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TRANSCRIPTION, CHROMATIN AND DISEASE

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Abstracts - MONDAY, AUGUST 18, 2008

TRANSCRIPTIONAL REGULATORY COMPLEXES

Conaway J; Stowers Institute for Medical Research, USA

No abstract

INSIGHTS INTO TRANSCRIPTIONAL COACTIVATOR FUNCTIONS THROUGH ANALYSIS IN BIOCHEMICALLY DEFINED IN VITRO SYSTEMS

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Various biochemical and genetic analyses have implicated a variety of coactivators in transcriptional activation. These include both chromatin remodeling/Histone modifying factors (including various Histone acetyltransferases and methyltransferases) that act at various stages of gene activation and factors (such as the 30-subunit Mediator complex and the TAF subunits of TFIID) that facilitate direct communication between promoter-bound activators and the general transcription machinery. We have employed biochemically defined systems, reconstituted either with DNA or with recombinant chromatin templates, to explore the functions of coactivators. Recent studies have shown (i) that the Mediator requirement for activator function in vitro is elicited by an RNA polymerase II-associated negative regulator, Gdown1, and that other coactivator requirements persist in the absence of Gdown1 and Mediator, (ii) that a TAF4-interacting activator acts to enhance TFIID binding, (iii) that elongation factors SII/TFIIS and PAF complex act synergistically to enhance activator- and p300-dependent transcription from recombinant chromatin templates in a completely defined system with over 90 distinct polypeptides, and through a mechanism involving direct SII-PAF complex interactions and cooperative binding to RNA polymerase II, and (v) that coactivators with acetyltransferase and methyltransferase activities exert both independent and synergistic effects through Histone modifications rather than (or in addition to) known modifications of non-Histone proteins/transcription factors. These results emphasize novel aspects of coactivator and elongation factor functions through chromatin-dependent and chromatin-independent mechanisms and illustrate the power of biochemical approaches for mechanistic analyses.

SWITCHING CORE TRANSCRIPTION MACHINERY: AN UNEXPECTED MECHANISM FOR MAINTAINING STEM CELL PLURIPOTENCY AND DRIVING DIFFERENTIATION

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It has generally been accepted that cell-type specific programs of gene transcription are largely governed by sequence specific enhancer and promoter binding factors. For example, embryonic stem cells are dependent on key DNA binding transcription factors such as Oct4, Sox2 and *nanog* to maintain its self-renewing pluripotent state. Likewise, the power of such transcription factors (i.e. Oct4, Sox2, Klf4, and Myc) to initiate reprogramming of fibroblast to iPS cells confirms their importance in directing cell specific programs. Similarly, emerging evidence suggests chromatin modifications play a role in at least maintaining, if not initiating cell fate. By contrast, the highly conserved components of the core transcriptional machinery are thought to be universal and largely invariant in different cell types. Therefore, basal factors such as the multi-subunit promoter recognition complex TFIID and various other co-activator complexes are not expected to play a significant role in directing differential programs of transcription and cell fate determination.
We will discuss several new findings that reveal a different paradigm for regulating cell specific transcriptional programs both in the maintenance of pluripotent stem cells and during skeletal muscle differentiation. In the case of self-renewing embryonic cells, we have recently isolated a novel stem cell-activator (SCC) that is required for Oct4/Sox2 mediated activation of transcription at the *nanog* promoter. Our analysis of skeletal myogenesis indicates that the prototypic core promoter recognition complex TFIID, a TBP/TAF assembly, must first be destroyed and replaced by a novel TRF3/TAF3 complex during differentiation of myoblast to myotubes, both in vitro and in vivo. These findings suggest that a significant aspect of transcriptional regulation driving cell fate likely involves switching major components of the core machinery in addition to employing classical sequence specific DNA binding factors. Indeed, it appears that differentiation and development of multiple cell types requires remodeling of the transcriptional pre-initiation complex (PIC) in a manner not previously anticipated.

RTR1 REGULATES THE TRANSITION FROM SERINE 5 TO SERINE 2 PHOSPHORYLATION ON THE RNA POLYMERASE II C-TERMINAL DOMAIN DURING TRANSCRIPTION ELONGATION *(ORAL POSTER)*

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Messenger RNA processing is coupled to RNA Polymerase II (RNAP II) transcription through the coordinated recruitment of accessory proteins to the Rpb1 C-terminal domain (CTD). Dynamic changes in CTD phosphorylation during transcription elongation are responsible for their recruitment, with serine 5 phosphorylation (S5-P) occurring towards the 5' end of genes and serine 2 phosphorylation (S2-P) occurring towards the 3' end. The proteins responsible for regulation of the transition state between the S5-P and S2-P CTD have remained elusive. Proteomic analysis of RNAP II complexes identified a conserved protein of unknown function, Rtr1. We show that Rtr1 associates with a transcriptionally active form of RNAP II whose CTD exists in

either the unmodified and/or S5-P form. Rtr1 localizes within coding regions, with its maximum levels of enrichment occurring between the peaks of S5-P and S2-P RNAP II. Upon deletion of Rtr1, the S5-P form of RNAP II accumulates in both whole cell extracts and throughout coding regions. These data show that Rtr1 is required to regulate the CTD phosphorylation state during early RNAP II elongation.

DISTINCTIVE CHROMATIN STRUCTURES RESIDE AT THE PROMOTERS OF TRANSCRIPTION FACTORS IMPORTANT FOR EMBRYO DEVELOPMENT IN HUMAN SPERM

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As nucleosomes are widely replaced by protamine in human sperm, the epigenetic contribution of the paternal genome to development would appear quite limited (beyond the known imprinted genes). However, our genome-wide approach finds the retained nucleosomes enriched at the promoters of many transcription factor families (Hox, Fox, Gata etc.) and signaling proteins involved in guiding embryo development (termed developmental promoters). Notably, these developmental promoters generally lack the testes-specific H2B variant tH2B, which we find enriched instead on genes involved in spermatogenesis. Importantly, we find that developmental promoters are hypomethylated in sperm. Furthermore, developmental promoters selectively acquire DNA methylation during differentiation, suggesting that development is accompanied by the targeting of DNA methylation to particular developmental promoters to silence their activity, possibly to restrict alternative cell fate decisions. Many developmental promoters also bear a moderate level of H3K27me, a modification associated with the polycomb complex PRC2. Finally, in ES cells, many transcription factor promoters are occupied by PRC2 and pluripotency factors (Oct4 or Nanog). We observe a striking overlap between these promoters and the developmental promoters in sperm that are hypomethylated and Histone bound, suggesting that common factors might help establish or maintain these distinctive chromatin states. These results suggest the formation of distinctive chromatin states in sperm selectively at the promoters of genes important for embryo development, which may poise these promoters for transcriptional competence and help enable developmental totipotency.

THE ROLE OF HISTONE MODIFICATIONS IN GENE TRANSCRIPTION

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Studies of protein complexes that modify histones for transcription has led to novel insights into the mechanistic roles of these modification in the transcription process. For example, the SAGA Histone acetyltransferase complex is recruited to the promoters of activated genes where it lays down a narrow patch of acetylated histones. The nucleosomes containing these histones are then targeted by the SWI/SNF nucleosome remodeling complex through interactions with its acetyl-ly sine binding bromodomain in the Swi2 subunit. This results in these nucleosomes becoming preferentially displaced by SWI/SNF to create a nucleosome-free gap for the assembly of the preinitiation transcription complex. During transcription, elongation histones are acetylated through a process that is still not clear but presumably assists the passage of RNA polymerase II. Histones are also co-

transcriptionally methylated on Histone H3 K36 by the Set2 methyltransferase, which binds the phosphorylated CTD of elongating pol. II. These methylated nucleosomes are subsequently recognized by the Rpd3S Histone deacetylase complex through a combinatorial action of a chromodomain and a PHD finger. This results in the removal of the co-transcriptional acetylation marks, which is necessary to restabilize the chromatin of transcribed regions. These are a couple examples of how the transcription machinery exploits Histone modifications to impart information onto the chromatin template for subsequent events in the transcription cycle.

CONTENDING WITH OBSTACLES TO TRANSCRIPTION: COLLISION AND DESTRUCTION

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After initiation, RNAPII faces multiple challenges as it traverses the coding region of a gene. Obstacles such as DNA damage, protein roadblocks, replication forks, and even other RNAPIIs on the same gene have to be dealt with in a manner that maintains both efficient gene transcription and genome integrity. Recent progress in work aimed at understanding obstacle-induced RNAPII ubiquitylation, RNAPII collision, as well as RNAPII association with new protein partners will be presented.

TRANSCRIPTION INITIATION EVENTS CONTROL LEVELS OF CYTOPLASMIC TY1 RNA BODIES *(ORAL POSTER)*

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Co-transcriptional loading of factors onto nascent RNA impacts the function of the resulting mRNA-protein (mRNP) particle. To address which facets of the transcription process impact mRNP fate, we have analyzed cellular mRNA distribution in *S. cerevisiae* strains harboring mutations in Rpb1p, the largest subunit of RNA polymerase II. In some mutants, poly(A)$^+$ RNAs strongly accumulate in a confined space of the cytoplasm. RNA and protein expressed from Ty1 retroviral-like elements co-localize with this structure, that we have consequently named the T-body. A visual screen reveals that deletion of most genes with proposed functions in Ty1 biology unexpectedly do not adversely affect T-body characteristics. In contrast, deletion of genes encoding the Mediator transcription initiation factor subunits Srb2p and Srb5p as well as the Ty1 transcriptional regulator Spt21p, greatly enhances T-body formation. Our data reveal a new cytoplasmic body putatively involved in assembly of Ty1 particles, and suggest that the cytoplasmic fate of mRNP can be affected by transcription initiation events.

ALLELE SPECIFIC EXPRESSION DUE TO MUTATIONS WITHIN THE CHROMATIN-ASSOCIATED FACTOR ATRX *(ORAL POSTER)*

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It is generally accepted that in healthy, non-cancerous diploid cells the majority of genes are expressed from both alleles. Exceptions to this include imprinted genes and X-inactivation in a female. We will present data, which shows that mutations in the chromatin remodeling protein ATRX have an allele-specific affect, often resulting in a shift from biallelic towards monoallelic expression at two identified target genes.
ATRX is an ATP-dependent chromatin remodeling protein, and mutations within the gene coding for this protein result in ATR-X syndrome, an X-linked mental retardation syndrome characterized by a characteristic facial appearance, abnormal growth and development, and varying levels of alpha thalassemia. The latter characteristic is highly variable between patients, and is due to down regulation of the alpha globin genes. We have recently identified another gene within 16p13.3, which is also down regulated in ATRX patients. ChIP-Chip of the ATRX protein confirms that both of these genes/loci are direct targets of ATRX. There is an association between the level of down regulation and the haplotype of the alpha globin locus, suggesting allele specific differences in regulation. In addition, the newly identified gene also displays allele specific expression. In many cases, allele specific expression of these genes occurs concomitantly with allele specific DNA methylation of the associated CpG islands.
The exact process by which mutations in *ATRX* result in allele specific differences in expression is not yet clear, however preliminary evidence suggests ATRX may recognize abnormal DNA structures, which are hypothesized to form within these genes.

CHROMATIN REMODELLING FOR TRANSCRIPTION

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Recent work along three lines will be presented:

- the mechanism of remodeling by RSC;
- the remodeling of promoter chromatin *in vitro*;
- and the basis for transcriptional silencing.

Poster No. I-1

THE ROLE OF THE HISTONE LYSINE DEMETHYLASE JMJD3 IN STRESS-INDUCED SENESCENCE

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Methylation of lysines within the H3 tail at position 4, 9, 27, 36 and 79 is important for the regulation of transcription and has long been regarded as stable modifications defining the epigenetic program of the cell. Lysines can be mono-, di-, or tri-methylated by specific histone methyltransferases. Trimethylated K4, K36 and K79 are in general considered to be marks of actively transcribed genes, whereas trimethylated K9 and K27 on histone H3 and K20 on histone H4 are enriched on transcriptionally silenced genes. The polycomb repressive complex 2 (PRC2), whose catalytic subunit EZH2 is an oncogene, mediates transcriptional repression by catalyzing the di- and tri-methylation of K27 (H3K27me2/me3).
Using a candidate approach we recently identified two JmjC-domain-containing proteins UTX and JMJD3 as being histone lysine demethylases with specificity

towards H3K27me3. We and others have found that high levels of H3K27me3 cover the *INK4A-ARF* tumor suppressor locus during normal cellular growth. When cells are undergoing stress-induced senescence H3K27me3 levels are reduced and transcription from the locus is initiated. The rapid reduction of H3K27me3 suggests that active demethylation could be involved in the process. Currently we are investigating the possible role of JMJD3 in this process. We have found that JMJD3 is strongly induced at both mRNA and protein levels in human diploid fibroblasts undergoing oncogene-induced senescence and importantly that JMJD3 is required for the transcriptional activation of the *INK4A-ARF* locus observed after oncogenic stress. We also found that shRNA-mediated inhibition of *Jmjd3* expression leads to immortalization of mouse embryonic fibroblasts (MEFs). Experiments are currently ongoing to understand the mechanism by which the JMJD3 protein is regulating the expression of the *INK4A-ARF* locus, and whether the protein is involved in tumorigenesis.

Poster No. I-2

A PROTEOMIC SURVEY OF CHROMATIN-ASSOCIATED HUMAN RNA POLYMERASE II

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RNA polymerase II (RNAP II) performs transcription in the context of chromatin, a nucleoprotein structure composed of DNA and histones. Although several independent studies examined the proteins associated with the high-salt-soluble human RNAP II, the proteomic composition of the native chromatin-associated form of human RNAP II still remains unclear. We have developed a novel strategy that has enabled us to separate and purify chromatin-associated and nucleoplasmic forms of human RNAP II complexes to virtual homogeneity under native conditions. We have performed unbiased proteomic analysis of these differentially purified protein complexes by using mass spectrometry. This approach enabled us to define the protein complexes that are specifically associated with the chromatin-associated human RNAP II and discover at least 6 novel proteins interacting with this form of the holoenzyme. Reciprocal proteomic analysis of the novel RNAP II associated factors from human cells confirmed the interactions, suggesting that our previous view of the human RNAP II transcription machinery was far from complete. We also provide evidence that our novel approach is useful for unbiased proteomic characterization of other chromatin-associated protein complexes in human cells.

Poster No. I-3

A PROPOSED FUNCTION OF THE HTH/MEIS TRANSCRIPTION FACTOR IN HETEROCHROMATIN FORMATION

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Normal chromosomal segregation during cell division requires the correct assembly of heterochromatin. A key structure in the process is the centromere, located in the centromeric heterochromatin, which contains many repetitive DNA sequences. In *Drosophila*, the preblastodermic syncytial nuclear divisions occur very fast, at a rate of 8-9 min. per round. In this short period of time chromosomes must condense, segregate and decondense, in conditions in which there is no transcription of the

zygotic genes; only maternally provided RNAs and proteins are present. The Homothorax (Hth)/Meis transcription factor is maternally provided and its function is necessary for the proper formation and function of the centromeres during preblastodermic divisions. Hth presumably acts together with its partner Extradenticle (Exd)/Pbx, so that embryos lacking either the exd or the hth maternally-derived transcript, show a similar phenotype: abnormal localization of the centromeric CID protein, the *Drosophila* homolog of CENP-A, and aberrant chromosomal segregation. We also find that in pre-blastodermic embryos Hth colocalize with RNA Polymerase II in the transcriptional pre-initiation complex, and that the transcription of centromeric heterochromatin repeats shows a 25 fold reduction in *hth* mutants. In this work we demonstrate a requirement of a homeodomain transcription factor in centromeric heterochromatin assembly in *Drosophila*. We suggest that Hth forms a physical association with RNA polymerase II that facilitates the transcription of heterochromatic DNA sequences, which is one of the first and essential steps for heterochromatin assembly.

Poster No. I-4

THE CRYO-EM RECONSTRUCTION OF RNA POLYMERASE II IN COMPLEX WITH ELONGATION FACTORS SPT4/5 IMPLIES A NOVEL MECHANISM FOR TRANSCRIPTIONAL ELONGATION

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The structure of the complex between RNA polymerase II and the highly conserved elongation factors Spt4/5 has been determined to 12 Å resolution using single-particle cryo-electron microscopy (spcEM). Upon binding to the elongation factors, the polymerase undergoes remarkable structural rearrangements that encompass large portions of the multiprotein complex. The most significant rearrangement is an opening of the central pore and funnel, leading to the formation of a channel wide enough to encompass an entire DNA-helix.

Based on the substantial structural rearrangements that we observe, we would like to propose a model for transcription where the initiation of transcription and first synthesis of RNA take place on the surface of the polymerase, as shown by previous X-ray crystallography studies, followed by an internalization of the entering DNA arm within the polymerase upon elongation. We propose that this transition can occur in the free polymerase, but is boosted by Spt4/5 and other elongation factors. Further, we also suggest that Spt4/5 and the elongation factor TFIIS lock the polymerase in the DNA-enclosed elongating conformation, thus preventing DNA escape and premature arrest.

Our reconstruction also suggests a new mechanism for the RNA polymerase II specific inhibitor a-amanitin. We propose that the inhibitor locks the polymerase in the initiating conformation, preventing the opening of the funnel and thus the transition into an elongating form.

The Cryo-EM reconstruction of RNA polymerase II in complex with the universal elongation factors Spt4/ suggests a new and general mechanism for the elongation process of eukaryotic transcription. Since Spt4/5 has been shown also to be involved in RNA polymerase I transcription, and part of the Spt5 subunit is conserved in both prokaryotes and archaea, the model for elongation that we present might be valid for all three kingdoms of life.

Poster No. I-5

STRUCTURAL REGULATION OF THE C-MYC GENE EXPRESSION

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Nuclear arrangement of transcriptionally active/inactive c-myc genes has been investigated in relationship to karyotype instability, cell differentiation and regarding to nuclear domains involved in RNA synthesis and processing. The c-myc loci and their transcription sites were positioned non-randomly within the interphase nucleus, even after cell differentiation (Harničarová et al., 2006). Using oligo-probes, designed to exon II and exon III of the c-myc gene, single c-myc transcription site was observed in several human carcinoma cell lines and in human embryonic stem cells. Conversely, human embryonal teratocarcinoma NTERA cells, with normal c-myc karyotype, were characterized by the presence of multiple c-myc RNA signals located in both the nucleoli and nucleoplasm (Bártová et al., 2008). In majority of cases, c-myc transcripts were associated with the transcription factories and SC35 domains and c-myc RNA signals were rarely located in close proximity to the promyelocytic leukemia (PML) bodies. Taken together, in comparison to coding sequences, c-myc RNA signals were positioned in the most internal parts of the interphase nuclei to be preferentially associated with the nucleoli and SC35 domains that probably play an important role in the c-myc RNA processing.

Acknowledgement:

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References:

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2. Bártová E., Harničarová A., Krejčí J., Strašák L. and Kozubek S. (2008) Single-cell c-myc gene expression in relationship to nuclear domains, accepted for publication in Chromosome Res., 16(2):325-43.

Poster No. I-6

THE STRUCTURE OF P-TEFB AND ITS COMPLEX WITH FLAVOPIRIDOL AND REGULATION BY PHOSPHORYLATION

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Positive transcription elongation factor b (P-TEFb) is a cyclin dependent kinase (CDK) complex consisting of CDK9 and cyclinT. It promotes transcriptional elongation of RNA polymerase II dependent genes through phosphorylation of the polymerase and elongation repressors. In order to understand the regulation of a transcriptional CDK by its cognate cyclin we determined the structure of human CDK9 in complex with cyclinT1. In the CDK9/CycT1 complex, CDK9 is in the active conformation and Thr186 (the threonine in the activation segment) is phosphorylated. We found distinct differences between CDK9/cylinT1 and the cell cycle CDK complex CDK2/cyclinA. In CDK9/cyclinT1, the cyclin is rotated by 26° with respect to cyclin A, showing for the first time plasticity in a CDK/cyclin interaction. This results in a reduced interaction surface of the CDK/cyclin complex. The C-terminal cyclinT1 helix has a different conformation in the complex as in the free cyclinT1 structure that may

be important for the interaction with its regulatory proteins HIV TAT and HEXIM. Flavopiridol, an anti-cancer drug, binds to the ATP site of CDK9 and induces unanticipated structural rearrangements that bury the inhibitor. These provide a rationale for the strong inhibition the inhibitor shows towards CDK9. CDK9 activity and recognition of regulatory proteins is governed by phosphorylation. We show that CDK9/CycT1 autophosphorylates its activation segment and C-terminus and analyze the effects on these phosphorylations on CDK9 activity.

Poster No. I-7

THE HISTONE DEMETHYLASES JMJD1A AND JMJD2B ARE TRANSCRIPTIONAL TARGETS OF HYPOXIA-INDUCIBLE FACTOR (HIF)

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Hypoxia is a reduction of oxygen availability in tissue and arises as a hallmark of vascular diseases, pulmonary disease and cancer. It leads to rapid activation of an adaptive transcriptional program that is governed by family of transcription factors termed "hypoxia-inducible factors" (HIF). Moreover, in a subset of cancers that have lost expression of the von Hippel Lindau tumor suppressor *VHL*, HIF accumulates irrespective of oxygen levels, which results in a constitutive expression of hypoxia-responsive genes. HIF target genes impact cellular metabolism, motility, apoptosis, genetic stability and promote angiogenesis, tissue invasion and tumor metastasis.
Posttranslational histone modifications serve to store epigenetic information and control both nucleosome assembly and recruitment of non-histone proteins. Histone methylation occurs on arginine and lysine residues and is involved in transcriptional activation and repression as well as in DNA repair. A recently discovered family of histone demethylases, the Jumonji C-domain (JmjC) proteins, is able to remove methyl groups from tri-, di– and monomethylated lysine and methylated arginine residues. Thereby, the JmjC proteins modulate gene expression in the context of i.e. stem cell renewal, cellular differentiation and hormone response.
Here we show that HIF binds to specific recognition sites in the genes encoding the JmjC proteins JMJD1A (KDM3A) and JMJD2B (KDM4B) and induces their expression. Accordingly, hypoxic cells and *VHL*-negative cancer cells express elevated levels of JMJD1A and JMJD2B mRNA and protein. Interestingly, studies on ectopically expressed JMJD1A and JMJD2B indicate that both proteins largely retain their lysine demethylase activity in cells at 1% oxygen. Based on these findings, we are currently assessing a possible impact of both JmjC proteins on gene regulation by HIF and tumor cell behavior and will present our recent results.

Poster No. I-8

REORGANIZATION OF CHROMATIN IS AN EARLY RESPONSE TO NITROGEN STARVATION IN *SCHIZOSACCHAROMYCES POMBE*

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There are several documented events of changes in subnuclear localization during gene activation. There are, however, different data on whether the nuclear periphery is a compartment for gene repression or activation, and whether genes are moved to the pores at the nuclear membrane (NM) or not during gene activation. Nitrogen

starvation of fission yeast cells serves as a good model system for studying gene induction since it causes fast regulation of hundreds of genes.
In this study we investigated the subnuclear localization of two clusters of genes repressed by nitrogen. The gene clusters localized at the nuclear periphery on the opposite side of the nucleus as compared to the spindle pole body (SPB). This constrained localization was dependent on the histone deacetylase Clr3, known to transcriptionally repress genes in these clusters. Nitrogen depletion induced drastic changes in subnuclear localization of the two clusters, already after 20 minutes, away from the NM towards the SPB. These data illustrates how interconnected events of gene activation and nuclear reorganization are, and indicates that relocalization to the nuclear pore is not a general mechanism of gene activation in yeast.

Poster No. I-9

LOSS OF ATAC-SPECIFIC HISTONE H4 LYSINE 12 ACETYLATION REDUCES JIL-1 BINDING TO CHROMATIN AND PHOSPHORYLATION OF HISTONE H3 AT SERINE 10

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Various combinations of posttranslational modifications of the N-terminal tails of nucleosomal histones serve as signals to govern chromatin-related processes. The relationship, however, among different types of histone modifications, most frequently acetylation, phosphorylation and methylation, and the order of their establishment has been explored only in a few cases.
Here we show that a reduced level of histone H4 lysine 12 acetylation by the *Drosophila melanogaster* ATAC histone acetyltransferase complex leads to a decrease in the phosphorylation of histone H3 serine 10 by the JIL-1 kinase. As JIL-1 activity antagonizes histone H3 lysine 9 di-methylation by Su(var)3-9, our observations demonstrate the interdependent actions of an acetyltransferase, kinase and methyltransferase. We demonstrate that in accord with the steps of modifications, mutations affecting ATAC subunits (*dGcn5*, *dAda2a* and *dAda3*) (i) decrease the level of histone H3 serine 10 phosphorylation, (ii) can be rescued partially by JIL-1 overproduction, (iii) enhance the spread of histone H3 lysine 9 di-methylation, and (iv) are suppressed by *Su(var)3-9* mutations. We propose that a reduced level of histone H4 lysine 12 acetylation by ATAC attenuates the phosphorylation of histone H3 serine 10 by JIL-1 due to a reduced binding of JIL-1 to hypoacetylated chromatin. Combined with our recent results on the dependence of ATAC access to chromatin on NURF function (*Carre et al. EMBO Rep. 2008*), these data show the delicate balance existing among chromatin remodeling and modifying complexes in regulating chromatin organization and function.

Poster No. I-10

CONTROL OF DNA METHYLATION BY THE LSD1 HISTONE DEMETHYLASE

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Chromatin structure and the modification status of histone tails have an important role in regulating gene expression. LSD1 (also known as BHC110 or KDM1) was the first enzyme identified that directly removes methyl marks from histones.

Here we show that LSD1 is connected to DNA methylation, a key epigenetic system involved in gene silencing. We show that LSD1 binds to DNA methyltransferases and associates with DNA methyltransferase activity *in vivo* system. Treatment of cells with 5-aza-2'-deoxycytidine, a DNA methyltransferase inhibitor, leads to derepression of LSD1 target genes. We also found that knockdown of either LSD1 or DNMTs results in reexpression of target genes. Further, we show that LSD1 is required for DNA methylation of LSD1-target promoters.
Our work thus suggests direct control of DNA methylation by LSD1, possibly uncovering a direct mechanistic link between a histone demethylase and the DNA methylation repressive system.

Poster No. I-11

EPIGENETIC REGULATION OF NEURAL STEM CELL STATE AND FATE BY THE HISTONE DEMETHYLASE JMJD2C/KDM4C

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It has been proposed that stimulation of neural stem cells (NSC) by fibroblast growth factor 2 (FGF2) is required for demethylation of tri-methylated (me3) lysine 9 on histone H3 (H3K9), and certain neural differentiation events in neural progenitors. We have previously showed that H3K9 is normally hypoacetylated in NSC, and increased acetylation of H3K9 (but not other residues) by HDAC inhibitors such as valproic acid (VPA) at a subset of genes is specifically associated with neuronal differentiation. A pre-requirement for this acetylation control mechanism is indeed that H3K9 is demethylated. Historically, histone methylation was considered a stable modification, but recently it has been shown that a certain family of proteins containing the so-called Jumonji domain (JmjC) can act as histone demethylases.
In this study, we have investigated the role of H3K9-demethylating enzymes in NSC derived from embryonic rodent cortices and, in most cases, expanded with FGF2 as monolayers. Experiments using RT-PCR and RT-qPCR revealed that the seven members of JMJD1 and JMJD2 families investigated were expressed in the NSCs.
We found that VPA induced neuronal differentiation of NSC associated with a decrease of H3K9me3 irrespective of FGF2 treatment. VPA-treated NSCs showed an increased expression of JMJD2C mRNA independent of de novo protein synthesis that correlated with the decrease in H3K9me3. Moreover, overexpression of JMJD2C in these cells led to a decrease in the methylation pattern of the same lysine. Putative target genes involved in control of NSC differentiation are currently being investigated in detail. Our results suggest that the histone demethylase JMJD2C play a crucial role for neuronal differentiation of neural stem cells.

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Poster No. I-12

CLASS I HDACS INHIBIT DIFFERENTIATION OF NEURAL STEM CELLS INTO THE OLIGODENDROCYTE LINEAGE

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Multiple sclerosis and several other neurological diseases are characterized by the defective myelination of neuronal axons. The transplantation of myelin-producing oligodendrocytes, derived from neural stem cells (NSCs), or the induction of terminal differentiation of the endogenous oligodendrocyte precursors are possible therapeutic approaches for treatment of these diseases. The study of the epigenetic regulation of these events might therefore allow the design of successful remyelination cell therapy protocols.
In this study, we investigated the involvement of epigenetic control mechanisms in oligodendrocyte specification/differentiation. Thyroid hormone (T3) is essential for these processes and regulates the transcription of specific genes by modulating the recruitment of transcriptional regulators, such as histone deacetylases (HDACs). T3 has been described to activate transcription by leading to the release of HDACs from promoters of certain genes. Intriguingly, however, HDACs have been suggested to be required for oligodendrocyte development. We determined that T3 can induce efficient specification and terminal differentiation of rat fetal NSCs into oligodendrocytes. In contrast, treatment of NSCs with the HDAC inhibitor valproic acid (VPA) initially repressed the expression of several oligodendrocytic genes. However, treatment of NSCs with VPA also led to a robust increase in expression of the transcription factor Sox10, which is essential for oligodendrocyte differentiation. Furthermore, sustained treatment with VPA and T3 led to the up regulation of late-oligodendrocyte genes and induced a striking morphological differentiation of the oligodendrocytes, with the formation of myelin plaques. This effect was dependent on the induction of Sox10 by VPA, as assessed by RNA interference. These effects of VPA could be due to HDAC inhibition and indeed class I HDACs were found to be present in regulatory regions of oligodendrocyte genes in NSCs and oligodendrocyte precursors, by chromatin immunoprecipitation. Moreover, the role of HDAC inhibition in the differentiation of oligodendrocytes was confirmed by RNA interference against specific class I HDACs. Thus, our results suggest that class I HDACs play distinct temporal roles in the transcriptional regulation of several oligodendrocytic genes. Therefore, modulation of HDAC activity might be an important tool for cell-based therapies in demyelinating diseases.

Poster No. I-13

INHERITED AND ACQUIRED EPIMUTATIONS IN HUMANS: LESSONS FROM CONGENITAL GROWTH DISORDERS

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DNA methylation is the main epigenetic mechanism regulating the monoallelic and parent of origin-dependent expression of imprinted genes. The overgrowth- and tumor-associated Beckwith-Wiedemann syndrome (BWS) and growth restriction-associated Silver Russell syndrome (SRS) are characterized by contrasting phenotypes and caused by different molecular defects of the same imprinted gene cluster. In this cluster, that is located at chromosome 11p15.5, the imprinted expression of *IGF2* and *H19* genes is controlled by the Imprinting Centre 1 (IC1), consisting in a methylation-sensitive chromatin insulator. Hyper- and hypo-methylation of this regulatory region are found in BWS and SRS patients, respectively, resulting in defective *IGF2/H19* expression. We demonstrated that the methylation imprinting defects arise from distinct molecular mechanisms. In BWS,

gain of methylation can result from maternally inherited deletions removing part of the IC1 region or arise independently from the sequence context. In the former case, the epimutations have high recurrence risk, while in the latter; they are generally not transmitted to the progeny. Sporadic epimutations of IC1 are also a common cause of SRS, since loss of IC1 methylation seems not to be associated with any deletion or mutation in cis. A common feature of all these cases is that the methylation defect is present in the mosaic form, suggesting that the epimutations arise as consequence of errors in the maintenance rather than in the establishment of genomic imprinting. Experiments are in progress to identify the epigenetic regulatory factors that are important for the imprinting maintenance and possibly involved in the etiology of these disorders.

Poster No. I-14

FUNCTIONAL DIVERSIFICATION AND TARGETING OF SWI/SNF CHROMATIN REMODELERS

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By regulating the structure of chromatin, ATP dependant chromatin remodelers play a critical role in the maintenance, expression and transmission of the eukaryotic genome. Moreover, aberrations in the functioning of these evolutionary conserved chromatin remodelers have been implicated in cancer development. Several distinct families of chromatin remodelers have been described; these include the Swi/Snf family. BAP and PBAP are the fly representatives of the two evolutionarily conserved major subclasses of SWI/SNF remodelers. Both complexes share 7 core subunits, including the Brahma (BRM) ATPase, but differ in a few signature subunits: Polybromo, SAYP and BAP170 specify PBAP, whereas OSA defines BAP. Although BAP and PBAP are clearly related to yeast SWI/SNF and RSC, some subunits, such as BAP170 and SAYP appear to be metazoan specific and absent in yeast. Here we describe two more metazoan-specific subunits part of BAP and PBAP respectively. Employing a combination of genomics, proteomics, and developmental genetics, we explore the functional roles of BAP and PBAP in cell cycle control and development. We will also present genome-wide binding and expression analysis to explore the transcription networks controlled by BAP or PBAP. In particular, we will discuss the roles of the signature subunit in SWI/SNF targeting. We found that mutations in genes encoding PBAP-specific subunits cause characteristic leg malformations and microcephaly, which are reminiscent of phenotypes caused by defective ecdysone signaling. These observations suggest that PBAP might be involved in ecdysone-inducible gene regulation *in vivo*. Therefore, we have compared the roles of BAP and PBAP in nuclear hormone receptors signaling during development. In summary, BAP and PBAP are essential chromatin remodeling factors that perform cooperative as well as unique function during development. Distinct subunits appear to be dedicated to specific regulatory pathways. Our analysis of BAP and PBAP suggests that they are dedicated to specific transcriptional pathways, rather than acting as true general factors.

Poster No. I-15

THE ROLE OF HISTONE DEACETYLASES (HDACS) IN INFLAMMATORY GENE CONTROL

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A commonly held view posits that histone acetylation mediates gene activation, whereas lack of/hypo acetylation tends to correlate with repression, the activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs) acting in concert to establish balance in levels of transcription. However in recent years, genome-wide analyses in yeast showed that HDACs are equally involved in both transcriptional activation and repression. Similarly, in mammalian cells, general inhibition of HDAC activity with a short trichostatin A (TSA) treatment results in a decreased expression of several rapidly inducible genes in response to various agonists. Gene expression profiling of cells treated with HDAC inhibitors (HDACi) also underscored the importance of HDACs as transcriptional activators. However, the contribution of individual HDAC to transcriptional activation and the mechanisms involved in HDAC-mediated gene expression are unknown. We investigate the role of individual class I HDACs namely HDAC 1, -2 and -3 using RNA interference(shRNA) to better understand the combinatorial control of inflammatory gene expression in 3T3 fibroblasts and primary macrophages. In addition to genes showing the expected de-repression of some genes, we observed 8/20 tested genes being down-regulated following individual HDAC depletion. The requirement for HDACs function in gene induction as opposed to the more commonly observed role as transcriptional repressors may either underlie an indirect consequence of impaired HDAC mediated repression or a direct involvement of HDAC 1 and HDAC 3 in inducible gene activity. Interestingly, some genes were antagonistically regulated by HDAC 1 and HDAC 3 suggesting that HDAC 1 and HDAC 3 might act as an antagonistic pair. Mechanistic basis for the antagonism between HDAC3 and HDAC1 as well as for the role of HDAC1/3 in activation are under investigation.

Poster No. I-16

A NOVEL HISTONE H1 PHOSPHORYLATION AT THE N-TERMINAL SERINE 35 ACCUMULATES DURING MITOSIS

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Post-translational modifications (PTMs) of histone proteins constitute the histone code which plays an important role in eukaryotic gene expression. In contrast to core histones H2A/H2B/H3/H4, little is known as regards H1 modification. The linker histone H1 is thought to help pack adjacent nucleosomes into higher order chromatin structure which is a critical event during cell cycle. H1 samples were analyzed on LTQ-FT for determining precursor ion mass with high accuracy and fast MS/MS analysis. A novel N-terminal serine 35 phosphorylation on both bovine H1 and human H1.2/1.3/1.4 from HeLa was identified. HeLa cells treated with nocodazol revealed that H1.4S35 phosphorylation accumulates during G2/M transition. Furthermore, using immunochemical approach, we observed that the S35-phosphorylated H1.4 is excluded from the condensed chromatin region during mitosis. An adjacent methylation site at lysine 33 on H1.4 was also identified, which exhibits mutually

exclusive existence from S35 phosphorylation. Unlike S35 phosphorylation, monomethylation at K33 on H1.4 is not affected by nocodazol in HeLa cells. The H1 peptide with S35E substitution which mimics S35 phosphorylation became a poor substrate for methylation. In contrast, the H1.4K33R mutant was highly phosphorylated at S35 in an unsynchronized condition, which implies a *cis*-crosstalk between K33 methylation and M phase-specific S35 phosphorylation on H1.

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Poster No. I-17
MODIFICATIONS OF HISTONE H3 ASSOCIATED WITH SENESCENCE AND DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS

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Modifications of histones and DNA methylation modulate chromatin assembly on gene regulatory sequences, and thereby transcription. Little is known on histone modifications marking promoters of lineage-specific genes in mesenchymal stem cells. Using chromatin immunoprecipitation (ChIP), we show that in undifferentiated human adipose tissue stem cells (ASCs) inactive adipogenic and myogenic promoters are enriched in a repressive combination of trimethylated H3K4 and H3K27 in absence of K9 acetylation or trimethylation. This combination is different in keratinocytes in which these promoters are enriched in trimethylated H3K4 and K27 but also harbor the heterochromatic H3K9 trimethylation. Sequential ChIP srgues for gene-dependent co-occupancy of H3K4m3 and H3K27m3 on single nucleosome in ASCs. The H3K4m3 and H3K27m3 marks are stable during long-term ASC culture even though cells lose ability to differentiate. Array comparative genomic hybridization indicates that epigenetic stability is superimposed onto an overall genetically stable genome. Adipogenic differentiation in early passage resolves the K4m3/K27m3 bivalency and results in H3K27m3 demethylation and H3K9 acetylation, selectively on adipogenic promoters. At senescence however, transcriptional up regulation is impaired, H3K4m3 and H3K27m3 are maintained and H3K9 acetylation is blocked. Furthermore, the polycomb repressor complex (PRC)1 and 2 markers Bmi1 and Ezh2, respectively, are targeted to all promoters examined, including the housekeeping *GAPDH* promoter, in senescent cells. Ezh2 and Bmi1 targeting correlates with H3K9 deacetylation on promoters, increased intracellular levels of Ezh2 and phosphorylated Bmi1, and decreased cellular acetylated H3K9 levels. The seemingly lack of promoter-specificity of Ezh2 and Bmi1 targeting in senescent ASCs suggests the establishment of a global polycomb-mediated epigenetic program aiming at shutting down transcription.

Poster No. I-18
TRANSCRIPTIONAL ELONGATION IS MODULATED BY UNCONVENTIONAL MYOSIN VI, THAT SHUTTLES BETWEEN NUCLEUS AND CYTOPLASM

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The nuclear role of actin and myosins has recently started to be unveiled. We have shown that unconventional myosin VI, the only myosin moving towards the pointed end of actin filaments, i.e. in the opposite direction to all other myosins, is present in

the nucleus where it modulates RNAP II transcription[1]. We studied the mechanism underlying this function as well as the capability of the protein to exchange between the cytoplasm and the nucleus.
Endogenous or GFP-tagged myosin VI is massively recruited to the nucleus upon transcriptional induction of HepG2 cells. It is present on actively transcribed genes together with the elongating (CTD-S2p), but not the initiating (CTD-S5p), form of RNAP II. Stable antisense-expressing cells show a substantial reduction of BrU-labeled nascent transcripts and a specific decrease of the RNAP II-CTD-S2p signal. Moreover, myosin VI specifically co-immunoprecipitates with CDK9 (p-TEFb), but not with CDK7 (TFIIH), strongly suggesting a role for myosin VI in transcriptional elongation.
The cytoplasmic vs. nuclear localization of myosin VI before/after transcriptional induction or LMB treatment of HepG2 cells, suggest the presence of functional NLS and NES. We have identified such signals 'in silico', mutated the relevant amino acids and tested their function. NES-mutated GFP-myosin VI accumulates in the nucleus in the absence of transcriptional induction, whereas NLS-mutated GFP-myosin VI is not recruited to the nucleus in the presence of a transcriptional stimulus. Thus, the nucleo-cytoplasmic shuttling of myosin VI is modulated in vivo by functional NES/NLS signals.

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Poster No. I-19

IDENTIFICATION OF KINASE SIGNALLING PATHWAYS THAT REGULATE HISTONE METHYLATION

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Posttranslational histone modifications are the core of epigenetic regulation of gene expression, in particular histone lysine methylation. The methylated lysine residues create docking sites for protein complexes, which when recruited to the histones regulate chromatin structure and gene transcription. The lysine methylation status of histones results from the balanced action of many proteins. Methylation is catalyzed by a number of histone methyltransferases and demethylation is catalyzed mainly by Jumonji C-domain proteins. Moreover, these enzymes typically function within large, regulatory protein complexes. Although a significant number of proteins modulating histone lysine methylation have been identified, the cellular signal transduction pathways which regulate these marks are poorly understood. Since protein phosphorylation is the most general regulatory mechanism in eukaryotes, we reasoned that kinases are key effectors of cellular signaling pathways that regulate histone lysine methylation.
To identify kinase signaling pathways which control histone lysine methylation, we first established a dsRNA library against all 241 kinases (the kinome) in the fruit fly, a model organism with minimal redundancy within kinase signaling pathways. We then performed robotic, high-throughput RNA interference screens in fruit fly S2 cells for kinases regulating the repressing H3 lysines 27 and 9 trimethylation marks and the activating H3 lysine 4 trimethylation mark.
At this point, we have identified several, interesting candidate hits for kinases which may be involved in establishing histone lysine methylation. We are in the process of

further validating and characterizing these kinase hits. We hope that these studies may shed light on the poorly understood issue how epigenetic marks and the regulation of gene expression is controlled by cellular signaling pathways.

BENZON SYMPOSIUM No. 55

TRANSCRIPTION, CHROMATIN AND DISEASE

AUGUST 18 – 21, 2008, COPENHAGEN, DENMARK

Organizing committee:
Kristian Helin (Copenhagen), Jesper Q. Svejstrup (London) & Arne Svejgaard (Copenhagen)

Abstracts - TUESDAY, AUGUST 19, 2008

CHROMATIN ASSEMBLY FACTORS AND THE CHALLENGES OF DNA REPLICATION AND REPAIR

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Inheritance and maintenance of the DNA sequence and its organization into chromatin are central for eukaryotic life. To orchestrate DNA-replication and –repair processes in the context of chromatin is a challenge.

Factors have been isolated from cell extracts that stimulate early steps in chromatin assembly *in vitro*. One such factor, chromatin assembly factor-1 (CAF-1), facilitates nucleosome formation coupled to DNA synthesis. It is thought to participate in a marking system at the crossroads of DNA replication and repair to monitor genome integrity and to define particular epigenetic states. We have now identified a chromatin assembly pathway independent of DNA synthesis involving the HIRA protein. Notably, CAF-1 is part of the Histone H3 complex, H3.1 complex (replicative form) and HIRA of the H3.3 complex (replacement form) (Tagami et al, 2004, Nakatani et al, 2004). In addition, another Histone chaperone, Asf1, has to be integrated in a network of interactions leading to nucleosome deposition. A major goal in our laboratory is now to better integrate the function of these factors *in vivo* during development and also in connection with replication, repair and control of Histone pools.

We will discuss our recent findings on these topics and the interrelationships with other assembly factors.

A ROLE FOR CHROMATIN REMODELING COMPLEXES AND ARCHITECTURAL FACTORS IN MRNA SPLICING *(ORAL POSTER)*

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The regulatory role of chromatin requires interaction between many chromosomal proteins. We employed a genetic approach to study the functional interplay between the chromatin remodeling complexes RSC or SWI/SNF and the architectural factors Nhp6p. *rsc*- or *swi*- mutations in combination with an *nhp6ΔΔ* double deletion result in synthetic sickness. Interestingly, *rsc nhp6ΔΔ* triple mutant cells have reduced amounts of the U4/U6 snRNP dimer and accumulate unspliced transcripts after a two hours incubation at 37°C, strongly suggesting a defect in pre-mRNA splicing. We selected multi-copy suppressors of the growth defect of an *rsc8-ts16 nhp6ΔΔ* strain

and isolated *MRN1*, multi-copy suppressor of *rsc nhp6ΔΔ*. Multi-copy *MRN1* also suppresses the ts-phenotype of *snt309Δ*, *prp3-1*, *prp4-1*, *prp11-1 and prp22* splicing deficient mutants. Furthermore, *2μ-MRN1*, suppresses the splicing defects in both *rsc nhp6ΔΔ* and *snt309Δ* cells. Interestingly, the growth and splicing defects in *snt309Δ* cells are also suppressed by deletion of *RRP6*, a nuclear exosome component. Studying Mrn1p function in transcription *in vivo* we show that Mrn1p localizes to the nucleus in a *mex67-5* dependent manner, that Mrn1p binds *ACT1* pre-mRNA and that it associates with the RNA Polymerase II subunit Rpb3p.

We propose i) that destabilization of the U4/U6 snRNP dimer in *rsc nhp6ΔΔ* cells results in pre-mRNA accumulation and ii) that suppression of the splicing defect by over-expressed Mrn1p is mediated through its binding of pre-mRNA protecting unspliced mRNA from exosome degradation.

THE SWI/SNF COMPLEX REGULATES ALTERNATIVE SPLICING *(ORAL POSTER)*

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The SWI/SNF complex is a chromatin remodeling machinery that facilitates access of transcriptional regulators to promoter sequences. This complex regulates many genes but seems particularly important for the control of cell growth, and several of its subunits including hSNF5/INI1 and the catalytic subunits Brg1 and Brm are either tumor suppressors or markers of bad prognosis.
Interestingly, the SWI/SNF complex is also present inside the coding region of several genes and we recently showed that it functions as a regulator of alternative splicing. In particular, on the CD44 gene, the presence of the SWI/SNF complex on the central cluster of variant exons can be correlated with RNA polymerase II pausing, a phenomenon known to favor the use of weaker splice donor sites and thereby to promote inclusion of alternative exons.
We now show that a native spliceosome, purified while assembled with pre-mRNA, contains all the core subunits of SWI/SNF. Likewise, immunoprecipitations show that Brm bound to chromatin interacts with the U1 and U5 splicing snRNAs, suggesting that the SWI/SNF complex may also affect exon inclusion by facilitating recruitment of the splicing machinery.
In addition, we have performed a genome wide analysis of the impact of the SWI/SNF complex on alternative splicing that provides many new insights on how this complex participates in the regulation of cell growth.

POLYCOMB REPRESSIVE COMPLEXES ARE REQUIRED TO MAINTAIN COMPACT CHROMATIN STRUCTURE AT HOX LOCI

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It is generally considered that inactive chromatin has a more closed or compact chromatin structure than active chromatin and that chromatin compaction can inhibit gene expression. However, the mechanisms that mediate changes in higher-order chromatin structure are poorly understood. We have investigated the role of the linker Histone H1 and the polycomb repressive complexes PRC1 and 2 in maintaining the compact chromatin state of Hox loci in embryonic stem (ES) cells. Using cells deficient in H1, Eed (PRC2) and Ring1b (PRC1) we show that whilst the reduction of H1 levels leads to a global decompaction of chromatin, this is not specific to the Hox loci. In contrast, loss of PRC1 or 2 activity leads to a visible decompaction of Hox loci in undifferentiated ES cells compared to wild-type, and this is not seen at other regions of the genome. We suggest that, consistent with in vitro studies of polycomb protein function, that PRC complexes act in vivo to establish a compact chromatin structure that inhibits gene expression at developmentally regulated loci.

UNRAVELING OCCUPANCY AND ORGANIZATION OF THE GENOME

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The spatial organization of chromatin and the protein composition of chromatin are both known to change during regulatory events, frequently in a causal manner. We have used two new technologies to find unanticipated regulatory effects in the spatial organization of chromatin in human cells and in the protein composition of telomeres. We have used a microarray-based technology to map structure across 500 kb of the HOX clusters during differentiation of human ES cells and across the INK4A/ARF locus during Polycomb-Group (PcG) regulation of those genes. Transcription of the HOX clusters increases over broad regions while chromatin structural changes are limited to punctate regions within both the expressed and the repressed domains of the HOX clusters. This contrasts with previous studies that have shown changes in covalent modifications (e.g., Histone methylation) to occur in large blocks across these clusters. Structural changes in the INK4A/ARF locus also occur over regions much more narrowly defined than where covalent changes occur. Thus structural changes in chromatin cannot be directly inferred from the location of covalent modifications of histones. This technology is being used to map key regulatory elements that respond to PcG function.

We have extensively modified a FISH protocol to develop a technique that isolates specific loci as chromatin in sufficient purity to identify proteins directly using MS/MS. Applying this technology to human telomeres, we identified ~90% of all proteins previously shown to bind telomeres, including proteins with low level expression. We compared HeLa telomeres with ALT telomeres and identified a family of orphan nuclear receptors that binds specifically to ALT telomeres. This binding appears to be mediated by a change in the sequence of ALT telomeres. Based upon knockdown studies, we propose that this family of transcription factors targets ALT telomeres to PML bodies, where they cluster to undergo the efficient recombination needed to allow their maintenance.

DOSAGE COMPENSATION AND CHROMATIN REGULATION

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Dosage compensation, mediated by the MSL complex, regulates X chromosomal gene expression in *Drosophila*. High-resolution, genome-wide chromatin profiling and gene expression analysis revealed differential behavior of chromatin modifying enzyme MOF on X chromosomal versus on an autosomal genes. We found that on the male X chromosome, where MSL1 and MSL3 are preferentially associated with the 3'end of dosage compensated genes, MOF displays bimodal distribution binding primarily to promoters as well as the 3' ends of genes. While on autosomal genes as well as MSL1/MSL3 independent X chromosomal genes in males and females, MOF binding was restricted to promoters. Binding of MOF to autosomes is functional, as H4K16Ac and the transcription levels of a number of genes are affected in MOF-depleted cells. MOF is therefore involved not only in the onset of dosage compensation, but also acts as a regulator of gene expression in the *Drosophila* genome.

ROLE AND REGULATION OF HISTONE METHYLATION AT MOUSE IMPRINTED GENE LOCI *(ORAL POSTER)*

Feil R; Institute of Molecular Genetics, CNRS, France

Our group is interested in the role and regulation of Histone methylation at 'imprinting control regions' (ICRs), the sequence elements that control imprinted chromosomal domains in mammals. For somatic cells, we reported earlier that the DNA-methylated allele of ICRs is consistently associated with trimethylation at H3 lysine-9 (H3K9me3) and H4K20me3. We now explore which Histone methyltransferases control the allelic H4K20me3 at ICRs. We find that Suv4-20h1/h2 are recruited to the ICRs' DNA-methylated allele and are essential for the allelic H4K20me3. Indirectly, their expression influences levels of H4K20me1 and H3K9me3 as well. H4K20me1, however, is present on both parental alleles and is mediated by Pr-Set7. These data highlight a regulatory pathway that implicates recruitment of Pr-Set7 and Suv4-20h1/h2 to promote first biallelic H4K20me1, and, then, H4K20me3 specifically on the DNA-methylated allele. Currently, we are determining which HMT(s) regulate the allelic H3K9me3 at ICRs in somatic. We are also developing approaches to study Histone methylation at imprinted loci in germ cells. In a related theme, we explore the role of Histone methylation in the control of tissues-specific imprinting. As one of our developmental models, we study the mouse *Grb10* gene, which displays brain-specific paternal expression. The brain-specific promoter region corresponds to the putative ICR of this gene, and was found to be marked by 'bivalent chromatin' (enriched in both H3K4me2 and H3K27me3) on the paternal allele from early embryonic stages onwards. During development, this bivalent chromatin is maintained in all somatic tissues, but is lost in adult brain. Our data support a model in which bivalent chromatin controls the paternal expression of *Grb10*, in association with neuronal specific factors. This reveals a novel mechanism of tissue-specific imprinting and provides one of the first examples of the implication of bivalent chromatin in the control of gene expression in a fully differentiated tissue.

CHARACTERISATION OF A NEW LYSINE METHYLATION SITE THAT MARKS HETEROCHROMATIN AND IS DYNAMICALLY REMODELLED DURING REPROGRAMMING AND DEVELOPMENT *(ORAL POSTER)*

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Histone modifications are central components of the epigenome, however the repertoire of known modification is far from complete. Here we characterise a new Histone methylation site. We show that this mark is specifically enriched in pericentric heterochromatin and localises to repeated sequences and inactive genes. We have followed the dynamics of this new mark during the two major epigenetic reprogramming periods: primordial germ cell (PGC) formation and early mouse development. We find that the methylation is highly dynamic, it is present in the PGCs at the time of specification, before reprogramming, but it disappears during the reprogramming. The mark is re-established in the growing oocyte and in the developing sperm. In the early mouse embryo it is specifically enriched at heterochromatic regions of the maternal pronucleus. The methylation is then rapidly removed from the maternal chromatin suggesting an important role in development.

Altogether, these findings establish a new Histone modification that marks repressive chromatin and that is dynamically reprogrammed during development. We propose that this modification might have a role in locking heterochromatin. This characterisation of a new modification expands considerably the complexity of Histone modifications.

CHROMATIN MODIFICATIONS AND THEIR FUNCTION

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Modifications of chromatin play an important role in many biological processes. We are currently characterising at the genomic level, the profile of lysine and arginine modifications in human cells and attempting to correlate their position and timing of appearance with gene expression. We are also characterising the function of a non-covalent modification, proline isomerisation. We have previously shown that the FPR4 enzyme in yeast is able to regulate transcription. We have recently identified a human homologue, which is able to accomplish similar functions and is miss-regulated in ovarian cancer, Finally, we have discovered a novel enzymatic activity which regulated chromatin by proteolytic cleavage of the Histone H3 tail. This activity, present in yeast, clips the tail of H3 at the promoter of genes, which are induced during sporulation.

BEYOND THE DOUBLE HELIX: WRITING AND READING THE "HISTONE CODE"

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The human genome is estimated to contain 30,000 – 40,000 unique genes; the DNA sequence and chromosomal location of all these genes are becoming widely known. A central challenge facing the biomedical community is how to derive medically-valuable knowledge about the function of these genes from the now-available DNA sequence data. Though every gene exists within every cell in the human body, only a small percentage of genes are activated in any given cell. To manage this genetic

information efficiently, nature has evolved a sophisticated system that facilitates access to specific genes. This system relies on a DNA-Histone protein complex called chromatin to efficiently package the genetic information that exists within each cell. This packaging system makes certain genes more readily accessible to transcription factors and other machinery that must engage our genetic template. Chromatin modifications, and the regulation of the enzymes responsible for adding or subtracting them, are poised to take center stage in the study of cancer in the current post-genomic or epigenomic era. Moreover, the implications of chromatin and its modification are beginning to gain appreciation in clinical oncology. The identification of altered DNA methylation and Histone acetylase activity in a range of human cancers, coupled with the use of HDAC inhibitors in the treatment of leukemia, make a compelling argument. It is clear that the regulatory signals provided by chromatin modifications will revolutionize our view of cancer as new models of "epigenetic carcinogenesis" are advanced. We favor the view that there exists an epigenetic indexing system for our genome, or a "Histone or epigenetic code," that represents a fundamental regulatory mechanism that acts outside of the DNA itself. We predict that this "code" impacts on most, if not all, chromatin-templated processes with far-reaching consequences for cell fate decisions and for normal and pathological development (for reviews and references, see below).

Most of our current research is centered on chromatin and its regulation though post-translational modification of Histone (and non-Histone) proteins. That said, other mechanisms such as the existence of DNA methylation and of small non-coding RNAs are also likely, if not certainly, involved. How epigenetic states, "ON" or "OFF", are inherited from one generation to the next is a central question that we, and others, are currently addressing.

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Poster No. II-1

VISUALIZATION OF A MASSIVE TBP-BINDING COUPLED HISTONE-FOLD DOMAIN REARRANGEMENT WITHIN THE GENERAL TRANSCRIPTION FACTOR IID

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The general transcription factor IID (TFIID) is required for initiation of RNA polymerase II-dependent transcription at many eukaryotic promoters. TFIID comprises the TATA-binding protein (TBP) and several conserved TBP-associated factors (TAFs). Recognition of the core promoter by TFIID assists assembly of a pre-initiation complex. Using cryo-electron microscopy in combination with new methods for *ab initio* reconstruction and heterogeneity analysis, we have produced density maps at ~8 Å resolution of two conformational states of TFIID, containing, and lacking TBP. Fitting of the atomic structures of the TBP-$TAF1_{N\text{-terminus}}$ and histone-fold domain (HFD) $(TAF4\text{-}TAF12)_2$ assemblies into the TFIID reconstruction help account for the known TAF-inhibition of promoter-TBP interactions and supports a coherent HFD arrangement within TFIID. We report that TBP-binding is coupled to a conformational transition of the TAFs, which includes a massive HFD rearrangement.

Poster No. II-2

A LARGE DNA ENTRY/EXIT ANGLE IMPOSES A MINIMUM OF CONFORMATIONAL CONSTRAINTS ON THE NUCLEOSOMAL FILAMENT

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The conformational freedom of nucleosomal filaments is limited by collisions between the nucleosomes. Model building of filaments with straight linkers has recently shown that conformational constraints imposed by the basic geometry of the filament are mainly caused by the DNA entry/exit angle (α) (1). Only right-handed helices are sterically allowed at $\alpha < 60°$, while $\alpha \geq \sim 90°$ permits the formation of right- and left-handed solenoids as well as non-helical conformations within the same filament. Interconversion of these two types of fibers by fluctuations in α, i. g. as a result of linker histone exchange or bending of the linkers, is sterically restricted because of collisions between nucleosomes owing to local changes in the coiling direction.
Conformations of nucleosomal filaments in heterochromatin of rat thymocytes and hen erythrocytes were studied by permeabilization or lysis of cells in the presence of a fixative using different buffers, fixatives and detergents, followed by transmission electron microscopy. Fast Fourier transformation of the images showed that the chromatin was preserved as irregular smooth and kinked filaments with loops and short right- and left-handed solenoids with an estimated DNA entry/exit angle of ~ 90° - 110°. 30 nm fibers were formed by compaction of the filaments in response to changes in the ion concentrations. The similarity between chromatin decondensed before, during and after fixation indicate that these structures are closely related to the conformation of nucleosomal filaments *in vivo*, suggesting that the basic geometry of the nucleosomal filament imposes a minimum of constraints on its dynamic behavior and structural plasticity.

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Poster No. II-3

TOWARDS OPENING UP THE YEAST *PHO5* AND *PHO8* PROMOTERS *IN VITRO*

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The yeast *PHO5* and *PHO8* promoters are used as model systems to study chromatin changes that occur during gene induction and repression. Under repressive conditions, the promoters are organized into positioned nucleosomes inhibiting access of DNA binding factors and gene activation. Phosphate starvation of yeast cells leads to the activation of the genes: both promoters undergo chromatin remodeling triggered by the transcription factor (TF) Pho4 resulting in eviction of histones, the promoter regions become accessible for the transcription machinery.
The molecular mechanism underlying the changes in the nucleosomal promoter structure upon induction is still not fully understood from *in vivo* studies. We use an *in vitro* system to recapitulate the series of events that lead from the repressed promoter nucleosomes to the loss of histone-DNA contacts. Yeast extract is added to chromatin reconstituted by salt dialysis leading to *in vivo*-like positioned nucleosomes. Promoter nucleosomes are shifted using yeast mutant extract lacking the TF Pho4 and Pho2. This shift to the closed state is dependent on ATP and yeast extract. Addition of Pho4 and Pho2 leads to a DNaseI hypersensitive site (HS) of very similar appearance as seen with the open *PHO5* promoter *in vivo*. This remodeling is energy dependent as apyrase treatment before TF addition does not change the closed promoter pattern. Up to now, we are able to generate a HS at the *PHO5* promoter *in vitro*. Nevertheless, the promoter remodeling is not as complete as in the *in vivo* case. We try to increase the extent of remodeling by adding different purified components like chromatin remodeling complexes. Upon addition of Pho4 to the *PHO8* promoter, the linker region becomes more pronounced due to Pho4 binding, although there is no extensive remodeling like in the *PHO5* promoter. The experiments were performed using endogenous histones from Drosophila embryos and the results resemble remarkably the *in vivo* situation. We were now able to repeat the experiments with recombinant yeast histones but face the difficulty that we cannot reconstitute the closed pattern of both promoters. We try to resolve if this is a technical or biological issue. Chances are that the positioning of the nucleosomes depends on histone modifications.

Poster No. II-4

POLYCOMB REPRESSIVE COMPLEXES ARE REQUIRED TO MAINTAIN COMPACT CHROMATIN STRUCTURE AT HOX LOCI

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Genes responsible for multilineage differentiation in embryonic stem (ES) cells are poised for transcription. Upon activation, we have shown that Hox gene loci undergo extensive chromatin remodeling in murine ES cells and embryos. Proteins known to mediate compaction of chromatin fibers include H1 and polycomb repressive complexes (PRC). Triple knockout H1 ES cells have previously been shown to have more decondensed chromatin fibers and more variable chromatin structure while PRC can inhibit chromatin remodeling and compact chromatin *in vitro*. However, little is

known about the chromatin compaction of poised developmental genes *in vivo*. Here we have used interphase FISH to measure the chromatin compaction of Hox loci in murine ES cells that are genetically deficient in components of PRC1 (Ring1B), PRC2 (Eed) or linker histone H1. Loss of three somatic H1 genes (H1c,d and e) had no specific effect on the chromatin of the Hoxb and d clusters. Conversely, we observed specific decondensation of Hox in ES cells depleted for Eed. These cells are deficient in histone H3K27tri methylation, a mark shown to be bound by Polycomb *in vitro*. To determine whether the decondensation was due to lack of PRC2 or failed recruitment of PRC2 we used ES cells depleted for Ring1B. Interestingly, we can also detect a more open chromatin structure for Hox loci in the Ring1B knockout cells whereas a control locus stayed the same. Our study suggests that the repressive role of PRC is conferred by PRC1 mediated chromatin compaction.

Poster No. II-5

A UNIQUE CHROMATIN SIGNATURE FOR RNA POLII PAUSING IN A HUMAN DISEASE

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In the devastating neurogenetic disease Friedreich's ataxia, GAA-repeat expansions within intron 1 of the *Fxn* locus trigger gene silencing. We have identified an unusual site within the first exon of the *Fxn* gene where there is an accumulation of RNA polymerase II reminiscent of 'stalling' of the polymerase. Co-incident with this, we find at this site HP1gamma, CTCF, Mecp2 and Nelfb, as well as a dip in histone H3 K4 methylation (accompanied by recruitment of a histone demethylase) relative to the rest of the locus. In different patients with varying disease severity and hence *Fxn* expression we find distinctive epigenotypes. These results suggest that RNA pol II pausing can be regulated by chromatin configuration, indicating a novel potential therapeutic avenue in this disease. To the best of our knowledge this is the first description of 1) a genetic disease where RNA pol II pausing appears to be crucial and 2) a link between RNA pol II and chromatin configuration.

Poster No. II-6

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF A *FACTORY* OF 4 HISTONE H3 LYSINE 9- SPECIFIC METHYLASES: ROLE IN CELL FATE REGULATION

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Histone lysine methylation plays a central role in the regulation of chromatin structure and gene expression. Histone lysine residues can be mono-, di-, or trimethylated, inducing different biological responses. As for example, highly condensed heterochromatic regions show a high degree of trimethylated H3K9, whereas silent euchromatic regions are preferentially enriched in mono- and dimethylated H3K9. In mammals, histone lysine methylation is mediated by some 50 histones methyltransferases (HMTs), but only a small number are specific of H3K9. Among the most studied HMTs are heterochromatinian trimethylases Suv39h1 and SETDB1, and euchromatinian dimethylases G9A and GLP.

Here, we have characterized the biochemical composition of the Suv39h1 complex in HeLa cells. Surprisingly, we have found 3 other H3K9 specific HMTs in Suv39h1 complex, namely: G9A, GLP and SETDB1. Our results were confirmed by biochemical exhaustive characterization of the reciprocal complexes corresponding to G9A, GLP and SETDB1. These interactions were also confirmed *in vitro* by GST pull-down assays and in living cells by co-IP experiments in MEFs, indicating that the four H3K9 specific HMTs interact together both in cancer (HeLa) and in normal cells. Finally, we have shown by ChIP and RNAi experiments a functional cooperation of this H3K9 HMTs complex in the regulation of a totipotency model gene, *oct3/4*. Indeed, they cooperate in the silencing of *oct3/4* gene during retinoic acid-induced differentiation of embryonic carcinoma cells (P19). In addition, their knock-down reactivates *oct3/4* in differentiated C2C12 myoblastic cells.
Taken together, our data strongly suggest the existence of a nuclear H3K9 methylases « factory » composed of 4 HMTs: Suv39h1, SETDB1, G9A and GLP. Future investigations will be to understand its role in global epigenetic regulation of normal or pathological cell fate.

Poster No. II-7

CHROMATIN CHANGES INDUCED BY LAMIN A/C DEFICIENCY AND THE HISTONE DEACETYLASE INHIBITOR TRICHOSTATIN A

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Recent studies of chromatin biology have shown that histone code dictates the type and structure of chromatin.
Bearing in mind the importance of A-type lamins for chromatin arrangement we studied the effect of histone hyperacetylation, induced by TSA, in lamin A/C deficient fibroblasts. In our study, mouse embryonic lamin A/C positive (LMNA+/+) and lamin A/C negative (LMNA-/-) fibroblasts were used. Lamin A/C deficiency caused condensation of chromosome territories and the nuclear reorganization of centromeric heterochromatin, which was accompanied by the appearance of a chain-like morphology of HP1beta foci. Conversely, HDAC inhibition induced de-condensation of chromosome territories, which compensated the effect of lamin A/C deficiency on chromosome regions. The amount of heterochromatin in the area associated with the nuclear membrane was significantly reduced in LMNA-/- cells when compared with LMNA+/+ fibroblasts. TSA also decreased the amount of peripheral heterochromatin, similarly as lamin A/C deficiency. In both LMNA+/+ and LMNA-/- cells, physically larger chromosomes were positioned more peripherally as compared with the smaller ones, even after TSA treatment.
Our observations lead to the suggestion that lamin A/C deficiency causes not only reorganization of chromatin as well as of some chromatin-associated domains, but has an impact on the extent of chromosome condensation. Moreover, HDAC inhibition can compensate the lamin A/C dependent chromatin changes, thus interaction between lamins and specifically modified histones plays an important role in the higher-order chromatin organization, which influences transcriptional activity.

Poster No. II-8

UPREGULATION OF MICRORNA EXPRESSION IN THE MFC-7 BREAST CANCER CELL LINE BY DRUGS THAT TARGETS THE EPIGENOME

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Introduction: Hypermethylation of promoter CpG islands has been shown to down regulate the transcription of tumor suppressor genes and microRNAs (miRs), and recent data suggest that histone H3K27 trimethylation (H3K27me3) may be an alternative mechanism of tumor suppressor inactivation in cancer. DNA methyltransferase inhibitors (DNMTi) can reactivate the transcription of silenced genes and restore normal cell growth and differentiation. Similarly, it has been suggested that inhibition of the S-Adenosylhomocysteine (SAH) hydrolase by 3-Deazaneplanocine A (DZNep) may inhibit the trimethylation of H3K27. *Aims:* To investigate if miRs are down regulated by DNA methylation and/or histone H3K27me3 in the MCF7 breast cancer cell line. *Methods:* MCF7 cells were treated with 1μM of the DNMTi 5-aza-2'-deoxycytidine (5-aza-CdR) for 72 hours. In another experiment MFC-7 cells were treated by 5 μM of DZNep for 72 hours. Total RNA was hybridized to Exiqon miRCURY 9.2 arrays. *Results:* Multiple testing showed that 21 out of 576 (~3%) human miRs examined were significantly upregulated by treatment with 5-Aza-CdR, while only two miRs (the miR221/222 cluster) were significantly upregulated by DZNep treatment. Interestingly, 14 of the miRs upregulated by 5-aza-CdR belonged to the miR-515 cluster. Upregulation of the miRs was confirmed by real time Q-PCR. A CpG island is located just upstream of the cluster. *Further analyses:* We are currently performing 5'-RACE to identify the transcription start sites of the miR 515 and the miR 221/222 clusters in order to evaluate the epigenetic modifications at these promoters. Potential oncogenic miR targets identified using database search for the members of the 515 cluster include NRAS, MAPK3, CCND1, AKT1 and EGFR. The miRs 221/222 have previously been implicated in S-phase regulation. The mRNA and protein expression levels in treated vs. untreated cells will be investigated.

Poster No. II-9

HISTONE DYNAMICS AND REPLICATION FORK PROGRESSION

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In eukaryotes, DNA packaged into chromatin presents a dual challenge for the process of DNA replication. First, the DNA template needs to be efficiently accessed and, second, chromatin structure must be reproduced on the daughter strands. This involves nucleosome disruption ahead of the replication fork, followed by reassembly behind the fork through parental histone transfer and new histone incorporation. A major unresolved issue concerns how histone dynamics are coordinated with fork progression to maintain chromosomal stability. We have characterized a complex in which the human histone chaperone Asf1 and MCM, the putative replicative helicase, are connected via a H3-H4 bridge. Defective DNA unwinding at replication sites upon Asf1 knock-down provides a functional link between the replicative helicase and Asf1. Similar defects arise upon overproduction of new histone H3-H4 due to interference with Asf1 function. Based on these data we propose that Asf1, as a histone

acceptor/donor, handles parental and new histones at the replication fork via an Asf1-(H3-H4)-MCM intermediate. The implication of these findings for fine-tuning replication fork progression with histone dynamics will be discussed.

Poster No. II-10

EPIGENETIC REGULATION OF LINEAGE SPECIFIC DNA *DE NOVO* METHYLATION IN MOUSE PREIMPLANTATION EMBRYOS

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The trophectoderm (TE) is the first cellular lineage to undergo differentiation during mammalian embryogenesis. The trophectoderm forms the most external cell layer of the blastocyst and its developmental potential is restricted after implantation to extra-embryonic tissues composing the trophoblast and then the placenta. The inner cell mass (ICM) is the other cell type present in blastocyst and will give rise to the embryo proper. Following the wave of demethylation inherent to preimplantation development, the ICM undergoes *de novo* methylation around 3.5dpc, while the TE stays globally demethylated. We are aiming towards the elucidation of the mechanisms leading to DNA hypomethylation during trophoblast differentiation, focusing on the epigenetic decision underlying the initial specification of a subset of blastomeres into trophectoderm precursors prior to blastocyst formation and the resistance of these cells towards *de novo* methylation.
We first studied the expression pattern of the enzymes responsible for *de novo* methylation, the DNA-methyltransferases Dnmt3A, Dnmt3B and their co-factor Dnmt3L during preimplantation development. Transcripts and proteins were found prior to and at the time of blastocyst formation and *de novo* methylation. We moreover evidenced an interplay between maternal transcript store and zygotic transcription modulating specifically Dnmt3A, 3B and 3L availability at each stage. We did not find any exclusion of Dnmt proteins from the TE-committed cell nuclei in morulae and blastocysts around the time of *de novo* methylation. We found however that TE cells presented with a higher state of H3K4 methylation compared to ICM cells, and that a subset of H3K4 demethylases are coincidently specifically expressed in the ICM and excluded from the TE. This observation points towards an association between H3K4 demethylation and *de novo* DNA methylation in the ICM, while persistence of H3K4 methylation may act as a protection mark from the activity of the *de novo* methylating machinery in the trophectoderm lineage.

Poster No. II-11

THE HINGE REGION REGULATES DNA BINDING, NUCLEAR TRANSLOCATION, TRANSACTIVATION AND INTRANUCLEAR MOBILITY OF THE ANDROGEN RECEPTOR

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The androgen receptor (AR) gene can undergo mutations during development and treatment of prostate cancer. Two such point mutations (R629Q and K630T) have been described in the part of the AR involved in DNA binding and nuclear translocation, namely the hinge region. Despite a decreased nuclear translocation, these mutant ARs display increased transactivating potencies. A deletion analysis of the hinge region revealed that deletion of residues 629 to 636 resulted in a stronger androgen response even though this mutant displays an extremely low affinity for

androgen response elements. This superactivity is independent of nuclear localization. Surprisingly, the AR activation functions, AF1 and AF2, are not dramatically affected when the inhibitory region (629-RKLKKLGN-636) is deleted, although the coactivator TIF2 had a stronger potentiating effect in absence of this motif. The ligand-dependent interaction between the amino-terminal domain and the ligand-binding domain (N/C interaction), which is important for transactivation by the AR, is strongly enhanced by deletion of the inhibitory region. A point mutation analysis of this 629-RKLKKLGN-636 motif showed that residues R629 and K630 are imperative for the ligand-induced nuclear translocation of the AR, whereas the overall charged nature regulates high affinity interactions with DNA response elements. Mutant proteins that have reduced DNA binding affinity surprisingly display an increase in activity as a result of an increased intranuclear mobility. The AR appears to have two immobile fractions with retention times of 12 and 0.1 seconds, respectively. The more active AR mutants with reduced DNA binding affinity posses only the second immobile fraction. Apparently, the first immobile fraction regulates the transcriptional activity of the AR in an inhibitory manner. In conclusion, the description of prostate cancer mutations has led to the discovery of a complex role of the hinge region in nuclear localization, DNA binding, co-activator recruitment, N/C interaction and intranuclear mobility of the AR.

Poster No. II-12

DISTINCT, SEQUENTIALLY ACTING UBIQUITIN LIGASES ARE REQUIRED FOR RNA POLYMERASE II POLY-UBIQUITYLATION

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Transcriptional arrest triggers ubiquitylation and degradation of RNA polymerase II (RNAPII). In yeast, this ubiquitylation requires the ubiquitin ligase Rsp5. Surprisingly, we found that Rsp5-mediated poly-ubiquitylation of RNAPII occurs via ubiquitin K63 links, and cells carrying a K63R mutation in ubiquitin can still degrade RNAPII. Moreover, Rsp5 associates with a ubiquitin protease, Ubp2, and together these proteins only mono-ubiquitylate RNAPII. Mutant Rsp5, *rsp5-1*, yeast extracts cannot poly-ubiquitylate RNAPII, but can do so if polymerase is mono-ubiquitylated prior to incubation, further indicating that Rsp5 is required only for initial RNAPII mono-ubiquitylation. Recent data suggest that the yeast Elongin C, within a cullin E3 complex, is required for RNAPII poly-ubiquitylation and degradation. Together, these data suggest a model in which RNAPII poly-ubiquitylation and degradation requires two distinct, sequentially acting ubiquitin ligases. We are currently using genetic and biochemical assays to test this model.

Poster No. II-13

IE2-MEDIATED CHROMATIN REMODELING AND PCAF CONTRIBUTE TO LATENCY AND ACTIVATION OF THE HUMAN CYTOMEGALOVIRUS

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The switch of viral latency to activation has been a good model to study chromatin remodeling. When reactivated from latent state, the human cytomegalovirus (HCMV) causes serious diseases in immunocompromised individuals such as AIDS patients. In this study we show that the viral immediate early two (IE2) protein mediated local chromatin structure changes via recruiting distinct corepressors to the viral major immediate early promoter (MIEP) in HCMV latently infected, undifferentiated N-teratocarcinoma (NT2) cells, resulting in the inhibition of the MIEP activity in a cis-repression sequence (crs)-dependent manner. IE2 binding to crs recruited histone H3 K9 methyltransferases as well as the Mi-2/NuRD corepressor complex which involves distinct activities such as ATP-dependent chromatin remodeling, histone deacetylation and binding to methylated CpG. Consistently, IE2 mediated local DNA methylation during latency. In contrast, in retinoic acid (RA)-differentiated NT2 cells which support lytic HCMV infection, IE2 bound to MIEP in a crs-independent manner and, instead of interacting with corepressors, recruited the coactivator PCAF which was greatly increased upon RA treatment. Knocking down PCAF in differentiated NT2 cells strongly repressed the viral growth. Together these studies demonstrate that IE2-mediated chromatin remodeling and PCAF regulated the MIEP activity, indicating that both viral and cellular factors are involved in HCMV latency and activation.

Poster No. II-14

EZH1 (ENHANCER OF ZESTE HOMOLOG 1) AND H3 LYSINE 27 MONO-METHYLATION CONTROL VERTEBRATE SKELETAL MUSCLE CELL DIFFERENTIATION

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Cell lineage-specific transcriptional programs are maintained by epigenetic mechanisms involving interplay of Polycomb (PcG) and trithorax (trxG) group proteins. Deregulated expression of PcG and trxG proteins may lead to altered developmental programs, deregulation of cell proliferation, lack of stem cell renewal, and cancer. SET-domain confers a histone methyltransferase activity to several PcG and trxG proteins, which act in large protein complexes to modify the local properties of chromatin. Mammals have two homologs of Drosophila SET-containing Enhancer of zeste E(z), Ezh1 and Ezh2 (Enhancer of zeste 1 and 2, respectively). Whereas Ezh2 is a well-established methyltransferase (mainly specific for H3 lysine K27) , the function of Ezh1 remains largely elusive.

Using skeletal muscle cell differentiation model C2C12 cells, we observed that reduced level of Ezh1 results in strong defects in cell differentiation. In particular, a significant delay in transcriptional upregulation of myogenin, a key early differentiation marker, was observed. ChIP analyses suggested that myogenin, but not late muscle cell differentiation markers like MCK, is indeed targeted by Ezh1. A genome-wide decrease in H3K27 mono-methylation level was detected after siRNA knock-down of Ezh1. Moreover, the lack of H3K27m1 in Ezh1 knock-down cells resulted in reduced recruitment of MyoD to promoter and low H3K4m3 level at myogenin gene, resulting in defects in transcriptional activation. Taken together, our data suggest Ezh1 acts as a trxG-like protein, much unlike PcG Ezh2.

Poster No. II-15

DETERMINATION OF POST-TRANSLATIONAL MODIFICATIONS OF HISTONE H3 USING CHROMATOGRAPHY, ION MOBILITY TANDEM MASS SPECTROMETRY AND CUSTOMIZED BIOINFORMATICS TOOLS

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Epigenetic processes are regulated by DNA methylation, exchange of histone variants and modifications on histone proteins. Histones are reversibly modified and most of these post translational modifications (PTMs) are located on the N-terminal tail of the histones. It is important to develop analytical methods to detect multiple PTMs on histones to establish the patterns and biological consequences linked to these PTMs.
Mass spectrometry combined with chromatographic separation is a key technology in investigations of PTMs on histones. Here, long N-terminal peptides of histones H3 were separated by reversed-phase high performance liquid chromatography (RP-HPLC), hydrophilic interaction chromatography (HILIC). And, each fraction was analyzed by ion mobility – tandem mass spectrometry (IMS-MS/MS). The Synapt ion mobility MS is a modified quadruple orthogonal time-of-flight (Q-oaTOF) MS which has a drift cell before TOF analyzer. Ions are separated based on their charge and the collisional section between molecules and collision gas. Acquired data were analyzed by commercial bioinformatics tools, and in-house developed software called 'mobilPTM'. The current version of the 'mobilPTM' contributes to significantly reduce analysis time for mapping modifications on histone tails by enabling semi-automatic search of different combination of modifications.
The established experimental and computational strategy is now applied to the study of modifications on histone tails obtained from TSA treated cells. We will describe that our analytical strategy is an efficient way to link the biological phenomenon to different combinatorial modifications on histone tails.

Poster No. II-16

ROLE OF THE SUMO E3 LIGASE PIAS IN CHROMOSOME AND NUCLEAR ORGANIZATION IN D. MELANOGASTER

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PIAS proteins were first identified as components of the JAK/STAT signal transduction pathway. In *Drosophila*, the only member of the PIAS family, *Su(var)2-10*, was found to be a suppressor of position effect variegation (PEV) (Reuter and Wolff, 1981). It is the only member of the PIAS-protein family in flies. Further studies of dPIAS suggested diverse roles in chromosome function and nuclear organization (Hari et al., 2001). This is consistent with the demonstration that PIAS proteins encode an E3 SUMO Ligase, which is part of the SUMO conjugating system (Heun, 2007). We found that in *Drosophila* there are at least 15 PIAS isoforms that result from alternative splicing. These isoforms share a central core, but differ in their N- and C-termini. Interestingly, they show different patterns of expression, are present in distinct complexes, and appear to have different target proteins.
The aim of this project is to determine how dPIAS mediated SUMOylation regulates heterochromatin formation and nuclear organization. We are currently trying to identify the dPIAS isoforms involved in PEV and their corresponding target proteins. Then we will determine the effect of SUMOylation on the function of the target proteins.

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Poster No. II-17

VISUALIZATION OF CYCLIN-DEPENDENT KINASE 8 MODULE AND RNA POLYMERASE II BINDING TO MEDIATOR BY ELECTRON CRYO-MICROSCOPY

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The Mediator complex is a central molecule in eukaryotic transcription regulation, comparable in importance to RNA polymerase II (pol II) itself. The mechanisms by which Mediator regulates transcription are poorly understood, but the high degree of conservation of Mediator subunits from yeast to man suggest that common underlying mechanisms exist. Using cryo-electron microscopy in combination with new methods for *ab initio* reconstruction, we have produced density maps of Mediator in complex with pol II (holoenzyme) and of Mediator in complex with the Cdk8-module (L-Mediator). A model is proposed for the repressive role of Cdk8-module-containing Mediator complexes, where regulation of transcription is achieved by mutually exclusive binding of the Cdk8-module and pol II to Mediator. Moreover, fitting of a Cdk8/CycC model based on homology to the human Cdk2/CycC and yeast CycC crystal structures to the L-Mediator reconstruction reveals an interaction to Cdk8 reminiscent of that between the cell-cycle inhibitor p19 and Cdk6. Our data help to account for the structural basis of the regulatory role of eukaryotic Mediator complexes.

Poster No. II-18

LARGE SCALE DNA METHYLATION PROFILING IN HUMAN TISSUES

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We have performed genomic scale DNA methylation profiling on numerous benign and malignant human tissues from the three embryonic layers (endoderm, ectoderm, and mesoderm), as well as extra-embryonic (trophoblast) derivation. Data analysis identified abundant loci of lineage-specific wild-type and malignant methylation, including both CpG island and off-island targets. Correlation of differential methylation with gene expression indicates distinct properties for on- and off-island targets. Comparison of matched frozen and formalin-fixed paraffin-embedded (FFPE) samples illustrates the technical feasibility of performing large-scale DNA methylation profiling in archival pathology samples. Overall, the results demonstrate that DNA methylation profiling is powerful cell-lineage and -phenotype classification methodology that is readily achievable in archival pathology specimens.

Poster No. II-19

HISTONE MODIFICATIONS ASSOCIATED WITH TRANSCRIPTION ACTIVATION AND SILENCING AS REVEALED BY A PANEL OF SPECIFIC MONOCLONAL ANTIBODIES

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Post-translational histone modifications and the variant replacement play critical roles in the regulation of gene expression and genome integrity. The modifications of histone H3, including acetylation and methylation, are particularly important in the gene activation and silencing. The modification-specific antibodies have allowed the detection of those modifications in given gene loci by chromatin immunoprecipitation (ChIP); however, most commercially available antibodies are rabbit polyclonals and so the specificity can vary from lot to lot. We therefore generated and characterized a panel of mouse monoclonal antibodies that specifically recognize different modifications on lysine residues on histone H3. These antibodies can be used for a variety of applications including ChIP and immunofluorescence, and thus may facilitate epigenomic studies on healthy and diseased cells. We will present our recent ChIP and ChIP-on-chip data using these antibodies on the dynamics of histone modifications in response to serum activation as well as on the mechanism of maintaining a transcriptionally active (escape) gene from the global silencing by X chromosome inactivation.

References:

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Poster No. II-20

CSB ATPASE ACTIVITY COUPLES TFIIB BINDING TO RNA POL II RECRUITMENT

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Cockayne syndrome (CS) is an autosomal recessive disorder caused by mutation in CSA and CSB genes. CS displays an array of clinical symptoms including mental and physical growth retardation, ocular abnormalities such as retinal degeneration and cataracts, sensorineural hearing loss and mild hypersensitivity towards sunlight. One of the hallmarks of CS cells is their inability to resume transcription after UV irradiation, which has been commonly ascribed to a defect in transcription coupled repair (TCR), responsible for the rapid removal of transcription-blocking lesions during elongation. However, accumulating data support another role for CSB, namely during formation of the transcription pre-initiation complex (PIC).

Having established different cell lines, expressing CSB with mutations in what was previously defined as putative helicase domains, we here examine the fate in the transcription process of either housekeeping genes, p53 dependent genes or nuclear receptor dependent genes. Tracking the mRNA levels after UV irradiation indicates a slightly higher DHFR expression in wild type cells compared to the CSB mutants,

whereas the p53 dependent gene GADD45 shows the same expression in all cell lines. Chromatin Immuno Precipitation assays clearly demonstrate that the recruitment of RNA pol II and the general transcription factor TFIIB to the promoter of DHFR is depending on the presence of CSB. But unlike the binding of TFIIB, the recruitment of RNA Pol II requires the ATPase activity of CSB. Additional experiments further show that the N-terminal acidic domain of CSB is stabilizing RNA pol II at the promoter.

Poster No. II-21

REVERSAL OF RNA POLYMERASE II UBIQUITYLATION BY THE UBIQUITIN PROTEASE UBP3

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The final outcome of protein poly-ubiquitylation is often proteasome-mediated proteolysis, meaning that 'proof-reading' of ubiquitylation by ubiquitin proteases (UBPs) is crucial. Transcriptional arrest can trigger ubiquitin-mediated proteolysis of RNA polymerase II (RNAPII) so a UBP reversing RNAPII ubiquitylation might be expected. Here, we show that Ubp3 de-ubiquitylates RNAPII in yeast. Genetic characterization of *ubp3* cells is consistent with a role in transcript elongation, and Ubp3 can be purified with RNAPII, Def1, and the elongation factors Spt5 and TFIIF. This Ubp3 complex de-ubiquitylates both mono- and poly-ubiquitylated RNAPII *in vitro*, and *ubp3* cells have elevated levels of ubiquitylated RNAPII *in vivo.* Moreover, RNAP II is degraded faster in an *ubp3* mutant after UV-irradiation. Problems posed by damage-arrested RNAPII are resolved either by removing the damage or degrading the polymerase. In agreement with this, cells with compromised DNA repair are better equipped to survive UV-damage when *UPB3* is deleted.

Poster No. II-22

PROTEOMIC SCREEN FOR REGULATORS OF DNA DAMAGE-MODIFIED CHROMATIN

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The packaging of the eukaryotic genome into highly condensed chromatin makes it inaccessible to processes such as transcription and replication. Modulation of chromatin is therefore essential to these processes, as they require access of enzymes and regulatory factors to their sites of action. Recent evidence indicates that modulations of chromatin structure are also important to many aspects of the DNA damage response. However, these mechanisms are still poorly understood.
Factors known to be important in the DNA damage response, such as mediator of DNA damage checkpoint protein-1 (MDC1) and p53 binding protein 1 (53BP1), gain increased affinity for chromatin in response to ionizing radiation (IR). Chromatin affinity was therefore used as the basis for a quantitative mass-spectrometry screen in order to identify proteins that play an, as yet, undescribed role in the DNA damage response. We employed the powerful Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) method to obtain accurate quantization of proteins, present on chromatin after salt extraction, by mass spectrometry. Proteins that show a

significant change in binding affinity after IR were identified using the analysis of variance (ANOVA) statistical method.
Amongst the candidate, proteins identified were several components of the nucleosome remodeling and histone deacetylase complex (NURD). Subunits of NURD, such as MTA2, have previously been described as being involved in the DNA damage response. Our data reveals that other subunits of the NURD complex may also play a role in the activation of the DNA damage response and in the recovery from double stranded breaks.

Poster No. II-23
ROLE OF H3K27 LYSINE DEMETHYLASES DURING DEVELOPMENT
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A strict regulation of gene expression is essential for cellular identity and differentiation during development of eukaryotic organisms. At the level of DNA, gene expression can be regulated by changes in the structure of chromatin through, for example, post-translational modifications of histone tails. Among the vast variety of known histone tail modifications, histone methylation has been shown to be involved in important cellular processes such as heterochromatin formation, X-chromosome inactivation, transcriptional regulation and DNA repair. Moreover, aberrant histone methylation has been linked to several diseases such as cancer.
We are particularly interested in the methylation of histone lysines and more specifically in the methylation state of lysine 27 at histone 3 (H3K27). The H3K27me3 (tri-methyl) mark, associated to transcriptional repression, is a unique mark for stem cell state and decreases during differentiation into somatic cells. While the Polycomb group proteins are known to mediate tri-methylation of H3K27, the Jumonji C (JmjC) domain-containing proteins, UTX and JMJD3, were recently shown to catalyze its demethylation. However, little is known about the biological functions of these enzymes *in vivo*.
In *C. elegans*, four homologs of the human H3K27 demethylases are identified (D2021.1/utx-1, F18E9.5, C29F7.6, F23D12.5). To determine their *in vivo* role(s), mutants and RNA interfered animals of all these homologs were analyzed. The resulting phenotypes, associated to loss or reduction of H3K27me3 demethylase activity, point to functional roles during embryonic and early larval development, in vulva and gonad formation as well as in fertility. Results from gene expression micro arrays, identifying genes which expression is affected by the loss of histone demethylase activity, will also be presented at the meeting.

Poster No. II-24
CHROMATIN INSULATOR BARRIER PROTEIN BGP1-ASSOCIATED PROTEIN COMPLEXES
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Chromatin insulator elements can protect genes from their chromosomal environment by acting as barriers to the encroachment of chromatin condensation processes. Four protein-binding 'footprints' of HS4 are required for its barrier activity. Of these, USF binds to FIV to recruit a number of 'active' histone modifications that were found to act as a chain terminator to the propagation of repressive histone

modifying processes. USF mediated manipulation of the histone code is necessary, but not sufficient for barrier activity. The three remaining footprints of HS4 (FI, FIII and FV) are all essential for barrier activity. We now find that Beta Globin Protein 1 (BGP1) interacts with each of these sites. Mutation of either BGP1 site leads to the *de novo* DNA methylation of an insulated transgenic promoter. The USF binding site is not required for protection from DNA methylation.

We wish to determine how BGP1 mediates HS4 barrier activity. We have embarked upon the identification of the proteins that interact with BGP1. We have utilized a modified TAP epitope tag to purify chicken and human BGP1 protein complexes. We are currently identifying the proteins complexed with BGP1 by LC-MALDI-TOF. Preliminary analysis indicates that the BRG1-chromatin remodeling protein associates with BGP1. Chromatin immunoprecipitation (CHIP) shows that BRG1 is in the same complex with BGP1 and recruited to HS4. We propose that barrier elements in vertebrates must be capable of preventing DNA methylation in addition to blocking the propagation of silencing histone modifications, as either of these mechanisms is sufficient to direct the establishment of an epigenetically stable silent chromatin state.

BENZON SYMPOSIUM No. 55

TRANSCRIPTION, CHROMATIN AND DISEASE

AUGUST 18 – 21, 2008, COPENHAGEN, DENMARK

Organizing committee:
Kristian Helin (Copenhagen), Jesper Q. Svejstrup (London) & Arne Svejgaard (Copenhagen)

Abstracts - WEDNESDAY, AUGUST 20, 2008

STRUCTURAL AND FUNCTIONAL ANALYSIS OF FISSION YEAST MEDIATOR

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The Mediator complex connects gene specific regulatory proteins to the basal RNA polymerase II (pol II) transcription machinery. Together Cdk8, CycC, Med12, and Med13, form a module (the Cdk8 module), which prevents interactions with RNA polymerase II. We have previously used electron microscopy and single particle reconstruction to demonstrate that the Cdk8 module forms a distinct structural entity, which binds to the head and middle region of Mediator. The Cdk8 module acts as a molecular lid, which sterically block pol II interactions with Mediator, independently of the Cdk8-dependent kinase activity. We will here present further data concerning the molecular architecture of the Cdk8 module and its interactions with other parts of the Mediator. We will also discuss recent findings, which reveal a role for Cdk8 kinase activity in the precise timing of cell-cycle-dependent transcription in fission yeast.

We will also demonstrate that Med15 exists in a protein complex together with Hrp1 (Chd1 in budding yeast), an ATP-dependent chromatin remodeling protein. The Med15/Hrp1 subcomplex is not a stable component of the core Mediator complex, but can interact with Mediator in the presence of the Cdk8 module. Deletion of med15+ and hrp1+ cause very similar effects on global steady-state levels of mRNA and genome-wide analyses demonstrate that Med15 associates with a distinct subset of Hrp1-bound gene promoters. Interestingly, loss of Med15 or Hrp1 leads to increased nucleosomal density at Med15 target sites. Based on these findings, we suggest that Med15 recruit Hrp1 to specific target sites, where it promotes nucleosomal loss. Our findings therefore indicate that Mediator may directly influence Histone density at regulated promoters.

READING THE GENE IN COMPANY OF SOME DNA REPAIR FACTORS

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Protein coding gene transcription is a multi-step process that begins with the recruitment of basal and specific transcription factors (such as nuclear receptors (NRs)) at the promoter. This is followed by chromatin remodelling, the formation of a pre-initiation complex, together with the recruitment of RNA polymerase II (RNA pol II), in order to begin RNA synthesis. Upon gene-activation, XPA, XPG, XPF, RPA, CSB, and TFIIH, all factors that participate in Nucleotide Excision Repair (NER), assembled at the gene promoter together with the transcription factors, and escort the elongating RNA polymerase II even past the transcription termination site. This

complex, which contained RNA pol II and TFIIH, was different from a "repair complex," since the latter did not contain the CAK module of TFIIH. Cells with mutations in XPB, XPD, XPF, XPA, and CSB, were unable to assemble this newly identified transcription complex loaded with repair factors upon gene activation and displayed an altered transcriptional response. In conclusion, we have identified a novel link between RNA pol II and NER factors, which may explain the severe clinical features of patients with DNA repair syndromes. We also uncovered a new function for NER factors as transcriptional modulators, which is unrelated to their role in DNA repair that will be also described.

POLYCOMB REPRESSORS CONTROLLING STEM CELL FATE: IMPLICATIONS FOR CANCER AND DEVELOPMENT

van Lohuizen M; Division of Molecular Genetics, The Netherlands Cancer Institute, The Netherlands

Repressive Polycomb-group (Pc-G) protein complexes and the counteracting Trithorax-group (Trx-G) of nucleosome remodeling factors are involved in the dynamic maintenance of proper gene expression patterns during development, acting at the level of chromatin structure. As such, they are important controllers of cell fate. When deregulated, these master switches of gene expression are strongly implicated in formation of a diverse set of cancers. An example is the Pc-G gene Bmi1 which is overexpressed in medulloblastoma, Non small cell lung cancer, hepatocellular carcinoma and breast cancer and Glioma and is causally implicated in leukemia. We and others have recently implicated Bmi1/Pc-G as a critical regulator of stem cell fate in hemapoietic stem cells, neural stem cells, mammary epithelial precursor cells and ES cells. In addition, we have shown that Bmi1 is regulated by the Shh pathway and that the Ink4a/ARF tumors suppressors are critical Bmi1 target genes in stem cells and in cancer formation. However our recent work on brain cancer (Glioma) points to important ink4a/ARF-independent Bmi1 targets involved in adhesion and motility. Comprehensive profiling of Polycomb target genes in Drosophila revealed its crucial conserved role in repressing lineage differentiation pathways and morphogens, including Wg, Hh, Delta and Notch. Furthermore, we have characterized in detail an essential E3-ubiquitin ligase activity in the PRC1 Polycomb complex that consists of a functional Ring1B-Bmi1 heterodimer. This E3 ligase activity is required for maintenance of Polycomb repression in normal- and cancer stem cells and hence offers potential novel ways to target cancer stem cells or tumor re-initiating cells in which the activity of this E3 ligase is hyperactivated. This is further substantiated by a novel way by which the activity of the Ring1B.Bmi1 E3 ligase is controlled. The implications of these findings for stem cell biology, development and cancer will be discussed.

THE HISTONE H2B-SPECIFIC UBIQUITIN LIGASE RNF20/HBRE1 ACTS AS A PUTATIVE TUMOR SUPPRESSOR THROUGH SELECTIVE REGULATION OF GENE EXPRESSION *(ORAL POSTER)*

Shema E, Tirosh I, Aylon Y, Huang J, Ye C, Berger SL & Oren M; Molecular Cell Biology, Weizmann Institute of Science, Israel

Histone monoubiquitylation is implicated in critical regulatory processes. We explored the roles of histone H2B ubiquitylation in human cells by reducing

the expression of hBRE1/RNF20, the major H2B-specific E3 ubiquitin ligase. While H2B ubiquitylation is broadly associated with transcribed genes, only a subset of genes was transcriptionally affected by RNF20 depletion and abrogation of H2B ubiquitylation. Gene expression dependent on RNF20 includes histones H2A and H2B and the p53 tumor suppressor. In contrast, RNF20 suppresses the expression of several proto-oncogenes, which reside preferentially in closed chromatin and are modestly transcribed despite bearing marks usually associated with high transcription rates. Remarkably, RNF20 depletion augmented the transcriptional effects of epidermal growth factor (EGF), increased cell migration, and elicited transformation and tumorigenesis. Furthermore, frequent *RNF20* promoter hypermethylation was observed in tumors. RNF20 may thus be a putative tumor suppressor, acting through selective regulation of a distinct subset of genes.

TRANSCRIPTION FACTOR-INDUCED GLOBAL EPIGENETIC REMODELING TO PLURIPOTENT STEM CELLS

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Ectopic expression of the four transcription factors Oct4, Sox2, cMyc, and Klf4 is sufficient to confer a pluripotent state to the human or murine somatic cell genome, generating induced pluripotent stem (iPS) cells. iPS cell lines are morphologically and functionally very similar to embryonic stem (ES) cells derived from the inner cell mass of pre-implantation embryos, and can be induced to differentiate into the three embryonic germ layers indicating pluripotency. Nuclear reprogramming by these four factors globally resets the epigenetic and transcriptional programs of the differentiated cell to the pluripotent ES cell state. How the four factors induce global epigenetic remodeling has remained unclear. Our data suggest that all four transcription factors are central regulators of the transcriptional network that specifies ES cell identity.

EPIGENETICS OF ACUTE PROMYELOCITYC LEUKEMIA

Pelicci PG; Department of Experimental Oncology, European Institute of Oncology, Italy

Molecular investigations on Acute Promyelocytic Leukemia (APL) have opened the way to modern concepts of anti-cancer treatment. APL has been the first example of a neoplastic disease that can be specifically treated by targeting therapy to the transforming protein (molecular treatment) and represents a unique model for differentiation therapy. Indeed, the APL oncogene (PML-RAR) is responsible for the high sensitivity of the blasts to the differentiative action of retinoic acid (RA) both in vivo and in vitro. The dissection of the molecular mechanisms underlying PML-RAR activities (chromatin recruitment of Histone deacetylases, Histone and DNA methyltransferases) has demonstrated that epigenetic modifications of DNA (methylation) and chromatin (acetylation and methylation of histones) may contribute to cancer. This has allowed the concept of epigenetic treatment of cancer to be introduced and validated. Recent work from our lab has demonstrated that HDAC-i induce apoptosis of leukemic blasts, that apoptosis is p53-independent and

depends upon activation of the death receptor pathway (TRAIL and Fas signalling pathway). The effects of HDAC-I treatment on RA-target genes in PML-RAR cells was negligible, thus suggesting that HDACi might target alternative mechanisms of PML-RAR activity. Indeed, members of the TRAIL and Fas pathway are not direct RA-targets. We are currently investigating whether PML-RAR regulates transcription of genes which do not possess RA-responsive elements (RARE). Many genes regulated by RA and/or PML/RARa do not contain a RARE. However, these genes are clustered in long stretches of co-regulation spanning regions up to 1 Megabase in length.These clusters are found within regions particularly enriched with RARE consensus sequences that, surprisingly, are included within Alu repeats. Such a striking co-localization of RAR- and/or PML/RARa- regulated genes, RARE consensuses and Alu sequences suggests that the insertion of potentially thousands of Alu repeats containing binding sites for NHRs throughout the primate genome is likely to have played a functionally important role in the evolution of regulation of the primate gene expression.

ANTAGONISTIC TRANSCRIPTION CONTROL THROUGH HISTONE (DE)-UBIQUITYLATION

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Maintenance of gene transcription status plays a critical role in development and disease. The evolutionary conserved Polycomb- (PcG) and trithorax group (trxG) proteins respectively maintain the repressed or activated status of many developmental genes. These opposing co-regulators function by changing the structure of chromatin, in part through covalent post-translational Histone modifications. Here, we will discuss the antagonistic functions of Histone H2A and H2B ubiquitylation in gene control. We identified a novel PcG Silencing complex, dRAF, which couples Histone H2A ubiquitylation to Histone H3 demethylation. dRAF is distinct from PRC1 and comprises the Histone H2A E3 ubiquitin ligase dRING, PSC and 4 additional subunits, two of which have enzymatic activities. In agreement with a role as a repressor *in vivo*, the signature subunit dRAF1 acts as an enhancer of *Pc* but a suppressor of the trxG Histone methyltransferase genes *trx* and *Ash1*. Importantly, cellular depletion and *in vitro* reconstitution assays revealed that dRAF1 not only mediates removal of the ASH1-mediated active Histone H3 methyl mark, but also allows efficient H2A ubiquitylation by dRING-PSC. Whereas Histone H2A ubiquitylation is associated with gene silencing, Histone H2B ubiquitylation correlates with transcription activation. We will describe the effects of Histone H2B (de)ubiquitylation during developmental gene control. In particular, we will focus on gene silencing by the GMP synthetase – Ubiquitin Specific Protease 7 (USP7-GMPS) co-repressor complex. We will discuss the implications of our results for understanding of how Histone (de)ubiquitylation impacts transcription control.

ROLE OF CTCF/COHESIN BINDING SITES IN REGULATION OF GENE EXPRESSION *(ORAL POSTER)*

De Gobbi M[1], Hughes J[1], Vernimmen D[1], Sloane-Stanley JA[1], Sharpe JA[1], Yokomori K[2], Dunham I[3], Wood WG[1], Gibbons RJ[1], Higgs DR[1]; [1]Weatherall Institute of Molecular Medicine, Oxford University,United Kingdom; [2]Department of Biological Chemistry, School of Medicine, University of California, USA; [3]The Wellcome Trust Sanger Institute,United Kingdom

Recent advances in studying genome-wide protein-DNA interactions has enabled the localization of transcription factors, co-factors and chromatin associated proteins in mammalian genomes. Among them, the multifunctional, CCCTC-binding factor (CTCF) has recently been studied in some detail. CTCF is known to act as either a transcriptional activator or a repressor at different gene loci. Moreover, since it shows *in vitro* enhancer blocking activity, CTCF is considered the prototype vertebrate insulator protein. It is of interest that recently it has been shown that CTCF co-localizes with the cohesin complex (SMC1, SMC3, RAD21 and SCC3) and it has been suggested that CTCF/cohesin binding sites may mediate some long-range chromatin interactions. Nevertheless, the molecular mechanisms underlying CTCF's diverse functions remain unknown.
Here we have used a tiled micro array to analyze the pattern of CTCF and RAD21 binding across the telomeric region of human chromosome 16, where the alpha globin locus lies. Typical CTCF/cohesin binding sites within conserved sequences, associated with DNAse1 hypersensitive sites were identified in this locus. We have examined these sites looking for correlation with function. However, we found that binding does not correlate with alpha globin gene expression (ES cells, primary erythroblasts and lymphoblastoid cell lines). Furthermore, their localization does not fit with the expected behavior of a canonical insulator, as one well characterized site lies between the alpha globin genes and their major regulatory element (HS-40). Chromosome conformation capture (3C) data, together with natural mutants and animal model studies where CTCF elements are deleted, have been characterized to clarify the role of CTCF/RAD21 in its natural chromosomal environment.

MOLECULAR ARCHITECTURE OF HUMAN CTCF AND THE INSULATOSOME *(ORAL POSTER)*

Martinez SR, Holdorf MM, Campbell AE, Kliegman JI & Miranda JJ; Cellular and Molecular Pharmacology, University of California, San Francisco, USA

Multi-protein, sequence-specific, DNA-binding complexes regulate the chromatin structure and transcriptional activation of genes as autonomously functioning loci throughout the human genome. The genetic units are generated in part by insulator proteins flanking a locus to protect promoters from activation by enhancers of other loci. Such enhancer blocking is distinct from repression of gene activity. Repression is a genetic switch with one logical criterion, that the acting cis element is an enhancer. Insulation bears two criteria, not simply that any enhancer is blocked, but also that the acting cis element be in a certain spatial position, thus allowing a privileged enhancer to function. We have characterized the molecular architecture of human CTCF, an insulator protein. By determining biophysical properties such as structure and oligomeric state, as well as mapping known cancer-specific mutations, we can propose a model of CTCF functional architecture. This protein, however, may not act alone on insulator elements. A Histone chaperone, chromatin remodeling enzyme, and cohesin may also function in insulation. We have therefore profiled the protein-binding sites of putative cofactors at high resolution by chromatic immunoprecipitation followed by next-generation deep sequencing. The composition and arrangement of proteins on insulators is thus identified, defining the molecular organization and assembly of a complex we refer to as the insulatosome.

HISTONE H3 K56 ACETYLATION MARKS THE CORE TRANSCRIPTIONAL NETWORK FOR PLURIPOTENCY IN HUMAN EMBRYONIC STEM CELLS

Xie W[1], Song C[1], Sperling A[1], Xu F[1], Sridharan R[1,], Conway A[2], Plath K[1], Clark A[2] & Grunstein M[1]; [1]Molecular Biology Institute and Department of Biological Chemistry, Geffen School of Medicine at UCLA, USA; [2]Department of Molecular, Cell and Developmental Biology at UCLA, USA

Histone post-translational modifications play important roles in regulating DNA based functions in eukaryotes [1]. Of these modifications, Histone H3 lysine 56 acetylation (H3 K56Ac) is especially intriguing in that it occurs not at the extended N termini but in the helical core of yeast Histone H3 in a cell cycle specific manner. Thus, yeast K56Ac is largely global in that it occurs on newly synthesized Histone H3 that is assembled as DNA is replicated in S phase. K56Ac opens yeast chromatin and has been shown to regulate Histone gene transcription, DNA replication and repair and epigenetic silencing. While K56Ac is abundant in yeast and flies, it has been unclear whether this important modification is conserved in mammals. Here we show that K56Ac is not cell cycle regulated and global in human cells but is gene specific and is enriched at promoters of the transcriptional pluripotency network that is regulated by NANOG, SOX2 and OCT4 in human embryonic stem cells (hESCs). Relocation of K56Ac then occurs to other genes in somatic cells. Thus, K56Ac state more accurately reflects the epigenetic differences of hESCs versus somatic cells than other Histone modifications such as H3 lysine 4 tri-methylation and lysine 9 acetylation that are related to gene activity. These results suggest a gene specific recruitment mechanism for human enzymes that acetylate K56 to open chromatin and allow the underlying genes of the pluripotency network to be poised for activity or transcribed.

Poster No. III-1

LONG-RANGE ACTIVATION OF FKBP51 GENE VIA DISTAL INTRONIC ANDROGEN RECEPTOR-BINDING SITES IN PROSTATE CANCER CELLS

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Androgen receptor (AR) is a ligand-regulated transcription factor that is frequently overexpressed in hormone-refractory prostate cancers. Perturbations in the transcriptional programs of AR are important, but poorly understood, events in prostate cancer. FK506-binding immunophilin 51 (FKBP51) gene is overexpressed in prostate cancer specimen and significantly down-regulated in response to reduction of tissue androgens. Here, we have characterized the mechanisms of androgen-dependent regulation of FKBP51 in VCaP human prostate cancer cells containing amplified AR levels. FKBP51 mRNA displayed rapid and strong induction (~10-fold at 2 h, ≥50-fold at 12 h) in response to androgen. Experiments with cycloheximide and actinomycin D indicated that FKBP51 is directly targeted by androgen-bound AR and the regulation occurs merely at the level of transcription. Interestingly, in silico analysis localized the highest scoring androgen response elements (AREs) to the 5th intron (I5) of FKBP51 gene. Three of the putative AREs bound AR in vitro and mediated androgen induction as isolated elements in reporter assays in prostate cancer cells. Chromatin immunoprecipitation assays in VCaP cells confirmed androgen-induced loading of AR onto two I5 ARE-containing regions at ~80 kb and ~90 kb from the transcription start site (TSS). Binding of holo-AR to the latter regions facilitated recruitment of RNA polymerase II (PolII) onto the FKBP51 TSS

region. FoxA1, GATA2 or Mediator component MED1/TRAP220 that have recently been implicated to be important for the function of some other androgen-regulated enhancers, do not appear to be critical for the regulation of FKBP51 by androgens, as their ablation by RNAi did not influence the expression of FKBP51. Chromatin conformation capture assays are under way to reveal the mechanism(s) of long-range communication between the holo-AR and transcription apparatus in the activation of FKBP51 gene.

Poster No. III-2

THE BROMODOMAIN PROTEIN BRD7 IS A NOVEL COFACTOR OF THE P53 TUMOR SUPPRESSOR.

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Prevention of cancer development by the tumor suppressor p53 relies substantially on its ability to regulate transcription of numerous target genes, as highlighted by the frequency of tumor-acquired mutations within p53 DNA-binding domain. However, mechanisms by which p53 function is subverted in human cancers lacking these mutations remain poorly defined.
By a yeast two-hybrid screening we have identified the bromodomain-containing protein Brd7 as a novel interactor of the p53 family proteins. Brd7 protein is associated with chromatin and binds to acetylated core histones through its bromodomain. We have shown that Brd7 is required for the ability of both p53 and p73 to promote transcription of p21, while not being involved in the induction of apoptosis-related p53 target genes. Consistently, inhibition of Brd7 expression by RNA interference is able to revert cell cycle arrest induced by p53 upon genotoxic treatment of human tumor cells. In addition, we have demonstrated that silencing of Brd7 can prevent RasV12-induced senescence of primary human fibroblasts, suggesting an involvement of Brd7 in the establishment of this antitumor mechanism by p53 in response to activated oncogenes.
In fact, it has been previously shown that Brd7 expression is frequently low in Nasal-Pharyngeal Carcinoma (NPC) relative to normal tissue, while its ectopic expression in NPC cells leads to inhibition of cell cycle progression. We have observed that Brd7 expression is down-regulated also in breast tumors, and that this event is significantly more frequent in cancers bearing wild-type p53: this is in agreement with a crucial role being played by Brd7 in tumor suppression by the p53 pathway.

Poster No. III-3

A COMPARATIVE STUDY OF A FAS APOPTOSIS RESISTANT B-CELL LINE FROM A SEVERELY NEUTROPENIC PATIENT

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Kostmann's Syndrome is an inherited disorder in which white blood cells of the granulocyte type can not complete their development. The resulting lack of these cells prevents the natural immune responses from controlling bacterial infections. The affected patients, mostly children, die young from bacterial infections. The estimated frequency of this disorder is 1-2 cases per million with an equal distribution for gender. Mortality has been reduced by treatment with granulocyte-

colony stimulating factor (G-CSF) and death has been reported at 8% after 10 years on G-CSF therapy.
While the exact genetic lesions that contribute to the premature death or maturation arrest in the granulocytes in these patients is unknown, two groups of patients with different genetic mutations have been identified among Kostmann patients. In one group, both normal copies of a gene protecting cells from spontaneous cell death, HAX-1, are damaged. In the second group, one copy of the gene for a secreted protease, Elastase1, is dys-functional. *This may render inflammatory processes less effective.*
Our studies of B-cells from Kostmann patients now suggest a third possible pathway for premature death of immune cells in this syndrome. These B-cells showed an elevated level of the signals that activate the programme for spontaneous cell death, as compared to B-cells from normal control patients. B-cells are known to depend during their development on signals delivered by a cell surface receptor, Fas. Since addition of antibodies directed against the Fas receptor on the cell surface were effective in counteracting the abnormal death signals in the Kostmann cells, we propose to further explore the use of similar therapeutic antibodies directed against this receptor, to find a therapy that might improve the clinical condition and quality of life for Kostmann patients.
This line of investigation is promising because such antibodies (as Rituximab) have proven beneficial in the treatment of other immune disorders, systemic lupus erythematosus, rheumatoid anthritis, immune thrombocytopenic purpura and autoimmune heamolysis.

Poster No. III-4

TRANSCRIPTIONAL REGULATION OF KCC2; ROLE OF UPSTREAM STIMULATING FACTORS

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The neuron-specific K^+-Cl^- -cotransporter (KCC2) is identified as the main mechanism for pumping Cl- out of neurons, and it is thus necessary for the fast hyperpolarizing response to neurotransmitters g-aminobutyric acid (GABA) and glycine. The mammalian KCC2 gene (alias Slc12a5) generates two isoforms by using alternative promoters and first exons. Expression of both KCC2 isoforms is regulated in a neuron-specific fashion. Expression of the major isoform, KCC2b, is strongly upregulated during neuronal maturation. In contrast, the overall expression of the minor isoform, KCC2a, remains relatively constant during development. Expression of KCC2b is modulated by neuronal activity, trauma and neurotrophic factors and downregulation has been observed after seizure activity, ischemia and axonal injury.
Our aim is to identify important transcriptional mechanisms regulating KCC2b expression in normal and pathological conditions. Analysis of the KCC2b promoter region has revealed several candidate transcription factor binding sites that are highly conserved in mammalian KCC2b genes. Earlier we have shown an important role of the early growth response 4 (Egr4) transcription factor in the developmental upregulation of KCC2b gene expression. In the present study, we have focused on the regulatory influence of the upstream stimulating factors (USFs) through an E-box control element in the KCC2b promoter. The USF proteins are known to be important for brain function by regulating transcription in response to neuronal activity. Binding of endogenous USF1 and USF2 to the E-box element in the context of the KCC2b promoter was demonstrated in electrophoretic mobility shift assay (EMSA) and

supershift assay as well as by chromatin immunoprecipitation in neurons. The importance of the E-box site in the KCC2 promoter driven transcription was confirmed by site-directed mutagenesis of the E-box element and analysis of the promoter activity in cell lines and neurons.

Poster No. III-5

STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE POLYCOMB REPRESSIVE COMPLEX 1 PROTEINS

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The Polycomb group (PcG) proteins are involved in the maintenance of the correct transcription patterns of homeotic genes that are regulators of the cell behavior and the cell fate. The PcG proteins assemble into at least two complexes, the Polycomb repressive complex 2 (PRC2) and the Polycomb repressive complex 1 (PRC1), the first is responsible for the initiation and the second for the maintenance of the repressive state of the transcription.
Our work is focusing on the proteins belonging to the PRC1, on their structural and functional characterization. The PRC1 complex consists of proteins belongings to different families. Recent studies showed that PRC1 contains RING1A and RING1B, which act as an E3 ligase that monoubiquitinates the Lys119 of histone H2A. Other members of the complex, namely BMI1 and MEL18 interact with them and modulate their ubiquitination activity. Anyway very little is known about these interactions and how these proteins carried out their functions *in vivo*. Moreover, the role of the many other proteins in the core of the PRC1 and how this complex is able to maintain the repressive state of the transcription is still to be understood.
Our interest is to deepen the knowledge on the PRC1, mainly about the ubiquitination activity but also regarding the role of other members of the complex. Our group uses crystallographic techniques to investigate the three-dimensional structure of proteins. With this information and with further biochemical and biophysical analysis we want to study PRC1 core proteins, their interactions and their function in the cell.

Poster No. III-6

A NOVEL POLYCOMB GROUP SILENCER COMPLEX COUPLES HISTONE H2A UBIQUITYLATION TO HISTONE H3 DEMETHYLATION

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Maintenance of gene transcription status plays a critical role in development and disease. The evolutionary conserved Polycomb- (PcG) and trithorax group (trxG) proteins respectively maintain the repressed or activated status of many developmental and disease-associated genes. These opposing co-regulators function by changing the structure of chromatin, in part through covalent post-translational histone modifications. Previously, a unidirectional regulatory pathway in which ubiquitination of H2B is a prerequisite for histone H3 Lysine 4 methylation was shown to correlate with active transcription. Here, we provide evidence for an analogous mechanism at play during transcriptional silencing: H2A ubiquitylation coupled to demethylation of histone H3. This trans-histone pathway was uncovered through the

identification of dRING associated factors (dRAF), a novel Polycomb group silencing complex harboring the histone H2A E3 ubiquitin ligase dRING, PSC and 4 additional subunits, two of which have enzymatic activities. Genome wide expression analysis revealed that dRAF and Polycomb repressive complex 1 (PRC1) share many transcriptional targets. In agreement with a role as a repressor in vivo, the signature subunit dRAF1 acts as an enhancer of Pc, but a suppressor of the trxG histone methyltransferase genes trx and Ash1. Importantly, cellular depletion and in vitro reconstitution assays revealed that dRAF1 not only mediates removal of the ASH1-mediated active histone H3 methyl mark, but also allows efficient H2A ubiquitylation by dRING-PSC. These observations suggest that dRAF, rather than PRC1, is responsible for bulk H2A ubiquitylation in cells. dRAF only mediates histone H2A ubiquitylation but does not influence histone H2B ubiquitylation, demonstrating its high selectivity. In conclusion, dRAF removes an active methyl mark from histone H3, and adds a repressive ubiquitin mark to H2A. These findings provide an example of coordinate trans-histone regulation by a PcG complex to mediate gene repression.

Poster No. III-7

CHROMATIN MODIFICATION SIGNATURES PREDICT DISEASE TYPE AND PROGNOSIS IN ACUTE LEUKEMIA.

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Transcription factor alterations and associated chromatin modifications play a crucial role in the pathogenesis of acute leukemia. We analyzed acetylation (H3Ac) and lysine 9 trimethylation (H3K9me3) of Histone H3 in a large cohort of primary acute leukemia (n=145) and control samples (n=39) by ChIP-Chip. Genome wide patterns of both histone modifications clearly allowed to separate AML from ALL and AML from normal CD34+ hematopoietic progenitor cells. Transcription factor binding sites for leukemogenic oncogenes such as c-myb and Meis1 were overrepresented in regions altered in AML specimens. A H3K9me3 pattern derived signature predicted survival in AML patients. Its combination with clinical covariates further improved survival prediction in an independent AML sample set. Pathogenetically relevant genes could be identified by altered chromatin modifications in AML compared to healthy CD34+ progenitor cells. Taken together, global patterns of chromatin modifications are leukemia type specific, predict patients´ prognosis and are associated with pathogenetically relevant genes. These data provide evidence for leukemia specific chromatin signatures and for the prognostic and pathogenetic relevance of chromatin modification patterns in AML.

Poster No. III-8

CELL-AUTONOMOUS AND NON-CELL-AUTONOMOUS FUNCTIONS OF *C. ELEGANS HPL-2*/HP1 IN ANTAGONIZING EGF SIGNALING DURING VULVAL CELL FATE SPECIFICATION

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The *Caenorhabditis elegans* vulva represents a simple developmental system in which to study how chromatin factors influence developmental processes dependent on signaling pathways. The synthetic multivulva genes (synMuv) encode conserved chromatin factors, which antagonize vulval cell fate specification. Until recently, the prevailing view was that this group of genes is part of an inhibitory signal from the hypodermis (hyp7) which opposes the action of the inductive Ras signaling pathway, thereby preventing inappropriate adoption of vulval fates. However, it has been recently show that some synMuv genes, including the Rb homologue *lin-35*, are likely to function within the hyp7 syncytium to repress ectopic expression of the vulval inducer LIN-3/EGF, thereby preventing ectopic induction of vulval precursor cells (VPC).
Genetic studies suggest that the *C. elegans* HP1 orthologue *hpl-2*, in addition to acting in the synMuv pathway, might be required more broadly for the transcriptional repression of genes necessary for proper vulval development. Biochemical analysis identified two synMuv complexes in embryonic extracts: one containing LIN-35Rb, LIN-9 and RbAp48/LIN-53, as well as a number of other conserved proteins, and a second complex with a composition similar to mammalian NuRD. HPL-2/HP1 was not detected in either one of these complexes. To gain insight into how *hpl-2/*HP1 interacts with other synMuv genes, we carried out experiments to establish its cellular focus. Surprisingly, we find that HPL-2/HP1 activity is required in both VPCs and HYP7 for correct vulval development. In HYP7, HPL-2 acts through *lin-3/*EGF, as shown for LIN-35Rb. However, LIN-3/EGF is not likely to be a relevant target for HPL-2/HP1 in VPCs. Our results suggest that in VPCs, HPL-2/HP1 may act cell autonomously to antagonize *lin-3*/EGF signaling from HYP7 at the level of transcription of target genes, which may include *lin-39*/HOX. This latter function is likely to be independent of *lin-35* Rb and other synMuv genes whose focus for vulval cell fate specification is in the hypodermis.

Poster No. III-9

ANDROGEN RECEPTOR REGULATES *ETS-LIKE TRANSCRIPTION FACTOR 4* IN PROSTATE CANCER CELLS

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Transcriptional control by androgens *via* androgen receptor (AR) is strongly involved in prostate cancer development, but the critical target genes have remained elusive. Since ETS domain transcription factor *ETS-like transcription factor 4* (*ELK4*) has been recently listed among genes overexpressed in prostate cancer and as perturbed regulation of at least two other ETS factor genes *EGR* and *ETV1* has been implicated in a significant number of prostate cancer samples, we chose to analyze potential androgen regulation of *ELK4* (also known as *serum response factor accessory protein 1*, *SAP-1*) in human prostate cancer cells. *In silico* screening identified three putative AR response elements (AREs) within -10 kb from the transcription start site of *ELK4*. Both ARE1 at -167/-153 and ARE2 at -481/-467 bound AR *in vitro* and mediated androgen induction as isolated elements in transcription assays in non-prostate cells. However, merely the ARE2 that cooperates with its proximal forkhead box A1 (FoxA1)-binding site was critical for the AR-dependent activation of *ELK4* promoter in prostate cancer cells. Preferential loading of holo-AR onto the ARE2 and concomitant recruitment of RNA polymerase II onto the *ELK4* promoter was confirmed in prostate cancer cells by chromatin immunoprecipitation. Database searches indicated that the

expression of *ELK4* is markedly increased in prostate cancers relative to normal prostates. Moreover, prostate cancer tissue immunostainings showed that nuclear ELK4 levels are significantly increased in androgen-refractory prostate cancers compared to untreated tumors. Reduction of the amount of ELK4 in LNCaP cells by RNAi retarded cell growth. In conclusion, *ELK4* is a direct AR target in prostate cancer cells. Androgens may thus contribute to the growth of prostate cancer *via* influencing ELK4 levels.

Poster No. III-10

COORDINATED REGULATION OF REPRESSION BY THE RBP2 DEMETHYLASE AND THE PRC2 COMPLEX

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Polycomb Group (PcG) proteins are transcriptional repressors essential for development and for cellular proliferation. PcG proteins play a central role in the establishment of specific transcription programs that regulate cell fate determination during development by the direct regulation of the expression of a large number of genes essential for proper development.
The Polycomb Repressive Complex 2 (PRC2), through its subunit EZH2, catalyzes the tri-methylation (me3) of Histone H3 on Lysine (K) 27. The mechanisms by which the PRC2 complex and H3K27 methylation induce transcriptional silencing are still unknown, but the PRC2 activity is required for different epigenetic phenomena such as X-chromosome inactivation and genes imprinting, is essential for development and is frequently deregulated in human tumors.
The discovery of Histone Lysine Demethylases that catalyze the removal of methyl groups from Lysine residues. This discovery has introduced an additional layer of complexity in the mechanisms of epigenetic regulation of transcription. Recently, others and we have identified RBP2 (JARID1A) as a specific H3K4me3 demethylase that is required for the expression of homeotic genes and worm development.
At the meeting, we will present data demonstrating a functional interplay in mouse Embryonic Stem (ES) cells between the PRC2 complex and the H3K4me3 demethylase Rbp2. By genome-wide location analysis we have found that Rbp2 is associated with a large number of PcG target genes in mouse ES cells. We will show that the PRC2 complex recruits Rbp2 to its target genes and that this interaction is required for PRC2-mediated repressive activity during ES cell differentiation. Taken together, our results demonstrate an elegant mechanism for repression of developmental genes by the coordinated regulation of epigenetic marks involved in repression and activation of transcription.

Poster No. III-11

CHARACTERIZATION OF THE RSC1 AND RSC2 COMPLEXES IN *S. CEREVISIAE*

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RSC is an ATP-dependent chromatin-remodeling complex that plays an important role in cellular processes such as chromosomal segregation and transcriptional elongation. In yeast, the rsc1 and rsc2 subunits can be separated into two distinct complexes. We propose that these two complexes function in different biological

pathways. Our goals are to characterize the components of the RSC1 and RSC2 complexes and to determine the biological function of these complexes.
Purification of each complex through tagged RSC1 or RSC2 followed by mass spectrometry analysis showed clear differences in composition. cDNA micro array analysis of *RSC1* or *RSC2* deletion mutants indicated that the RSC1 and RSC2 complexes have both overlapping and distinct roles in the regulation of gene expression. Finally, deletion of both *RSC1* and the gene coding for the Set2 histone methyltransferase was lethal, while the *RSC2 SET2* double deletion mutant was viable. Preliminary genome-wide data shows that rsc1 occupancy changes more dramatically that that of rsc2 in the *SET2* deletion background, which agrees with the genetic findings. These data suggest that the RSC1 complex plays a role in transcriptional elongation *in vivo*.

Poster No. III-12

THE ROLE OF THE JUMONJI 2 (JMJD2) HISTONE DEMETHYLASES IN CELL PROLIFERATION AND CANCER

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DNA is wrapped around histone proteins forming an organized structure called chromatin. Post-translational modifications of histones are crucial for transcriptional regulation, as well as for maintenance of genomic stability and for cell proliferation and differentiation. Genes encoding histone-modifying enzymes are thus commonly found deregulated in human cancers.
Others and we recently showed that the Jumonji 2 (JMJD2) proteins are histone demethylases specific for tri- and di-methylated lysine 9 and lysine 36 on histone H3 (H3K9 and H3K36). Methylation of H3K9 is associated with heterochromatic areas of the genome, which are condensed and transcriptionally inactive. Methylated H3K36 is, on the other hand, primarily detected within the transcribed regions of active genes. Several lines of evidence indicate that the JMJD2 demethylases could be involved in malignant transformation. The expression of the JMJD2 histone demethylases is highly elevated in a subset of human cancers, and knockdown of JMJD2C (a.k.a. Gene Amplified in Squamous cell Carcinoma 1 (GASC1)) retards growth of tumor cell lines. Furthermore mice deficient for one of the key enzymes responsible for H3K9 di- and tri-methylation are tumor prone.
To elucidate the role of the JMJD2 histone demethylases in cell proliferation and cancer, we have performed screens to identify protein interaction partners and genes regulated by the two JMJD2 proteins, JMJD2A and GASC1/JMJD2C. Specific antibodies have been used to identify promoter regions directly bound by JMJD2 proteins in a genome-wide location analysis. Complementary to this, changes in gene expression profiles caused by down-regulation of the JMJD2 proteins have been monitored. Target genes were identified in the esophageal carcinoma cell line, Kyse150, which has the *GASC1* gene amplified. Interestingly, our data suggests that the JMJD2 proteins regulate a substantial number of genes involved in cell proliferation and mitosis, and consistent with this down-regulation of GASC1 appears to affect genomic stability.

Poster No. III-13

INHIBITION OF P53 FUNCTION BY THE HEPATITIS C VIRUS CORE PROTEIN-MEDIATED DOWNREGULATION OF HISTONE METHYLTRANSFERASE ACTIVITY

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Posttranslational modification of histone proteins constitutes the histone code, which plays an important role in eukaryotic gene expression as well as viral pathogenesis. Previous studies have shown that viral proteins functionally interact with histone acetyltransferases/deacetylases. Whether cellular histone methyltransferases (HMTs) are also targeted by viral oncoproteins remains unclear. Here we demonstrate that the core protein of the hepatitis C virus (HCV) which causes chronic hepatitis, cirrhosis, fibrosis and hepatocellular carcinoma in infected individuals, inhibited core histone methylation by p53 coactivator HMTs, the coactivator-associated arginine methyltransferase 1 (CARM1), protein arginine methyltransferase 1 (PRMT1), and SET9. In human hepatoma cell line HuH 7, the reporter assays indicated that core protein repressed CARM1- and PRMT1-enhanced p21 promoter activities as well as p21 synthesis. In addition, we showed the *in vitro* and in vivo interactions between core protein and HMTs. Using the chromatin-immunoprecipitation (ChIP) analysis, we further demonstrated that core protein was recruited to the p21 promoter and inhibited the active histone pattern induced by these three HMTs, such as histone H3 acetylation and H3K4 mono-methylation. Taken together, our results indicate that HCV core protein inhibits HMT activities *in vitro* and *in vivo,* which in turn resulted in the inhibition of p53 function.

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Poster No. III-14

***CHLAMYDIA TRACHOMATIS* PROTEIN CT737 IS A HISTONE METHYLTRANSFERASE AND A POTENTIAL MEDIATOR OF GENE REGULATION**

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Chlamydia trachomatis is an obligate intracellular pathogen that primarily infects human epithelial cells and is the most commonly acquired sexually transmitted disease. Ct737 is a chlamydial protein identified as a putative secreted protein, thus giving it the potential to act as a bacterial effector during infection. The protein is of particular interest due to its high degree of homology with SET domain proteins, which regulate gene expression and replication by transferring methyl groups to the lysines of histones. We hypothesize that Ct737 is a novel methyltransferase that may target mammalian histones during infection. In support of this hypothesis, we found Flag- and GFP-tagged Ct737 expressed in HeLa cells localized to the nucleus as seen by immunoflourescence. Cross-linking proteins in transfected cells prior to lysis revealed a shift in the molecular weight of full-length Ct737, suggesting that it exists in a complex with other nuclear proteins. In addition, purified recombinant GST-tagged Ct737 methylated histones *in vitro* and the methyltransferase inhibitor 5'-deoxy-5'-methylthioadenosine (MTA) dramatically inhibited the progression of infection in Chlamydia infected HeLa cells. We have also found the protein is able to

automethylate, which may indicate a form of autoregulation. Interestingly, Chlamydia have a unique biphasic life cycle in which the infectious form is characterized by a lack of metabolic activity due to highly condensed chromatin, and replication can occur only after decondensation once the bacteria have entered a host cell. The Chlamydial histone-like protein 1 (Hc1) plays a critical role in this transition. We have found a methylated protein in Chlamydial lysates of the same molecular weight as Hc1. Thus, Ct737 has the potential to methylate both mammalian histones and bacterial proteins during infection. We are currently investigating the biological implications of these modifications.

Poster No. III-15

NOVEL TARGETS FOR THE MITF TRANSCRIPTION FACTOR IN MELANOMAS

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Malignant melanomas are chemotherapy-resistant cancers with high mortality. Recent advances in our understanding of the disease at the molecular level have indicated that in addition to the loss of cell cycle checkpoints which may be common to all cancers, malignant melanoma shares many characteristics in common with developmental precursors to melanocytes, the mature pigment producing cells of the skin and hair follicles. The development of melanocytes depends on the presence of the microphthalmia-associated transcription factor (MITF), a member of the basic helix-loop-helix-leucine zipper (bHLH-LZ) transcription factor family. MITF has been shown to regulate a broad range of genes, ranging from genes important for pigment production, to genes involved in cell cycle regulation, migration and survival. Nevertheless, the known MITF target genes do not explain all the roles of MITF in melanocyte development and melanoma progression.
We have used a novel microarray approach to characterize MITF target genes in melanomas. First we identified genes which correlate with the expression of MITF in human melanoma samples. Second, we compared the genes expressed in a stably MITF-transfected line of SKmel28 cells with the genes expressed in SKmel28 cells without MITF. Third we compared the two lists of genes to obtain a list of genes potentially regulated by Mitf.
A number of the genes thus identified are known Mitf target genes, whereas the rest represent novel targets. Among the novel targets are genes involved in cell migration and may therefore be important for the metastasis of melanoma. This data is being confirmed experimentally using chromatin immunoprecipitation (ChIP) for the new potential targets, followed by qPCR, as well as co-transfection studies of MITF with the respective promoter constructs. This will help us to reveal real target genes and pathways activated by MITF in melanoma.

Poster No. III-16

ETS1 INTERACTS WITH THE *NKX3.1* 5' PROMOTER IN PROSTATE CANCER CELLS

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NKX3.1 is a member of the NK2 class of homeodomain proteins critical for prostatic epithelial cell proliferation and differentiation. Located on 8p21, a chromosomal region frequently disrupted in human prostate tumors, NKX3.1 has been proposed to function as a prostate-specific tumor suppressor. Expression of *NKX3.1* is regulated by androgens in both the normal prostate and in prostate cancer cells, as well as retinoic acid in the prostate cancer cell line, LNCaP. Analysis of the initial 2062bp of the *NKX3.1* promoter using a series of deletion reporter constructs identified regions of both positive (-2062 to -993) and negative (-993 to -200) transcriptional activity. Characterization of sequences harboring positive transcriptional activity revealed that a 76bp sequence between -1069 and -993 contained strong enhancer activity when coupled with an SV40 promoter in a luciferase reporter vector. Subsequent bioinformatics analyses of *cis*-acting elements operating within this region highlighted the presence of two putative ETS1 binding sites between -1016 and -993, which displayed 84% and 72% homology to the ETS1 consensus binding sequence, respectively. Electromobility shift assays (EMSA) utilizing recombinant GST-ETS1 and nuclear proteins from LNCaP cells demonstrated *in vitro* ETS1 interaction with this 23bp sequence of the *NKX3.1* 5' promoter, and ongoing studies are investigating ETS1 regulation of *NKX3.1* in prostate cancer cells. ETS1 is a member of a large family of transcription factors that play important roles in development, with recent genomic studies indicating enrichment of ETS1 binding sequences in androgen target genes. The increased expression of ETS1 in human prostate tumors together with results from this study suggests that ETS1 may regulate *NKX3.1* expression in prostate cancer cells.

Poster No. III-17

LOCAL COHERENCE IN GENETIC INTERACTION PATTERNS REVEALS PREVALENT FUNCTIONAL VERSATILITY

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Epistatic or genetic interactions, representing the effects of mutating one gene on the phenotypes caused by mutations in one or more distinct genes, can be very helpful for uncovering functional relationships between genes. Recently, the Epistasis Miniarray Profile (E-MAP) method has emerged as a powerful approach for identifying such interactions systematically, and has been successfully employed to investigate the functional organization of genes involved in various aspects of chromosome biology, including transcription and DNA repair, in the yeast *S. Cerevisiae* (Collins et al. Nature **446:** 806-810, 2007). As part of this approach, hierarchical clustering is used to partition genes into groups on the basis of the similarity between their global interaction profiles, and the resulting descriptions assign each gene to only one group, thereby ignoring the multi-functional roles played by most genes. Here we present an original Local Coherence Detection (LCD) algorithm for identifying groups of functionally related genes from E-MAP data in a manner that allows individual genes to be assigned to more than one functional group. This enables investigation of the pleiotropic nature of gene function, a goal that cannot be achieved with hierarchical clustering. The performance of the LCD algorithm is illustrated by applying it to the E-MAP dataset. In addition to identifying the majority of the functional modules and many protein complexes reported previously, LCD uncovers many recently documented and novel multi-functional relationships between genes

and gene groups. Our algorithm hence represents a valuable tool for uncovering new roles for genes with annotated functions and for mapping groups of genes and proteins into pathways.

Poster No. III-18

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Poster No. III-19

MITOCHONDRIAL TRANSCRIPTION TERMINATION FACTOR (mTERF) DNA BINDING SITES REVEALED BY LIGATION-MEDIATED PCR (LM-PCR)

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The mammalian mitochondrial genome is organized in a highly compact manner, with virtually no spacers between its 37 genes, and a major non-coding region (NCR) shorter than 1 kb which contains the transcription promoters. This circular genome is transcribed by a phage-type RNA polymerase into polycistronic transcripts which, in mammals, encompass the entire genome on both strands. In addition, initiation from a different promoter gives rise to a truncated transcript encompassing just the rRNAs (plus two tRNAs) and thus defines a distinct mitochondrial rDNA transcription unit. Termination at the 3´ end of the rDNA is brought about by a transcription termination factor, mTERF. mTERF binds sequence-specifically with high affinity to a sequence element within the coding sequence of $tRNA^{Leu(UUR)}$, located immediately downstream of the rDNA. In addition to its role in transcription termination, other roles such as regulation of DNA replication and mitochondrial protein synthesis have been proposed. Based on this, we have studied the effect of over-expression of mTERF in human cultured cells using ligation-mediated PCR (LM-PCR). This technique has been used to reveal persistent free 5´ ends of DNA that are the result of replication fork stalling at mTERF binding sites. Results show that, along with the expected binding site in of $tRNA^{Leu(UUR)}$, mTERF can also bind to other sites in the mitochondrial genome and a consensus binding sequence has been derived. However, significant differences are observed between $tRNA^{Leu(UUR)}$ and the other binding sites, suggesting the possibility of further control point and/or non-transcriptional roles.

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Poster No. III-20

MOUSE MODELS TO STUDY MEDIATOR FUNCTIONS IN TRANSCRIPTIONAL ACTIVATION

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The vertebrate Mediator is commonly seen as a molecular bridge that connects information from transcription factors, bound to the regulatory regions of genes, and the RNA polymerase II machinery assembled on the promoter of these genes. The

Mediator is a large complex of around 30 subunits, some of which seem to play a structural role in the complex and are therefore generally required for transcription. Other subunits however, are thought to be responsible for specific interactions with defined transcription factors.
Our goal is to create mouse models where these Mediator subunits can be specifically downregulated using an inducible shRNA system integrated in the mouse ROSA26 locus. We have designed constructs that can be easily integrated in the mouse genome by Recombination Mediated Cassette Exchange. These constructs carry shRNA's targeting our genes of interest under the control of RNA polymerase II tissue specific promoters as well as a doxycyclin responsive silencer. Thus, this system provides temporal and tissue specific control of the gene knockdown.
We are mostly interested in two Mediator subunits, Med1 (Trap220) and Med12 (Trap230). Med1 is one of the best-characterized Mediator subunits and it has been shown to be essential for nuclear receptors activated transcription. Med1 deficiency leads to embryonic lethality in mice. Our system will allow us to study the role of Med1 as a coactivator during mouse development and identify processes where nuclear receptors are essential. Med12's function in the Mediator complex is less clear and there is currently no mouse model available but its ability to bind β-catenin suggests a role in wnt signaling. Sox9 and Gli3, a Sonic Hedgehog effector, have also been shown to bind to Med12 and the activation of their target genes is dependent on this interaction.
We have generated embryonic stem (ES) cell lines carrying inducible constructs to downregulate Med1 and Med12, and are currently in the process of generating mouse lines using these cells.

Poster No. III-21

YIN YANG 1 IS A CRITICAL REPRESSOR OF MATRIX METALLOPROTEINASE-9 EXPRESSION IN BRAIN NEURONS

Rylski M[1], Amborska R[1], Zybura K[1], Mioduszewska B[1], Michaluk P[1,2], Jaworski J[3] & Kaczmarek L[1]; [1]Department of Molecular and Cellular Neurobiology, Nencki Institute, Poland; [2]Department of Physiological Chemistry and Centre for Biomedical Genetics, University Medical Center Utrecht, The Netherlands; [3]Laboratory of Molecular and Cellular Neurobiology, International Institute of Molecular and Cell Biology, Poland

Matrix Metalloproteinase-9 (MMP-9) is aberrantly upregulated in many disorders of the central nervous system, participating in a substantial exacerbation of their harmful potential. However, the mechanisms regulating MMP-9 gene expression in neurons are largely unknown. We demonstrate that the transcription factor YY1, a Polycomb group protein, controls MMP-9 gene expression in brain neurons *in vivo* via transcriptional and epigenetic mechanisms. YY1 occupies the proximal *Mmp-9* promoter in a manner dependent on the transcriptional co-regulator CtBP1 and strongly represses its activity due to HDAC3-dependent hypoacetylation of histones H3 and H4. Consequently, neuronal depolarization, which triggers CtBP1 dissociation from the proximal *Mmp-9* promoter, results in YY1 and HDAC3 release, histone H3 and H4 hyperacetylation, and initiation of *Mmp-9* transcription, despite increased overall levels of YY1 expression. We also show that the transcriptional activity of hippocampal YY1 is modified by ubiquitination. Monoubiquitinated, but not native, YY1 is able to bind to HDAC3 and CtBP1 in rat hippocampus, and thus, to regulate *Mmp-9* transcription. In conclusion, our data suggest that ubiquitinated YY1 plays an important role in a control of neuronal gene expression.

Poster No. III-22

SWI/SNF ATPASES IN RNA PROCESSES

Ryme J[1], Tyagi A[2], Visa N[2] & Farrants A-K Ö[1]; [1]The Wenner-Gren Institute, Stockholm University, Sweden; [2]Dept. of Molecular Biology and Functional Genomics, Stockholm University, Sweden

The SWI/SNF chromatin remodeling complexes has the ability to alter the contacts between DNA and histones by using the energy from ATP hydrolysis. The unwinding of the DNA is one important regulation of gene expression in all eukaryotic cells and the ATPase subunits of the complexes have been found both in yeast, insects and mammals.

In man, two different ATPase subunits have been identified: the Brahma protein, BRM and the Brahma related gene 1, BRG1. Recent studies have proposed an involvement of the SWI/SNF complexes in coordination of transcription and splicing of pre-mRNA. These two processes are performed in close vicinity and it has been shown that both BRG1 and BRM interact with pre-mRNA splicing factors and that BRM is involved in splice site regulation.

Here we combine studies of the mammalian BRG1 subunit with the ortholog of BRG1 and BRM protein in Chironomus tentans, Ct-BRM, in order to determine whether the ATPase subunits are associated with the chromatin template and/or the nascent pre-mRNPs.

Biochemical fractionation and immuno-EM studies indicate that a fraction of the ATPase protein is associated with the nascent pre-mRNP complexes both in insects and in human cells.

We have also observed an interaction between hBRG1 and the U2 and U5 snRNA.

BENZON SYMPOSIUM No. 55

TRANSCRIPTION, CHROMATIN AND DISEASE

AUGUST 18 – 21, 2008, COPENHAGEN, DENMARK

Organizing committee:
Kristian Helin (Copenhagen), Jesper Q. Svejstrup (London) & Arne Svejgaard (Copenhagen)

Abstracts - THURSDAY, AUGUST 21, 2008

EPIGENIC CONTROL BY HISTONE METHYLATION

Jenuwein T; Research Institute of Molecular Pathology, Vienna Biocenter, Austria

Epigenetic mechanisms, such as Histone modifications, control eukaryotic development beyond DNA-stored information. Intriguingly, there is an under-representation of repressive marks in quiescent (resting) cells, stem cells and regenerating cells, but a selective accumulation of aberrant Histone lysine methylation profiles in aging, 'stressed' and tumor cells, particularly for the H3K9, H3K27 and H4K20 methyl marks. To examine this notion in functional detail, we have generated mutant mice that lack crucial HMTases, such as e.g. the Suv39h and Suv4-20h enzymes. In addition, we have been characterizing jumonjiC-containing proteins that represent Histone lysine demethylases with the potential to remove H3K9me3 marks. We have also screened chemical libraries (in collaboration with Boehringer Ingelheim, Ridgefield USA) and identified a small molecule inhibitor for the G9a HMTase. We have done extensive profiling by ChIP-chip micro-arrays for many Histone modifications in chromatin from ES cells and from a variety of differentiated cells. Our data indicate that distinct Histone lysine methylation profiles contribute to the epigenetic 'make-up' of stem cells vs. more committed cells. Surprisingly, epigenetic variation appears to reside in repeat-associated heterochromatic islands and much less at annotated genes. Together, these functional approaches promise to yield new insights into the plasticity of cell fate decisions and will provide novel strategies to modulate epigenetic control in normal and aberrant development.

CONTROL OF STEM CELL DIFFERENTIATION AND CANCER BY HISTONE METHYLATION

Agger K, Christensen J, Cloos PAC, Hansen KH, Kleine-Kohlbrecher D, Pasini D, Pedersen MT, Rudkjær L & Helin K; Biotech Research and Innovation Centre (BRIC) and Centre for Epigenetics, University of Copenhagen, Denmark

A major goal of the research in our laboratory is to identify and characterize genes involved in the regulation of normal proliferation and differentiation that also contribute to the development of human cancer. Consistent with an essential role of epigenetics in controlling cell-fate decisions, we have identified several oncogenes that function as epigenetic modifiers.

At the meeting, a couple of stories will be presented demonstrating the role of two families of proteins in controlling cell proliferation and differentiation. The Polycomb group proteins (PcGs) were until recently best known for their essential role in development, however, several reports have established that PcGs are frequently

deregulated in human tumors. Others and we have demonstrated that the EZH2 Histone methyl transferase is an oncogene, which regulates the expression of a large number of genes dictating cell-fate decisions. I will introduce you to these results and present some data giving mechanistic insights into how these proteins control cellular senescence and stem cell differentiation.

Others and we have recently discovered a group of proteins that catalyze the demethylation of methylated lysines. Members of this Jumonji demethylase family have been found overexpressed in human cancer and mutated in neurological disorders. Results will be presented at the meeting describing the functional characterization of some of these very exciting proteins that also present strong candidate targets for drug development.

NUCLEAR EXOSOME IS REQUIRED FOR TRANSCRIPTIONAL SILENCING OF THE HIV-1 PROMOTER *(ORAL POSTER)*

Rousset E, Chable-Bessia C, Benkirane M & Kiernan R; Laboratoire de Virologie Moleculaire, Institut de Génétique Humaine, France

Following integration into the host cell genome, HIV-1 provirus becomes transcriptionally silent in a subset of T-cells, which likely contributes to clinical latency. The mechanism of transcriptional silencing is not understood but it appears to involve the recruitment of chromatin remodelling and transcriptional repressor complexes as well as HP1-gamma-dependent heterochromatin formation to the integrated provirus. Silenced LTRs permit basal transcription in which a low level of a short transcript, TAR, is synthesized. Nuclear exosome has been implicated in RNAi-dependent heterochromatin formation and silencing in yeast. We now show that the catalytic subunit of nuclear exosome, PM-scl100/Exosc10/Rrp6, is required for HIV-1 silencing using a LTR gene reporter system as well as during HIV-1 infection of T-cells. Knockdown of Rrp6 using siRNA induced a greater than 50-fold increase in transcriptional output from the LTR. Levels of initiated as well as elongated transcripts were increased. Consistent with this, significant recruitment of RNAPII to the promoter was detected by ChIP. Rrp6 was found to be associated with HIV-1 chromatin, and was reduced following knockdown, which supports previous observations that Rrp6 is chromatin-associated. In addition, several Histone modifications were associated with Rrp6-dependent de-repression. While H3K4Me3 was increased as might be expected, we also noted a significant increase in H3K9Me3. HP1-gamma, which is associated with HIV-1 silencing, was not displaced by de-repression, but instead became phosphorylated. Our data suggest a model in which synthesis of a short, basal transcript recruits Rrp6 that is, in turn, required to maintain a state of transcriptional silencing at the integrated HIV-1 promoter. Such a mechanism that depends on ongoing, low level transcription to mediate transcriptional silencing may be particularly suitable for inducible genes.

GENOME-WIDE CHIP-SEQ PROFILING OF PPARG/RXR TARGET SITES AND GENE PROGRAM DURING 3T3-L1 ADIPOCYTE DIFFERENTIATION *(ORAL POSTER)*

Pedersen TÅ[1], Nielsen R[1], Hagenbeek D[2] , Alako B[2], Siersbæk R[1], Megens E[2], Moulos P[2], Stunnenberg H[2] & Mandrup S[1]; [1]Dept. Biochemistry and Molecular Biology, University of Southern Denmark, Denmark. [2]Dept. of Molecular Biology, Radboud University Nijmegen, The Netherlands

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors which bind to DNA as heterodimers with members of the retinoid X receptor family. Numerous gain-of-function and loss of function experiments have unequivocally shown that PPARγ is a master regulator of and obligate for adipocyte differentiation. In addition to driving the adipogenic process, PPARγ activates directly a large number of genes involved in lipid storage and lipid turnover.

Using ChIP-sequencing we have generated a genome-wide map of PPARγ-RXR binding to chromatin as well as the activation of associated target genes during differentiation of murine 3T3-L1 adipocytes. Our analysis shows that target sites/genes attain RXR and PPARγ occupancy at different time points. Most of the known target genes have multiple binding sites within few kb from the promoter, and around 50% of these target sites are located in introns. These results provide an excellent basis for extensive analyses of the regulatory network controlled by PPARγ during adipocyte differentiation and in the mature adipocytes.

Acknowledgement:

This work is supported by grants to the EU FP6 STREP project X-TRA-NET, EU FP6 IP HEROIC and grants from the Lundbeck Foundation and the Danish Natural Science Research Council.

ROLE OF HISTONE DEMETHYLASES IN DEVELOPMENT AND CELLULAR METABOLISM

Zhang Y; Howard Hughes Medical Institute & University of North Carolina-Chapel Hill, USA

Posttranslational Histone modifications play an important role in regulating chromatin dynamics and function. One of the modifications, methylation, occurs on both lysine and arginine residues and participates in diverse range of biological processes including heterochromatin formation, X-chromosome inactivation, and transcriptional regulation. While acetylation, phosphorylation, and ubiquitylation are dynamically regulated by enzymes that catalyze the addition and removal of a particular modification, enzymes that are capable of removing methyl groups were not known until recently. To understand the biological function of the JmjC domain-containing Histone demethylases, we have used a loss of function approach, including RNAi and targeted gene deletion. Our results revealed important roles of Histone demethylases in cellular metabolism, germ cell development, and stem cell pluripotency.

HISTONE DE-METHYLATION

Shi Y; Department of Pathology, Harvard Medical School, USA

Histone methylation is a fundamental regulatory mechanism, which impacts transcription, DNA recombination, replication and damage response, heterochromatin, and epigenetic state of the cell. Histone methylation patterns correlate with stem cell maintenance and differentiation, and altered Histone methylation is linked to numerous human diseases including cancer and neurological disorders. Histone methylation was discovered in the 1960s and was viewed as a stable and irreversible modification until the recent discovery of Histone demethylases. In this presentation, I will discuss our efforts of understanding the biochemical and molecular mechanisms by which Histone demethylases regulate

Histone methylation dynamics and transcription, and their roles in development and human diseases.

Poster No. IV-1

CANCELLED

Poster No. IV-2
STRUCTURE AND FUNCTION OF THE YAF9 YEATS DOMAIN
Schulze JM[1], Wang A[1], Skordalakes E[3], Gin JW[2], Berger JM[2], Rine J[2] & Kobor MS[1]; [1]Centre for Molecular Medicine and Therapeutics, University of British Columbia, Canada; [2]Department of Molecular and Cell Biology, University of California Berkeley, USA; [3]The Wistar Institute, Gene Expression and Regulation Program, USA

The structure of chromatin can be modified in several ways, including post-translational modifications of histones, ATP-dependent chromatin remodeling, and incorporation of histone variants. Most chromatin modifying enzymes contain subunits with signature domains important for function, such as bromodomains for recognizing acetylated lysine, and chromodomains for recognizing methylated lysine. The Yaf9 protein from *Saccharomyces cerevisiae* is a subunit of the essential histone acetyltransferase NuA4 and the chromatin remodeling complex SWR1-Com, which is responsible for deposition of the histone variant H2A.Z into chromatin. Yaf9 is one of three proteins in yeast that contains the structurally uncharacterized YEATS domain, found in proteins from multiple chromatin remodeling and transcription complexes. The structure of the Yaf9 YEATS domain was determined by X-ray crystallography and was found to consist largely of beta-sheets and belonged to the immunoglobulin family. YEATS domain function was conserved between Yaf9 and human GAS41, a protein encoded by a gene highly over-expressed in early stages of gliomas. The phenotypes caused by mutations that altered amino acids on the surface of the YEATS domain revealed that the YEATS domain was important for protein function in cells exposed to genotoxic stressors and for the incorporation of H2A.Z with chromatin by SWR1-Com. In addition, the structure of the YEATS domain was highly similar to the structure of the conserved histone deposition protein Asf1. Genetic data suggested that Yaf9 and Asf1 share a common function important to cell replication.

Poster No. IV-3
ACTIVE MOTIF: INNOVATIVE (HIGH-QUALITY) TOOLS FOR EPIGENETIC RESEARCH
Sanz A, Meents D, Calomme C, Heyman J & Scott D; Research & Development, Active Motif Inc., USA

Epigenetics refers to the study of heritable changes in phenotypes that have no underlying changes in genotype. The control of epigenetic modifications and their downstream effects on gene expression operates at all levels of the cellular machinery. Stimuli are converted into signaling pathways leading to the nucleus, where they influence the enzymes that modify chromatin structure. DNA methylation, histone variants and post-translational modifications, nucleosome

positioning factors, boundary setting elements, and chromatin loop and domain organizer complexes are all elements of the epigenome.
Alterations in methylation, imprinting and chromatin are ubiquitous in cancer. A clear link between epigenetic changes and cancer, as well as other diseases, has in many case been established. New technologies are then needed to study some questions of higher order chromatin organization and function.
Active Motif has recently developed a wide range of molecular biologic techniques to further our understanding of epigenetic phenomena, including a rapid chromatin immunoprecipitation (ChIP) magnetic procedure, a simplified method for assembling chromatin onto DNA, sensitive tools to analyze DNA methylation from limited amounts of cell or tissue samples, and an exclusive histone purification kit. Moreover, Active Motif offers a broad line of mono- and polyclonal antibodies directed against a variety of histone modifications (acetylated, methylated, & phosphorylated) and transcription factors, many of which have been ChIP-validated.

Poster No. IV-4
THE HISTONE CHAPERONE VPS75 FUNCTIONS INDEPENDENTLY OF RTT109 IN TRANSCRIPTION
Selth LA[1], Lorch Y[2], Kornberg RD[2] & Svejstrup JQ[1]; [1] Cancer Research UK, London Research Institute, United Kingdom; [2]Department of Structural Biology, Stanford University School of Medicine, USA

Acetylation of histone H3 at lysine 56 (H3K56) occurs on newly synthesized DNA and is important for genome integrity. It is catalyzed by Rtt109, a recently-identified HAT that requires one of two histone chaperones, Asf1 and Vps75, for efficient activity. Despite appearing to be a more potent stimulator of Rtt109-mediated acetylation, deletion of *VPS75* does not significantly affect bulk H3K56 acetylation *in vivo* and does not increase sensitivity of yeast to genotoxic agents. These data suggest that Vps75 may have additional cellular roles outside of H3K56 acetylation. Here, we present biochemical, genomic and genetic evidence to indicate that Vps75 functions in histone exchange during RNA polymerase II transcription, and that this activity is independent of Rtt109.

Poster No. IV-5
TRANSCRIPTIONAL PROOFREADING AND REACTIVATION OF STALLED RNA POLYMERASE II
Sigurdsson S & Svejstrup JQ; Cancer Research UK, London Research Institute, United Kingdom

Pausing or arrest of RNA polymerase II (RNAPII) complexes occurs frequently during transcription in response to various obstacles such as nucleosomes, DNA damage and intrinsic secondary structures in the DNA. When RNAPII encounters obstacles during transcription it backtracks on the DNA template, displacing the RNA 3' end from the active site of RNAPII. For continued transcription the RNAPII has to realign its active center with the 3' end of the nascent RNA molecule. The transcription elongation factor TFIIS stimulates transcription by activating RNAPII from the backtracked state. TFIIS binds to the surface of RNAPII and extends into a pore on RNAPII. Highly conserved amino acids in an acidic hairpin of TFIIS stabilize the binding of a second Mg^{2+} ion, which is important for stimulating the intrinsic nuclease activity of RNAPII.

Subsequently, the active center of RNAPII and the RNA 3' end are aligned by endonucleolytic cleavage of the RNA.
Misincorporation during transcription is believed to be relatively common due to low selectivity of RNAPII. Similarly to its function during transcription elongation, TFIIS facilitates the removal of misincorporated nucleotides by stimulating the nuclease activity of RNAPII and is therefore important in transcriptional proofreading. Interestingly, cells lacking TFIIS are viable, suggesting that self-cleavage by RNAPII itself might be sufficient in most situations.
My studies are aimed at studying the transcription elongation factor TFIIS, in particular its role in reactivating paused RNAPII and proofreading during transcription. I will present preliminary data suggesting that the intrinsic proofreading and reactivation of RNAPII is very important for cell survival, an important idea that has been overlooked previously.

Poster No. IV-6

IDENTIFICATION AND CHARACTERIZATION OF NOVEL HDAC1-ASSOCIATED PROTEINS

Smith KT, Martin-Brown SA, Florens L, Washburn MP & Workman JL; Stowers Institute, USA

There are several zinc-dependent histone deacetylases in humans, which share a structurally similar catalytic domain. Inhibitors to these enzymes are being tested as anti-cancer drugs. However, these inhibitors target the conserved catalytic domain of the HDACs and do not discriminate among these distinct proteins. Individual HDACs reside in multi-subunit protein complexes, and a few known examples show that some of these HDAC-associated proteins are associated with and required for the catalytic activity of a particular HDAC. We propose that a single HDAC can be inhibited in the cell by targeting HDAC-associated proteins rather than the HDACs themselves. We have taken a proteomics approach to identify proteins differentially associated with HDAC1 and HDAC3 in human embryonic kidney 293T cells. Through this analysis, we have identified several interesting candidate proteins and have begun to follow up potential new HDAC1-interacting proteins. Interestingly, we have found one of these proteins to be a novel component of at least two distinct Sin3A histone deacetylase complexes. Currently, we are working to determine its function in these complexes using biochemical analyses. We are also testing the conservation of these interactions in cancer cell lines and determining if this HDAC-interacting protein has a role in cell growth.

Poster No. IV-7

DNA METHYLATION PROFILING IN MESENCHYMAL STEM CELLS FROM ADIPOSE, BONE MARROW AND MUSCLE TISSUES

Sørensen AL, Vekterud K & Collas P; Institute of Basic Medical Sciences, University of Oslo, Norway

Adipose tissue stem cells (ASCs) can differentiate into multiple cell types; yet their capacity to differentiate into non-adipogenic mesodermal pathways is restricted. We show here that there is an epigenetic basis for this restricted differentiation capacity. DNA methylation was determined on lineage-specific promoters by bisulfite sequencing and genome-wide by methyl DNA immunoprecipititation (MeDIP) and promoter array hybridization in mesenchymal stem cells (MSCs) of various tissues.

Bisulfite sequencing shows that the adipogenic *FABP4* and *PPARG2* promoters are differentially methylated in ASCs and bone marrow (BM)MSCs relative to Wharton's jelly (WJ) MSCs and muscle progenitor cells (MPCs). In contrast, the myogenin (*MYOG*) promoter is hypomethylated in WJMSCs and MPCs relative to ASCs and BMMSCs, while exon 1 is methylated in all cell types. In hematopoietic stem cells (HSCs), all promoters are hypermethylated. Differential methylation correlates with distinct differentiation capacities: ASCs and BMMSCs differentiate efficiently into adipocytes but not into multinucleated myogenin positive myocytes, whereas MPCs display poor adipogenic differentiation. The endothelial *CD31* promoter is heavily methylated in ASCS, BMMSCs, WJMSCs and MPCs, in agreement with their poor endothelial differentiation potential. *CD31* is however unmethylated in HSCs, in which its expression can be induced. Methylation patterns in adipocytes, muscle and endothelial cells argue that ASCs (and BMMSCs) are epigenetically pre-programmed for adipogenesis, while MPCs have a methylation pattern predictive of myogenic potential. Bisulfite sequencing corroborates genome-wide methylation profiling. MeDIP reveals similarity in methylation profiles between MSCs from adipose tissue, bone marrow and muscle, reflecting the mesodermal origin of these cells. Data on similarities and differences in methylation profiles between these cell types, in relation to lineage commitment and gene expression, will be presented. Collectively, our results put forward the working hypothesis that DNA methylation patterns on lineage-specific promoters may be indicators of lineage differentiation capacity.

Poster No. IV-8

ROLE OF SET8 IN CELL CYCLE PROGRESSION AND THE DNA DAMAGE RESPONSE

Jørgensen S[1], Elvers I[3], Trelle MB[4], Menzel T[1], Eskildsen M[1], Jensen ON[4], Helleday T[3,5], Helin K[1,2] & Sørensen CS[1]; [1]Biotech Research and Innovation Centre (BRIC) and [2]Centre for Epigenetics, University of Copenhagen, Denmark; [3]Department of Genetics, Microbiology, and Toxicology, Stockholm University, Sweden; [4]Centre for Epigenetics, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Denmark; [5]Radiation Oncology and Biology, University of Oxford, United Kingdom

Chromatin structure and function is influenced by histone posttranslational modifications. SET8 (also known as PR-Set7 and SETD8) is a histone methyltransferase that catalyzes the monomethylation of histone H4-K20. However, a function for SET8 in mammalian cell proliferation has not been determined. We show that siRNA inhibition of SET8 expression leads to decreased cell proliferation and accumulation of cells in S phase. This is accompanied by DNA double-strand break (DSB) induction and recruitment of the DNA repair proteins such as replication protein A, RAD51, and 53BP1 to damaged regions. SET8 depletion causes DNA damage specifically during replication, which induces a CHK1-mediated S-phase checkpoint. Furthermore, we find that SET8 interacts with proliferating cell nuclear antigen through a conserved motif, and SET8 is required for DNA replication fork progression. We also show that SET8 is required to control homologous recombination repair, a key DNA repair pathway for cells in S-phase. Co-depletion of RAD51, an important homologous recombination repair protein, abrogates the DNA damage after SET8 depletion. Current and future studies aim at investigating SET8 function following radiation DNA damage as well as replication stress brought about by treatments with either UV or X-ray irradiation (IR), hydroxyurea, and aphidicolin.

In conclusion, our findings reveal a novel critical role of SET8 to promote faithful DNA replication and DNA repair.

Poster No. IV-9

ROLE OF PAD4 IN ESTROGEN-REGULATED TRANSCRIPTION AND EPITHELIAL-TO-MESENCHYMAL TRANSITION

Stadler SC[1], Wang Y[2], Cherrington BD[3], Zhang X[3], Allis CD[1] & Coonrod SA[3]; [1]Laboratory of Chromatin Biology, The Rockefeller University, USA; [2]Department of Biochemistry and Molecular Biology, Pennsylvania State University, USA; [3]Baker Institute for Animal Health, Cornell University, USA

Induction of transcription requires an ordered recruitment of coregulators and specific histone modifications at the promoter. The methylation of histone H4 arginine 3 (Arg3) by protein arginine methyltransferase I (PRMT1) is the first mark to occur in estrogen-receptor (ER) regulated gene activation and is essential for subsequent modifications. However, the mechanism by which arginine methylation marks are removed during down-regulation is poorly understood.
We have recently found that the peptidylarginine deiminase PAD4 also targets histone methylarginine residues for citrullination and have been testing the hypothesis that PAD4 may function as an ER transcriptional regulator via citrullination of either histone arginine or methylarginine residues at ER target genes. In support of this prediction, we have found that in MCF7 breast cancer cells, conversion of histone H4MeArg3 to H4Cit3 on the estrogen-responsive pS2 gene correlates with recruitment of PAD4 to the promoter. Further, we found that knockdown of PAD4 in MCF7 cells results in a down-regulation of several ER target genes. Interestingly, we observed that the phenotype of PAD4-depleted cells is distinct from wild type MCF7 cells. PAD4-depleted cells display a more fibroblast-like mesenchymal phenotype and show a reduction in levels of epithelial markers such as E-cadherin and beta-catenin and an increase in levels of mesenchymal markers such as vimentin. We also found that PAD4-depleted MCF7 cells show increased cell motility and invasiveness. These findings correlated with distinct changes in gene expression patterns in microarray analysis. Our results suggest that PAD4 may function as an ER co-regulator in breast cancer cells and might play a direct role in tumor suppression by blocking the epithelial-to-mesenchymal transition, the first step of invasion and metastasis.

Poster No. IV-10

P38 MAPK SIGNALING REGULATES POLYCOMB (PCG) ACTIVITY DURING MUSCLE DIFFERENTIATION

Stojic L[1], Prezioso C[1], Jasencakova Z[2] & Orlando V[1]; [1]Dulbecco Telethon Institute, Institute of Genetics and Biophysics, Italy; [2]Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Denmark

The Polycomb group (PcG) and trithorax group (trxG) genes are part of widely conserved cell memory system that prevents changes in cell identity by maintaining transcription patterns, throughout development and in adulthood. The balanced action of PcG and trxG genes controls the expression of developmentally and cell cycle regulated genes. Polycomb proteins form repressive complexes (PRCs) that function in the initiation and maintenance of gene silencing by catalyzing histone H3 methylation (H3K27) at target loci. EZH2, a member of PRC2 complex, was shown to

regulate muscle gene expression acting as a classical PcG repressor in muscle progenitor cells. To date, several aspects of PcG function in muscle differentiation are still unknown, such as the role of SUZ12 another member of PRC2 complex required for EZH2 histone methyltransferase activity, and cellular signals that remove PRC2 complex from muscle genes promoting muscle gene expression and cell differentiation.
We observed that Suz12 is recruited to the promoter of several muscle-specific genes. Notably, while some of these genes are up-regulated after depletion of SUZ12, in keeping with a repressive function of this PcG protein, others are down-regulated under the same experimental conditions. This suggests the role of SUZ12, in both transcriptional repression and activation. Moreover, we found that the differentiation-activated p38 pathway is involved in displacement of PRC2 complex from its target genes in muscle progenitor cells by regulating the PRC2 activity.

Poster No. IV-11

MEC1-DEPENDENT PHOSPHORYLATION OF RAD26P INCREASES THE EFFICIENCY OF NUCLEOTIDE EXCISION REPAIR

Taschner MJ[1], Teng Y[2], Waters R[2] & Svejstrup JQ[1]; [1] Cancer Research UK, London Research Institute, United Kingdom; [2]Department of Pathology, Cardiff University, United Kingdom

The nucleotide excision repair (NER) pathway deals with a variety of helix-distorting DNA lesions, including UV-induced Cyclopurine-pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4 PPs). It is divided into two subpathways, transcription-coupled repair (TCR) which leads to fast repair of the transcribed strand of genes, and global genome repair (GGR) which repairs the rest of the genome at a slower rate. While the overall mechanism of NER is well characterized and has been reconstituted using purified components, the exact mechanism of the fast TCR pathway remains elusive. We have characterized a Mec1-dependent phosphorylation of the TCR factor Rad26, identified the phosphorylated amino acid residues and created point mutants of the Rad26 phosphorylation sites. Even though genetic experiments indicate that Rad26 phosphorylation is not absolutely required for TCR to occur, we show using a more sensitive NER assay that Rad26 phosphorylation increases the efficiency of damage removal in the transcribed strand of a gene and possibly also affects repair of the non-transcribed strand. The biochemical basis for this increased efficiency as well as a possible conservation of this mechanism in human cells is currently being investigated.

Poster No. IV-12

DOMINANT AND REDUNDANT FUNCTIONS OF TFIID IN THE REGULATION OF HEPATIC GENES

Tatarakis A[1], Margaritis T[2], Martinez-Jimenez CP[1], Kouskouti A[1], Mohan II WS[3], Haroniti A[1], Kafetzopoulos D[2], Tora L[3] & Talianidis I[1]; [1]Biomedical Sciences Research Center Al. Fleming, Greece; [2]Institute of Molecular Biology & Biotechnology, FORTH, Greece; [3]Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS UMR7104, France

To study the in vivo role of TFIID in the transcriptional regulation of hepatic genes we generated mice with liver-specific disruption of *TAF10* gene. Inactivation of TAF10 in hepatocytes resulted in the dissociation of TFIID into individual components. This

correlated with the down regulation of most hepatocyte-specific genes during embryonic life and a defect in liver organogenesis. Unexpectedly however, the transcription of less than 5% of active genes was affected by TAF10 inactivation and TFIID disassembly in adult liver. The extent of changes in transcription of the affected genes was dependent on the timing of their activation during liver development, relative to that of TAF10 inactivation. Furthermore, TFIID dissociation from promoters leads to the re-expression of several postnatally silenced hepatic genes. Promoter occupancy analyses, combined with expression profiling demonstrate that TFIID is required for the initial activation or postnatal repression of genes, while it is dispensable for maintaining ongoing transcription.

Poster No. IV-13

MOLECULAR INSIGHTS INTO AP1 TRANSCRIPTIONAL ACTIVITY REPRESSION UPON SUMOYLATION

Tempé D; Institute of Molecular Genetics of Montpellier, CNRS, France

The AP1 transcription factor family is implicated in a variety of cellular transcriptional responses to stimuli such as mitogens and stresses. Its deregulation is also associated with various pathologies, in particular cancer. Therefore, AP1 activity is tightly regulated at both the transcriptional and post-translational levels. In particular, we have shown that c-Fos and c-Jun, the best-characterized components of this heterodimeric complex, are both subjected to SUMOylation on specific lysines. This modification leads to a repression of their transcriptional potency in reporter gene assays. Moreover, the activating phosphorylation of c-Fos on Threonine 232 inhibits its SUMOylation.
In line with our results, several recent studies have involved SUMOylation of transcription factors as a repressive post-translational modification. As only few direct molecular evidence have been proposed to explain these observations, our current study aims to uncover the molecular mechanisms responsible for SUMO-mediated c-Fos transcriptional repression. In particular, we are focusing our work on chromatin modifications and transcriptional complexes remodeling at the level of AP1 endogenous target promoters. One strategy involves a biochemical approach using an *in vitro* SUMOylated recombinant c-Fos/c-Jun complex, in order to screen for binding partners specific of this post-translational modification, expected to mediate its repressive function. Complementarily, we developing a polyclonal antibody raised against the c-Fos/SUMO conjugation site, which will be used in a cellular model using ChIP-based assays.

Poster No. IV-14

STRUCTURE DETERMINATION OF OCT-4, A KEY REGULATOR IN EMBRYONIC STEM CELL DIFFERENTIATION

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Embryonic stem cells are derived from the inner cell mass of the blastocyst, and are able to differentiate indefinitely in vitro, to form all three germ cell layers (pluripotency). These properties make stem cells extremely valuable for novel therapies in regenerative medicine. Stem cells therapies are anticipated to help in many serious or terminal disorders commonly treated in western countries, such as Parkinson’s disease, diabetes, and cardio vascular diseases.

Four transcription factors (Oct-4, Sox2, c-Myc, Klf4) are required to retain stem cell pluripotency in mice, permitting normal embryonic development. In vitro experiments have demonstrated, that embryonic fibroblasts are "reprogrammable" to a stem cell-like state using these four factors. Amongst these four factors Oct-4 is known to be one of the key regulators of self-renewal and pluripotency. Unlike the other factors, Oct-4 has shown to be indispensable. To date, studies have been focused on *in vivo* analysis, yielding valuable data on the function of Oct-4 in living cells. However, the structural properties and biochemical mechanisms, that regulate repression and induction of a particular set of genes needed for differentiation, are largely unknown.
In order to elucidate the structural properties, that allow a wide variety of DNA motifs to be recognized, we have crystallized the Oct-4 DNA binding domain in complex with the PORE binding motif. Crystals are produced in reproducible manner and the structure has been solved with high resolution limits up to 2.8 Å. This complex gives a new insight of Oct-4 DNA binding properties and helps to interpret its unique properties in development.

Poster No. IV-15
TRANSCRIPTIONAL NETWORK REGULATED BY P63
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p63 is a transcription factor required for the development and maintenance of ectodermal tissues, including skin, limb and, in general, multilayered epithelia. The identification of its target genes is fundamental for understanding the complex network of gene regulation governing the development of these epithelia. Our lab employed two strategies for this task: expression profiling of si RNA p63 depleted human keratinocytes (HaCat cells) (1) and ChIP on chIP analysis on two different platforms (CpG island and promoter arrays) with specific p63 antibodies in HaCat cells (2). The analysis of the chip-chip data with different criteria revealed a list of almost 1000 putative targets Independent chIP validations confirmed that the majority of these new targets are bound by p63 in vivo and a subset of these genes are changing in expression during in vitro differentiation of primary human keratinocytes. Functional classification revealed some unexpected GO categories enrichments which are at the moment under selective validation. Moreover, a deeper analysis has been performed on several transcription factors identified as regulated by p63, such as C/EBPd (3) and HBP1. A more comprehensive regulatory network is now emerging with p63 situated in the center of the multilayered epithelia developmental cascade and tightly interplaying with its own targets.

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Poster No. IV-16
IDENTIFICATION OF A SWI/SNF-LIKE HELICASE AS A NOVEL COFACTOR FOR E2F-MEDIATED TRANSCRIPTION
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E2F transcription factors are key regulators of the metazoan cell cycle and their action is tightly regulated since aberrant E2F activity is a key feature of human tumors. During the last decades the actions of E2Fs have been elucidated in great detail by many *in vitro* and *in vivo* approaches. Nevertheless, the exact chromatin-based mechanisms of E2F-mediated transcription have only started to being addressed.
To identify novel E2F-interacting proteins, which might exert a function on chromatin, we used a GST pulldown approach coupled to analysis of bound proteins by mass spectrometry. By this approach, we were able to identify about 40 proteins bound by the GST-E2F3b fusion protein.
One of the identified proteins is a SWI/SNF-like helicase.
This interaction is confirmed by independent GST pulldowns and Co-IP assays. In these assays, the SWI/SNF-like helicase preferentially interacts with the splice variant E2F3b. More importantly, we were able to show by ChIPs that this helicase associates with direct E2F target genes, such as *MCM4, PCNA* or *CCNB1*. Similar to the *E2f3* null phenotype, we were able to severely reduce cell proliferation and G1/S transition of synchronized NIH 3T3 cells by depletion of this helicase using retroviral shRNAs. In addition, overexpression of a helicase point mutant with an abolished ATPase activity is able to nearly completely block the activation of E2F target genes after serum induction. This indicates that this mutant acts in a dominant-negative fashion and that ATP-dependent chromatin remodeling by this helicase is essential for proper activation of E2F target genes.
Currently, we are investigating which biochemical function this helicase performs on E2F-regulated promoters and whether this activity plays a role in oncogenic transformation of cells. Ultimately, we wish to clarify if this novel cofactor is required for E2F3-mediated growth in human tumors.

Poster No. IV-17

THE ELONGATOR SUBUNIT ELP3 IS IMPORTANT FOR ECDYSONE-INDUCED TRANSCRIPTION AND LARVAL DEVELOPMENT IN DROSOPHILA

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The Elongator complex has been implicated in both transcription and tRNA modification, yet *Saccharomyces cerevisiae* cells lacking an *ELP* gene are viable and only display relatively mild growth phenotypes. We investigated the biological importance of the *ELP3* gene in a multi-cellular organism, *Drosophila melanogaster*. Flies that lack Elp3 protein or over-express an Elp3 variant carrying a point mutation in the histone acetyltransferase domain are inviable. During early development, larval growth is dramatically impaired, with progression to the 3rd instar delayed around 24 hours, and pupariation only occurring at day 14 after egg laying. Consistent with this, expression of ecdysone-induced genes is severely reduced and delayed in *Elp3* mutant larvae. Growth of larval discs and salivary glands is severely impaired, and melanotic tumors occur in *Elp3* mutant larvae from day 4 onwards and reach a penetrance of ~90%. Molecular characterization of tumors and genetic interaction studies suggest that Elp3 acts in a tumor-promoting pathway independently of the Toll- and the JAK/STAT pathways. Together, these data demonstrate that *Drosophila* Elp3 is essential for viability, normal development, and melanotic tumor suppression in fruit flies.

Poster No. IV-18

THE C-TERMINUS OF H2A.Z IS IMPORTANT FOR ITS DEPOSITION AND FUNCTION

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The eukaryotic genome is packaged into chromatin, which is dynamic and responsible for epigenetic inheritance. Chromatin consists of four abundant canonical histones and also minor histone variants that can replace the canonical histones in specific neighborhoods of the genome and thereby profoundly regulate chromatin function. H2A.Z, or Htz1 in *Saccharomyces cerevisiae*, is a H2A variant that is conserved from yeast to humans. H2A.Z has roles in transcription repression and activation, chromosome segregation, DNA replication, repair and boundary formation for heterochromatin.

It has been previously found that one of the key differences between H2A and its variant H2A.Z is the higher affinity of the SWR1 complex, an ATP-dependent chromatin remodeling complex, for H2A.Z. The SWR1-COM is dedicated to H2A.Z deposition into euchromatin. This is in contrast to the equal affinity of the Kap114 and Nap1 proteins, which function as nuclear import and histone chaperone, respectively.

To understand the roles of SWR1-Com, Kap114, and Nap1 in H2A.Z chromatin deposition in detail, H2A.Z was truncated at the C-terminus. The H2A.Z mutants were tested for binding to the SWR1-Com, Kap114 and Nap1, its chromatin deposition, drug sensitivity, and chromosome loss. The SWR1-Com and Kap114 binding sites in H2A.Z was localized to the proposed docking domain at the C-terminus of the histone variant. The C-terminal mutants exhibited differential sensitivity to drugs and varying degrees of H2A.Z deposition in chromatin. These findings suggest that the C-terminus of H2A.Z is important in regulating its deposition and function.

Poster No. IV-19

POSTTRANSLATIONAL MODIFICATIONS OF METHYL-DNA-BINDING PROTEIN KAISO

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DNA methylation leads to heritable and stable gene repression, mediated in part by methyl-DNA binding proteins. These proteins recruit co-repressors to the chromatin and modify it. Methylation of CpG has well known developmental role and also it is important for somatic cells – in health (imprinting, maintenance of X inactivation, silencing of "junk" DNA) and disease (silencing of tumor suppressor genes). As it was shown early for one of the methyl-DNA-binding protein MBD1, the transcriptional repression by this protein is regulated by sumoylation. In addition, there are many examples about phosphorylation and sumoylation, and they influence on the repression properties of various transcription factors.

Kaiso is methyl-DNA binding protein that contains BTB/POZ domain. Kaiso uses three zinc finger motif to bind methylated CGCGs, also it can bind consensus Kaiso Binding Site (KBS) TCCTGCNA.

Here we show that Kaiso is phosphorylated and sumoylated. Analyses of deletion mutants of Kaiso have shown that these modifications lie within BTB/POZ domain. Using mutation analyses, we detected one phosphorylation site and one sumoylation site in BTB/POZ domain. It must be mentioned that these two modifications are

independent. Mutation of sumoylation site did not influence on phosphorylation and vice versa. Moreover using cross-linking technique we observed that BTB domain of Kaiso can dimerize. The BTB domain with mutation in phosphorylation and sumoylation site also can form the dimer. BTB domain is responsible for the repression properties of protein. Therefore, we suspect that these modifications may influence directly on the pattern of co-repressors that interact with Kaiso.

Poster No. IV-20

HAIR FOLLICLE REGENERATION *DE NOVO* IN ADULT KAISO-DEFICIENT MICE SKIN AFTER WOUNDING

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The mammalian hair follicle is 'mini-organ' that undergoes cyclic regeneration throughout adult life, and is an important model for organ regeneration. Classical works have documented that wounding induces an embryonic phenotype in adult mouse skin, involving periodic Wnt/β-catenin cycle.

Methyl-DNA-binding protein Kaiso is a member of BTB/POZ family proteins. It contains two functional domains: N – terminal BTB/POZ domain and C-terminal zinc-finger (ZF) domain of C_2H_2 type. Kaiso is a bifunctional transcriptional repressor protein that binds a core sequence (CTGCNA) and the methylated DNA (mCGmCG) via ZF domain. Promoter regions of Wnt target genes, *Siamois* and *Wnt11*, contain CTGCNA – Kaiso binding site. Till now we do not know the role of Kaiso in Wnt signaling pathway: either Kaiso acts as inhibitor non-canonical Wnt pathway via direct binding with *Wnt11* or it acts as specific regulator of the canonical Wnt pathway via direct protein-protein interactions with TCF/LEF factors.

The aim of our work is to determine the rate of regeneration and regulation of Wnt expression in adult Kaiso-deficient mice skin against C57BL6 mice skin after wounding. Using mouse wound healing model, a 0.63 cm^2 dorsal wound we observed that Kaiso-deficient mice develop alopecia before 14 day (area in wound and around of wound) in comparison with wild-type mice. We detected by RT-PCR decreasing the expression of particular genes, such as *KRT17* (cytokeratin 17), *Lef1*, *BMP4* in Kaiso-deficient mice. It is interesting that expression of *Wnt11* and *Wnt4* was significantly increased in Kaiso-deficient mice in comparison with wild-type mice. We suggest that Kaiso inhibits canonical Wnt signaling pathway via protein-protein interactions with factors that are not involved in Wnt pathway.