phosphorylation and regulation of CBP by IKK might play a role in the anti-apoptosis mediated by NF-κB via the reciprocal regulation of NF-κB and p300. Our study provides a new insight into the molecular mechanism by which IKK regulates the transcriptional potential of CBP.

K2-016P
The Krüppel-like zinc-finger protein ZNF224 represses aldolase A gene transcription by interacting with the KAP1 corepressor protein
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Transcription factors belonging to the Krüppel-like zinc finger class of proteins participate in the regulation of cell differentiation and development. Although a large number of these proteins have been identified, little is known about their structure and function. Recently, we have purified by DNA affinity chromatography a novel KRAB-ZFP, ZNF224. This zinc-finger protein of about 82 KDa, contains the box A (45 aa) of Krüppel-associated box (KRAB) domain at the N-terminus, and 19 Cys2His2 zinc-finger motifs at the C-terminus. We showed that ZNF224 specifically interacts through its zinc fingers motifs to the negative regulatory element AldA-NRE, located in the promoter region of the human aldolase A gene. Moreover, it is able to repress transcription of the chimeric reporter CAT gene bearing the AldA-NRE fused to the heterologous promoter of the heavy chain ferritin gene [1]. To elucidate the biological role of ZNF224 in transcriptional modulation of gene expression, here we demonstrate, by chromatin immunoprecipitation and transient transfection assays, that ZNF224 binds in vivo to the distal promoter of aldolase A gene and represses its own transcription. Moreover, transient cotransfection experiments show that the ZNF224-mediated transcriptional repression requires the 45-amino-acid long KRAB-A domain. The ability of KRAB-containing ZNF224 protein to repress transcription is dependent on specific interaction with KAP1 corepressor molecule, as indicated by cotransfection and immunoprecipitation assays. Finally, we demonstrate, by selective treatment of HDAC1 inhibitor trichostatin A, that ZNF224-mediated repression is dependent on histone deacetylases. Our data strongly indicate that the protein ZNF224 serves as a bridging molecule to recruit KAP1 and other factors of the chromatin remodeling machinery to AldA-NRE sites in the specific promoter in order to repress gene transcription.

K2-017P
Chromatin-based gene silencing by RNA interference machinery in the Drosophila germline
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RNA-interference (RNAi) is an ability of double-stranded RNA (dsRNA) molecules to cause selective gene silencing. Long dsRNA is processed by Dicer nuclease into short 21–27 nt RNAs. Short RNAs when incorporated into ribonucleoprotein
complexes induce repression of homologous genes by one of the three known pathways, including repression of mRNA translation, cleavage of mRNA and repression on a chromatin level. Mechanism of transposons silencing shares traits with RNAi. dsRNA and short RNAs corresponding to transposons were detected in different organisms including Drosophila melanogaster. We show, that mutations in several Drosophila genes encoding proteins required for RNAi cause derepression of endogenous retrotransposons and a reporter construct driven by LTR of copia retrotransposon in the germline. Derepression of retrotransposons due to the RNAi mutations is accompanied with chromatin decompaction and the gain of acetylated histones implicating RNA silencing complexes in a modulation of histones (de) acetylation in oocytes. We also demonstrate, that Su (Ste) repeats producing short RNAs cause transcriptional silencing of homologous Stellate genes in testes. These data suggest that short RNAs operate as guides for heterochromatin formation in the Drosophila germline.

K2-018P

Epigenetic cell fingerprinting
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Using a previously described methylation tag approach, we tested methylation patterns of 50 young HERV-K (HML-2) LTRs in 10 different human cell lines. The investigated LTRs could be subdivided into three groups: “constitutively” unmethylated, “constitutively” methylated and mosaically methylated, that is methylated in some cell lines, but unmethylated in the others. We used these LTRs to classify cell types by cluster analysis, and showed that similar cell lines were grouped into the same cluster. Therefore, the LTR methylation pattern is a characteristic feature of the cell line that can be used for epigenetic cell fingerprinting. Expression of 20 genes located in close vicinity to 14 analysed LTRs was studied by RT-PCR. Generally, the results suggest a positive correlation between the LTRs’ hypomethylation and gene expression.

K2-019P

The activation of human cancer/testis antigen gene, XAGE-1, in tumor cells is correlated with CpG island hypomethylation
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Expression of the XAGE-1 antigen is restricted to germ cells of the testis and a variety of neoplastic tissues. To date, the molecular mechanism for regulating expression of this cancer/testis antigen gene has been unknown. To evaluate methylation as a potential mechanism for regulating expression of this gene, we first correlated gene methylation status (measured by sequencing of bisulfide modified DNA and COBRA) to expression of XAGE-1 mRNA in normal and cancerous cells. This analysis revealed dense methylation of the CpG island in the XAGE-1 gene promoter for the normal and cancerous cells that do not express this gene, but loss of this methylation in normal testis, cancer cell lines, and the primary gastric cancers where the gene is highly expressed. Further supporting the role of methylation in regulating expression of XAGE-1 were observations that treatment of two heavily methylated cell lines, SNU620 and HT29, with 5-aza-deoxoyctydine resulted in demethylation of XAGE-1 promoter and corresponding expression of this gene. Finally, we cloned various segments of the CpG-rich XAGE-1 gene promoter linked to a luciferase reporter construct and transiently transfected this construct into HCT116 cells. These experiments confirmed transcriptional regulatory activity for the promoter region that incorporates the CpG island and demonstrated that in vitro methylation of this island results in loss of promoter activity. Collectively, these studies indicate that XAGE-1 expression in normal and cancerous tissues is regulated by methylation of the CpG island in the gene promoter.

K2-020P

Expression of dsRNA-specific antibodies in transgenic plants and their effect on the progress of virus infection
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dsRNA-mediated gene silencing is a well known protective mechanism which inhibits viral gene expression and virus multiplication in plants. Many viruses developed specific proteins to interfere with gene silencing and are therefore able to escape this highly efficient protective mechanism. The influence of such virus proteins was investigated in several laboratories by mutagenesis studies or by expressing anti-silencing proteins in transgenic plants. It was shown that the proteins may influence gene silencing in different ways even in the absence of the original virus [reviewed by Baulcombe, Nature 2004; 431: 356]. The aim of our experiments was to find out whether the expression of dsRNA-specific antibodies in transgenic plants has an effect on virus multiplication and/or on host-mediated gene silencing. We used an antibody, which specifically recognises dsRNA, independent of the nucleotide composition and the sequence of the antigen. The Fab was expressed as single-chain Fv-fragment (scFv) and the highest expression levels were observed when it was targeted to the endoplasmic reticulum. Expression of dsRNA-specific scFv in tobacco resulted in alteration of the symptoms, virus multiplication and/or of virus spread. In potato virus Y (PVY) infected N. tabacum cv. Xanthi virus symptoms occurred earlier than in non-transgenic control and were more severe. Infection by tobacco mosaic virus (TMV) resulted in lower lesion number and delayed appearance of the lesions on the infected leaf, but despite the presence of the N resistance gene systemic spread of the virus could also be observed. No such effects were observed in plants expressing a single-stranded RNA specific scFv. The possible mechanisms will be discussed. Acknowledgment: Supported by OTKA grant T032861.
K2-021P
DNA bending as a determinant of the differential binding stoichiometry of the human isoforms of Ets-1, p51 and p42, on the palindromic Ets binding sites of the stromelysin-1 promoter
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Abstracts

K2-023P
HMG I (Y) proteins: emerging roles in the transcription of herpes simplex virus immediate-early genes
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The HMG I (Y) proteins are architectural components of the mammalian chromatin. They possess multiple copies of the conserved DNA-binding motif called the “AT-hook” through which they bind the minor groove of the AT-rich DNA regions. HMG I (Y) proteins do not have a direct effect on gene expression but they can regulate the activities of other transcription factors. This is achieved through specific interactions of the HMG I (Y) proteins with both DNA and protein targets. These interactions include both structural changes in chromatin substrate and the formation of stereospecific complexes, called enhanceosomes, on the promoter/enhancer regions of genes whose transcription is being regulated by HMG I (Y) proteins. We have shown previously that HMG I (Y) proteins interact with a regulatory region of the α4 (IE-3) gene of herpes simplex virus type 1 (HSV-1). HMG I (Y) proteins bind to a regulatory region of the α4 (IE-3) gene of herpes simplex virus type 1 (HSV-1). HMG I (Y) proteins bind to a regulatory region of the α4 (IE-3) gene of herpes simplex virus type 1 (HSV-1). HMG I (Y) proteins interact with all alpha promoters and affect the binding and/or the activities of known viral transcriptional regulators, i.e. ICP4, VP16, ICP0. These data indicate that the architectural proteins HMG I (Y) do regulate, in a complex way, the activities of all immediate early promoters of HSV-1.
K2–024P
Trimethyl-guanosine synthase, TGS1, the enzyme forming the cap-structure of small non-coding RNAs has an essential function in Drosophila melanogaster development
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The Drosophila melanogaster gene, dtl encodes a 60-kDa protein (DTL) with RNA-binding activity and a methyltransferase (MTase) domain. DTL is expressed throughout the developmental stages of Drosophila. The expression of the gene is regulated by a complex regulatory region located partly within the coding region of the gene adjacent to dtl. The dtl mRNA has two ORFs (uORF and dORF). The product of dORF is a 60-kDa protein that is translated from an internal AUG located 538 bp downstream from the 5' end of the message. This product of dtl is responsible for the formation of the 2',3',7-trimethylguanosine (m3G) 5' cap structure of snRNAs and snoRNAs of Drosophila. Recently described homologues of DTL include PIMT, an RNA-binding protein, which interacts with and enhances nuclear receptor co-activator function, and TGS1, the methyltransferase involved in the formation of the m3G cap of non-coding small RNAs. In Drosophila, in contrast to yeast, trimethyl-guanosine synthase activity is essential: deletion in the dORF or point mutation in the putative MTase active site results in a reduced pool of m3G cap-containing RNAs and lethality in the late larval, early pupal stages. Interestingly, the 5' region of the dtl message also has coding capacity (uORF) for a 178 amino acid protein, which has no other known homologues, but in Drosophila species. Mutations within the uORF did not affect the MTase activity and m3Gcap formation, but result in lethality in a late stage of development.

K3 – Nuclear Hormone Receptors

K3–001
Isoforms of nuclear receptors and of their coregulators diversify the transcriptional response.
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Nuclear receptors function as ligand-regulated transcription factors. Many nuclear receptors are bimodal in their transcriptional properties and can either repress or activate target gene expression by alternate recruitment of auxiliary factors denoted corepressors and coactivators. Nuclear receptors are often encoded by multiple genetic loci and through alternative mRNA splicing to create a series of interrelated isoforms or isotypes. These isoforms are evolutionarily conserved, are differentially expressed in different tissues, and can perform distinct functions. We report here that the different isoforms of a given nuclear receptor, such as the retinoic acid receptor (RAR), differ in the ability to recruit corepressors and to repress transcription. We provide evidence that these different transcriptional properties reflect differences in the conformation of the unliganded receptors that permit, or prevent access of the corepressor to its docking surface on the receptor. Notably the SMRT and N-CoR corepressors are similarly expressed from multiple loci and through alternative mRNA splicing to create distinct corepressor isoforms. We describe how these corepressor isoforms differ in their response to cellular kinase signaling pathways and in their affinities for different nuclear receptor partners. These different corepressor isoforms are expressed at different levels in different tissues. Our results indicate that the interactions between the nuclear receptors and their auxiliary proteins are diversified through the expression of multiple isoforms. The existence of these isoforms permits receptor and corepressor function to be individually tailored to the requirements of different cell types and in response to different signaling events.

K3–002
Functional consequences of the dynamic folding of the glucocorticoid receptor
N-terminal transcription activating domain (AF1)
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The N-terminal regions of steroid hormone receptors (SHRs), ligand-activated transcription factors, appear to contain unfolded regions, these coincide with strong transcription-activating domains within the N-terminal portions of the SHRs. The AF1 (enh2/tau1) of the glucocorticoid receptor (GR) is representative of such domains. In the human GR (hGR) AF1 is defined functionally by amino acids 77-262 and its core (AFc) by a.a.187-242. Recombinant AF1/AFc show little evidence of structure, even when expressed within a larger transcriptionally active form of the GR. AF1 interacts selectively with other proteins, to affect transcription. This implies that AF1 must achieve one or more structures. Three separable conditions promote the functional folding of the hGR AF1/AFc: incubation in osmolytes, binding of the GR DNA-binding domain (DBD) to a GR binding element in DNA, and direct interaction with the TATA box binding protein (TBP). The osmolyte trimethylamine N-oxide (TMAO) best promotes AF1 folding in vitro. The folded AF1 is functional, as seen in its enhanced binding to TBP and to known GR Coactivators. When amino acids 1–500 of the GR are expressed, to include the DBD but not the ligand-binding domain, the protein is constitutively active. AF1 still lacks structure, but it folds either with incubation in TMAO or with an oligonucleotide sequence for high affinity binding to the DBD. Since this is located 200 a.a away from AF1, the DNA site seems to act as a ligand, to cause an allosteric fold in AF1. Thirdly, in vitro, approximately equimolar TBP and AF1 bind with gain of structure in AF1. In vivo, CV-1 cells transfected with GR and a requisite GR-specific binding site, FRET experiments showAF1-dependent interaction with TBP.
K3–003
Deciphering the specificity of response element-specific transcriptional regulatory complexes
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The glucocorticoid receptor (GR) can activate or repress transcription depending on bound ligand, the sequence and architecture of the glucocorticoid response elements (GREs), and the availability and activity of interacting cofactors. We are examining the functional surfaces of GR and coregulators, and the mechanisms of regulation, that operate in different GRE and ligand contexts. At the collagenase-3 GRE in U2OS.G cells, GRIP1 is a GR corepressor via a repression domain (RD1) distinct from its known activation domains; the other p160 proteins, SRC1 and RAC3, lack RD1 and were inactive as corepressors of collagenase-3. In contrast, both GRIP1 and SRC1 were corepressors at the osteocalcin GRE, and GRIP1 RD1 was dispensable. In general, we could efficiently distinguish the composition and activities of regulatory complexes at different response elements by comparing functional domains of as few as two factors (e.g. GR and GRIP) at those response elements. In A549 cells, GR represses interleukin-8 (IL-8) transcription by tethering to the bound NFκB that activates the gene, and inhibiting phosphorylation of serine-2 in the RNA polymerase II C-terminal domain (CTD). In contrast to our findings in U2OS.G cells, GRIP1 appears not to be a GR corepressor of IL-8 in A549 cells. Rather, GR inhibits recruitment of P-TEFb, a kinase thought to phosphorylate CTD ser-2. We conclude that transcriptional regulatory complexes assemble on demand in a cell- and response element-specific manner, and that the rules for differential assembly and mechanisms can be discerned by reductionist approaches without prior identification of all the components in a given complex.

K3–005
Role of the RIP140 corepressor in adipose biology and ovarian function
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RIP140 is a transcriptional corepressor for nuclear receptors that plays a crucial role in adipose biology and female reproduction. Mice devoid of RIP140 are lean and show resistance to high-fat diet induced obesity and hepatic steatosis. In addition, RIP140 is essential in the ovary for ovulation. A major role for RIP140 in adipose tissue is to block the expression of genes involved in energy dissipation and mitochondrial uncoupling, including uncoupling protein 1 and carnitine palmitoyltransferase 1b. The analysis of RIP140 null embryonic fibroblasts, which can be induced to undergo adipogenesis in vitro, indicates that the corepressor plays an intrinsic role in differentiated adipocytes. The use of specific PPAR agonists and antagonists identifies both PPARα and PPARγ in mediating RIP140 signalling in these cells. Expression profiling identifies markers of both white and brown adipose tissue suggesting that RIP140 not only regulates adipocyte functions but also plays a role in selecting pathways towards adipocyte differentiation. In the ovary we have found that the ovulatory failure is accompanied by impaired expression of genes required for cumulus cell expansion following the LH surge. The impairment is preceded by the upregulation of genes responsible for the production of the extracellular matrix and cell-cell interactions. Thus it appears that the RIP140 corepressor plays a crucial role in preventing the expression of genes at specific stages of follicular development that would otherwise impair its function.

K3–004
Repression by nuclear hormone receptors
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Transcriptional repression is a critical function of certain nuclear receptors (NRs). The Nuclear Receptor Corepressor (N-CoR) and Silencing Mediator for Retinoid and Thyroid Receptors (SMRT) are related proteins that serve as corepressors for unliganded NRs. SMRT and N-CoR both function as part of multikinase repression machines. Among other functions, the corepressor complexes modify chromatin by promoting histone deacetylation, which has been correlated with repressed gene expression. Several histone deacetylases (HDACs) have been shown to interact with N-CoR and SMRT. We have been particularly interested in HDAC3, which is tightly bound to SMRT and N-CoR in a core repression complex. HDAC3 is recruited to target genes and is required for repression by several NRs. The enzymatic activity of HDAC3 requires interaction with a Deacetylase Activating Domain (DAD) that is conserved in SMRT and N-CoR. Evidence will be presented in support of a major role of the DAD domain in mediating repression by NRs. The DAD contains a SANT motif that is required for its function. SMRT and N-CoR also contain a second SANT motif that constitutes a histone interaction domain (HID). The HID synergizes with the DAD to promote histone deacetylation and preferentially binds to hypoacetylated histones. We will provide evidence for specific and interrelated functions of the HID and DAD which support a feed-forward model of repression.

K3–006
Recruitment and assembly of the nuclear receptor repression complex
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Ligand-regulated nuclear hormone receptors act as molecular switches activating transcription of their target genes in response to binding cognate ligands. This is achieved through the cyclical recruitment of various activation and chromatin remodelling complexes. In the absence of ligand many nuclear receptors actively repress basal transcription through the recruitment of a large (1-2MDa) multi-protein repression complex. One of two homologous co-repressor proteins (SMRT and N-CoR) interact directly with the nuclear receptor and serve as a platform for the recruitment of several histone deacetylase enzymes (HDACs) along with other proteins whose function is less well-understood. It is thought that the core of the repression complex is formed from four proteins: SMRT/N-CoR, TBL1, GPS2 and HDAC3. HDAC3 is activated through binding to SMRT/N-CoR and modifies chromatin structure through the removal of acetyl groups from lysine residues in the N-terminal tails of the core histones. TBL1 plays a role in interaction with histones tails and also recruits proteasome components necessary for the recycling of the repression complex. The role of GPS2 is not well understood. Other components of the complex include SHARP, HDAC1 and HDAC4. Our recent research has focused on understanding (i) the mechanism of recruitment and dissociation of this repression complex from nuclear receptors and (ii) the assembly and function of the repression complex itself.
K3–007P
Peroxisome proliferator-activated receptor alpha protects against alcohol-induced liver damage
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The mechanisms underlying alcoholic liver disease are not completely understood, but lipid accumulation seems to be central to the cause of this disease. The peroxisome proliferator-activated receptor alpha (PPARalpha) plays an important role in the control of lipid homeostasis, metabolism of bioactive molecules, and modulation of inflammatory responses. To investigate the roles of PPARalpha in alcoholic liver injury, wild-type and PPARalpha-null mice were continuously fed a diet containing 4% ethanol and liver injury was analyzed. PPARalpha-null mice fed ethanol exhibited marked hepatomegaly, hepatic inflammation, cell toxicity, fibrosis, apoptosis, and mitochondrial swelling. Some of these hepatic abnormalities were consistent with those of patients with alcoholic liver injury and were not found in wild-type mice. Next, the molecular mechanisms of ethanol-induced liver injury in PPARalpha-null mice were investigated and changes related to ethanol and acetaldehyde metabolism, oxidative stress, inflammation, hepatocyte proliferation, fibrosis, and mitochondrial permeability transition occurrence occurred specifically in PPARalpha-null mice as compared with wild-type mice. In conclusion, these studies suggest a protective role for PPARalpha in alcoholic liver disease. Humans may be more susceptible to liver toxicity induced by ethanol as PPARalpha expression in human liver is considerably lower compared to that of rodents.

K3–008P
Association of rat liver glucocorticoid receptor with Hsp90 and Hsp70 upon mercury intoxication
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Glucocorticoid receptor is an inducible transcription factor activated by hormone binding. Hormone-free glucocorticoid receptor occurs in the form of multiprotein heterocomplexes comprising heat shock proteins Hsp90 and Hsp70 and other proteins required for maintaining the receptor in a conformation capable of hormone binding. Present study investigated the influence of mercury on association of the glucocorticoid receptor with Hsp90 and Hsp70, in the rat liver. To examine effects of mercury, intact male Wistar rats received single i.p. injection of HgCl₂ (1, 2 or 3 mg Hg/kg b.w.) and were sacrificed 4 h later. The glucocorticoid receptor heterocomplexes with Hsp90 and Hsp70 were immunopurified from the liver cytosol of rats administered with different doses of mercury. Amounts of co-immunopurified apo-receptor, Hsp90 and Hsp70 were then determined by quantitative Western blotting. The ratio between the amount of heat shock protein Hsp90 or Hsp70 and the amount of apo-receptor within immunopurified heterocomplexes was found to increase in response to mercury administration. On the other hand, levels of Hsp90 and Hsp70 in hepatic cytosol remained unaltered. The finding that mercury stimulates association of the two heat shock proteins with the glucocorticoid receptor, rendering the cytosolic heat shock protein levels unchanged, suggests that mercury affects mechanisms controlling assembly of the receptor heterocomplexes.

K3–009P
Effects of glucocorticoids in proliferation and apoptosis induced by hydrogen peroxide in human endothelial cells.
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Glucocorticoids are well known as anti-inflammatory agents, widely used for the treatment of patients with various disorders including allergic disease, autoimmune disease and lymphoproliferative disorders. Excess of glucocorticoid often elicits adverse effects on the vascular system, such as hypertension and atherosclerosis. Glucocorticoids act on cells both in unspecific and specific ways, the latter being mediated by glucocorticoid receptor (GR), which is present also in endothelial cells. We assessed the impact of dexamethasone, prednisone and hydrocortisone, in the range of concentrations of 10 nM to 1000 nM on the proliferation and redox state of human endothelial cells. Our results show that glucocorticoids stimulate proliferation of primary endothelial cells HUVEC, but not immortalized cell line HUVEC-ST. The proliferation was estimated after 96 h by MTT assay and fluorometrically by total nucleic acid amount. Cell cycle was analyzed by flow cytometry by propidium iodide staining. We also found that 24 h preincubation of cells with glucocorticoids protects HUVEC but not HUVEC-ST against apoptosis induced by H₂O₂. Apoptotic changes of cells and protective effect of analyzed steroid hormones were observed 24 h after treatment with hydrogen peroxide by measurement of activation of caspase 3. Condensation of chromatin was visualized by Hoechst 33258/PI staining. Changes were also visible in total antioxidant capacity (TAC) of the cells assessed by a modified ABTS⁺ decolorization assay.

K3–010P
Identification and organ distribution pattern of peroxisome proliferator activated receptors in brown trout (Salmo trutta f. fario)
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It was shown in vitellogenic Salmo trutta females that along with a volume decrease of the hepatocytic peroxisomes, the activities of some liver peroxisomal enzymes were also reduced [1]. Being peroxisome proliferator activated receptors (PPARs) involved in peroxisomal metabolism regulation [2], it is possible that they might be implicated in the alterations observed in brown trout liver. We identified three PPAR isotypes, encoded by separate genes and showing different tissue distribution. cDNA fragments
encoding PPARα, β, and γ were amplified by PCR, and deduced sequences of the correspondent peptides were compared with other species sequences. Both the 183 amino acid sequence from PPARα and the 103 amino acid sequence from PPARβ shared high levels of homology with the correspondent peptides of other fishes and terrestrial vertebrates, whereas PPARγ 108 amino acid sequence showed much less similarity with non-fish PPARγ. Two sequences for PPARβ were found. After homology analysis, they both turned out to be closer to the PPARβ isoform of other species. According to semiquantitative RT-PCR, PPARα both turned out to be close to the PPARα sequence showed much less similarity with non-fish PPARα. Two isoforms of PPARγ were identified in the same sample. The 183 amino acid sequence from PPARγ is the least one, while the 103 amino acid sequence from PPARγ is the most strongly expressed, whereas PPARγ is the least one.

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**References**


**K3–011P**

**Characterization of a novel retinoid-x receptor antagonist, LG1208**

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Through the genetic processes they have control over, nuclear receptors have an effect on virtually all aspects of the life of multicellular organisms, including embryogenesis, homeostasis, reproduction, cell growth and death. Their gene-regulatory power and selectivity has prompted intense research on these key factors. Retinoid-X Receptor (RXR) is a member of the nuclear receptor transcription factor superfamily, which regulates the activation of their target genes in a ligand dependent manner. According to the general model, in absence of ligand they recruit co-repressor molecules that are dissociated upon ligand binding. RXR is present as a common heterodimeric partner for several types of nuclear receptors as an indication of their major role in these systems. Despite the extensive medical application of retinoids, due to their teratogenic effects and toxicity it has become necessary to design and improve therapeutically identical synthetic ligands that lack these side effects. Our aim was to examine and characterize a novel synthetic potential RXR-ligand, LG1208. We applied mammalian two-hybrid system to detect protein-protein interactions. After transfecting the appropriate plasmids by cationic liposomes into CV-1 fibroblasts, we treated them with ligands and 18 h later lysated the cells. Then we measured the β-galactosidase and luciferase activity of the lysates. By this method it was possible to carry out both activation- and interaction-studies, so we could analyze the ligand dependent activation ability and the receptor – co-regulator interactions. As a result of our studies we characterized the ligand in several permissive and non-permissive RXR-heterodimers. In the activation assays LG1208 behaved as a competitive antagonist. With the interaction assays we revealed the changes of co-regulator binding that lies behind the antagonistic effect. Characterizing and understanding the mode of action of such ligand can result in a useful research tool to understand and model the complex function of nuclear receptors.

**K3–012P**

**Genome-wide location analysis of nuclear receptors**

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Genomic location analysis of transcription factors involves combination of chromatin immunoprecipitation (ChIP) with high throughput DNA detection methods such as microarrays or serial sequencing of concatemerized tags. Genomic microarrays use non-transcribed loci of the genome as probes. About 56% of human genes are associated with CpG islands (Antequera and Bird, 1993). These CpG enriched loci of the human genome are likely to be enriched in regulatory elements. For our initial study we employed such a microarray that contains 12 000 CpG islands and analyzed fragments bound by PPAR gamma antibody. We analyzed the enrichment of PPAR gamma bound DNA fragments in rosiglitazone treated Monomac6 (MM6) cells. Several new putative binding sites of PPAR gamma were identified. By using in silico analysis, putative response elements of PPAR receptors could be identified in these locations. By comparing genomic location analysis of PPAR gamma to global gene expression data we can regroup the primary data of the two experimental approaches. Several genes that were not identified as PPAR regulated by gene expression analysis due to their low expression levels present PPAR gamma enrichment on their regulatory regions. On the other hand genes with enrichment of PPAR gamma on their regulatory elements were not expressed at all, based on global gene expression data. Further analysis of the epigenetic context of these genomic elements need to be performed. We propose that genomic location analysis can complement global expression profiling in order to further refine our understanding of the relationship between PPAR gamma regulated gene networks and genomic binding sites.

**K3–013P**

**Glucocorticoid hormone elicited tyrosine-phosphorylation events involving T cell specific kinases**

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The glucocorticoid hormone (GC) plays an important role in the regulation of T-cell activation; maturation and apoptotic processes. However, the cross-talk between the GC induced signalling mechanisms and T-cell receptor (TcR) signal transducing pathways needs to be elucidated. After binding its ligand, the GC receptor (GR) dissociates the HSP-90 complex and it translocates into the nucleus, acting as a transcription factor. The GR may also participate intracytoplasmic interactions mediating non-genomic GC effects. We examined the rapid effects of GC exposure on in vitro cultured human Jurkat cells. Our results showed that Dexamethasone (DX), a GR agonist, when applied at high dose (10 µM), induced rapid (within 5 min) tyrosine-phosphorylation events in Jurkat cells. The tyrosine-phosphorylation events after anti-CD3 antibody stimulation was strongly inhibited, when a short DX pre-treatment was applied. Moreover, the phosphorylation status of ZAP-70, a T-cell specific tyrosine kinase, which plays a central role in augmenting the TcR-elicited signal in T cells, was also investigated. We demonstrate that high dose DX exposure induced a rapid ZAP-70 tyrosine-phosphorylation in Jurkat T cells. ZAP-70 phosphorylation induced by DX could be inhibited by RU486 (GR antagonist), suggesting the involve-
ment of GR in this process. After DX treatment ZAP-70 phosphorylation did not occur in the p56-lck kinase-deficient Jurkat cell line JCaM1.6, suggesting that this process was p56-lck dependent. Our results show that DX, at a high dose, can rapidly influence the initial tyrosine-phosphorylation events of the CD3 signalling pathway in Jurkat cells, thereby it may modify the TcR-derived signals. Lck and ZAP-70 represent an important molecular link between the TcR and GC signalling pathways.

K3–014P
Analysis of the subcellular distribution of DHR38 in mammalian cells
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Drosophila Hormone Receptor (DHR38), Ecdysteroid receptor (EcR) and Ultraspiracle (Usp) are members of the steroid superfamily in Drosophila melanogaster. EcR and Usp are components of functional ecdysone receptor, which regulates all the major developmental transitions in insects in response to the 20-hydroxyecdysone. DHR38 is homologous to the vertebrate nervous-growth factor-induced clone B (NGFI-B) – type orphan receptors. In addition to binding to the specific response elements as a monomer, DHR38 interacts with Usp and this complex responds to a distinct class of ecdysteroids independently of EcR. Our latest research, in CHO-K1 cells, suggested that DHR38 possesses nuclear localization sequence (NLS). In order to determine NLS, we examined fragments of the receptor tagged with yellow fluorescent protein (YFP). Only the DNA binding domain (DBD) tagged with YFP was present in the nucleus. No NLS was found in the N-terminal (A/B) and the ligand binding (LBD) domains. From our previous investigations, we already have determined the subcellular distribution of EcR and Usp in mammalian cells. We were interested whether DHR38 can influence the subcellular distribution of these proteins. Therefore, we coexpressed DHR38 tagged with cyan fluorescent protein (CFP-DHR38) and Usp tagged with YFP (YFP-Usp) or CFP-DHR38 and EcR tagged with YFP (YFP-EcR) in CHO-K1 cells. Our preliminary results suggest that DHR38 coexpressed with EcR or Usp changes the distribution pattern of the EcR/Usp heterodimer components.

K3–015P
Correlation between glucocorticoid receptor binding parameters, blood pressure and body mass index in healthy human population
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The explanation of molecular basis of glucocorticoid resistance and involvement of glucocorticoid hormones in various diseases, including obesity and hypertension, requires investigation of glucocorticoid receptor (GR) functional properties both in health and in disease. In this study we used peripheral blood mononuclear cells (PBMC) of healthy human subjects and applied radioligand binding assay to examine correlation between the number of the receptor sites per cell and the affinity for the ligand, as well as relationship between these equilibrium binding parameters and body mass index, blood pressure and age. It was found that the only statistically significant correlation was that between the number of glucocorticoid binding sites per cell and equilibrium dissociation constant, Kd (r = 0.84, P < 0.0001). Since Kd represents a reciprocal measure of the receptor affinity for the hormone, this observation implies the existence of a compensatory mechanism providing lower glucocorticoid receptor affinity in individuals that have more binding sites in circulating mononuclear cells and vice versa. This compensatory phenomenon together with considerable interindividual variation (the number of receptor sites per cell ranging from 1391 to 15133, CV = 58.62%; and Kd from 2.5 to 98.6 nmol/liter, CV = 80.87%) reflects plasticity of the glucocorticoid system. The results open the question whether this compensatory mechanism observed in healthy human subjects persists in pathophysiological states associated with glucocorticoid hormones action and suggest that tissue sensitivity to glucocorticoids might be better predicted by the sign and magnitude of correlation between the two receptor equilibrium binding parameters than by each of them separately.

K3-016P
Analysis of the distribution of ultraspiracle and ecdysteroid receptor in mammalian cells
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The constitutive androstane receptor (CAR) exhibits transcriptional activity in the absence of exogenous ligand. This is in contrast to classical nuclear receptors whose activity is derived from interactions with small-molecule ligands. However, there are two classes of non-physiological ligands that can modulate the activity of murine CAR. These are exemplified by the testosterone metabolite, 5α-androst-16-en-3α-ol (androstenol), which represses mCAR activity. The second class of ligands, exemplified by TCP-OBOP [1,4-bis(3,5-dichlorophenyl)-benzen]-, can both activate CAR and alleviate the effects of the androstenol-mediated repression of CAR activity. The three active states of CAR are most likely to be a consequence of different structural conformations that are linked by an allosteric pathway, highly active (TCPOBOP) > active (apo) > inactive (androstenol). These conformations give the protein the ability to discriminate between various ligands and co-regulatory proteins. We present data from X-ray crystallography and Fluorescence Spectroscopy to determine the structural basis of ligand recognition and the structural changes that accompany ligand and co-regulator protein binding.

K3-017P
Analysis of the distribution of ultraspiracle and ecdysteroid receptor in mammalian cells
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The steroid hormone, 20-hydroxyecdysone (20E), controls insect metamorphosis by initiating molting process. The functional
Abstracts

20E receptor is a heterodimer composed of two members of the nuclear receptor superfamily – the ecdysteroid receptor (EcR) and Ultraspiracle (USP). In order to study the subcellular distribution of EcR and Usp, we expressed these receptors as fusion proteins with variants of green fluorescent protein in Chinese Hamster Ovary (CHO-K1) cells. Heterologous expression of EcR and Usp in mammalian cells facilitated analysis of their individual subcellular distribution in the absence of endogenous expression of these two receptors. Usp tagged with yellow fluorescent protein (YFP-Usp) was almost exclusively localized in the nucleus in contrast to EcR tagged with YFP (YFP-EcR) which was localized in the nucleus as well as in the cytoplasm. Interestingly, coexpression of YFP-EcR and Usp tagged with cyan fluorescent protein (CFP-Usp) resulted in nuclear colocalization of both proteins in nearly all transfected cells. Additionally fluorescence recovery after photobleaching (FRAP) revealed that the mobility of Usp within the nucleus is decreased in case of coexpression with EcR. The presence of both EcR and Usp in the nucleus indicates that they possess the nuclear localization sequences (NLS). To identify NLS within EcR and Usp, cDNA constructs with various regions of both receptors tagged with YFP were generated and analyzed in CHO-K1 cells.

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K3-018P
Modulation of Zac1 transcriptional activities in HeLa cells by trichostatin A (TSA) and histone deacetylase 1 (HDAC1)
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Zac1 is a novel seven-zinc-finger protein which may possess the specific DNA element binding ability and intrinsic transactivation activity. Zac1 not only promotes cell cycle arrest and apoptosis, but it also acts as transcriptional cofactors for nuclear receptors and p53. Our previous study indicates that the N- and C-terminal regions of Zac1 involve in the down-regulation of its putative transactivation activity in HeLa cells. Subsequent results in this study demonstrated that a histone deacetylase inhibitor, trichostatin A (TSA), dramatically enhanced the transactivation activity of Zac1 in HeLa, CV-1 and 293 cells. The enhancements of Zac1 transactivation activity by TSA were suppressed by the addition of over-expressed histone deacetylase 1 (HDAC1), not HDAC4. Zac1 physically interacted with the catalytic domain of HDAC1 and, however, the catalytic activity was not required for this physical interaction. In addition, Zac1 was able to act synergistically with the effect of TSA to modulate the p21 promoter activity and estrogen receptor transcriptional activity in HeLa cells. Taken together, our observations demonstrate that the functional role (positive or negative role) of Zac1 in some transcriptional systems depend on specific molecules (coactivators or corepressors) recruited by Zac1’s functional complexes in cells.

K3-019P
Characterization of a novel steroid receptor-binding protein, SRB-RGS
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A novel steroid receptor-binding protein, SRB-RGS, suppressed the estrogen receptor (ER) alpha-mediated and other promoter-driven transcriptional activities [1]. This study describes the interaction of the full-length SRB-RGS and ERs by using yeast two-hybrid system and a coimmunoprecipitation assay. SRB-RGS suppressed the transcriptional activities of ER beta and ER alpha + ER beta as well as those of ER alpha and the SV40 early promoter. It was found that the middle region and the RGS domain of SRB-RGS affected the activities of ER alpha and ER beta-mediated transcription, respectively. Green fluorescence of the fusion protein of EGFP and SRB-RGS was localized both in the nucleus and in the cytoplasm. This finding was confirmed by SDS-polyacrylamide electrophoresis (SDS-PAGE) analysis of subcellular fractionation of the COS-7 cells overexpressing SRB-RGS and pulse-labeled with [35S]methionine. Overexpression of SRB-RGS in the HeLa cells induced apoptosis in the cells. Intrinsic SRB-RGS was identified by SDS-PAGE analysis after immunoprecipitation subsequent to pulse-labeling of the estrogen responsive rat pituitary cell, MtT/Se, with [35S]methionine. For the identification, a confocal laser scanning microscope after immunostaining of HeLa cells was used, as well as Western blotting of the whole cell lysate from HeLa cells by using the anti-SRB-RGS antibody.

Reference

K3-020P
Regulation of the transcriptional activity of COUP TF II by MAPK-mediated phosphorylation
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Chicken Ovalbumin Upstream Promoter Transcription Factor II (COUP TF II) is an orphan member of the nuclear hormone receptor superfamily that strongly inhibits transcriptional activation mediated by a number of other nuclear receptors. COUP TF II represses transcription mainly via competition with other activators for common binding sites at promoters. Since no significant changes have been found in the protein levels of COUP TF II upon various extracellular stimuli, we were prompted to check whether regulatory mechanisms other than altered expression levels – such as post-translational modification – should be involved in COUP TF II-dependent gene regulation. We found that COUP TF II is a nuclear phosphoprotein that is phosphorylated both in vitro and in vivo by the Erk2 MAP kinase within its N-terminal transactivation domain. Erk2-mediated phosphorylation significantly increased the binding affinity of COUP TF II to the hormone responsive elements of both the β retinoic acid receptor (βRAR) and the apoprotein AI promoters. However, no such changes were observed with the apoprotein CHI promoter element, indicating that phosphorylation-related changes in its binding affinity are target site-specific. We also demonstrated by transient transfection assays that phosphorylation-dependent differences in the DNA-binding affinity of COUP TF II were
K3-021P  
**Differentiation of embryonic stem cells overexpressing mineralocorticoid receptor into functional cardiomyocytes: a cell-based model to investigate aldosterone action in the heart**

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The mineralocorticoid receptor (MR) is a transcription factor expressed in a wide variety of tissues including kidney, hippocampus and heart. Activated by its natural ligand aldosterone, MR regulates specific target gene expression involved in the control of ion homeostasis but also exerts other yet uncharacterized functions in the cardiovascular system. Recent clinical trials demonstrated that mineralocorticoid antagonists (spironolactone and eplerenone) caused striking benefits for patients with heart failure or myocardial infarction, thus renewing the interest to study mineralocorticoid action in cardiomyocytes. We previously showed that transgenic mice overexpressing human MR (hMR) under the control of its own P1 and P2 promoters developed mild dilated cardiomyopathy associated with tachycardia and arrhythmia. To further investigate the implication of MR in cardiac pathophysiology, we have established embryonic stem (ES) cell lines from wild type, or transgenic P1:hMR and P2:hMR blastocysts and differentiated them into functional contractile cardiomyocytes under a reproducible experimental protocol. Both endogenous mouse MR and recombinant hMR transgene were expressed during P1.hMR cell differentiation, simultaneously with cardiomyocyte marker genes thus validating our strategy. These original models of ES-derived differentiated cardiomyocytes present powerful systems to study aldosterone and MR overexpression effects on cardiomyocyte function and to identify new aldosterone target genes in the heart. Moreover, using hMR transcripts as a reporter gene, we will be able to analyze both P1 and P2 promoter activity under various differentiating conditions. This study is an original attempt to analyze steroid receptor functions using ES cell differentiation models.

K3-022P  
**Structural analysis of sequence variants of the glucocorticoid receptor ligand-binding domain**

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In recent years several mutations and sequence polymorphisms of the glucocorticoid receptor gene have been described. Here we also present the results of an *in silico* study, which revealed previously undescribed sequence variants of the glucocorticoid receptor gene. Although the three-dimensional structure of the DNA-binding domain of the glucocorticoid receptor has been known for several years, the crystal structure of the ligand-binding domain of the receptor has been published only recently. Using a comparative protein modeling, we analyzed the structural relevance of known mutations as well as novel sequence variants discovered by our *in silico* approach in the ligand-binding domain of the glucocorticoid receptor. We conclude that comparative protein modeling of these mutant receptor variants offers a useful means to predict the functional consequences of amino acid replacements and to correlate structural abnormalities with clinical findings.

K3-023P  
**ZN202 and the nuclear receptor network controls ABC transporters and HDL metabolism**

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In addition to the role of nuclear receptors in the transcriptional regulation of ABCA1 and ABCG1 there is ongoing evidence that the transcriptional repressor ZNF202 and other SCAN domain containing proteins, such as SDP1, play a pivotal role in controlling the expression of many important genes involved in HDL metabolism. The zinc finger protein ZNF202 is a transcriptional repressor that binds to promoter elements predominantly found in genes involved in HDL and triglyceride metabolism. We have shown that ZNF202 suppresses ABCA1 and ABCG1 gene expression via binding the universal corepressor KAP1. Investigating ZNF202 regulation and nuclear trafficking during macrophage differentiation and foam cell formation, we could demonstrate that ZNF202 expression is inversely correlated with its targets ABCA1, ABCG1 and apoE. In addition, nuclear translocation of ZNF202 by interaction with SDP1, provides a further level of control for ABCA1, ABCG1, and apoE gene expression. SDP1 which functions as a PPARG2 coactivator blocks binding of the universal corepressor KAP1 to ZNF202 and thereby can force expression of ZNF202 target genes. Data from ZNF202 knockout mice revealed an increase in pre-b-HDL as well as shift from fast migrating to slow migrating a-HDL in serum of ZNF202 knockout mice compared to controls. mRNA expression profiling in liver and colon of knockout animals revealed upregulation of known ZNF202 target genes and identified a significant increase in PPARD and HNF4a expression in both tissues of knockout mice. Thus, in addition to the recent findings in HDL metabolism, ZNF202 may orchestrate novel regulatory mechanisms controlling several genes of lipid and glucose homeostasis.

K3-024P  
**Regulation of MAPK p38 signaling cascade by thyroid hormone receptor: effect on NFκB signaling pathway**

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Nuclear receptors (NR) constitute a super-family of ligand-dependent transcription factors that activate or repress gene expression. In the last few years it has been shown that they are able to regulate the mitogen-activated protein kinase (MAPK) signaling cascade. MAPK activation is achieved by phosphorylation, while MAPK inactivation is mediated by protein phosphatases. Depending on the cell type, NR can either stimulate or inhibit MAPK activity and this regulation can be modulated by...
specific phosphatases. Thus, we and other groups have previously shown that the dual specificity phosphatase MKP-1 plays an important role in the inhibition of diverse MAPK by the glucocorticoid receptor. We have new data showing that the proinflammatory cytokine TNF promotes MKP-1 p38 activation in pituitary cells and the NR for thyroid hormone (TR) specifically prevents this activation. Moreover TR rapidly induces MKP-1 protein levels and this induction is sustained for at least 24 h. The effect of TR on MKP-1 induction is at post-transcriptional level since MKP-1 mRNA levels were unaffected after thyroid hormone treatment in these cells. Since NFκB is a target gene of MKP-1 through its downstream kinase MSK-1, the regulation of NFκB pathway by TR has been investigated in pituitary cells. As expected MKP-1 p38 is able to stimulate NFκB transactivation activation and TR prevents this effect. The TR-mediated regulation of NFκB appears to be at the level of the transactivation potential of the p65 subunit since no modifications on either κB degradation or NFκB DNA binding were observed in the presence of TR. These novel results could be important to understand the molecular mechanism by which the TR exerts part of its effects in pituitary cells.

**K3-025P**

**Orphan nuclear receptor Nur77 enhances stability of hypoxia-inducible factor-1alpha protein**

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Hypoxia-inducible factor-1α (HIF-1α) plays a central role in oxygen homeostasis by inducing the expression of a broad range of genes in a hypoxia-dependent manner. Here, we show that the orphan nuclear receptor, Nur77, is an important regulator of HIF-1α. Under hypoxic condition, Nur77 protein and transcripts were induced time-dependently. When Nur77 was exogenously introduced, it enhanced the transcriptional activity of HIF-1α whereas the dominant-negative Nur77 mutant abolished the function of HIF-1α. The HIF-1α protein was greatly increased and completely localized in the nucleus when coexpressed with Nur77. The N-terminal transactivation domain of Nur77 was required and sufficient for the activation of HIF-1α. The association of HIF-1α with von Hippel-Lindau protein was not affected whereas that with MDM2 was greatly reduced in the presence of Nur77. Further we found that the expression of MDM2 was repressed at transcription-level in the presence of Nur77 as well as under hypoxic condition. Finally, PD080509 decreased Nur77-induced HIF-1α stability and recovered MDM2 expression, indicating that the extracellular signal-regulated kinase pathway is critical in the Nur77-induced activation of HIF-1α. Together, our results demonstrate a novel function for Nur77 in the stabilization of HIF-1α and suggest a potential role of Nur77 in pathogenesis and therapy of human vascular diseases.

**K3-026P**

**Specific transcriptional activities of c-terminal truncated androgen receptor detected in hormone refractory prostate cancer**

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Androgen receptor (AR) mutations are involved in the failure of androgen ablation therapies of prostate cancer (Pca). The Q640X AR has been detected in a Pca that was resistant to androgen ablation. In this mutant AR, the substitution of glutamine in position 640 in the hinge region by a stop codon leads to a truncated receptor (75 kDa instead of 110 kDa), which is constituted of the AF-1, the DNA binding domain and a nuclear localization signal. We have previously shown that this mutant AR exhibits constitutive transcriptional activities from an MMTV-luciferase construct, and is exclusively localized in the nucleus when transfected in CV1 cells. However, how this truncated AR could lead to androgen independent growth of prostate cancer cells remains unknown. Using luciferase reporter assays, we have demonstrated that the Q640X AR led to a stronger activity from the MMTV promoter containing glucocorticoid response elements compared with the full-length human PSA promoter, which contains androgen response elements. Same results were obtained with GRE and ARE artificial promoters. In brief, the Q640X AR and wild type (wt) AR may have different transcriptional capacities. Moreover and surprisingly, we found that the Q640X AR exhibited strong transcriptional activities from minimal promoter with just the TATA box. In regards of these results, differences observed in transcriptional activities between Q640X and wt ARs might be due to the loss of multiple interactions sites for AR co-regulators in the truncated AR. Data from protein–protein interactions studies will be presented to sustain this hypothesis.

**K3-027P**

**Molecular evaluation of the high basal activity of human PPARs**

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 Peroxoxisome proliferator-activated receptors (PPARs) belong to the same nuclear receptor (NR) superfamily as vitamin D receptor (VDR) and constitutive androstane receptor (CAR). The latter NR has remarkably high activity as does the other NRs liver receptor homologue-1 (LRH-1) and estrogen related receptor 3 (ERR3). Detailed inspection of the human PPARdelta and PPARgamma apo-crystal structures revealed that the position of their helix 12, which is the most C-terminal alpha-helix of the PPAR ligand-binding domain, is very similar to that of LRH-1 and ERR3, suggesting that PPARs also have constitutive activity. Structure-function analysis of the PPARs indicated that all three PPARs display high basal activity and show ligand-independent co-activator association comparable to CAR. The molecular basis of these ligand-independent actions is a stabilization of helix 12. By detailed analysis of crystal structures, reporter gene, limited protease digestion and supershift assays we confirmed that PPARs do have high basal activity and that four different amino acid groups contribute to the stabilization of helix 12 of human PPARgamma. These are (i) K329 and E499 coupling a charge-type stabilization of helix 12 via co-activator bridge, (ii) E352, R425 and Y505 directly stabilizing the helix 12 via ionic interactions and hydrogen bonds, (iii) K347 and D503 interacting with each other as well as contacting the co-activator and (iv) H351, Y355 and Y501 forming a hydrogen bond network. With the exception of H351 and Y355, these amino acids are conserved within the PPAR subfamily. Finally, we addressed the functionality and evolution of the helix 12 by constructing phylogenetic trees. Interestingly, the trees demonstrated that the helix 12s of all three PPARs are the closest to CAR, suggesting a similar function and evolution of their helices and comparable high constitutive activity. Recognizing the constitutive activity of PPARs provides a new view on their physiological function.
K3-028P
Characterization of a nuclear estrogen receptor-like protein in the helminth parasite *Taenia crassiceps*

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In this study, for the first time we have identified an estradiol-17β receptor (ER) in the helminth cestode *Taenia crassiceps*. Previously, we have shown that the in vitro exposure of *Taenia crassiceps* cysticerci to 17-b estradiol (E2) stimulated their reproduction and infectivity. To determine the possible molecular mechanisms by which estradiol affect *Taenia crassiceps*, a specific nuclear estrogen receptor like was characterized. By RT-PCR using specific primers to amplify ER of total RNA, we found that *Taenia crassiceps* expressed estrogen receptor. Scatchard analysis revealed that one binding component with high affinity and low capacity for the ligand was present in the cytosol, but not in the nuclear extract of the cystercy. An inhibition of the steroid effect by the antiestrogen Tamoxifen showed that estradiol-17beta activity is prevened. By using antibodies anti ER (578–595), we localized by Western blotting one band of about 70 kDa. ER immunoreactivity has been localized in the nuclei of the parasite cells, in the nuclei of the epithelium lining the proximal portion of the oviduct and in the nuclei, and in the cytoplasm of the inner region of the oviducal gland and in the cytoplasm of the outer region of the oviducal gland. These data, taken together, provide evidence that in *Taenia crassiceps* the ER has biochemical and immunohistochemical characteristics resembling those of ER in vertebrates. These results demonstrate that estradiol act directly upon parasite reproduction by binding to a classic and specific ER receptor on the parasite.

K3-029P
No significant difference between intact and testosterone depleted or administrated male rats in spatial learning and memory

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Androgens have been shown to affect cognitive aspects of spatial memory. Testosterone which is the most important androgen, plays a role in the organization of behavior during development. Also, it has been shown that androgens cause sex related differences in learning and memory especially during neonatal period. In the current study, we assessed the effects of castration and testosterone enanthate (TE) administration on spatial cognition. Multiple doses of testosterone enanthate (20, 40, 80 and 120 mg/ kg) were examined on different groups using Morris water maze. Spatial memory was preserved in castrated rats. There was no difference among multiple doses and control groups, also. For control of the level of testosterone in the blood of castrated rat and intact rat, blood samples were collected from intact group and 7, 10, 12, 14, 21 days after castration. Testosterone levels were measured by RIA technique and compared among all groups. The level of testosterone after 7 days in castrated rats was 0 nmol/l while in castrated rats after 7 days were 2.69 ± 0.88 nmol/l and after 21 days were 0.02 ± 0.02 nmol/L. These data suggest that changes in the level of circulation androgen have no effect on spatial localization at least after puberty in male rats.

K3-030P
Pure recombinant N-terminal regions of ecdysteroid receptor A and B1 isoforms

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The functional receptor for the steroid hormone 20-hydroxyecdysone is a heterodimer of two members of the nuclear receptor superfamily, ecdysteroid receptor protein (EcR) and Ultraspiracle (Usp). The receptor regulates genes important for insect development and metamorphosis. It has been shown thatEcR from *Drosophila melanogaster* exists in three isoforms, EcRA, EcRB1 and EcRB2 which differ only in their amino-terminal A/B regions and are unequally distributed in various insect tissues. The functional differences between EcRα, EcRB1 and EcRB2 suggest that A/B domain itself plays a crucial role in the isoform-specificity. The N-terminal region of EcR has been also evinced to be important for transactivation. Despite these facts nothing is known about the structure and folding pattern of this domain. In order to investigate this problem N-terminally His-tagged A/B domains of EcRA and EcRB1 were cloned, overexpressed in *Escherichia coli* and purified. Gel filtration experiments demonstrated an extended shape of isolated EcRA-A/B and EcRB1-A/B which may suggest the lack of tertiary structure within the A/B domains. Dichroism spectroscopy (CD) analysis revealed that there exists very little secondary structure in the A/B region of both EcRA and EcRB1 isoforms *in vitro*. Thus, we conclude, that A/B domain of ecdysteroid receptor consists of large unstructured regions.

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K3-031P
Expression of low molecular weight isoforms (LMW) of cyclin E in human renal clear cell carcinoma (RCCC): possible associations with thyroid hormone receptors (TR)

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Cyclin E, a key mediator of G1/S checkpoint regulation, is controlled by E2F1 transcription factor. Transcription of E2F1 gene is regulated by TRs (thyroid hormone-dependent transcription
factors). The negative thyroid hormone response element (TRE) in E2F1 promoter is activated by TR in the absence and repressed in the presence of T3. This suggests that one of the mechanisms of T3-dependent regulation of proliferation is repression of cyclin E gene that promotes S-phase entry. Cyclin E is aberrantly regulated in a wide variety of cancers. It was recently reported that overexpression and generation of LMW might be associated with poor clinical outcome in breast cancer. TR abnormalities have been described in different cancers including RCCC. We hypothesize that mutated TRs contribute to kidney tumorigenesis due to ineffective inhibition of cyclin E gene. We have analyzed 19 RCCCs, 19 respective controls and seven healthy kidney tissues. Western blot analysis of total cyclin E gene expression revealed that the mean values of cyclin E protein were significantly higher in G2, G3 tumors vs. control tissues. Similarly, the mean levels of LMW expression were significantly higher in G1, G2 and G3 tumors vs. control tissues. Scatchard analysis showed statistically significant T3 maximal binding ($B_{\text{max}}$) decrease in G1, G2, G3 cancer tissues in comparison to healthy controls. Comparison of cyclin E expression and T3 binding results revealed that RCCCs has higher amount of total cyclin E and decreased T3 $B_{\text{max}}$. Gel retardation assays revealed weaker binding of TR to E2F1 TRE in RCCC. Our results suggest that cyclin E and its LMW isoforms play important role in RCCC tumorigenesis. Abnormal hormone binding to TR could result in aberrant regulation of E2F1 and increased level of cyclin E.

**K3-032P**

Characterization of nuclear receptors and their effects in mouse dendritic cells

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Myeloid dendritic cells (DCs) play an important role in the immune system, as professional antigen presenting cells they can stimulate naive T-cells and initiate the specific immune response. Previously we examined the transcriptional and functional changes in the human monocyte-derived DCs after nuclear receptor ligand treatment. Our data suggest that activation of PPARgamma (Peroxisome Proliferator Activated Receptor) or RAR (Retinoic Acid Receptor) is able to modulate DC differentiation. However an important question is if mouse DCs behave similarly or not. We chose C57BL/6 and NOD (Non-Obese Diabetic) mice for our experiments. First of all we determined if ligand treatment had the same effect in mouse cells like it had in human cells. We generated DCs with GM-CSF and IL-4 treatment from bone marrow cells and also isolated DCs from mouse spleen with immunomagnetic selection. These cell types were treated with PPAR specific agonists and retinoids. In order to identify spleen DCs and the bone marrow-derived DCs we measured the mouse DC specific marker CD11c on the cell surface by flow cytometry. Most of the cells were CD11c+ and the ligand treatment decreased the proportion of this cell population. We have also measured mRNA levels of PPARg and RAR target genes by ‘real-time’ quantitative PCR. The PPARg specific ligand (Rosiglitazone) induced their target gene FABP4 (Fatty Acid Binding Protein 4) expression, suggesting that PPARg is present and active in this mouse model. The RAR target gene TG2 expression increased in mouse DCs, but we found that the RAR specific agonist (AM580) could not enhance it further. These results suggest that PPARg and RAR specific agonists interfere with the differentiation of dendritic cells and may modulate the gene expression of this all type in mice.

**K3-033P**

Single-chain protein containing DNA-binding domains of Usp and EcR specifically recognizes the 20-hydroxyecdysone response element from the hsp27 gene promoter

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The functional 20-hydroxyecdysone receptor regulates *Drosophila* metamorphosis. The receptor is composed of two proteins, Usp and EcR, which are members of the nuclear receptor superfamily. To investigate the possibility of employing parts of this heterodimer to create an artificial transcription factor we constructed a single-chain protein containing Usp and EcR DNA-binding domains (UE-DDB). The N terminally His-tagged UE-DDB was overexpressed in *E. coli* cells and purified in two chromatographic steps. Electrophoretic mobility-shift assay (EMSA) demonstrated that the single-chain protein specifically binds the ecdysone response element from the hsp27 gene promoter. The results of DNase I footprinting assay indicate, that both domains of UE DBD specifically interact with this element, in the manner which is similar to that observed for the heterodimer of individual DNA-binding domains of Usp and EcR. We also created a fusion protein composed of UE-DDB and yellow fluorescent protein (YFP UE-DDB) to examine its distribution in mammalian K562 cells. Confocal microscopy analysis indicates nuclear localization of YFP UE-DDB. This is the first report indicating that covalently joined DNA-binding domains of functional ecdysone receptor may be used as DNA-binding domain in a potential artificial transcription factor.

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**K3-034P**

Nuclear receptors and ligand-dependent repression

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Our research group is interested in investigating the role of nuclear receptors (such as retinoid acid receptor, vitamin D receptor, PPAR etc.) in myeloid cells. Treatment with ligands of nuclear receptors, besides up-regulation of transcription of direct target genes, induces also down-regulation of a large number of genes. The mechanism of ligand-dependent induction is intensively investigated and well described. Much less is known about the ligand-dependent repression. Although a common mechanism is not known, several models are proposed and proved to contribute to repression. Competition can occur between nuclear receptors and other transcription factors for limiting amount of essential co-activators. Ligand activated nuclear receptors can also physically interact with other transcription factors (e.g. NFKB, AP-1) blocking their ability to bind DNA. Another interesting possibility has also emerged: induction of negative
K3-035P
Global gene expression study on PPARgamma ligand activated dendritic cells by Affymetrix microarrays and Taqman low density arrays
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Peroxisome Proliferator-Activated Receptor gamma (PPARγ) is a ligand dependent transcription factor that has well established role in the regulation of lipid metabolism and adipocyte differentiation. We found that PPARγ expression is dramatically increased in human monocyte-derived dendritic cells as compared to monocytes. Furthermore we detected a robust induction of a well-known target gene of PPARγ (FABP4) in the PPARγ specific ligand treated dendritic cells. These data prompted us to investigate the role of PPARγ activation in dendritic cells. To determine putative PPARγ regulated genes, we performed microarray analyses (HU133 plus chipsets) in developing dendritic cells: primer human cells, obtained from three different individual, were treated with vehicle (DMSO/ethanol) or with a PPARγ activator (1 μM Rosiglitazone) and they were harvested after 6, 24 h. We identified seven novel genes/transcripts which were upregulated at least twofold in the PPARγ activated dendritic cells. Then we conducted real-time PCR using Taqman low density arrays to confirm the affymetrix expression data. Applying this quantitative PCR technique we confirmed around 50% of the inductions obtained with affymetrix microarray analyses. These results indicate that PPARγ nuclear receptor can be activated during dendritic cell development and it is a valuable system for the identification of novel PPARγ target genes.

K3-036P
A novel corticosteroid-sensitive cell line derived from the inner ear expressing functional potassium channel
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Endolymph, a compartmentalized high K+/low Na+ fluid, participates to the mechanoelectrical transduction in the inner ear. Molecular mechanisms controlling endolymphatic ion homeostasis remain elusive, hampered by the lack of appropriate cellular models. We took advantage of the expression of the mineralocorticoid receptor (MR) in the inner ear to derive a new cell line by exploiting targeted oncogenesis strategy in which the expression of SV40 T antigen was driven by the proximal promoter of the human MR gene. The EC5v cell line, microdissected from the semicircular canals, grew as a monolayer of immortalized epithelial cells forming domes. MR expression was confirmed by RT-PCR and aldosterone binding assays (20 fmol/mg protein, KD = 0.37 ± 0.10 nM). The 11 beta-hydroxysteroid dehydrogenase type 2, a key enzyme responsible for MR selectivity, was clearly evidenced. EC5v cells exhibited on filters high transepithelial resistance (Rt = 1500 Ohms/cm²) and promoted K+ secretion and Na+ absorption. RT-PCR and Western blotting revealed expression of the Epithelial Sodium Channel subunits (ENaC), probably involved in Na+ transport. Quantitative PCR demonstrated that aldosterone stimulated early expression of the serum glucocorticoid-regulated kinase 1 and alpha ENaC, indicating that EC5v represents a novel corticosteroid-sensitive cell line.

86Rb transport assays and ionic measurements revealed apical secretion of K+ possibly through the IsK/KvLQT1 potassium channel whose transcripts and proteins were readily detected. Surprisingly, clofilium inhibited K+ transport only when cells were exposed apically to high K+/low Na+ fluid, suggesting that IsK/KvLQT1 actually functions as a strict apical to basolateral K+ channel. This first vestibular cellular model may contribute to better understand ionic transports in the inner ear and their dysfunctions responsible for vertigo and hearing loss in the Menière’s disease.

K3-037P
Fluorescence anisotropy-based automated high throughput screening of estrogen receptor beta ligands
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Estrogen receptor β-selective agonists as drugs may prove useful in the treatment of mood disorders. Screening of a subset of our chemical library was performed using a homogenous fluorescence anisotropy-based assay on 384-well microplates with an in-house-developed workstation. Assay plates contained dilutions of test compounds of the chemical library and 17β estradiol as a reference compound. All subsequent plate handling operations were performed fully automated in a custom-built robotic cell. After addition of the reagent (PanVera, Invitrogen), containing a fluorescently-labeled estrogen analogue and human recombinant estrogen receptor β, plates were incubated for 2 h. After plate identification, fluorescence anisotropy of each well was determined. Ligand binding, thus displacement of fluorescently labeled estrogen was indicated by a decrease in fluorescence anisotropy of the fluorophore. Assay quality was monitored by determining Z’ value, a measure of the ratio of signal window to the uncertainty of the measurement. The assay proved to be robust for automation and enabled the measurement of up to 14 000 compounds per day with Z’ values ranging between 0.55 and 0.80. Our data on assay performance show that with certain technical limitations fluorescence anisotropy-based assays are applicable for high throughput screening of compound libraries in the search for nuclear receptor ligands. Details of assay optimization and adaptation will be discussed.