EPIGENETIC REGULATION OF TRANSCRIPTION

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Nucleosome-Factor Interactions in vitro

J8-001 THE ANTIREPRESSION ROLE OF GENERAL CLASS III TRANSCRIPTION FACTORS, Marie-Claude Marsolier[1], Shigeo Tanaka[2], Magda Livingstone-Zatchej[2], Michael Grunstein[3], Fritz Thoma[2] and André Sentenac[1], [1]Service de Biochimie et de Génétique Moléculaire, CEA-Saclay, 91191 Gif-sur-Yvette, France, [2]Institut für Zellbiologie, Zurich, Switzerland, [3]Molecular Biology Institute, University of California, Los Angeles.

Accurate in vitro transcription of yeast U6 snRNA gene by RNA polymerase III in a purified system requires only TFIIIB components. But after nucleosome reconstitution or chromatin assembly, U6 snRNA synthesis becomes dependent on TFIIIC and on the integrity of a downstream B block element. As seen by electron microscopy, purified TFIIIC binds to the B block in an oriented manner and is able to induce DNA looping via a weak intragenic A block element. A B block-deficient U6 RNA gene harboring GAL4 binding sites (UAS_G) could be reactivated in vivo by fusing the GAL4 DNA binding domain to TFIIIC subunits. Therefore, B block recognition could be replaced by an unrelated DNA-protein interaction. UAS_G sequences were effective at various positions, including upstream of the initiation site. A precise location of the main anchoring site for TFIIIC, therefore, was not critical for antirepression and for gene activation.

Chromatin disruption, induced by histone H4 depletion, activated the in vivo transcription of promoter-deficient, but not of wild type, U6 RNA genes, revealing a competition between the formation of nucleosomes and the assembly of pol III transcription complexes which was much in favor of transcription factors. Analysis of the U6 gene locus by micrococcal nuclease digestion showed arrays of nucleosomes in the gene flanking regions, and a protection of the TATA box (the TFIIIB binding site). In contrast, a transcriptionally silent U6 gene with a defective B block showed a loss of TATA protection and a randomisation of nucleosomes in the flanking sequences. This underscored the reciprocal interferences between nucleosomal organization and transcriptional activity of the yeast U6 gene.

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J8-002 MECHANISMS MEDIATING TRANSCRIPTION FACTOR DISRUPTION OF NUCLEOSOMES, Rhea Utley, Phillip Walter, Li-Jung Juan, Michelle Vettese-Dadey, Christopher Adams, Jacques Côté, Thomas Owen-Hughes, David Steger and Jerry Workman. Department of Biochemistry and Molecular Biology and Center for Gene Regulation, The Pennsylvania State University, University Park, PA 16802.

Primary levels of chromatin structure (nucleosome arrays) are remodeled as a consequence of the function of transcription activators by mechanisms which do not require DNA replication. Multiple mechanisms facilitate transcription factor binding to nucleosomal DNA.

Transcription factor binding to nucleosomes is inherently cooperative and subject to nucleosome position effects[1,2]. Cooperative binding of GAL4-derivatives overcomes inhibition of binding from the core histone amino termini[1]. Cooperative nucleosome-binding between GAL4-derivatives, USF and NFkB in various combinations can increase the affinity of these factors by 2 orders of magnitude and thus appears to be a general mechanism which may play a significant role in the binding of multiple factors to complex regulatory elements in chromatin.

Factor binding to nucleosomes is also enhanced acetylation of the core histone amino termini. Removal these domains results in a non-cooperative mode of binding similar to that observed on naked DNA[1]. Immunoblots of factor bound and unbound nucleosome cores demonstrates enrichment of acetylated histones in the factor-bound population. Removal or acetylation of the core histone amino termini also enhances factor binding indirectly by reducing the affinity of histone H1, alleviating H1-mediated repression of factor binding at the nucleosome edge[3].

The binding of transcription factors to nucleosomes can be induced by histone-binding proteins. Nucleoplasmin stimulates nucleosome binding of GAL4, USF and SP1 by mediating the removal of H2A/H2B dimers from factor/nucleosome complexes[4]. Crosslinking of the histone octamer to prevent H2A/H2B dissociation abolishes stimulation of factor binding by nucleoplasmin.

The purified yeast SWI/SNF protein complex stimulates factor binding to nucleosomal DNA in a reaction requiring ATP hydrolysis[5]. The stimulatory activity of SWI/SNF is augmented by small concentrations of nucleoplasmin, which also appears to facilitate release of SWI/SNF-nucleosome interactions, and is reduced by crosslinking the histone octamer. These data are consistent with a model whereby SWI/SNF interacts with transcription factors and nucleosomal DNA utilizing the energy of ATP hydrolysis to disrupt the underlying nucleosome

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Chromatin Repression of Gene Function

J8-003 POSITION-EFFECT VARIEGATION: *CIS*-ACTING DETERMINANTS OF THE INACTIVE STATE, Steven Henikoff[1], Douglas R. Dorer[1], Joy F. Sabl[2], Jeffrey M. Jackson[1], and Paul B. Talbert[2], [1]Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, [2]Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle.

In *Drosophila,* chromosome rearrangements that juxtapose euchromatin and heterochromatin cause mosaic inactivation of neighboring genes, a phenomenon known as position-effect variegation (PEV). We have used PEV to explore *cis*-acting requirements for heterochromatin formation with the aid of a mini-*white* eye-pigment reporter gene. Variegated expression of mini-*white* occurs when it is present in repeat arrays. Variegation is particularly strong for repeated transposons at a euchromatic site near heterochromatin, but also results from repeats at a site distant from heterochromatin. Inactivation strengthens with increasing copy number. The variegated phenotypes are subject to the effects of genetic modifiers of PEV, including the Y chromosome dosage and specific *Su(var)* mutations. We argue that the repeated arrangement of these transposons induces formation of heterochromatin, and that pairing of repeats in general causes constitutive heterochromatin to condense. Our model can account for diverse gene silencing phenomena, such as those associated with transgene arrays and trinucleotide repeat expansions. In parallel work on the *brown* eye-pigment gene, we have found that classical PEV on a *brown* transgene is strengthened when *brown* is locally duplicated. Successive rounds of P transposase mutagenesis and phenotypic selection produced a series of PEV alleles with differences in phenotype that depended on copy number and orientation. These modifications of classical PEV by alterations at a single site are unexpected, and contradict current models for spreading of heterochromatin along the chromosome. Rather, local alterations affect the affinity of a gene region for nearby heterochromatin; we suggest that repeats pair with each other and "loop" into the heterochromatic chromocenter of the nucleus. Looping on a larger scale can account for the behavior of $brown^{Dominant}$, a heterochromatic insertion into *brown* that causes PEV. When $brown^{Dominant}$ is moved distally by breaks very distant from the insertion itself, PEV weakens, whereas movement proximally strengthens PEV. Thus the ability of a block of heterochromatin to inactivate a gene depends on how close it is to the bulk of heterochromatin in the cell. PEV might then be seen as a nuclear mislocalization phenomenon, with affected genes mislocalized into the condensed chromocenter formed by coalesced repeats, which are derived from the pericentric regions of all the chromosomes.

J8-004 A REPRESSIVE CHROMATIN DOMAIN CLOTHES THE *STE6* GENE IN α-CELLS. Robert T. Simpson*, Sharon Y. Roth, Julia P. Cooper, Hugh-George Patterton, and Yuko Tsukagoshi, Laboratory of Cellular and Developmental Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892. *Current address: Department of Biochemistry and Molecular Biology, 308 Althouse Lab, The Pennsylvania State University, University Park, PA 16802.

The **a**-cell specific *STE6* gene is repressed in *S. cerevisiae* α-cells. We have previously reported that a repressive chromatin structure exists adjacent to the α2 operator with a positioned nucleosome covering the TATA box of the *STE6* gene. An absolute concordance of complete repression of the gene and presence of the positioned nucleosome was demonstrated in various yeast mating type strains and strains expressing amino-terminal mutants of histone H4. Others reported that *SSN6* and *TUP1* are required for repression of **a**-cell specific genes. We disrupted these genes and found a concordant disruption of chromatin structure for the *STE6* gene; the altered chromatin structure occured even when the *STE6* TATA box was mutated and transcription reduced to less than 5% of wild type **a**-cell levels. Moving the TATA box into a short linker did not remove repression, suggesting that local chromatin structure might be sufficient but not necessary for repression. A striking finding in the initial investigations of the organized chromatin structure was an apparent propagation of the structure into the *STE6* gene. We have mapped chromatin for ~800 bp adjacent to the α2 operator at high resolution and, at lower resolution, for the entire *STE6* gene. An unique structure apparently is present for the entire structural gene. Close packed, dimeric nucleosomes are separated by long linkers which are half the length of one turn of DNA around the histone octamer. The organized structure begins 15 bp from the edge of the α2 operator and terminates near the 3' end of the gene, spanning over 4 kbp. Studies designed to assess the role of a consensus ARS sequence at the 3' end of the domain and its possible interactions with the Origin Recognition Complex (both required for repression at the silent mating type loci of yeast) will be presented. We are extending this work to examine the possibility of a similar chromatin organization for another yeast gene, *BAR1*, which is also repressed in α-cells. The structural implications of the [close-packed dimer nucleosome-long linker]$_n$ organization will be discussed in the context of possible interactions with Ssn6p and Tup1p, which possess motifs implicated in assembly of large protein complexes. We emphasize the need to postulate and define possible chromatin structures which might interact with other cellular components to build reasonable models for chromatin involved in silencing, locus control, heterochromatin, and conditional repression.

Replication on the Nuclear Matrix and Nucleosome Assembly (Joint)

J8-005 *IN VITRO* RECONSTITUTION OF *DROSOPHILA* HSP26 PROMOTER STRUCTURE AND FUNCTION IN CHROMATIN.
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Transcription of the *Drosophila* hsp26 gene is rapidly induced by activated Heat Shock Factor (HSF) in response to a variety of stresses. The basis for this quick response is its "preset" configuration in chromatin (Wallrath et al., *BioEssays* **16**, 165, 1994). Salient features are two accessible, DNase I-hypersensitive regions providing accessible binding sites for transcription factors, a positioned nucleosome between proximal and distal regulatory elements contributing to the promoter architecture (Thomas and Elgin, *EMBO J.* **7**, 2191, 1988) and a polymerase II molecule arrested in chromatin after having transcribed a short RNA. To study the functional consequences of specific chromatin structures we reconstituted the hsp26 promoter into chromatin*in vitro*. Chromatin reconstituted with physiological nuclesome spacing in a cell-free system derived from fly embryos (Becker and Wu, *MCB* **12**, 2241, 1992) displays highly dynamic properties due to an activity that utilizes energy to render entire arrays of nucleosomes mobile. Transcription factors, such as the GAGA factor (GA-F) and HSF, make use of this inherent flexibility to associate with chromatin and influence nucleosomal positions. Using the fly embryos extracts and a strategy for chromatin assembly and transcription on immobilized DNA (Sandaltzopoulos et al., *EMBO J.* **13**, 373, 1994) we have reconstituted prominent structural features of the hsp26 promoter, such as DNaseI-hypersensitivity, transcription factor accessibility and the positioning of a nucleosome between proximal and distal transcription factor binding sites. Transcription from the hsp26 promoter in chromatin requires the assembly of a preinitiation complex prior to nucleosome reconstitution. The presence of binding sites for GA-F are required for the stable persistence of this complex during the six hours of chromatin reconstitution. Efficient transcription of this reconstituted chromatin template nevertheless depends on activated HSF. The reconstitution of hsp26 promoter structure and function *in vitro* opens avenues for the analysis of the structural requirements for proper regulation of hsp26 gene transcription.

J8-006 **REPLICATING THE MAMMALIAN GENOME IN 3-D,** Ronald Berezney, Department of Biological Sciences, State University of New York, Buffalo, NY 14260.

We are using laser scanning confocal microscopy (LSCM) in conjunction with multi-dimensional computer image analysis (MDA) to study the three dimensional arrangement of DNA replication sites (RS) inside the cell nucleus of mammalian cells grown in culture. Appropriate pulse and pulse-chase labeling experiments will be described for studying the spatio-temporal arrangement of RS throughout the S-phase and in subsequent cell generations. MDA enables us to determine the x,y,z coordinates and volume contours for each individual replication site within the 3-D nuclear structure. Rotational analysis contributes additional valuable information about individual as well as neighboring RS. Recent progress will be presented for delineating the three-dimensional organization of groups of individual RS into neighboring domains.

Numerous studies have suggested that each RS is composed of multiple replicons. We are attempting to directly address this question by combining LSCM-MDA with FISH (fluorescence in situ hybridization). In this manner we hope to map DNA sequences (genes) at RS defined by specific x,y,z, coordinates. Initial results will be presented involving the replication of specific chromosomal domains and gene sequences.

J8-007 **POSSIBLE INVOLVEMENT OF THE NUCLEAR MATRIX IN REGULATING INITIATION OF REPLICATION IN THE DIHYDROFOLATE REDUCTASE DOMAIN IN CHO CELLS.** Joyce L. Hamlin, Pieter A. Dijkwel, Victor V. Levenson, Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, VA 22908.

Several years ago, we utilized an *in vivo* labelling protocol to roughly localize the origin of replication in the amplified dihydrofolate reductase domain (amplicon) of a methotrexate-resistant CHO cell line. Interestingly, this initiation zone lies in the spacer region between two convergently transcribed genes, both of which are active in the early S period. The intergenic region contains a matrix attachment site (MAR) in its center, but only about 10-15% of the DHFR amplicons actually appear to be attached to the matrix at this site. To gain a higher resolution picture of the initiation reaction, we have analyzed this locus by a two-dimensional (2-D) gel electrophoretic mapping technique. We found that initiation can occur at any of a large number of sites scattered throughout the 55 kb intergenic region, but slightly more often near two sites within this zone termed ori-β and ori-γ. However, initiation occurs in only 10-15% of the DHFR amplicons in any one cell cycle, with the remainder being replicated passively. These findings suggest: 1) that ori-β and ori-γ may correspond to classic *cis*-acting replicator elements that interact with initiator proteins; and 2) that transcription toward the MAR in the early S period may generate superhelical stress that destabilizes the entire intergenic region beginning at ori-β and ori-β; this then allows nascent strands to initiate at random locations within the intergenic zone. This model predicts that initiation can only occur in those copies of the amplicon in which transcription occurs and in which the MAR is attached to the matrix. Support for this model was obtained in experiments in which the replication pattern in a CHO cell line that contains a single, promoter-less DHFR gene was analyzed by the 2-D gel technique: initiation in the downstream origin no longer occurs, suggesting that initiation is obligately coupled to transcription in this replicon. Moreover, when the chromatin in the intergenic region was examined for the presence of micrococcal nuclease hypersensitive sites, a complex pattern was uncovered directly over ori-β and ori-γ, but only when analysis was restricted to that portion of the genome that partitions with the nuclear matrix. Thus, it seems that attachment to the nuclear matrix can play a critical role in determining whether a neighboring origin is actually functional.

J8-008 CHROMATIN ASSEMBLY IN VITRO, David J. Tremethick, John Curtin School of Medical Research, The Australian National University, Canberra, ACT 2601, Australia.

To understand the mechanism by which highly organised active chromatin structures are assembled, it is first necessary to understand the basic steps involved in assembling DNA into chromatin. Fractionation of an extract prepared from *X.laevis* ovaries, which can assemble plasmid DNA into authentic chromatin, is revealing the mechanism by which DNA is assembled into chromatin. In addition, substituting *X.laevis* components with counterparts purified from other vertebrate sources is also helping in elucidating the mechanism. When the N1/N2-(H3, H4) complex, isolated from the oocyte extract, is mixed with chicken histones H2A and H2B, plasmid DNA, and topoisomerase I, nucleosomes are assembled efficiently under physiological conditions in a two-step process. However, these assembled nucleosomes are spaced only in a very short manner (approximately 145 base pairs per particle). Recently, an ATP dependent activity was partially purified from the oocyte extract that could organise these nucleosomes into a regular array with a repeat length of 160 to 165 base pairs. In a second independent step, chicken histone H1 was able to increase the repeat length from 165 to 190 base pairs. Therefore, the assembly of DNA into chromatin *in vitro* appears to be a sequential process occurring in at least four steps. More recently, it was found that the phosphorylated form of HMGs 14 and 17 from human placenta can, at least in part, functionally replace the impure spacing activity to produce chromatin with a 160 base pair repeat. Extensive digestion of chromatin formed in the presence of HMGs 14 and 17 with micrococcal nuclease produces a DNA fragment 160 base pairs in length; a length similar to that obtained when histone H1-containing nucleosomes are similarly digested. This indicates that HMGs 14 and 17 may interact directly with DNA peripheral to the nucleosome core and/or that they may indirectly stabilise histone-DNA contacts in the internucleosomal core DNA region. To investigate the mechanism of how these proteins may prevent the close packing of nucleosomes, assembly reactions were carried out in which the relative amounts of HMGs 14 and 17, histones H2A and H2B, and the N1/N2-(H3, H4) complex were varied in assembly reactions. Under conditions in which histones H2A and H2B were limiting, and in the absence of HMGs 14 and 17, micrococcal nuclease digestion of the assembled product produced a ladder of DNA fragments that was poorly defined. The apparent repeat length for this chromatin template was around 125 base pairs. Extensive digestion produced DNA fragments between 100 and 110 base pairs in length; this DNA was probably associated with subnucleosomal particles that contained the H3/H4 tetramer plus only one H2A/H2B dimer. Most interestingly, when HMGs 14 and 17 were added to this assembly reaction, 'nucleosome-like' structures were reassembled as shown by the disappearance of subnucleosomal particles and the restoration of a regular, well defined ladder of DNA fragments (multiples of 145 base pairs) upon micrococcal nuclease digestion. Analysis of the protein composition of chromatin formed in the presence or absence of HMGs 14 and 17 reveals that HMGs 14 and 17 might be able to substitute for a histone H2A/H2B dimer in a H2A/H2B deficient nucleosome. In addition, HMG 14 or HMG 17 can individually space H2A/H2B deficient chromatin. The ability of these different chromatin templates to be transcribed *in vitro* was also examined. This work has shown that the association of HMGs 14 and 17 with nucleosomal particles deficient in histones H2A and H2B can create a template that is transcriptionally active. Whether the *X. laevis* spacing factor displays similar properties is under investigation.

Role of the Nuclear Matrix and Chromatin Transitions in the Regulation of Gene Expression (Joint)

J8-009 CHROMATIN STRUCTURE AND REGULATED EXPRESSION OF *DROSOPHILA HSP26* IN DIFFERENT CHROMOSOMAL ENVIRONMENTS, Sarah C.R. Elgin, C. Craig, H. Granok, B.A. Leibovitch, Q. Lu, C.D. Shaffer, M.J. Swede, J.M. Teare and L.L. Wallrath, Washington University, St. Louis, MO 63130 USA.

Our goal is to understand the contribution to gene regulation made by the organization of the 100Å chromatin fiber - the nucleosome array - and the contribution made by higher order packaging. We have analyzed the inducible activity and structural features of the *Drosophila hsp26* gene, examining both the sequence determinants of *hsp26* packaging in a euchromatic environment, and alterations in *hsp26* packaging in a heterochromatic environment. The *hsp26* promoter region includes two DNase I hypersensitive sites (DH sites), which encompass the heat shock regulatory elements (HSEs) and the TATA box; these DH sites are separated by a precisely positioned nucleosome. Analysis of a large number of *hsp26* transgenes with alterations (deletions, rearrangements, and point mutations) in the 5' regulatory region indicates that the $(CT)_n$ elements play a major role in establishing this chromatin structure, apparently through binding of the GAGA factor. A mutation in the TATA box that essentially eliminates inducible expression has only a minor effect on chromatin structure. The proximal $(CT)_n$ element includes both $(CT)_{3.5}$ and an inverted repeat with two segments of $(CT)_{4.5}$ that can shift to H-form DNA under conditions of supercoiling and acid pH. To test the significance of the potential H-form DNA, transgenes with substitutions in this region have been analyzed *in vivo*. The inverted repeat can be replaced with a short $(CT)_n$ site without loss of activity. Replacement with an $(CCTTT)_n$ inverted repeat decreases activity. The results indicate that the potential H-form DNA does not play a major role in *hsp26* regulation.

When a P-element construct containing a marked copy of *hsp26* and a *white* gene as a visible marker is mobilized in the *Drosophila* genome, flies recovered showing position effect variegation (PEV) of the eye color marker invariably have P-element inserts in/near the centromeres, along the fourth chromosome, or at/near the telomeres. These regions are associated with heterochromatin protein 1 (HP1); mutations in HP1 result in suppression of PEV. Mutant alleles of HP1 suppress variegation of the transgenes in the chromocenter and fourth chromosome, but not of the transgenes at the telomeres. *Su-var (2)1^{01}*, a mutation associated with increased histone acetylation, also suppresses variegation of transgenes at the chromocenter and along the fourth chromosome. The *hsp26* transgenes in variegating sites show reduced accessibility to restriction enzyme digestion at the HSEs; those at the chromocenter/fourth chromosome, but not those at the telomeres show reduced expression on heat shock. *hsp26* transgenes inserted near the chromocenter show a more regular nucleosome array compared to that seen for euchromatic transgenes. The evidence points to altered packaging as a means of gene inactivation at the chromocenter/fourth chromosome, but suggests a different source of variegation at the telomeres. Double immunofluorescence staining of the polytene chromosomes shows that HP1 and GAGA factor have very different distribution patterns, with few areas of overlap. Enhancers and suppressors of PEV, some of which have effects based on gene dosage, may compete to establish active or inactive packaging formats. This competition may involve two types of proteins, both those that interact directly with DNA, such as GAGA factor, and those that do not bind DNA but presumably act through multiprotein complexes, such as HP1.

J8-010 FUNCTIONAL ARCHITECTURE OF CHROMOSOMAL DNA DOMAINS, Sergey V. Razin[1,2], Olga V. Iarovaia[1,2], Maria Lagarkova[1],Irina I. Gromova[1,3], and R. Hancock[4], [1]Institute of Gene Biology, Russian Academy of Sciences, 117334 Moscow, Russia, [2]International Centre for Genetic Engineering and Biotechnology, Trieste, Italy, [3]Dept. Mol. Biol., University of Aarhus, Aarhus, Denmark, [4]Laval University Cancer Research Centre, Quebec, Canada G1R2J6.

We have developed a new approach for mapping the ends of DNA loops fixed at a nuclear matrix. Individual loops are excised from the genome by specific DNA cleavage with the high salt-insoluble topoisomerase II which is a component of the nuclear matrix and the chromosomal scaffold. The protocol includes preextraction of permeabilized cells with 2M NaCl solution followed by incubation in a topoisomerase II cleavage buffer supplemented with VM-26. Released loops are then separated by pulsed field gel electrophoresis and identified by hybridization with specific probes. Additional cleavage of released loops by rear-cutting restriction enzymes gives a possibility to map loop ends in cloned areas of the genome by indirect end labelling. Using the above-described approach we have demonstrated that ribosomal genes in human cells and in Chinese hamster cells are organized into loops equal in size to the size of individual rDNA repeat and separated by ~3 kb long matrix attachment areas in a non-transcribed spacers. We have also mapped several loops in c-myc gene amplicon in a human small cell lung cancer cell line. In all cases loops were separated by several kb long matrix attachment areas including multiple sites of DNA contact with the high salt insoluble topoisomerase II. In permeabilized cells the matrix attachment areas are preferentially sensitive to digestion with endogenous and exogenous nucleases including those specific to single stranded DNA. High salt extraction abolishes this preferential sensitivity. Matrix attachment areas are also found to constitute preferential targents for the endogenous nucleases mediating large-scale fragmentation of nuclear DNA at the initial steps of apoptosis. Basing on the above observations and literature data, we suggest a new model of DNA interaction with the nuclear matrix which describes the matrix as a system of internal nuclear channels.

J8-011 CONTRIBUTION OF CHROMATIN STRUCTURE AND THE NUCLEAR MATRIX TO TRANSCRIPTIONAL CONTROL OF CELL GROWTH AND TISSUE SPECIFIC GENES DURING PROLIFERATION AND DIFFERENTIATION, Gary S. Stein, Janet L. Stein, Jane B. Lian, Martin Montecino, and André van Wijnen, Dept. of Cell Biology and Cancer Center, University of Massachusetts Medical Center, 55 Lake Ave. N., Worcester, MA.

Structural parameters of the nucleus contribute to the developmental and hormonal control of cell growth and tissue-specific gene transcription by integrating regulatory activities at multiple independent promoter elements. Components of nuclear structure thereby support responsiveness to steroid hormone and growth factor-mediated signalling mechanisms which regulate transcription of genes functionally related to proliferation and differentiation. In proliferating cells transcriptional upregulation of the cell cycle regulated histone genes during S-phase is accompanied by a remodelling of chromatin structure and nucleosome organization. These cell cycle dependent modifications in structural parameters of the histone gene promoter may modulate accessibility of the proximal cell cycle regulatory element to transcription factor complexes containing cdc2, cyclin A, RB and IRF. Synergistic activity of distal regulatory sequences is facilitated. Association of the histone gene and transcription factors which enhance histone gene transcription with the nuclear matrix may further influence competency for expression in a cell cycle dependent manner. In postproliferative rat osteoblasts we have addressed developmental and steroid hormone-related modifications in structural parameters of the bone-specific osteocalcin gene promoter which are coupled with transcriptional control. Three levels of nuclear structure contribute to vitamin D regulation of osteocalcin gene transcription. First, DNase I hypersensitivity is directly correlated to developmental and vitamin D enhanced transcription. Hypersensitive sites flank the vitamin D response element and reside in proximity to the TGFβ, OC Box and TATA elements reflecting steroid hormone modulation of transcription factor interactions in the osteocalcin promoter. Second, selected changes in nucleosome positioning occur in the vitamin D response element in response to vitamin D only in osteoblasts expressing osteocalcin. Third, the nuclear matrix may support osteocalcin transcription during osteoblast differentiation by involvement in gene localization and targeting of transcription factors. We have characterized tissue-specific nuclear matrix proteins that bind with sequence specificity to sites flanking the vitamin D response element and TGFβ response element. Taken together, these modifications in structural parameters of the nucleus functionally relate to control of histone and osteocalcin gene expression by: 1) influencing competency of promoter elements for binding of cognate transcription factors; and 2) imposing structural constraints on promoter organization that integrate activities at basal and steroid hormone responsive elements in a developmental and tissue-specific manner requisite for control of genes during proliferation and differentiation.

Active Chromatin Domains and the Control of Higher Order Structure in the Cell Nucleus (Joint)

J8-012 Chromosomal structural loops and functional domains: SARs and Boundary Elements. Ulrich K. Laemmli, Keji Zhao, Craig Hart and Reiner Strick. Departments of Biochemistry and Molecular Biology, University of Geneva, Switzerland.

The long-term goal of this laboratory is to approach a structural/biochemical understanding of the chromatin loops of mitotic chromosomes and study the role of chromatin domains in gene expression. The topological loops of metaphase chromosomes are thought to be anchored by special highly A + T -rich regions of about 1 kb termed SARs (scaffold associated regions). Recent evidence strongly support this notion, this evidence and the role of SARs in chromosome banding and gene expression will be reviewed. The first part of the presentation will address in some detail the question of whether SARs are CIS elements involved in the dynamic structural transitions of chromosomes such as condensation and decondensation. The second part of this talk will focus on the DNA boundary elements (BE) which separate the chromatin fiber into functional domains. BE's are proposed to insulate one domain from the enhancer or repressor activities of flanking domains. The SCS and SCS' elements, which flank the Drosophila 87A7 hsp70 heat shock locus, were experimentally identified as BE's by Schedl's group. We will report about our progress to characterize proteins termed BEAFs (Boundary Element Associated Factors) which bind to these elements and are implicated in the insulation of chromosomal domains.

Global Transactivators

J8-013 GLOBAL ACTIVATORS AND REPRESSORS IN TRANSCRIPTIONAL CONTROL,
Marian Carlson, Columbia University College of Physicians & Surgeons, New York, NY 10032.

Transcription of many genes is under complex regulatory control in the budding yeast *Saccharomyces cerevisiae*. Global transcriptional activators, global repressors, and regulatory proteins that respond to specific signals must function in an integrated manner to achieve proper regulation of RNA polymerase II transcription. Work in our lab has focused on regulation of the *SUC2* (invertase) gene in response to glucose availability. In this case, specific regulatory signals are mediated by the SNF1 protein kinase pathway. In addition, a global activator and a global repressor are required for transcriptional control. Expression of *SUC2* in response to glucose starvation requires the SNF/SWI activator complex (including SNF2, SNF5, SNF6, SWI1, SWI3). The SNF/SWI complex appears to facilitate activation of many genes via affects on chromatin, and the complex is conserved in *Drosophila* and mammals. Glucose repression of *SUC2* also requires a global factor, the SSN6/TUP1 repressor complex, which is tethered to different promoters by gene-specific DNA-binding proteins. We have used genetic analysis and the two-hybrid system to address the roles of these global activators and repressors in regulation.

J8-014 THE SWI/SNF COMPLEX: A CHROMATIN REMODELING MACHINE, Craig L. Peterson[1], Jacque Côté[2], Janet Quinn[1], Jerry Workman[2], Emilie Richmond[1], Mark Wechser[1], Loree Burns[1], Laurie Boyer[1], and Kerri Pollard[1], [1]Program in Molecular Medicine and Department of Biochemistry and Molecular Biology, University of Massachusetts Medical Center, Worcester, [2]Center for Gene Regulation and Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park.

The SWI/SNF complex plays a key role in the regulation of eukaryotic gene expression. Five subunits of the yeast SWI/SNF complex, encoded by the *SWI1* (*ADR6*), *SWI2* (*SNF2*), *SWI3*, *SNF5*, and *SNF6* genes, were initially identified as positive regulators of two genes, *HO* and *SUC2*, and subsequently found to be required for the transcription of many other diversely regulated genes. Furthermore, many sequence-specific activators, including the yeast GAL4 protein, require one or more SWI/SNF products to enhance transcription in yeast. Genetic studies in yeast suggest that one role for the complex is to antagonize chromatin-mediated repression of transcription. Recently, biochemical studies with purified SWI/SNF complex have provided the first direct biochemical link between the function of the SWI/SNF complex and chromatin. Purified SWI/SNF complex has a striking effect on the binding of a transcriptional activator, GAL4-AH, to nucleosomal DNA in vitro. Addition of SWI/SNF complex and ATP stimulates the binding of GAL4 derivatives to a single recognition site on a nucleosome by a factor of 10 to 30. This reaction is absolutely dependent upon a hydrolyzable form of ATP, is independent of the translational positioning of the GAL4 binding sites on the nucleosome, and does not require a functional transcriptional activation domain. Current studies are focused on the biochemical mechanism of SWI/SNF action and on determining the role of each of the ten subunits of the complex.

J8-015 ANALYSIS OF HISTONES, TATA-BINDING PROTEIN, AND OTHER TRANSCRIPTION FACTORS OF YEAST, Fred Winston, Alex Bortvin, Lisa Gansheroff, Grant Hartzog, Joel Hirschhorn, Jon Madison, Inés Pinto, and Stephanie Ricupero-Hovasse, Department of Genetics, Harvard Medical School, Boston, MA 02115.

A large number of genes that are essential or important for transcription in the yeast *S. cerevisiae* have been identified by suppressors of Ty or Ty LTR insertion mutations in the promoter regions of genes. Analysis of these genes (designated *SPT* genes) has shown that most of them fall into one of two classes. The first class, the TBP class, includes the gene *SPT15*, which encodes TATA-binding protein (TBP), and three other genes of unknown function, *SPT3*, *SPT7*, and *SPT8*. Previously, both genetic and biochemical evidence demonstrated that TBP interacts with SPT3. Based on the *spt3* mutant phenotypes, these results suggested that SPT3 functions at specific promoters to help TBP function. Recent genetic results have demonstrated that SPT3 functionally interacts with another protein, MOT1, that also is known to interact with TBP. MOT1 has been shown by others to inhibit TBP binding to TATA *in vitro* and to behave as a repressor *in vivo* (1). Taken together, these results suggest that SPT3 may serve to overcome the general repression by MOT1 in a promoter-specific fashion. The second class of *SPT* genes, the histone class, includes the genes *HTA1/SPT11*, *HTB1/SPT12*, and at least three other genes of unknown function, *SPT4*, *SPT5*, and *SPT6*. Previous work has shown that a deletion of *HTA1-HTB1*, one of the two sets of genes in yeast that encode histones H2A and H2B, can suppress loss of the SNF/SWI complex, normally required for expression of many genes *in vivo*. In addition, these effects are exerted at the level of chromatin structure. These results, along with recent *in vitro* experiments from others (2,3,4), suggest the SNF/SWI activates transcription by remodeling chromatin. Recently, a new class of mutations in *HTA1* have been isolated that cause repression of transcription. Genetic analysis of these histone H2A mutants has suggested an additional step in activating transcription of a chromatin template that must occur after SNF/SWI function. Finally, analysis of *SPT4*, *SPT5*, and *SPT6* has indicated that these genes play an important role in establishment or maintenance of a repressed chromatin state. Recent evidence has shown that these genes may also be conserved: a human homolgue of *SPT4* has been identified and has been shown to function in yeast.

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Higher Order Structure and Gene Activation

J8-016 MECHANISMS OF HERITABLE GENE SILENCING DURING DEVELOPMENT OF DROSOPHILA, Renato Paro, Achim Breiling, Axel Möhrle, Valerio Orlando, Helen Strutt and Daniele Zink, ZMBH, University of Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, FRG

In Drosophila the genes of the Polycomb-group (PcG) and the trithorax-group (trxG) are responsible for maintaining respectively the inactive and the active state of factors involved in determinations (i.e. homeotic genes). There is growing evidence that the PcG uses epigenetic mechanisms to keep target genes silenced over many cell divisions. The PcG shows many similarities to another distinct class of Drosophila genes, the modifiers of position effect variegation. This class is considered to encode regulatory/structural parts of heterochromatin. The PcG seems to form heterochromatin-like repressive complexes. On parallel lines, the trithorax-group (trxG) is maintaining the active transcriptional state by counteracting the PcG.
The establishment of silencing complexes occurs through DNA elements termed PRE (PcG Regulated Elements). In line with the phenomena observed with heterochromatin, PRE have been interpreted as nucleation sites from where the repressive PcG-chromatin can spread and inactivate large chromosomal domains. We have tested the role of two PREs of the homeotic bithorax complex (BX-C), termed MCP and FAB-7, in an artificial environment. Constructs containing a yeast Gal4 activatable reporter gene were flanked by FAB-7 and/or MCP and tested in transgenic fly lines. These PREs were found to induce mosaic expression patterns of the reporter gene and the *white* reporter gene. We were able to follow the binding of the repressory Polycomb protein and the Gal4 protein at the site of insertion of the transgene in polytene chromosomes by immunolocalization. We find that the PcG complexes can prevent the Gal-4 protein from binding to its UAS target site. However, high doses of Gal4 can displace the PcG complex.
By applying a formaldehyde in vivo cross-linking method we have mapped the distribution of the Pc protein in the BX-C. Pc is found over extended chromosomal regions covering inactive genes, but is absent in regions of active homeotic genes. We applied this technology to map the distribution of brahma, a member of the trxG (in collaboration with C. Muchardt and M. Janiv, Paris). Using the *empty spiracle* gene as a target we find that the activating transcription factor, Abdominal-B, has a rather broad binding pattern upstream and downstream of the gene. brahma has a more restricted but overlapping pattern around a mapped enhancer element.
By using immunoprecipitations we have found that at least four proteins of the PcG (Pc, ph, Psc and Pcl) are associated in large multimeric complexes. We have mapped two relevant functional domains of the Pc protein. The C-terminal part seems to be interacting with other proteins. In immunoprecipitation experiments this part was shown to be necessary for the interaction of Pc with ph and Psc. The evolutionary conserved chromo domain in the N-terminus appears to interact with an RNA moiety. We have been able to purify the RNA part by applying semi-denaturing conditions. An initial partial characterization revealed a di-nucleotide repeat as well as an apparent localization of the encoding gene(s) in heterochromatic sequences. Heritable gene silencing is an important but little understood mechanism in pattern formation as well as in other biological phenomena that range from the inactivation of the mating type loci in yeast to X-chromosome inactivation in mammals. The Drosophila PcG appears to be a good tool to unravel the molecular mechanisms where such functions are maintained by higher order chromatin-structures.

J8-017 MECHANISMS REGULATING THE EXPRESSION OF THE ULTRABITHORAX GENE AND ITS MAINTENANCE BY THE PC-G PROTEINS, Vincenzo Pirrotta, Luca Rastelli, Chi Shing Chan, University of Geneva, CH1211 Geneva, Switzerland

The expression of homeotic genes in Drosophila is initiated in response to segmentation gene products which also delimit the segmental domain of activity of each gene. In the case of the *Ubx* gene, the *hunchback* and *tailless* proteins act as repressors preventing expression in the anterior half of the embryo and in the posterior terminal region. The segmentation genes act on a set on parasegmental enhancers distributed over a region of 80 kb both upstream and downstream of the promoter. The segmentation genes cease functioning in their segmental domains during germ band elongation and both activator and repressor functions fade away. Expression in the later embryo depends on different activators and expression in the imaginal discs is due to a second set of enhancers that have no positional input from the segmentation genes. Correct parasegmental expression of the *Ubx* gene in later development depends on the *Polycomb* Group (Pc-G) genes. We have identified a single major target for these gene products, the *Polycomb* Response Element (PRE). A complex of these gene products assembles at the PRE in the early embryo and establishes a repressed state in those cells in which the *Ubx* gene was repressed but not in cells in which the *Ubx* gene was active. The repressed state of the chromatin affects all *Ubx* enhancers and, in transposon constructs, it affects also other genes placed in the same construct. The repressed state is maintained through later development and determines the correct domain of expression of the imaginal disc enhancers. The PRE also represses the *white* gene present in the same transposon, resulting in a variegated expression in the eye. It can also repress genes flanking the insertion site of the transposon containing it. The complex formed at the PRE is strongly influenced by the nature of the flanking regions, by association with a homologously paired chromosome region, by temperature and by the presence or absence of factors such as the *trithorax* or *zeste* gene products which antagonize repression. These factors determine the nature or configuration of the repressive complex at the time of its formation in the early embryo in a way that affects its repressive function in later development. Thus, the temperature, level of *zeste* or *trx* products in the first ten hours of development affect the state of repression in the pupa or adult. The PRE is contained within a 1.5 kb fragment and can be subdivided into functionally more specialised regions: a core fragment of 660 bp interacts with *Pc, ph, Psc, Su(z)2* products and creates new binding sites for these Pc-G proteins, visible on polytene chromosomes; a flanking fragment of 550 bp interacts with and creates binding sites for *Su(z)2* but not for *Pc, ph* and *Psc*. The *Ubx* PRE contains binding sites for *trx* protein and GAGA factor and is immediately flanked by a set of *zeste* binding sites whose presence is required for the effect of *zeste* on PRE repression. A model for the establishment of an extended Pc-G complex that initiates at the PRE and extends by recruitment of flanking regions will be discussed.

Structure and Dynamics of the Chromatin Fiber

J8-018 CONFORMATIONAL DYNAMICS OF NUCLEOSOMAL AND SUBNUCLEOSOMAL ARRAYS: IMPLICATIONS FOR FORMATION OF NUCLEAR ULTRASTRUCTURE AND FOR EPIGENETIC CONTROL OF TRANSCRIPTION, Jeffrey C. Hansen, Terace M. Fletcher and Patricia M. Schwarz, Department of Biochemistry, The University of Texas Health Science Center, San Antonio, TX 78284-7760.

Transcriptional activation is associated with several changes in the chromatin organization of eukaryotic genes, including dissociation of H2A/H2B dimers from individual nucleosomes, acetylation of the c-terminal core histone tails, and selective depletion of histone octamer(s) at key sites within the nucleosomal array. Both the structural and the functional consequences of such changes have been studied *in vitro* using defined model systems reconstituted from 12 tandem repeats of *Lytechinus* 5S rDNA and saturating or subsaturating amounts of either intact histone octamers, trypsinized histone octamers, or H3/H4 tetramers. No linker histones are present in these reconstitutes. Structural dynamics were studied using quantitative agarose gel electrophoresis and analytical ultracentrifugation. *In vitro* transcription experiments performed in a *Xenopus* oocyte nuclear extract yielded estimates of the transcriptional activity of the same 5 S reconstitutes under identical buffer conditions. The solution-state behavior of a saturated 5 S array (12 nucleosomes/DNA) is shown in Fig. 1. The 29 S conformer is the exclusive species present in low salt TE buffer. The 55 S solenoidal species, which is observed in 1.0-2.0 mM $MgCl_2$ but not in NaCl, is the most compact structure that can be formed by a 12-mer nucleosomal array. Above 2.0 mM $MgCl_2$ (but not in NaCl) the nucleosomal arrays cooperatively self-associate to form a defined ~500 S particle, which then undergoes further association as the $MgCl_2$ is raised above 5 mM. All steps shown in Fig. 1 are reversible. These data will be discussed in the context of the observations that linker histones can be deleted from some eukaryotic cells without grossly affecting nuclear ultrastructure. The functional studies indicate that oligonucleosome species that sediment at <40 S can be transcribed by RNA polymerase III. However, conditions that lead to formation of the more highly folded 40-55 S species lead to complete and specific repression of transcription. Selective depletion of intact histone octamers, trypsinization of the core histone tails, and removal of H2A/H2B dimers each disrupts the ability of the nucleosomal arrays to form repressive folded structures, which suggests that modulation of the intrinsic folding behavior of nucleosomal arrays may be an important site of epigenetic regulation of transcription. The structural and functional consequences of differences in the surface charge density and conformational flexibility of the various nucleosomal and subnucleosomal arrays also will be discussed.

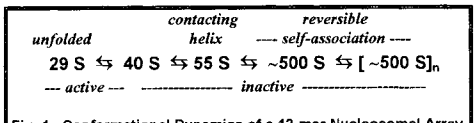

Fig. 1. Conformational Dynamics of a 12-mer Nucleosomal Array

J8-019 STRUCTURE AND LOCATION OF THE LINKER HISTONE H1 IN CHROMATIN, V. Ramakrishnan, Biology Department, Brookhaven National Laboratory, Upton, NY 11973.

The linker histone H1 and its variant H5 bind to the nucleosome and play an essential role in organizing chromatin higher-order structure. Much current evidence points to a role for H1 in the repression of chromatin, but detailed structural evidence regarding its structure and location in chromatin is only now emerging. The location of histone H1 in the 30 nm filament of chromatin has been determined by neutron scattering. These results and their implications for current models of chromatin structure will be described. The structure of the nucleosome binding domain of H5 and its counterpart H1, and their implications for DNA and nucleosome binding will also be discussed, as will. efforts to extend these results by biochemical studies on mutants of the linker histone.

Genome Imprinting

J8-020 ON THE ORIGIN OF CpG ISLANDS. Bird, A. and Macleod, D. Institute of Cell and Molecular Biology, University of Edinburgh, Kings Buildings, Edinburgh, EH9 3JR, U.K.

CpG islands are DNA sequences which are on average 1kb in size and are almost exclusively found at the 5' end of genes. They are GC-rich compared to bulk genomic DNA, and, unlike the rest of the genome, have CpG at the expected density. CpG islands lack methylation in germ cells, and in the vast majority of cases, in somatic cells as well. In mouse and man CpG island genes include all sequenced 'housekeeping' genes and many genes that show a tissue-restricted pattern of expression. In an attempt to find the mechanism by which CpG islands remain free of methylation we have undertaken a detailed examination of the mouse adenine phosphoribosyltransferase (*aprt*) gene. This housekeeping gene has a CpG island which extends over the gene promoter and includes the first two exons. We show that the island is free of methylation at all CpGs, whereas the flanks are methyated. Detailed patterns of methylation beyond the boundaries of the CpG island vary between cells. *In vivo* footprinting across the island region shows that three GC-boxes clustered at the 5' edge of the CpG island are occupied, most probably by Sp1. No other footprints are detected within the island region. Deletion or mutagenesis of the Sp1 sites causes *de novo* methylation of the CpG island in a transgenic mouse assay. Thus the peripherally located Sp1 sites are necessary to keep the *aprt* island methylation-free. We have extended this analysis to CpG islands associated with genes that are silent in most cell types. The results suggest a common origin for all CpG islands, based upon transcription.

J8-021 ESTABLISHMENT OF IMPRINTED METHYLATION PATTERNS DURING DEVELOPMENT, Ruth Shemer, Tal Kafri, Yehudit Birger, Xiaohong Gao and Aharon Razin, Department of Cellular Biochemistry, Hebrew University Hadassah Medical School, Jerusalem, Israel.

Allele specific methylation patterns have been observed in all imprinted genes studied so far[1]. It has not been clear, however, precisely when and how is this differential methylation established. Performing a rigorous analysis of the methylation status of specific sites in differentially methylated regions revealed that methylation inherited from the gametes is changed and an allele specific methylation pattern is gradually established starting in the zygote before the two pronuclei fuse. These methylation changes involve prompt demethylation and de novo methylation that occur within one cell cycle. To gain further insight into the mechanism responsible for allele specific methylation we studied the status of methylation of sites in several imprinted genes in diploid and haploid parthenogenetic preimplantation embryos, in E 11.5 mice with a maternal disomy of a distal region in chromosome 7 (MatDi7) and in gynogenetic and androgenetic ES cells. The sites proved to be methylated to the same extent in diploid parthenogenetic and in normal embryos. This observation clearly implies that methylation changes, demethylation and de novo methylation took place in the parthenogenetic embryo resulting in only one allele methylated, in spite of the fact that both alleles were identical. However, these methylation changes did not occur in haploid parthenogenetic embryos suggesting that two copies of the gene are required for such changes to occur. That the methylaton changes result in methylation of one allele we learned from Southern analysis of differentially methylated regions in parthenogenetic ES cells where several sites could be analyzed simultaneously. Same results were obtained in MatDi7 embryos in which the entire genome is normal carrying both maternal and paternal alleles except for a region in the distal part of chromosome 7 which carries a maternal disomy. Taking all our results together we conclude that differential methylation patterns do not derive from the gametes but are rather established during development following the eraser of the gametic methylation pattern. The differential methylation patterns of imprinted genes are obtained by a gene dosage adjustment mechanism resembling the methylation process that follows the random inactivation of one x-chromosome in eutherian female cells. Our methylation data are in accord with recent data obtained on the expression of the imprinted genes Igf2 and Igf2r in normal, gynogenetic and androgenetic preimplantation embryos[2].

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Imprinting II

J8-022 PARAMUTATION: AN ALLELIC INTERACTION THAT CAUSES HERITABLE CHANGES IN TRANSCRIPTION, *Vicki L.* Chandler, Garth I. Patterson, and Kenneth Kubo, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

Paramutation is an interaction between alleles that leads to a mitotically and meiotically heritable change in one of the alleles at high frequency. In maize, paramutation has been characterized at the *b*, *r*, and *pl* loci, which encode transcription factors that regulate the synthesis of the red and purple anthocyanin pigments. Paramutation of the *b* gene results when the lightly pigmented *B'* allele heritably alters the darkly pigmented *B-I* allele in a *B'/B-I* heterozygote, such that the *B-I* allele becomes *B'*. The interaction is allele specific; most *b* alleles have no effect on *B-I*, and are not affected by *B'*. Analyses of the expression and structure of *B-I* and *B'* have revealed that the *B'* and *B-I* pigment phenotypes are due to ~20 fold differences in transcription, but no structural or methylation differences between the two alleles have been detected (Patterson et al. Genetics 135:881-894). The association of paramutation with changes in *b* transcription combined with the fact that the ability to paramutate *B-I* is tightly linked to *B'* (<0.1 centimorgans), suggested that transcriptional regulatory sequences may be involved in paramutation. To more precisely define the sequences required for paramutation, we used *B'* and *B-I* to isolate intragenic recombinants with an allele that is insensitive to paramutation, *B-Peru*. Physical mapping of the recombinant alleles showed that recombination was not uniformly distributed within the *b* gene; the rate of crossovers is ~100-fold higher at the 5' end of the transcribed region relative to the more 3' transcribed region. The recombinant alleles were tested for color phenotype, and for the ability to cause and respond to paramutation. The ability to participate in paramutation and the control of tissue-specific expression both map to the 5' region of the gene. These results further demonstrate paramutation is a transcriptional phenomenon, and are consistent with the possibility that transcriptional regulatory sequences may play an important role in controlling or responding to paramutation.

Nucleosome-Factor Interactions in vitro; Chromatin Repression of Gene Function

J8-100 β-GLOBIN GENE TRANSCRIPTION IN SYNTHETIC NUCLEI, Michelle Craig Barton[1] and Beverly M. Emerson[2], [1]Dept. of Molecular Genetics, University of Cincinnati Medical Center and [2]The Salk Institute, La Jolla, CA

Regulated gene expression within a complex chromosomal locus requires multiple nuclear processes. We have analyzed the transcriptional properties of the cloned chick β-globin gene family when assembled into synthetic nuclei using Xenopus egg extracts. Assembly in an erythroid protein environment correctly recapitulates tissue-specific chromatin structure and long-range promoter-enhancer interaction within the chromosomal locus resulting in β-globin gene activation. Nucleosome-repressed β-globin templates can be transcriptionally activated by double-stranded DNA replication in the presence of staged erythroid proteins by remodeling the chromatin structure within the promoter region and establishing distal promoter-enhancer communication. The programmed transcriptional state of a gene, as encoded by its chromatin structure and long-range promoter-enhancer interactions, is stable to nuclear decondensation and DNA replication unless active remodeling occurs in the presence of specific DNA binding proteins.

J8-101 THE κ-ENHANCER AND MAR SEQUENCES DIRECT DEMETHYLATION IN B CELLS, Yehudit Bergman, Howard Cedar, Andrei Kirillov and Michal Lichtenstein, Department of Experimental Medicine and Cancer Research, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

We have studied the molecular mechanism of demethylation and its role in the regulation of κ-chain gene expression. Following transfection into B-cell cultures, this gene undergoes regional demethylation in a process that is developmentally regulated, in a lineage and stage specific manner. A germ line Vκ promoter is not required for the demethylation activity, and thus transcription itself is unlikely to be the cause of this demodification.

We identified a cis regulatory element that directs demethylation in a B-cell specific manner. This element contains the intronic κ-chain transcriptional enhancer, the matrix attachment region (MAR) and an unidentified element located 3' to Jκ5. This conclusion is based on the key observation that a complete rearranged κ gene construct undergoes cell type specific demodification when transfected into B-cells. In the absence of these elements, demethylation does not take place, but this reaction can be restored if a fragment containing the full complement of the demethylation elements is added back. In its natural location downstream to the Jκ5 sequence, this element induces bidirectional demodification of plasmid constructs in a distance-and orientation-independent manner. When the κ-enhancer and MAR sequences are present 5' to the Vκ promoter, methylation prevents expression even though the enhancer and MAR sequences are potentially active as transcriptional elements from this position. Thus, we have demonstrated that demethylation is required in order for the κ-chain gene to undergo transcription in B-cells. These results thus clearly show that κ enhancer and MAR sequences play a dual role in κ-chain gene activation, during B-cell development, first by inducing demethylation, and secondly by promoting tissue specific transcription.

References

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J8-102 THE ARCHITECTURE OF THE NUCLEOPROTEIN COMPLEX ORGANIZED BY SRY PROTEIN IS CRITICAL FOR SEX DETERMINATION

Marco E. Bianchi, Andrea Pontiggia, Rebecca Rimini, Vincent R. Harley, Peter N. Goodfellow and Robin Lovell-Badge
Dept. Genetics & Microbiology, University of Milano, Italy; DIBIT, San Raffaele Scientific Institute, Milano, Italy; Dept. of Genetics, Cambridge University, UK; Laboratory of Eukaryotic Molecular Genetics, MRC National Institute of Medical Research, London, UK

In mammals, sex determination is caused by the Y-chromosome gene *SRY*. The DNA binding domain of human SRY protein is similar to those of the chromatin protein HMG1. Like HMG1, SRY binds to kinked DNA structures, and produces a sharp bend of about 80° on binding to linear DNA. We have analysed the biochemical properties of mutant SRY proteins from five patients with complete gonadal dysgenesis. Mutant proteins fall in three categories: two bind and bend DNA almost normally, two bind inefficiently but bend DNA normally, and one binds DNA with almost normal affinity but produces a different angle. The mutations with moderate effect on complex formation can be transmitted to male progeny, the ones with severe effects on either binding or bending are *de novo*. The angle induced by SRY depends on the exact DNA sequence, and thus adds another level of discrimination in target site recognition. The data suggest that the exact spatial arrangement of the nucleoprotein complex organised by SRY in chromatin is essential for the expression of genes involved in testis differentiation.

J8-103 **EXTINCTION OF α1-ANTITRYPSIN GENE EXPRESSION IN CELL HYBRIDS IS INDEPENDENT OF TRANSACTIVATORS HNF1α AND HNF4,** **Gary A. Bulla, Pediatric Research Institute, Saint Louis University Health Sciences Center, St. Louis, MO 63110**

Extinction of tissue-specific gene expression in somatic cell hybrids is often associated with loss of essential *trans*-acting factors, suggesting that extinction is primarily a lack-of-activation phenotype. According to this model, ectopic expression of appropriate *trans*-acting factors should prevent extinction of target genes. To test this hypothesis, I tested whether extinction of the liver-specific α1-antitrypsin (α1AT) gene could be prevented by maintaining expression of it's two essential liver-enriched transcription factors Hepatocyte Nuclear Factors -1 (HNF1α) and -4 (HNF4). I show that hepatoma-like levels of HNF1α and HNF4 in fibroblast x hepatoma hybrids fails to prevent extinction of either rat or human α1AT loci. Furthermore, a human α1AT minilocus was also extinguished in these cell hybrids, despite strong activation of a transiently introduced α1AT promoter. These results show that extinction of the α1AT locus is independent of the loss of essential transactivators HNF1α and HNF4 and suggests that extinction of tissue-specific gene expression is a dominant phenotype acting through target sequences in the context of chromatin.

J8-104 NUCLEOSOME DEPLETION DEREPRESSES MMTV IN YEAST, Sebastián Chávez, Reyes Candau and Miguel Beato, Institut für Molekularbiologie und Tumorforschung, Philipps-Universität D35037-Marburg, Germany

Chromatin structure plays an essential role in transcription of the mouse mammary tumour virus (MMTV). MMTV is induced by glucocorticoids and progestins in a process mediated by the corresponding hormone-receptor and the nuclear factor I (NFI). Organization of the promoter region in chromatin seems to repress transcription of MMTV by restricting NFI accessibility. It has been proposed that binding of hormone receptor alters a nucleosome precisely located on the promoter region, releasing the repression imposed by chromatin. We have introduced the MMTV transcriptional system in *Saccharomyces cerevisiae* and tested that model by making use of the genetic tools available in yeast.

MMTV promoter, that is well organized on chromatin in yeast, is submitted in this organism to the same regulation than in animal cell: it is up to 100 fold induced by steroids, depending on the presence of hormone-receptor and NFI in the cell; and it is silent in the absence of hormone, even when NFI is expressed. A first conclusion from this picture is that histone H1, that is lacking in *Saccharomyces*, is neither required for MMTV repression nor for its hormonal induction.

When the MMTV system were introduced in the recombinant yeast strain UKY403 (1) and cells were partially nucleosome depleted by switching H4 synthesis off, basal transcription in the presence of NFI increased, demonstrating *in vivo* that chromatin represses MMTV transcription. A similar conclusion was obtained by using deleted MMTV promoters, lacking the sequence able to stably positioning a nucleosome on the NFI-binding site.

1. U.-J. Kim, M. Han, P. Kayne and M. Grunstein (1988) EMBO J. 7: 2211-2219.

J8-105 c-JUN DOWN REGULATES THE RAT α-FETOPROTEIN PROMOTER IN HEPG2 HEPATOMA CELLS WITHOUT BINDING TO DNA. J.L. Danan[1], B.Bois-Joyeux[1], M.Denissenko[1,2], S. Guesdon, R.Ikonomova[1], D. Bernuau[2], G.Feldmann[2] and H.Thomassin[1]; [1]CNRS UPR1511, 9 rue jules Hetzel, F92190 Meudon, France and [2]INSERM U327 Hop Bichat, F75018 Paris .

The α-fetoprotein (AFP) gene offers a very powerful model to gain insights into the molecular mechanisms which govern liver-specific gene expression in the course of development and cancerogenesis. It is indeed a potential target for several regulatory cascades. In the present work we have tested the effect of a phorbol ester (TPA) and of members of the Jun and Fos oncoproteins family on the functioning of the rat AFP promoter. For this, plasmids bearing the CAT gene under the control the rat AFP promoter (pAFP CAT) were transfected into HepG2 hepatoma cells.

Our results clearly show that TPA can lower (5-8 fold) the activity of the pAFP CAT plasmids in a dose dependent manner. In a second set of experiments, expression vectors for members of the Jun and fos families were cotransfected together with the AFP and Tk CAT plasmids.
c-Jun specifically down regulated (5 to 8 fold) the activity of the rat AFP promoter. Jun B and Jun D were much less effficient in doing so; c-Fos did not potentiate the effect of c-Jun

These effects appeared to be highly specific to the AFP promoter since neither TPA nor Jun proteins were able to modulate the CAT activity of a plasmid bearing the rat albumin promoter.

The mechanism does not require binding of Jun to the AFP promoter since c-jun proteins mutated in their DNA-binding domain and fusion proteins bearing the N-terminal part of c-jun fused to the Gal4 DNA-binding domain are still able to down regulate the AFP promoter. In agreement with these observations we did not observe any binding of purified c-Jun on the AFP promoter in DNase 1 footprinting experiments. The region of the AFP promoter where c-Jun exerces its effect still remains to be precisely mapped because all the deletion mutants of the AFP promoter (from -322 to -134) which exhibited residual activity were down regulated by c-Jun.

In views of these results, it is reasonable to think that members of the jun familiy can be key intermediates in some of the cascades of cellular events which result in the modulation of the AFP gene expression in the course of development and cancerogenesis.

J8-106 FACILITATION OF TRANSCRIPTION FACTOR BINDING TO NUCLEOSOMAL DNA BY HUMAN SWI/SNF , Anthony N. Imbalzano and Robert E. Kingston, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

Our research has focused on the identification of mechanisms by which nucleosome structure can be altered to facilitate transcription factor:DNA interactions. Binding of the basal pol II transcription factor TBP to nucleosomal DNA can be facilitated at the dyad axis of symmetry by hyperacetylating the core histones or by exposing the nucleosomes to human SWI/SNF (hSWI/SNF), which alters nucleosomal DNA structure in an ATP dependent manner. In both cases, binding of TBP is restricted by the rotational position of the TATA box on the nucleosome and requires greater than 100-fold more TBP than is required to bind to naked DNA. In addition, hSWI/SNF can increase the ability of GAL4 derivatives to bind to nucleosomal DNA, and derivatives containing an activation domain show more of an increase in binding than does the GAL4 DNA binding domain. Current experiments will address the mechanism of hSWI/SNF mediated alteration of nucleosome structure and will determine whether addition of other factors required for transcription (upstream activators, TFIIB, TFIID) will further increase the ability of TBP to bind to nucleosomes and/or will overcome the requirement for a specifically positioned TATA box.

J8-107 DIFFERENTIAL REPRESSION OF TRANSCRIPTION FACTOR BINDING BY HISTONE H1 IS REGULATED BY THE CORE HISTONE AMINO TERMINI, Li-Jung Juan*, Rhea T. Utley#, Christopher C. Adams#, Michelle Vettese-Dadey#, and Jerry L. Workman#, *InterCollege Program in Genetics, #Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802-4500

In order to investigate the interrelated roles of nucleosome cores and histone H1 in transcription repression, we have employed a purified system to analyze the function of H1 in the repression of transcription factor binding to nucleosomes. H1 binding to nucleosome cores resulted in the repression of USF binding to nucleosomes. By contrast, H1 only slightly inhibited the binding of GAL4-AH indicating that H1 differentially represses the binding of factors with different DNA binding domains. H1-mediated repression of factor binding was dependent on the core histone amino terminal tails. Removal of these domains alleviated H1-mediated repression and increased acetylation of these domains partly alleviated repression by H1. H1 binding assays suggest a less stable interaction of histone H1 with the core particle in the absence of the amino termini.

J8-108 STRUCTURAL CHARACTERIZATION AND TRANSCRIPTION OF A MODEL DINUCLEOSOME TEMPLATE , Kiyoe Ura and Alan Wolffe, Laboratory of Molecular Embryology, NICHD, NIH, Bethesda, MD 20892

In order to study the role of linker histone and core histone modification on gene expression, it is important to use a well-characterized nucleosomal template. DNA fragments containing two tandem repeats of the nucleosome positioning sequence from the *Xenopus borealis* somatic 5S RNA gene were constructed and reconstituted into dinucleosomes by salt dialysis. The structure of the dinucleosomes was analyzed using DNase I and hydroxyl radical footprinting, and micrococcal nuclease mapping. We also investigated transcriptional activity of the dinucleosomes, both with and without linker histones using *Xenopus* oocyte nuclear extract.

J8-109 TRYPSIN HYDROLYSIS OF CHROMATIN FIBERS REVISITED BY SCANNING FORCE MICROSCOPY, Sanford H. Leuba[1], Carlos Bustamante[1,2,3], Ken van Holde[4], and Jordanka Zlatanova[4,5], [1]Institute of Molecular Biology, [2]Department of Chemistry, and [3]Howard Hughes Medical Institute, University of Oregon, Eugene, OR 97403 [4]Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331-7305 and [5]Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria.

Chromatin fibers have the following arrangements: i) the DNA wraps 1.75 turns around an octamer of the core histones (H3, H2B, H2A, and H4) to form a core particle; ii) core particles are linked by linker DNA to form oligonucleosomes; and iii) the linker histones (H1/H5 family) bind to the linker DNA to form chromatin fibers. The central 80 aa globular domain of H1/H5 (GH1 and GH5) is thought to bind to the DNA entering/exiting nucleosomes. Mild immobilized-trypsin hydrolysis of chromatin fibers was monitored by SDS-PAGE and Scanning Force Microscopy (SFM). At early points of hydrolysis when 50% of the linker histones were not intact commensurate with the appearance of GH1/GH5 on the gels, SFM images showed irregular, three-dimensional chromatin fibers similar to those to which no trypsin had been added. Only when core histone H3 got attacked were changes in the fiber structure observed in SFM. At 50% degradation of intact H3, the linker DNA between some nucleosomes became apparent. Moreover, the nucleosomes in the extended stretches had a zig-zag arrangement. Later times of hydrolysis when all linker histones were cut yet some core histone H3 was still intact, the fibers still exhibited some three-dimensional arrangement of clusters of nucleosomes. Finally, when all H3 had been hydrolyzed and GH1/GH5 were present, the fibers formed a flat two-dimensional zig-zag of nucleosomes. These studies suggest i) that intact core histone H3 is necessary for the three-dimensional arrangement of nucleosomes; and ii) the linker histone globular domain fixes the angle of the DNA entering and exiting the nucleosome. However, fixing the angle at the entry/exit point by the globular domain is not sufficient in itself for preserving the three-dimensional arrangement of nucleosomes in the chromatin fiber.

J8-110 ROTATIONAL & TRANSLATIONAL NUCLEOSOME POSITIONING OF A GLUCOCORTICOID RESPONSE ELEMENT HAS DISTINCT EFFECTS ON GLUCOCORTICOID RECEPTOR AFFINITY, Qiao Li, Ulla Björk and Örjan Wrange, Dept. of Cell and Molecular Biology, Medical Nobel Institute, Karolinska Institutet, S-171 77 Stockholm, Sweden

In eukaryotic nucleus, DNA is packed into nucleosome which is the fundamental repeated unit of chromatin. This is believed to impede the recognition of DNA response elements by transcription factors in gene regulation. However, the transcription factor must gain access to its corresponding DNA element to initiate gene activation. The glucocorticoid receptor (GR) recognises its cognate response element (GRE) on DNA by making base-specific contacts in two consecutive major grooves on the same side of the DNA axis. We employed GR-GRE interaction as a model system to study the mechanism of factor-DNA recognition in a nucleosome context. A DNA bending sequence, the TG motif (Schrader and Crothers, 1989), was used to direct a single GRE into various defined rotational and translational positions in *in vitro* reconstituted nucleosomes. The effects of different rotational setting of the GRE on GR-GRE affinity were evaluated by DNase I footprinting and DMS methylation protection analysis. We conclude that rotational setting of the GRE in a nucleosome may have a major impact on GR-GRE affinity and that the effects of rotational positioning of the GRE is influenced by the translational positions of the GRE in the nucleosome. Since strictly positioned nucleosomes are found in an increasing number of different promoters/regulatory DNA elements it is important to understand how different transcription factors may recognise their cognate DNA elements when placed within a nucleosomal context. Our data suggests that nucleosome positioning plays a regulatory role in gene regulation.

J8-111 ANTI-REPRESSION AND DIRECT ACTIVATION ARE DISTINCT PROPERTIES OF GAL4-VP16.

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The regulation of transcription is fundamental to maintaining normal health and development. However, the mechanisms by which transcription factors activate transcription of the gene are not clear. Two possible modes of activation can occur: direct activation, in which the transcription factor directly stimulates the recruitment of active TFIID complexes to the promoter; and anti-repression, in which the transcription factor allows the TFIID complex to compete more efficiently with repressors, such as chromatin proteins, for binding to the promoter.

We have examined activation with *in vitro* transcription assays in which preinitiation complex formation occurs either in the absence of repressors or in competition with the binding of histone H1. Using partially purified basic transcription factors, the potent activator GAL4-VP16 was able to stimulate transcription 5-10-fold from a template containing specific GAL4 binding sites, and monoclonal antibodies raised against the VP16 activation moïety failed to inhibit this direct activation. The addition of histone H1 to the factors during preinitiation complex formation led to a complete repression of transcription in the absence of activator. The inclusion of GAL4-VP16 was able to prevent this repression, confirming its ability to act as an anti-repressor, as well as a direct activator. In contrast to the assays conducted in the absence of repressors, however, the monoclonal antibodies were strong inhibitors of GAL4-VP16-activated transcription in the presence of histone H1. Thus, the antibodies allow us to distinguish between the direct activation and anti-repression functions of GAL4-VP16, indicating that they function through distinct molecular mechanisms.

J8-112 *IN VIVO* FOOTPRINTING OF THE MURINE OCT3/4 GENE IN EC CELLS: Saverio Minucci[1], Ian Sylvester[2], Dina Zand[1], Anup Dey[1], Hans Schöler[2] and KeikoOzato[1], [1]Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland 20892 and [2]Gene Expression Programme, EMBL, 69012 Heidelberg, Germany

Oct 3/4, a member of the POU family of transcription factors, is expressed in the totipotent (ES) and pluripotent (EC) stem cells of the early mouse embryo, and is down-regulated when such cells differentiate. For this reason, Oct 3/4 is believed to play an important role in the first steps of the genetic cascade that regulates mammalian embryogenesis. In EC and ES cells, expression of Oct3/4 mRNA is rapidly down-regulated following RA treatment. In the present study, we have analyzed by dimethylsulphate-mediated genomic footprinting the *in vivo* occupancy of the upstream region of the Oct 3/4 gene in P19 embryonal carcinoma cells. We found that in undifferentiated cells, factor occupancy is detected both at an high affinity binding site for Sp1 in the proximal promoter region and at the retinoic acid-repressible enhancer at -1132 to -889. The *in vivo* occupancy at both sites becomes undetectable after all-trans RA treatment, in parallel with the greatly reduced steady-state RNA levels. In murine L fibroblasts, that do not express the Oct 3/4 gene, factor binding is not detected. These data indicate that coordinated occupancy of distinct regulatory regions takes place in undifferentiated cells expressing the Oct 3/4 gene, and that RA treatment leads to coordinated cessation of factor occupancy of this gene.

J8-113 THE NUCLEOSOMAL ORGANIZATION OF THE FIRST INTRON REGION IS INVOLVED IN EGFR GENE REGULATION IN BREAST CANCER CELLS, Jane M. Murphey, Kenneth R. McGaffin and Susan A. Chrysogelos, Department of Biochemistry and Molecular Biology and Lombardi Cancer Center, Georgetown University, Washington, DC 20007

Human breast cancer cells exhibit a wide range of epidermal growth factor receptor (EGFR) expression, with estrogen independent breast cancer typically characterized by high levels of EGFR. We have mapped the positions of DNase I hypersensitive sites (DH sites) in several human breast cancer cell lines whose EGFR levels differ by more than two orders of magnitude. A group of DH sites in the EGFR first intron was seen in high EGFR expressors, but not in low EGFR expressors, implicating this region in the control of EGFR overexpression. The regulatory ability of the hypersensitive regions of the EGFR first intron were examined with several CAT constructs and we observed that individual intron regions showed similar regulatory activity in cell lines that express either high or low levels of EGFR. However, when we transiently transfected a β-globin reporter construct that contains the 4kb region of EGFR that extends from the promoter through the hypersensitive regions of the first intron, we observed that this "native" configuration of the regulatory region of EGFR is able to reproduce the characteristic expression of EGFR in different cell lines. We next examined the nucleosomal organization of the first intron region of EGFR. Our most significant finding was that the nucleosomes were shifted by approximately 80bp when comparing high and low expressors. Additionally, there appeared to be a nucleosome "missing" in the EGFR first intron in high EGFR cells, as well as a region of disrupted nucleosomes. The differences in nucleosomal organization relative to the DH sites in these cell lines suggests that nucleosome phasing may control regulatory factor access to specific regions of the first intron that are involved in the up-regulation of EGFR. In other words, the higher order configuration of the EGFR gene determines its transcriptional state.

J8-114 SPECIFIC BINDING OF B4 AND/OR HMG-1 TO THE NUCLEOSOME, Karl Nightingale, Raymond Reeves* and Alan P. Wolffe, Laboratory of Molecular Embryology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892 *Department of Biochemistry & Biophysics, Washington State University, Pullman. WA 99164

Chromatin undergoes dramatic changes during *Xenopus* embryogenesis in both the type and abundance of its associated non-histone proteins. Here we examine two of these proteins: the putative linker histone B4, and a *Xenopus* homolog of HMG-1. Both proteins are highly abundant prior to MBT, but disappear by the end of gastrulation, correlating with the appearance and rapid increase in levels of somatic histone H1. We assess the binding of these proteins to chromatin by using reconstituted 5S mononucleosomes as a model system and examine both the specificity and nature of the interactions by a variety of assays.

J8-115 NEGATIVE REGULATION OF TRANSCRIPTION BY THE YEAST GAL11 PROTEIN INVOLVES ALTERATIONS IN CHROMATIN STRUCTURE Masafumi Nishizawa, Dept. Microbiol., Keio Univ. Sch. Med., Shinjuku, Tokyo 160, Japan

Gal11 is an auxiliary transcription factor that functions either positively or negatively and genetic analysis suggests that it functions by mediating the interaction of sequence-specific activators with the basal transcription machinery. Using reporters bearing various UAS and different core promoter structures, I found that Gal11's requirement for stimulation of transcription differs with a combination of DNA-bound activators and the structure of target promoters, that effect of GAL11 is not a function of the distance between UAS and the TATA box, and that negative regulation by *GAL11* involves alterations in chromatin structure. By chloroquine gel electrophoresis, *GAL11* appears to affect chromatin structure of a circular plasmid.

GAL11 is also involved in the regional regulation of transcription in which chromatin structure plays a major role: a *gal11*Δ mutation causes a partial relief of transcriptional repression at telomeres. The defective position effect in a *gal11*Δ strain was suppressed by overproduction of Sir3 whereas overexpression of *GAL11* failed to restore the telomere position effect in a *sir3*Δ strain. Hyperproduced Gal11 can partially suppress the defective silencing at *HMR* in a *sir1*Δ mutant. Overproduced Sir3 also could restore silencing at *HMR* in *sir1*Δ cells. In contrast, *SIR1* in a multicopy plasmid relieved the telomere position effect, especially in a *gal11*Δ mutant. Taken together, these results suggest that Gal11's role in transcriptional regulation involves at least in part alterations in chromatin structure.

J8-116 STEROID HORMONE-INDUCED CHANGES IN CHROMATIN STRUCTURE AT THE MMTV PROMOTER ASSOCIATED WITH INDUCTION OF PROMOTER ACTIVITY, Steven K. Nordeen, Marissa L. Moyer, Eva Zaniewski*, Christy Fryer*, Trevor K. Archer*, Dept. of Pathology, Univ. Colorado Health Sci.Ctr., Denver, CO, 80262 and *Dept. of Ob/Gyn, and Biochemistry, Univ. Western Ontario, London, ON, N6A 4L6

The DNA binding domains of glucocorticoid receptors and progesterone receptors are highly homologous and the two receptors exhibit identical DNA sequence recognition properties. Nonetheless, in the T47D(A1-2) mammary carcinoma cell line which has been engineered to express comparable levels of glucocorticoid (100,000/cell) and progesterone (200,000/cell) receptors, glucocorticoids are able to induce far greater expression of a stably integrated luciferase reporter gene under control of a truncated mouse mammary tumor virus (MMTV) promoter. Despite the fact that the truncated promoter contains only 224 basepairs 5' of the transcription initiation site, the sequences appear to direct assembly of a positioned nucleosome. Analyses of restriction enzyme accessibility of the MMTV DNA in isolated nuclei indicate a rapid induction of accessibility after administration of glucocorticoids, peaking in 30-60 min, that is accompanied by increased transcription factor loading as assessed by exonuclease III footprinting. Thus the ability of glucocorticoids to induce alterations in the chromatin environment of the promoter presages its ability to induce gene expression. Upon continued exposure to glucocorticoids, nuclease accessibility declines, returning to basal levels. Administration of additional hormone does not reinduce accessibility. In contrast to glucocorticoids, progestins mediate no increase in nuclease accessibility and little induction of luciferase gene expression. Although little induction is observed, progesterone receptors appear to be occupying target sites in the MMTV promoter since progestins and Type II antiprogestins can block glucocorticoid-mediated induction. Thus we find that changes in chromatin correlate with the ability of steroid hormones to induce expression of a target promoter.

J8-117 **INSULATION OF A HETEROLOGOUS GENE IN TRANSGENIC MICE BY SEQUENCES FLANKING THE K18 GENE BUT NOT BY SATB1 BINDING SITES.** Robert G. Oshima, Nickolay Neznanov and Terumi Kohwi-Shigematsu. La Jolla Cancer Research Foundation. La Jolla, CA. 92037

The keratin 18 (K18) gene is expressed in an integration site-independent and copy number-dependent fashion in transgenic mice. The 2.5 kb and 3.5 kb of DNA which flank the K18 gene were added to a fusion reporter gene consisting of the mouse metalothionein promoter driving the human growth hormone gene (NNMThGH). Expression of the hGH gene in transgenic mouse tissues was compared to either the control reporter gene or the same gene flanked by synthetic binding sites for the SATB1 nuclear matrix protein. RNA of the control MThGH gene was expressed in brain, heart, intestine, kidney, liver and testes confirming previous studies with this construct. However, not all of three independently derived transgenic mice expressed the gene in every tissue and the level of expression was not correlated with the copy number. In contrast, the MThGH gene insulated by the K18 flanking sequences was expressed in the same tissues in every of four independent transgenic animals at levels correlated with the copy number except for intestine. The average level of expression of the K18 insulated gene ranged from 8.4 to 49 fold higher than the control on a per gene basis. Circulated serum hGH protein was nearly 5 mg/ml in the highest expressing mouse. Five additional transgenic mice derived from the MThGH flanked by binding sites for the SATB1 nuclear matrix attachment protein did not express detectable MThGH RNA in brain, kidney or intestine except for one animal. However expression was nearly as efficient in testes as the K18 insulated gene, and 4/5 animals expressed detected levels of RNA in liver. These results demonstrate that the flanking sequences of the K18 gene are capable of insulating a heterologous transgene from position effects and appear not to adversely effect the tissue specific expression of the gene. In contrast, tandemly repeated synthetic nuclear matrix attachment sites did not appear to facilitate expression except in testes.

J8-118 METHYLATION-ASSOCIATED TRANSCRIPTIONAL REPRESSION OF HSP70 GENES IN MOUSE CELL LINES, B. Phillips and J.J. Gorzowski, Dept. of Obstetrics & Gynecology, Northwestern Univ. Med. Schl., Chicago, IL 60611

Several mouse cell lines fail to exhibit the marked transcriptional activation of the hsp70.1 gene which is normally seen after heat shock. In these lines, there is normal activation of HSF1, the transcription factor which mediates the heat shock response by binding to heat shock elements (HSE) in the promoters of heat shock genes. Furthermore, transcription of another heat shock gene, hsp90, is stimulated normally by heat shock in these same lines. The deficiency thus appears to reside in the hsp70.1 gene itself.

Genomic footprinting analysis revealed that HSF fails to bind to the endogenous hsp70.1 promoter in these cell lines during heat shock, thus explaining the lack of transcriptional responsiveness. Surprisingly, of four randomly chosen murine cell lines with heat shock responsive hsp70.1 genes, only one line, F9 embryonal carcinoma cells, exhibited a pronounced occupancy of the proximal and distal HSEs during heat shock, similar to that seen in primary mouse embryo fibroblasts (MEF). In the other three lines, the interaction was detectable but much less striking. In all of the cell lines examined, there was a very good correlation between the occupancy of Sp1 sites in the proximal hsp70 promoter and the binding of HSF during heat shock, suggesting that the binding of HSF reflects differences in the overall accessibility of the hsp70 promoter to transcription factors.

Southern blot analysis of a 1.2 kb region centered at the transcription start site of hsp70.1 revealed a clear correlation between the extent to which the methylation sensitive restriction enzymes Hpa2 and HhaI were able to cut at their sites and the accessibility of the promoter to Sp1 and HSF. These sites were completely unmethylated only in F9 cells and MEF and appeared to be fully methylated in all cell lines which lack hsp70 heat shock responsiveness. In the three cell lines which exhibit reduced promoter accessibility yet retain heat shock responsiveness, both hsp70.1 alleles are methylated, although there are some unmethylated sites in one or both alleles. Furthermore, in all cell lines exhibiting methylation of the hsp70.1 gene, the adjacent hsp70.3 gene is transcriptionally repressed and appears to be methylated as well. Thus, modifications of hsp70 chromatin structure which attenuate or block accessibility of the promoter to transcription factors occur frequently in cultured mouse cells.

J8-119 TARGETING THE X-LINKED HPRT GENE IN CELLS WITH TWO ACTIVE X CHROMOSOMES: A CELL CULTURE SYSTEM FOR THE ANALYSIS OF X CHROMOSOME INACTIVATION, Helen H. Tai, Karen Jardine, Michael W. McBurney, Departments of Biochemistry and Medicine, University of Ottawa, Ottawa, Ont., Canada K1G 8M5

Although female (XX) mammals have twice the number of X-linked genes as males (XY), the level of expression of these genes is the same in both sexes since one of the X chromosomes is inactivated in female somatic cells. Both X chromosomes are initially active in cells of female embryos until the early blastocyst stage when X inactivation is first detected. Either X chromosome is inactivated at random in cells of the inner cell mass that give rise to the embryo proper. The timing of X inactivation in the embryo seems to coincide with tissue differentiation.

Embryonal carcinoma (EC) cells resemble cells of the early embryo. P10 is a female EC cell line with two active X chromosomes which can be induced to differentiate with retinoic acid. This differentiation is accompanied by the inactivation of one X chromosome in each cell. Homologous recombination was used to target the X-linked HPRT gene in P10 cells. The selectable marker, β geo (confers G418 resistance), was used in a replacement vector. A clone with one intact HPRT allele (HAT resistant) and one targeted allele (G418 resistant) was screened using genomic Southerns. The cell line derived from this clone has two active X chromosomes so it is resistant to both HAT and G418 (selects for the targeted allele). Treatment of these cells with retinoic acid induces inactivation of one of the X chromosomes so cells are resistant to either HAT or G418 but not both. Therefore, this cell line can be used to study the X inactivation process as media additives can be used to distinguish cells that undergo normal X inactivation from cells that have been altered such that they do not inactivate an X.

J8-120 **THE FUNCTION OF ACETYLATED HISTONE AMINO TERMINI IN TRANSCRIPTION FACTOR BINDING TO NUCLEOSOMAL DNA, Michelle Vettese-Dadey and Jerry Workman, Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802**

Cooperative binding of a transcription factor, GAL4-AH, to nucleosomal DNA occurred in response to inhibition from the core histone amino termini. The binding of GAL4-AH to nucleosome cores containing multiple GAL4-AH binding sites initiated at the end of a nucleosome core and proceeded in a cooperative manner until all sites were occupied. However, following tryptic removal of the core histone amino termini, GAL4-AH binding appeared to be noncooperative, similar to binding naked DNA. Binding of GAL4-AH to a single site at different positions on trypsinized versus intact nucleosome cores indicated that inhibition of GAL4-AH binding was largely mediated by the histone amino termini. This effect occurred primarily at sites well within the core. Trypsinization of the amino termini is thought to mimic the post-translational acetylation of lysine residues within the tails. Consistent with this idea, acetylation of the amino termini enhanced GAL4-AH binding. Preferred binding of GAL4-AH to acetylated nucleosomes was detected by Western blots of mobility shift gels and immunostaining with anti-acetyl lysine, anti-acetyl H4Ac16 or anti-acetyl H4Ac5. Similarly, hyperacetylated nucleosomes containing the HIV-1 LTR show strong enhancement of USF binding. These data illustrate that factor binding to nucleosomes is augmented by two independent mechanisms, cooperative binding and histone acetylation.

J8-121 ANALYSIS OF NOVEL MUTATIONS IN HISTONES H3 AND H4, Mark A. Wechser and Craig L. Peterson, Department of Molecular Medicine, UMASS Medical Center, Worcester, MA 01605

We are analyzing a set of novel mutations, called *sin* alleles, in the genes encoding histones H3 and H4. *Sin* mutations alter invariant residues located within the central domains of both histone proteins. These mutations define a novel domain of the histone octamer that functions as a negative regulator of transcription.

We are using genetic and biochemical approaches to test the hypothesis that *sin* mutations cause an alteration in the structure of nucleosomes that impairs their ability to repress transcription. We have demonstrated that *sin* mutations derepress *PHO5* transcription to 25% of wild-type induced levels. We also find that bulk chromatin containing these mutant histones is much more sensitive to micrococcal nuclease digestion. We have successfully isolated nucleosomes from *SIN+* and *sin-* strains for further characterization.

This work has three specific aims: (1) to test whether *sin* alleles define discrete domains of histone H3 and H4 proteins, (2) to test whether *sin* mutations alter nucleosome structure, and (3) to identify nonhistone proteins that functionally interact with the histone octamer.

J8-122 EFFECT OF HISTONE ACETYLATION ON TRANSCRIPTION FACTOR BINDING, Jaya G. Yodh, Anthony N. Imbalzano, and Robert E. Kingston, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

A strong correlation exists between hyperacetylation of core histones and increased levels of transcription in eukaryotes. Our laboratory is studying this phenomenon at the level of transcription factor binding to nucleosomal templates. We have examined the binding of TATA Binding Protein (TBP) to the TATA box in three different rotational positions on a phased mononucleosome, and have shown that a nonacetylated nucleosome inhibits binding of TBP to any of these templates (Imbalzano et. al., (1994) Nature 370, 481-485). We substituted bulk-actylated histone octamers for nonacetylated core histones in nucleosome reconstitutions and found that this facilitates binding of TBP in a rotationally-dependent manner. Nucleosome pools generated from bulk-acetylated histones are highly heterogeneous with respect to their acetylation state (each of the four core histones can contain from one to four acetyl groups on their N-terminal tails). In order to more fully understand the mechanistic role of the number and position of acetyl groups in the facilitation of TBP binding, we have purified homogeneous populations of histone H4 which are tri- and tetra-acetylated and will use them to reconstitute nucleosomes with defined levels of acetylation. We hope to analyze, by a combination of EMSA and footprinting techniques, the binding of both TBP and the upstream activator, GAL4, to these specifically acetylated nucleosomes.

Replication on the Nuclear Matrix and Nucleosome Assembly; Role of the Nuclear Matrix and Chromatin Transitions in the Regulation of Gene Expression

J8-200 **A ROLE FOR NUCLEAR FACTORS AND NUCLEOSOME REARRANGEMENT IN CHROMATIN STRUCTURAL CHANGES AT THE MOUSE KAPPA IMMUNOGLOBULIN 3' ENHANCER**, **Veronica C. Blasquez, Ma. Corazon N. Roque, and Patricia A. Smith, Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556.**

Transcription of the mouse kappa immunoglobulin gene is controlled by two enhancers: the intronic enhancer (Ei) that occurs between the joining (Jk) and constant (Ck) gene segments and the 3' enhancer (E3') which is located 8.5 kb downstream of the gene. To understand the role of E3' in the activation of the kappa immunoglobulin locus, we have analyzed its chromatin structure in a number of cultured B-cell lines arrested at various stages of differentiation. These studies indicate that the DNAse I hypersensitive pattern of digestion at E3' undergoes developmental stage-specific changes in structure. To elucidate the molecular basis of these chromatin alterations, we have carried out in vivo footprinting experiments and micrococcal nuclease digestion analyses. Our results indicate the combined effect of protein factor binding and dissociation and nucleosomal rearrangement in the induction of the chromatin changes observed. We present a model depicting a dynamic E3' chromatin structure that correlates with the functional state of the kappa gene.

J8-201 CHARACTERIZATION AND ISOLATION OF A SILENCER PROTEIN AND AN ANTISILENCER PROTEIN INVOLVED IN VIMENTIN GENE EXPRESSION, Indira Carey, Alma Bracete and Zendra E. Zehner, Department of Biochemistry and Molecular Biophysics, MCV/VCU, Richmond, VA 23298-0614

Vimentin is an intermediate filament protein normally expressed in cells of mesenchymal origin. The vimentin gene has a complex expression pattern, since it is developmentally and cell-cycle regulated, as well as serum, phorbol ester, TGF-β and FGF inducible. In addition, the gene is expressed in many cultured cells, regardless of origin. In chicken, there are three copies of a silencer element which downregulate vimentin gene expression in fibroblasts. We have also described a unique element which overides the silencers and restores gene expression in fibroblasts. This element has been termed an antisilencer because it functions only in the presence of the silencers. Interestingly, the binding activities of the silencer and antisilencer proteins correlate with vimentin gene expression during development, as well as upon TPA induction. In order to define the mechanism of vimentin gene expression, and to study the interaction of these proteins, we have purified both factors after Sephacryl S-300, DEAE-Sephadex and Hydroxylapatite chromatography, followed by DNA affinity chromatography. Two major polypeptides are purified by the silencer affinity column. These correspond to 86 kDa and 70 kDa in size. Amino acid sequencing of peptides generated by tryptic digest revealed that these two proteins are identical to the Ku autoantigens. These polypeptides have been implicated in the recruitment of a kinase which phosphorylates the CTD of RNA Polymerase II. Additionally, they copurify with a binding activity for the TRA element of the human transferrin receptor gene. Hence, they appear to play a role in transcriptional regulation. Further directives will examine the mechanism by which the Ku proteins are involved in vimentin gene expression. The antisilencer affinity column purifies three major polypeptides of 105, 97 and 90 kDa in size. Southwestern blot analysis indicates that the largest protein is probably the antisilencer. Initial information reveals that the N-terminus of this polypeptide is unblocked, therefore we are initiating sequencing of this protein, which will enable us to either identify it, or to begin cloning of the cDNA.

J8-202 CHROMATIN SUBSTRUCTURE: STRUCTURAL STATES OF THE NUCLEOSOME AND GENE EXPRESSION

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Nucleosome structure and its changes are now being recognized as important in fundamental cellular processes. Recent studies indicate that the nucleosome is a biochemically active structure: it participates in facilitating or inhibiting transcription[1], reflects the physiological state of the cell, and is even implicated as a crucial element in human disease[2]. Despite studies of this macromolecular complex which indicate a dynamic structure that changes with ionic environment, various post-translational modifications and gene activation, all current X-ray and neutron diffraction studies have consistently resulted in one structure, suggestive of a static conformation[3]. In contrast, studies using electron microscopy have yielded a different structure[4]. These differences have been addressed by us and resolved in a detailed study of nucleosome structure with respect to ionic environment. Using spectroscopic electron microscopy, novel analyses of nucleosome structure[5], and newly developed 3D reconstruction techniques[6,7] we find a series of conformational states in which nucleosome structure ranges in shape from prolate to oblate and from highly extended to compact. This study of nucleosome conformation with ionic environment also forms a structural basis for our analysis of the 3D structure of hyper-acetylated nucleosomes purified from transcriptionally active chromatin via affinity chromatography[8]. The 3D structure of this type of nucleosome indicates a dramatically more open and accessible structure. In summary, the results of our investigation of nucleosome structure with ionic environment and gene-expression indicate transitions in nucleosome structure and a dynamic nature for the particle in accord with genetic and biochemical studies[1].

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J8-203 **DNASE IN VIVO FOOTPRINTING REVEALS TISSUE-SPECIFIC HYPERSENSITIVITY OF A MIRROR-REPEAT SEQUENCE WITHIN THE OVALBUMIN STEROID-DEPENDENT REGULATORY ELEMENT. Diane M. Dean, Paul S. Jones, and Michel M. Sanders, Department of Biochemistry, University of Minnesota, Minneapolis, MN 55455**

Induction of the chicken ovalbumin gene by steroid hormones requires the steroid-dependent regulatory element (SDRE: -900 to -780). Within the SDRE there exists a ten base pair near perfect mirror repeat (-897 to -877). Chloramphenicol acetyltransferase (CAT) reporter constructs were tested by transient tranfection assay in primary oviduct tissue culture cells. Removal of the repeat completely abolished the response to steroids and deletion of one half of the repeat resulted in a 50% reduction. *In vivo* DMS footprinting of this region revealed an estrogen-dependent protection of the central guanine residues -885 and -889 and hypersensitivity of several adenine residues within and flanking the repeat. CAT constructs with point mutations based on the DMS footprint show reduce steroid responsiveness. To further examine the DNA configuration of the mirror repeat, *in vivo* DNase footprinting was performed on nuclei isolated from estrogen-stimulated and -withdrawn oviduct and from liver. All three tissues exhibit protection of the mirror repeat when compared to naked DNA. However, estrogen-dependent and tissue-specific hypersensitivity was observed within the repeat at base -889 and flanking it at -905 and -864.These data are consistent with the hypothesis that an estrogen-responsive transcription factor(s) acts on the SDRE resulting in a tissue-specific and steroid-dependent chromatin arrangement.

J8-204 APPROPRIATE ACTIVITY OF THE PROMOTER FOR HUMAN CD21 REQUIRES CHROMOSOMAL INTEGRATION. Dehoff, M., [1]Pham, C., [1]O'Connor, S., [1]Jacobi, S., Makar, K., and Holers, V. M. University of Colorado Health Sciences Center, Denver, CO and [1]Washington University School of Medicine, St. Louis, MO, USA.

Human Complement Receptor 2 (CR2/CD21) is expressed during the late-pre and mature stages of B lymphocyte development. We have identified in the CD21 gene DNAse hypersensitive sites near the transcriptional initiation site and within the first intron. The 5' promoter region of CD21 contains a number of binding sites for ubiquitous transcriptional activators (SP1, E-box/USF and AP-1/AP-2). Transient transfection using CD21 -5 kb to +75 bp fused to CAT or neomycin reporter genes reveals no cell or stage specificity, as the promoter is active in all cell lineages tested (B and T lymphocyte, erythrocyte, monocyte). Likewise, gel shift analyses using these activator regions showed no differences across these cell lines. Constructs containing the -5 kB to +75 bp CD21 promoter region driving the neomycin reporter gene, with a 2 kB segment of the first intron containing the hypersensitive sites, were then transiently transfected into human cell lines. Expression was again not appropriately regulated. Cell and stage appropriate expression did, however, occur in stably transfected cell lines with this construct. This expression pattern was confirmed in transgenic mice utilizing the same construct, as neomycin RNA was expressed in spleen, but not in kidney or liver. These data strongly suggest that cell and stage specific regulation of expression of the human CD21 gene is conferred through activity of intronic elements that require chromosomal integration.

J8-205 HISTONE DOMAINS REQUIRED FOR CHROMATIN ASSEMBLY, Lita A. Freeman and Alan P. Wolffe, Laboratory of Molecular Embryology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.

We are interested in the mechanisms of histone assembly into replicating chromatin. Nucleosome assembly following the replication fork is staged: first a tetramer of histones H3 and H4 is deposited onto newly synthesized DNA, then two histone H2A/H2B dimers are assembled to form the octameric core, and finally histone H1 is added to the outside of the nucleosome. The X-ray structure of the histone octamer delineates histone domains involved in histone-histone and histone-DNA interactions. We have mutated these histone domains and examined the ability of mutant histones to incorporate into replicating chromatin during early *Xenopus laevis* development.

J8-206 EXTRACORPOREAL PHOTOCHEMOTHERAPY FOR CUTANEOUS T-CELL LYMPHOMA - DO RESPONSES IN PATIENTS RESULT FROM THE CONCERTED EFFECTS OF TRANSCRIPTION FACTOR INDUCTION AND ADDUCT REPAIR?
F. P. Gasparro, Yale University Photobiology Laboratory, Department of Dermatology, New Haven CT 06510

Since the inception of this modality (also called photopheresis) in 1982, the elucidation of the mechanism underlying clinical response in CTCL patients has remained elusive. Because only a small fraction of malignant cells are treated during a single phototherapy session, it has been assumed that an auto-vaccination process was occurring. How these effects could be linked to the well-characterized formation of psoralen-DNA photoadducts has confounded many investigators. The collective consideration of phenomenological observations have now redirected our approach. It has been shown that proteins and lipids are also modified by 8-MOP and that increased amounts of TNF-α and H_2O_2 are produced during photopheresis. In addition, *in vitro* studies have shown that 8-MOP/UVA alter cytokine secretion patterns and enhances the expression of class I MHC molecules on the surface of treated cells within 24 hrs. These are valuable clues that indicate a common cellular regulation process may be activated by 8-MOP/UVA. Cellular stress (*e.g.*, as a result 8-MOP/UVA induced photomodification of biomolecules and perhaps the generation of reactive oxygen species) is a potent inducer of transcription factors (*e.g.*, NF-κB). The genes for TNF-α and class I MHC both contain NF-κB sites which if occupied lead to the enhanced synthesis of their respective gene products. Since *de novo* protein synthesis is not required for their activation, stress-induced transcription factors are rapidly upregulated and may occur within the 3 hr photopheresis period. These events coupled with the intiation of the repair of 8-MOP DNA photoadducts (and the concomitant effects on chromatin structure)may lead to the expression of greater amounts of cytokines and class I MHC molecules which could stimulate the patient's formerly quiescent immune system.

J8-207 CHROMATIN AND GLUCOCORTICOID RECEPTOR FUNCTION. T. Grange, M.-L. Espinas, J. Roux and R. Pictet, INSTITUT J. MONOD, Univ. Paris 7, 2 Place Jussieu, 75251 PARIS CEDEX 05, FRANCE.

The glucocorticoid receptor (GR) activates transcription through interaction with regulatory sequences termed glucocorticoid-responsive units (GRUs). The GRUs are constituted of the assembly of binding sites for the GR and other transcription factors. In many cases, the GR is able to trigger a local alteration of the nucleosomal structure that allows the recruitment of the other transcription factors. We show here that various regulatory pathways can affect the modality of transcription factor recruitment at a GRU.

The model system analyzed is the -2,500 GRU of the rat tyrosine aminotransferase gene (TAT). An array of phased nucleosomes are present over and around the GRU and GR activation leads to the structural alteration of two nucleosomes positioned over this GRU. The consequences of this chromatin transition vary in different hepatoma cell lines.

In H4II cells, the liver-enriched transcription factor HNF3 interacts in a strictly glucocorticoid-dependent manner with this GRU, the "intact" positioned nucleosomes are able to interfere with HNF3 interaction. The glucocorticoid-dependent interaction of the GR with DNA is not detectable per se. However regular but transient interaction of the GR is necessary to maintain HNF3 interaction.

In Fto2B cells, HNF3 is able to interact with DNA in the absence of glucocorticoids, despite the presence of "intact" nucleosomes. The chromatin transition triggered by the activated GR improves slightly HNF3 recruitment. Furthermore, protein kinase A (PKA) controls the duration of the interaction of the GR with DNA. PKA must be activated to allow the GR interaction to last long enough to be detectable per se. PKA stimulation also improves slightly HNF3 recruitment irrespective of the chromatin structure of the GRU. Finally, PKA enhances the glucocorticoid response in an HNF3-dependent manner.

In conclusion, different regulatory pathways exert their effect by affecting either chromatin structural transition or the functional consequences of these transitions.

J8-208 TRANSCRIPTIONAL REGULATION OF THE NOVEL ACUTE PHASE PROTEIN LIPOPOLYSACCHARIDE BINDING PROTEIN (LBP), Carsten Kirschning, Norbert Lamping, Anett Unbehaun, Dagmar Pfeil, Friedhelm Herrmann and Ralf R. Schumann, Max-Delbrück-Center for Molecular Medicine (MDC), D-13122 Berlin, Germany

Lipopolysaccharide Binding Protein (LBP) is a 58/60 kD glycoprotein synthesized in the liver and secreted into the blood stream. It binds with high affinity to Lipopolysaccharide (LPS) of Gram-negative bacteria and mediates the secretorical response of macrophages by transferring LPS to the CD14 molecule.
The expression of LBP during the acute phase increases 10- to 100-fold as we show by Northern blotting and nuclear run-on experiments in an animal model, and by in-vitro stimulation of hepatoma cell lines with IL-1, IL-6, LIF and TNF. We found a maximum transcript accumulation 24 hours after stimulation, that was due to both, transcriptional and post-transcriptional mechanisms. The 5´ region of the gene was sequenced by us and the mechanism of LBP expression was analyzed by primer extension and luciferase reporter gene assays. Furthermore, characterization of the entire gene and DNase I hypersensitivity tests were performed. A singular transcription start site and a TATA box upstream were found, constituting a typical promoter structure. Two STAT-3 binding sites, characteristic for acute phase promoters were determined to be crucial for transcriptional activation of the LBP gene. Other hepatocyte- and acute phase-typical recognition elements like AP-1, NFkB, GCRE and ets-1, furthermore were identified within the LBP promoter. The coding region of LBP, consisting of 16 exons, contained several putative regulatory elements that are also currently under investigation. DNase-I hypersensitivity testing revealed the LBP gene region to be in an active chromatin structure in HUH-7 cells as opposed to control cells, not secreting LBP. These results suggest, additionally to classical transcriptional mechanisms, an involvement of a change in the chromatin structure in the activation of the LBP gene to be of importance in LBP transcript induction.

J8-209 **COMPETITION BETWEEN HISTONES AND SEQUENCE-SPECIFIC DNA-BINDING PROTEINS IN YEAST.** Randall H. Morse*, Kellie B. Cummings, Rachael Jones, Nicholas Kent, Steven Hanes, Benjamin D. Hall[1], and Craig L. Peterson[2], Wadsworth Center, NY State Dept. of Health, Albany, NY 12201-2002. [1]Dept. of Genetics, U. of Washington, Seattle WA [2]U. Mass. Medical Center, Worcester, MA

Histones and sequence-specific DNA binding proteins compete for binding to DNA in vivo. We are studying the variables which influence the outcome of this competition using stable episomes in yeast. Binding sites for transcription factors are engineered in plasmid sites which are packaged into positioned nucleosomes in yeast, and chromatin structure is then monitored in the presence and absence of the given factor. Using this strategy, we are investigating the binding of GAL4 and Drosophila bicoid protein to DNA in chromatin. In the case of GAL4, we have found that binding to a site in a positioned nucleosome perturbs chromatin structure, and that a secondary perturbation is caused by an unmasked activation domain. We are currently investigating whether TATA-binding protein or RNA polymerase II are involved in this secondary, activation-domain dependent perturbation of chromatin structure. We are also exploring the effect of the SWI/SNF complex on factor binding to nucleosomal and non-nucleosomal sites. Finally, we have examined interaction of TFIIIC with a weakened binding site in vivo; our results suggest that binding site affinity is an important variable in determining whether a factor can successfully compete with the histones for occupancy of a site in vivo.

J8-210 **GLUCOCORTICOID INDUCIBLE RNA TRANSCRIPTION FROM BPDE ADDUCTED TEMPLATE DNA**

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We have measured transcription from a construct derived from mouse mammary tumor virus, in which most of the viral sequence between the two long terminal repeats (LTR) is replaced by thymidine kinase (*tk*) gene from herpes simplex virus. The *tk* gene lacks its own promoter and is transcribed from the glucocorticoid inducible promoter located in the upstream LTR. The mouse cell line L1.4-3 contains a single 1½ tandem copy of this construct [Zaret & Yamamoto 1984, *Cell* 38:29-38] and provides a very useful system to study chromatin structure and its function in regulating transcription. Benzo[a]pyrenediol epoxide (BPDE), a potent chemical carcinogen which forms bulky chemical adducts to DNA, can both perturb the chromatin structure and inhibit replication and transcription by DNA and RNA polymerases, respectively. We find >70% inhibition of *tk* mRNA levels in cells treated with a final concentration of 3 μM (+)*anti*-BPDE for 1 hour. Moreover, this inhibition follows single-hit kinetics suggesting that BPDE adducts to DNA template are directly responsible for this effect. We are currently investigating whether this effect is due to adducts formed in the promoter region (thus inhibiting initiation of transcription by hormone-receptor complex, *i.e.*, changing the chromatin structure required for this interaction) or simply due to adducts formed in the path of RNA polymerase. Preliminary studies indicate that *tk* transcription returns to >60% of normal levels within 24 hours, if cells are allowed to repair in a quiescent state.
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J8-211 EPIGENETIC REGULATION OF HSP 70-1 GENE TRANSCRIPTION AT ONSET OF ZYGOTIC ACTIVITY IN THE MOUSE , Renard J.P., Chastant S., Thompson E., and Christians, E. Unité de Biologie du Développement, INRA Jouy en Josas, 78352, France.

Activation of the mouse embryonic genome at the 2-cell stage is characterized by the synthesis of several a-amanitin-sensitive polypeptides, some of which belong to the multigenic hsp 70 family. We show that a member of this family, the *HSP 70.1* gene, is highly transcribed at the onset of zygotic genome activation. Transcription of this gene began as early as the 1-cell stage. Expression of the gene continued through the early 2-cell stage but is repressed before the completion of the second round of DNA replication.Interestingly, the coincidence of repression of HSP70.1 transcription with the second round of DNA replication was not found for other transcription-dependent polypeptides synthesized at the 2-cell stage. The level of transcription of HSP 70-1 appears to be modulated by in vitro culture conditions and by nucleo cytoplasmic exchanges occuring at the one cell stage as evidenced by nuclear transfer experiments.

J8-212 THE INVOLVEMENT OF A NUCLEAR MATRIX ATTACHMENT REGION IN REGULATING THE CD4 GENE, John F. Sands, Rhonda K. Hansen, and Paula M. McCready, Department of Microbiology and Molecular Genetics, Loma Linda University, Loma Linda, CA 92350

The chromosomal locus for the murine CD4 gene has been extensively analyzed for possible transcriptional control elements using DNase I hypersensitive site assays, nuclear matrix attachment site analysis, repetitive sequence analysis, and deletion clones that systematically remove DNA from the 15 kb immediate 5' of the promoter and from the large 9 kb first intron. Numerous positive and negative *cis*-acting elements have been identified. One of the positive *cis*-acting elements is a DNA sequence located about 13 kb 5' of the CD4 promoter. Between this 5' enhancer and the CD4 promoter is large region of DNA that negatively effects this enhancer's ability to stimulate the promoter. In transient transfection assays, the deletion of the negative intervening sequence between the CD4 promoter and the 5' enhancer is essential for the 5' enhancer to work productively on the CD4 promoter. The 5' enhancer is located in the midst of a nuclear matrix attachment region which could only be detected in $CD4^+CD8^+$ cells. This 5' enhancer has been shown to be essential for expression of the CD4 gene at the $CD4^+CD8^+$ stage of thymocyte development. Recently, a MAR/SAR DNA binding protein, SATB1, expressed primarily in $CD4^+CD8^+$ thymocytes, has been identified (Cell 70:631-645). We find that the chromosomal configuration of the CD4 gene in double positive cells is in a much more open state than in $CD4^+CD8^-$, $CD4^-CD8^+$, or $CD4^-CD8^-$ cells suggesting that the chromatin at the CD4 locus may have been altered as a result of the interaction of the 5' MAR with the stage-specific SATB1 protein. We also propose that the MAR/enhancer *cis*-acting element located about 13 kb 5' of the CD4 gene may be working in conjunction with another MAR sequence located immediately upstream of the CD4 promoter to juxta-position the 5' enhancer next to the CD4 promoter during the $CD4^+CD8^+$ stage of thymocyte development thereby activating the CD4 promoter.

J8-213 NUCLEAR MATRIX ATTACHMENT REGIONS (MARs) IN PLANTS: AFFINITY FOR THE NUCLEAR MATRIX AND EFFECT ON TRANSIENT AND STABLE GENE EXPRESSION, Steven Spiker[1], George C. Allen[2], Gerald E. Hall, Jr.[1], Susan Michalowski[1], Winnell H. Newman[1], William F. Thompson[2] and Arthur K. Weissinger[3], Departments of Genetics[1], Botany[2] and Crop Science[3], North Carolina State University, Raleigh, NC 27695

We have isolated a number of DNA sequences based upon their ability to bind specifically to the nuclear matrix of tobacco (*Nicotiana tobaccum*). We have determined the sequences of these *circa* 1 kb fragments and measured their affinities for the isolated nuclear matrix by an exogenous rebinding assay. Affinities range from "strong" (90% bound to nuclear matrix in a standard binding assay) to "weak" (10% bound to nuclear matrix in a standard binding assay). We determined the effects of flanking MARs on stable transcription of a GUS (β-glucuronidase) reporter gene in tobacco cells transformed by microprojectile bombardment. When a weak MAR was used, a 12-fold increase in average level of gene expression was observed. Despite the overall increase in gene expression, copy number dependent expression was not attained. When a strong MAR was used, an over 60-fold increase in average level of stable gene expression was observed. Still, copy number dependent expression was not attained. These results contrast with those in animal systems in which flanking MARs do sponsor copy number dependent gene expression. In transient expression assays in electroporated tobacco protoplasts, the MARs had only slight positive effects on levels of gene expression.

Active Chromatin Domains and the Control of Higher Order Structure in the Cell Nucleus; Global Transactivators

J8-300 FACILITATED ENHANCER ACTIVATION IN TRANSGENIC CHROMATIN BY EXTENDED ENHANCER-FLANKING SEQUENCES Bruce J. Aronow, Catherine A. Ebert, David P. Witte, and John J. Hutton Division of Basic Science Research, Department of Pediatrics, University of Cincinnati College of Medicine, Children's Hospital Medical Center, Cincinnati, Ohio 45229

We have defined novel gene regulatory elements, termed "facilitators", in transgenic mice. These elements bilaterally flank, by up to 1 kb, a T-cell specific enhancer in the human adenosine deaminase (ADA) gene. Facilitators were essential for gene copy-proportional and integration site-independent reporter expression in transgenic thymocytes, but had no effect on the enhancer in transfected T-cells. Both segments were required. Individual segments had no activity. Lack of facilitators caused positional susceptibility and prevented DNAse I hypersensitive site formation at the enhancer. The segments were required to be at opposed ends of the enhancer and could not be grouped together. Reversing the orientation of a facilitator segment caused a partial loss of function, suggesting involvement of a stereospecific chromatin structure. Trans-acting factor access to the enhancer was modeled by exposing nuclei to restriction endonuclease. The enhancer domain within the intact regulatory region was accessible to endonuclease in a tissue and cell type specific fashion. However, unlike DNAse I hypersensitivity and gene expression, accessibility to the endonuclease could occur without the facilitator segments suggesting that an accessible chromatin domain is an intermediate state in the activational pathway. These results suggest that facilitators: (1) are distinct from yet positionally constrained to the enhancer, (2) participate in a chromatin structure transition necessary for DNAse I hypersensitivity and function of the enhancer, and (3) act after cell type-specific accessibility to the enhancer sequences is established by factors that do not require the facilitators to be present.

J8-301 RETINOBLASTOMA PROTEIN (RB) REGULATION OF HLA CLASS II GENE EXPRESSION. George Blanck[1], Yanmei Lu[1], David Marler[1] and Hong-Ji Xu[2]. [1]Department of Biochemistry and Molecular Biology, University of South Florida College of Medicine, Tampa, FL 33612 and [2]Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77382.

The HLA class II proteins bind antigen peptides for presentation to CD4+ T-cells. These proteins are highly polymorphic, and certain alleles are more common among individuals with autoimmune diseases, such as multiple sclerosis and juvenile onset diabetes. Also, the class II proteins have been shown to play a role in tumor rejection by the immune system. Recent work from our lab indicates that the interferon-γ (IFN-γ) activation of HLA class II proteins in RB-defective cells is either very weak or nonexistent. Replacement of RB in two RB- cell types restores IFN-γ inducibility. Thus, RB defects may not only contribute to loss of growth control, but also to a reduction in tumor immunogenecity. For one tumor type, class II inducibility by IFN-γ was maintained in a series of revertants (RBrev) from RB+ transformants, which had lost RB, suggesting that the RB effect is epigenetic. Long-term passage of RB+ transformants and RBrev clones ultimately led to loss of class II inducibility by IFN-γ. Current work is directed toward understanding the molecular mechanism of the direct and epigenetic effects of RB on class II inducibility by IFN-γ.

J8-302 FLUORESCENCE MICROSCOPY OF POLYCOMB-GROUP PROTEINS IN THE EMBRYONIC DEVELOPMENT OF *DROSOPHILA*

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For proper development of the *Drosophila* embryo, homeotic genes have to be stably repressed in certain defined regions of the organism. This is achieved through an epigenetic mechanism involving the gene products of the Polycomb-group (Pc-group). These nuclear proteins are thought to assemble into multimeric complexes which repress target genes either by packaging into heterochromatin-like complexes or by sequestering them into inactive compartments within the interphase cell nucleus. The pattern of repression is clonally inherited within a cell lineage Studies using high resolution multiwavelength confocal microscopy of living and fixed embryos should provide insights into some of these processes:

(i) distribution of Pc-group proteins within embryonic nuclei in relation to each other, to DNA concentration and to other chromosomal proteins.

(ii) dynamics of the distribution of Pc-group proteins in living embryos.

(iii) pattern of Pc-group proteins in embryonic nuclei of identical lineage.

We are currently performing experiments in this direction and the results will be presented.

J8-303 **MULTIPLE INITIATORS AND C/EBP BINDING SITES ARE RESPONSIBLE FOR THE TRANSCRIPTION INITIATION OF THE RAT XANTHINE DEHYDROGENASE/ OXIDASE PROMOTER.** Chow, C., Clark, M., Rinaldo, J., Chalkley, R. Dept. of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232.

Xanthine dehydrogenase/oxidase (XDH/XO) has been proposed to play a role in ischemia-reperfusion injury and inflammation. It can be up-regulated by lowered oxygen tension as well as by acute-phase mediators including interferons and glucocorticoids. To understand the regulation of the gene in the molecular level, we have cloned and identified the promoter from the rat genome. The 5' end of the gene is extensively intronic. Using primer extension, RNase protection, and anchor-PCR, multiple transcriptional start sites have been identified. However, neither a TATA box nor an Sp1 binding site are present in the promoter. The absence of a TATA box has led to a search for initiator elements around the transcription initiation sites of the gene. Sequence similarity between the XDH/XO initiators and known initiator-driven genes was identified. Footprint analyses have revealed at least six footprints for *trans*-acting factor interactions. Two of these footprints mapped to the two most upstream transcriptional start sites of the gene. To identify the nature of the other *trans*-acting factors upstream of the initiators, gel mobility assays were performed. Results have indicated that there are several C/EBP binding sites. In the presence of antibodies specifically against C/EBP-α and/or C/EBP-β binding proteins, "super-shifts" of the DNA-protein complexes can be observed. C/EBP-δ antibody, however, does not recognize any DNA-protein complex. Deletional analysis of the promoter and transient transfection studies imply a potential cross-talk between the upstream elements and the downstream initiators. Deletion of these upstream elements abolishs the transcriptional activity; however, transcriptional activity can be restored by an Sp1 binding site that has been constructed upstream of the initiator elements. Transient transfection and in vitro transcription studies have implied an additional downstream regulatory factor present in the coding region of the gene. Cooperation between this downstream factor and the other upstream elements seem to be required for the physiologically correct level of initiation from each initiator region and possibly for the ability to respond to acute phase mediators.

J8-304 CHARACTERIZATION AND DNA-BINDING PROPERTIES OF THE CHROMOSOMAL HIGH MOBILITY GROUP PROTEIN cHMGI

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Rapidly proliferating cells of mammalian organisms contain large amounts of nonhistone chromosomal high-mobility group (HMG) proteins I and Y. This abundant appearance of these proteins in cells has been correlated with neoplastic transformation, however the functions of these proteins remain unclear. We are interested in elucidating the role of the HMGI/Y proteins by exploiting genetical and cytological properties of Dipteran insects: *Drosophila* and *Chironomus*. In this study we describe a chromosomal HMG protein from *C. tentans* with striking homology to the mammalian HMGI protein.

The *Chironomus* HMGI (cHMGI) protein was purified and chemically characterized from an epithelial cell line. cDNA clones encoding this protein were isolated and sequenced. The comparison of the primary structure of the insect cHMGI and human HMGI revealed the presence of three K/RXRGRP motifs which crucial role in DNA-binding has previously been demonstrated. In the gene each of the motifs is encoded by a different exon, a situation similar to the human gene. Using synthetic peptides we have shown that two of the motifs are necessary for high affinity binding of cHMGI to the minor groove of AT-rich DNA. The protein is also able to bind specifically to four-way junction DNA. The identification and characterization of HMGI proteins in insects with polytene chromosomes opens new possibilities for studying functions of this group of chromosomal proteins.

J8-305 AN NFAT SITE IN THE GM-CSF ENHANCER FORMS AN INDUCIBLE DNASE I HYPERSENSITIVE SITE, Peter.N. Cockerill, M.Frances Shannon, Andrew G. Bert, Gillian W. Cockerill, Gregory R. Ryan, Fay Jenkins and Mathew A. Vadas, Division of Human Immunology, Hanson Centre for Cancer Research, Institute of Medical and Veterinary Science, Frome Road, Adelaide 5000, Australia.

The closely linked interleukin-3 (IL3) and granulocyte-macrophage colony stimulating factor (GM-CSF) genes are are co-induced in T lymphocytes upon activation of the T cell receptor. GM-CSF, but not IL3, can also be induced in many other cell types such as endothelial cells by pro-inflammatory stimuli. To identify DNA elements that mediate the inducible and tissue-specific regulation of these genes we mapped DNase I hypersensitive (DH) sites across the GM-CSF/IL-3 locus. We located an inducible DH site, 3 kilobases upstream of the GM-CSF gene, which appeared just before the onset of IL3 and GM-CSF transcription in T cells and was also induced in all other cell types that could be induced to express GM-CSF. This DH site functioned as a strong enhancer of both the GM-CSF and IL3 promoters in T cells, and the GM-CSF promoter in endothelial cells, responding to protein kinase C and Ca^{2+} mediated signals. We have also detected five T cell specific DH sites upstream of the IL3 gene which are likely to control the T cell specific expression of IL-3. One of these sites functions as an inducible enhancer of the IL-3 promoter. Three ubiquitous constitutive DH sites are also located between the IL-3 and GM-CSF genes. These sites have no detectable enhancer activity but might function to partition the locus into differentially regulated domains.

The essential core of the GM-CSF enhancer lies within the inducible DH site and contains a novel type of NFAT site that binds NFATp with high affinity. Adjacent to the NFATp binding site is a CRE-like element that binds either CREB or AP-1 family proteins co-operatively with NFATp to form higher order NFAT complexes. Upon stable transfection into T cells, the high affinity NFATp-binding motif is sufficient to form an inducible DH site, but is insufficient for efficient enhancer function. We therefore hypothesise that NFATp functions in a highly co-operative manner to remodel chromatin and enhance the binding of AP-1 and CREB family proteins that interact directly with the transcription initiation complex.

J8-306 **Analysis of the relative affinities of several members of different regulatory elements for their respective transcriptional factors.**

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In T lymphocytes, the patterns of active genes are modified, through transition from resting to activated state. In the quiescent T cells some genes are constitutively expressed (TcR, CD4 or CD8 and $p56^{lck}$ genes) and can be subjected to down-regulation whereas other genes are silent and induced following stimulation (lymphokine and HIV genes). A computer analysis demonstrated that the promoters of the induced and down-regulated genes aforementioned shared cis acting elements interacting with several transcription factors (including NFκB, NFAT, huGATA3, Decanucleotide and Ets1). These target elements, showing weak to great variations in sequence, can differ in their affinity and specificity for a given factor (usually member of a protein family) and thus influence the transcriptional response. Indeed, the efficiency of transcription is governed by the fine tuning between transcriptional factors and their corresponding binding sequences.

That is the reason why the differences in binding capacities of two transcriptional factors, NFκB and huGATA3, for their respective regulatory elements present in several promoters, have been studied by electrophoretic mobility shift assay (EMSA) and surface plasmon resonance (SPR) (BIAcore™, Pharmacia Biosensor). Variations in binding capacities of 2 κB elements (from the IL-2Rα and the TcRVγ9 promoters) showing significant differences in the target sequence itself, were observed by the two technical approaches. Nevertheless, SPR was able to reveal small differences, in DNA interaction with purified NFκB p50 homodimer (A. Israël) and huGATA3 (P.H. Romeo), not detected by EMSA. Indeed, small changes in the flanking sequences of HIV κB elements were able, in and of themselves, to influence their binding capacities for the p50 homodimer.

In addition to purified factors, the SPR approach was used to analyse the specific DNA interactions with transcriptional factors present within the complex protein environment of the nuclear extracts, providing that the proteins actually complexed to the DNA are characterized. Indeed, the proteins interacting with the immobilized target elements, were collected and characterized as the genuine transcriptional factors by SDS PAGE, EMSA and Western-blotting.

We think that the development of this technology will contribute to establish the hierarchy of affinity of various members of different regulatory elements for their respective transcriptional factors. Finally, SPR and *in vivo* footprinting experiments will allow the characterization of the factors and the regulatory elements actually involved in the regulation of the constitutive and inducible genes throughout activation of the T cells.

J8-307 THE TRANSCRIPTION FACTOR MYOD CAUSES NUCLEOSOME DISPLACEMENT WHILE DIRECTLY ACTIVATING MYOGENIN, Anthony Gerber and Stephen Tapscott, Department of Pathology, University of Washington; and Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109

A chimera between MyoD and the hormone binding domain of the estrogen receptor was stably introduced into mouse fibroblast $10T^{1}/_{2}$ cells to generate a fibroblast cell line that can be induced to form skeletal muscle with addition of beta estradiol. Myogenin message could be detected in these cells within three hours following exposure to beta estradiol. Accumulation of myogenin message was associated with an alteration in chromatin structure at the myogenin promotor. In particular, a *Ban* I restriction endonuclease site adjacent to the proximal MyoD binding site was specifically cleaved when nuclei from induced cells were exposed to the enzyme, but not in nuclei from uninduced cells. Additionally, *Pst* I and *Pvu* II sites further upstream became sensitive to digestion following induction, presumably reflecting a change in nucleosome positioning which allowed greater enzyme access to the myogenin promotor in nuclei from induced cells. The time course of these events suggests that nucleosome displacement and transcriptional activation are tightly coupled. Furthermore, similar results are obtained when induction is carried out in the presence of cycloheximide, indicating that MyoD can participate directly in transcriptional activation and nucleosome displacement without the benefit of *de novo* protein synthesis.

J8-308 LYMPHOID PROTEINS FOUND TO INTERACT WITH LYMPHOID ENHANCER FACTOR, Katherine L. Guttridge and Marian L. Waterman, Department of Microbiology and Molecular Genetics, University of California, Irvine, Irvine CA 92717

Our lab is investigating the mechanisms by which Lymphoid Enhancer Factor (LEF-1) regulates gene expression in T cells. LEF-1 consensus binding sites are found in the promoters and/or enhancers of lymphoid genes, such as T Cell receptor (TCR) variant subunits α,β,γ,δ, the invariant subunits CD3 γ,δ,ε, as well as $p53^{lck}$, CD4 and the HIV-1 and -2 LTR. LEF-1 is a context-dependent transcription factor. In the TCRα gene, LEF-1 cooperates with two flanking elements (a site for the ubiquitous CREB/ATF family, and a second, less-well characterized site named TCF-2 which may include a member of the *ets* family) to create a powerful enhancer; LEF-1 does not act on its own to increase transcription rates from this site. LEF-1 sites are not always found next to CREB or TCF-2 elements, thus LEF-1 may cooperate with a variety of other factors to regulate gene expression in lymphoid cells. The C-terminal half contains an HMG box, which serves as a DNA binding/bending domain. The N-terminal half of LEF-1 contains an activation domain that is active only in T cells, and when fused to a heterologous DNA binding domain can cooperate with the flanking elements of the TCRα core to enhance gene transcription. This suggests that the activation domain makes contacts with other proteins to regulate transcription, and that at least some of these proteins must themselves be lymphoid-specific. To identify these interacting proteins, we have fused the N-terminal 308 amino acids, which contain the activation domain, to the DNA binding domain of the yeast protein Gal4, and are using this construct in a yeast two-hybrid screen to isolate cDNAs that encode proteins binding directly to the LEF-1 transactivation domain. Our screen has identified 90 positive clones, and we are currently testing these isolated clones with mutant LEF-1/Gal4 fusion proteins in yeast to determine their specificity. Results from these experiments will be presented.

J8-309 THE PRODUCT OF THE *VIVIPAROUS-1* GENE ENHANCES THE DNA-BINDING ACTIVITY OF SEVERAL TRANSCRIPTION FACTORS, Alison Hill, Andre Nantel, Ralph S. Quantrano, Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280

The maize VP1 protein is an embryo-specific regulator of gene expression which effects the regulation of a subset of Abscisic Acid (ABA)-dependent genes that are required for the seed maturation program. In addition to its role in the regulation of some ABA-dependent genes, VP1 has pleiotropic effects on seed development that are not related to ABA. VP1 is a 690 amino acid polypeptide with no detectable sequence homology to other proteins except its homologues in *Arabidopsis* (abi3) and rice (osvp1). In transient expression studies, VP1 has been shown to transactivate gene expression through at least two distinct promoter elements; the G-boxes and Sph1 element of the ABA-inducible Em promoter and the Sph1 box from the C1 promoter. We have investigated how Vp1 can transactivate gene expression through diverse promoter elements by analyzing its association *in vitro* with EmBP-1, a factor that binds the Em promoter. We demonstrate that VP1 can greatly enhance the DNA-binding activity of EmBP-1 to the ABRE in a gel retardation assay. The enhancement by VP1 is most pronounced when the target bears a low affinity binding site for the bZip factor or when the factor itself is at low concentration. Deletion of a small but highly conserved region (BR2) eliminates the enhancement while a 40 amino acid fragment containing BR2 can substitute for VP1. Attempts at showing a physical interaction between VP1 and EmBP-1 did not yield convincing results, but a weak interaction between BR2 with DNA is demonstrated by U.V. crosslinking. In addition, the capacity of VP1 to enhance DNA-binding activity has also been observed on transcription factors as diverse as opaque-2, max, Sp1 and NF-κB.This lack of specificity might explain why a mutation in the *Vp1* locus has pleiotropic effects.

J8-310 **AFFINITY ENRICHMENT AND FUNCTIONAL CHARACTERIZATION OF TRAX1, A NOVEL TRANSCRIPTION ACTIVATOR AND X1 SEQUENCE BINDING PROTEIN OF HLA-DRA.** YOSHIE ITOH-LINDSTROM, B. MATIJA PETERLIN, AND JENNY P.-Y. TING, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7295

The promoters of all class II major histocompatibility (MHC) genes contain a positive regulatory motif, the X element. The DNA binding proteins specific for this element are presumed to play a critical role in gene expression although there is a paucity of functional studies supporting this role. In this study, the X box binding proteins of HLA-DRA were affinity-purified from HeLa nuclear extracts. Fractions 46 - 48 contained an X box binding activity and determined by electrophoretic mobility shift assays (EMSAs) to be specific for the X1 element. This X1 sequence binding protein, TRAX1, was shown to be a specific transcriptional activator of the HLA-DRA promoter using an *in vitro* transcription assay. By UV cross-linking analysis, the approximate molecular weight of TRAX1 including the bound DNA was determined to be 40 Kd. When the TRAX1 complex was incubated with antibodies against a known recombinant X-box binding protein, RFX1, and tested in EMSAs, TRAX1 was neither shifted nor blocked by the antibody. Further analysis with methylation interference showed that TRAX1 bound to the 5' end of the X1 sequence at -109 and -108 and created the hypersensitive sites at -114, -113, and -97. This methylation interference pattern is distinct from the known X1 binding proteins, RFX1, RFX, NF-Xc, nor NF-X. Taken together, our results indicate that TRAX1 is a novel X1 sequence binding protein and transcription activator of HLA-DRA.

J8-311 MULTIPLE CONTROL ELEMENTS ARE FOUND IN DISTAL 5' FLANKING SEQUENCES OF THE HUMAN EMBRYONIC (EPSILON) GLOBIN GENE, Jin Li, Alan N. Schechter, Constance T. Noguchi, Lab of Chemical Biology, NIDDK, 10/9N307, National Institutes of Health, Bethesda, MD 20892

The developmental and tissue-specific control of human hemoglobin synthesis is a complex process involving two switches which control the sequential expression of specific globin genes from embryonic (epsilon) to fetal (gamma) and to adult (beta). A number of trans-acting proteins and cis-acting DNA elements have been found to be part of the switching machinery, including a silencer located in the region between -250 and -300 bp 5' to the epsilon-globin gene capsite, and positive control regions extending up to about 0.8 kb. In order to characterize other cis-acting element(s) involved in regulating epsilon globin gene expression, we made progressive deletions extending up to 6 Kb 5' of the epsilon-globin gene. These deletion mutants were assayed by transient transfection into human erythroid K562 and non-erythroid HeLa cells. The functional assay reveals marked variation in expression of the reporter gene with different 5' upstream deletions. The region between 2.8 kb and 5.8 kb which contains DNase I hypersensitive site I (HSI) of the globin locus control region (LCR) exhibits positive control in K562 cells, but negative control in HeLa cells. The region between 2.8 kb and 0.9 kb 5' exhibits negative control in K562 cells, but deletion of this region has no effect in HeLa cells. Truncating deletion mutants between 1.9 kb and 3 kb have been used to localize further these positive and negative control regions. These results suggest that the upstream 5' flanking region of the epsilon globin gene has multiple control elements which may function as both negative and positive regulators of gene expression and that developmental and possibly tissue specific expression of this gene results in part from interactions with these factors.

J8-312 CHANGES of HISTONE DEACETYLASE(S) BEHAVIOUR by Its ASSOCIATION with NUCLEAR MATRIX in CHICKEN ERYTHROCYTE

W Li and JR Davie, Dept of Biochemistry and Molecular Biology, University of Manitoba, Winnipeg, Manitoba, Canada, R3E 0W3.

The reversible posttranslational acetylation of core histones (H1, H2, H3 and H4) of chromatin is associated with a number of nuclear functions including DNA replication, transcription and DNA repair in eucaryote cells. The dynamic acetylation of core histones is controlled by histone acetyltransferases and histone deacetylases (HD). Two isoforms of HD were separated by Q sepharose ion exchange and gel filtration chromatography from chicken erythrocyte. The estimated molecular size of HD1 is about 45-65 KDa and it prefers deacetylating free H3.H4 relative to (^{3}H)H2A.H2B. HD2 is a multisubunit protein with molecular size of 200-240 KDa and its deacetylation preference for H3.H4 is much less obvious in comparison with that of HD1. HD1 and HD2 also differ in pH optimum, inhibition pattern to sodium chloride and sodium butyrate. It is found that HD2 can be dissociated into HD1 by 1.6 M of sodium chloride and 1% 2-mercaptoethanol. The enzymatic properties of HD associated with nuclear matrix show remarkable difference from that of both HD1 and HD2, particularly in its striking preference for H2A.H2B. Furthermore, HD activity associated with nuclear matrix can be solubilized and behaves like HD1 and HD2 on chromatography and substrate preferences. These results suggest that by association with other nuclear proteins and nuclear matrix,creating multisubunit protein and association with nuclear matrix, HD develops multiple isoforms with different enzymatic property and fulfils multiple functional demands in the nuclei.

J8-313 **IDENTIFICATION OF A LOCUS CONTROL REGION FOR THE HUMAN GROWTH HORMONE GENE CLUSTER,** Stephen A. Liebhaber[1,2,3], Beverly Jones[1,2], Bob R. Monks[2,3], and Nancy E. Cooke[1,2], Departments of Medicine[1], Genetics[2], and the Howard Hughes Medical Institute[3], University of Pennsylvania School of Medicine, Philadelphia, PA 19104, U.S.A.

Locus control regions (LCRs) are considered to mediate localized decondensation of chromatin facilitating access of *trans*-acting factors to promoters and interactions between the usually distal LCR and proximal promoter elements. LCRs have been defined in relatively few systems and detailed characterizations have been limited. The *human growth hormone* (*hGH*) locus contains five genes. Expression of *hGH-N*, the most 5′ gene, is strictly limited to the pituitary, while the remaining genes are expressed selectively in the placenta. In transgenic mice, *hGH-N* with up to 7.5 kb of 5′-flanking sequences was expressed only sporadically and with loss of tissue specificity indicating that it was subject to position effects. Four DNase I hypersensitive sites (HS) were identified in pituitary chromatin 14 kb to 32 kb 5′ of *hGH-N*. Extension of the transgene to encompass these HS resulted in loss of position effects with consequent high-level, pituitary-specific, copy-number dependent transgene expression. The four HS could be divided into two subsets that separately mediated position-independent pituitary expression although demonstrating relaxed tissue specificity. The proximal set also displayed potent chromatin-dependent enhancer activity. We conclude that the *hGH* gene cluster requires an LCR composed of these two HS subsets that must interact to establish the full site-of-integration independent transcriptional activation and tight tissue specificity of *hGH-N* expression *in vivo*.

J8-314 MULTIPLE ELEMENTS IN THE SV40 PROMOTER CAN CONFER NUCLEASE HYPERSENSITIVITY, Barry Milavetz, RaeJean Hermansen, and Michael Friez, Dept of Biochem. and Mol. Biol., School of Medicine, University of North Dakota, Grand Forks, ND 58202

We have analyzed the Simian Virus 40 (SV40) promoter for the presence of DNA elements capable of conferring restriction endonuclease hypersensitivity in chromatin using an SV40 based recombinant reporter system. The reporter system consists of a polylinker of various unique restriction endonucleases introduced into SV40 at nt2666. The restriction sites were used both for the introduction of test DNA sequences from the SV40 promoter and for measuring the effects of the introduced sequences on the restriction endonuclease sensitivity at each of the restriction sites. For initial studies the SV40 promoter was divided into three functional regions; the earl domain nt5149-5232, the enhancer domain nt103-177, and the late domain nt255-424. Subsequently, each of the domains was subjected to a deletional analysis to further localize the elements responsible for the conferred nuclease hypersensitivity. In the early domain an element responsible for conferred hypersensitivity was localized to nt5164-5187. In the enhancer domain an element was found between nt113-133. In the late domain two elements were found; one between nt255-310 and second between nt372-424. Although we have not yet demonstrated that specific proteins are involved in conferring nuclease hypersensitivity, it is interesting that the enhancer element coincides with the Jun binding site and the late elements contain the binding sites for two of the Late Promoter Binding proteins. No protein has yet been identified which binds to the element localized to the early domain.

J8-315 REGULATION OF IL-2RECEPTOR α GENE TRANSCRIPTION : BIPARTITE DISTRIBUTION OF INTERLEUKIN RESPONSE ELEMENTS, Markus Nabholz, Elisabetta Soldaini, Peter Sperisen, Maria Pla, San-Ming Wang, Philipp Bucher and Patrick Reichenbach, Swiss Institute for Experimental Cancer Research (ISREC), CH-1066 Epalinges, Switzerland

Interleukin 2 receptor α (IL-2Rα) expression controls the capacity of T lymphocytes to proliferate in response to IL-2. Expression of the IL-2Rα gene is controlled principally by changes in the rate of transcription, in response to a number of stimuli, including antigen, IL-1 and IL-2. We have mapped the *cis*-acting elements in the mouse gene that mediate these responses. The results indicate that induction of IL-2Rα transcription is a two-stage process. First, antigen or IL-1 induce a rapid but transient increase in transcription. During the second stage maximal and sustained promoter activity is stimulated by IL-2. The two phases are reflected by a bipartite distribution of *cis*-acting elements controlling the signal responses. Analysis of transgenic mice has shown that the segment between nt -2539 and +93 confers correct lymphocyte specific, inducible expression on a reporter gene. Mapping of DNaseI hypersensitive sites (DHS) in normal cells has revealed two lymphocyte specific consitutive sites at -0.1 and -5.8 kb, as well as a site at -1.3 kb that appears in T lymphocytes upon induction of IL-2Rα gene expression by ConA and IL-2. Transient transfection analysis has shown that the response to IL-1 is controlled by several elements, including an NF-κB site, in the promoter proximal region (nt -585 to -54). Responsiveness to IL-2, on the other hand, depends on a 48 nt segment 1.3 kb upstream of the promoter which overlaps with the inducible DHS. Scanning of this segment by substitution mutations indicates that IL-2 responsiveness depends on binding sites for three different transcription factors.

J8-316 **CHANGES IN CHROMATIN LOOP CONFORMATION DUE TO HISTONE REMOVAL,** J.Y.Ostashevsky, SUNY-HSCB, Brooklyn, NY 11203

We studied chromatin unfolding due to salt- and intercalating dye-induced histone removal. Changes in chromatin loop size of fixed V79 nucleoids were monitored with fluorescent microscopy and flow cytometry 90° light scattering. In the absence of dyes (ethidium and propidium), removal of histones H1 (0.2-0.6M NaCl) and H2A/H2B (0.7-1.2M NaCl) are respectively accompanied by small and very large increases in loop size and by binding of Na^+ ions to DNA. In contrast, removal of histones H3/H4 (1.5-2.0M NaCl) is accompanied by small decrease in loop size and by the release of Na^+ ions from DNA. Bound intercalating dyes enhance histone removal. Due to this enhancement, chromatin conformational transitions occur at lower salt concentrations in presence than in absence of dye. Removal of H3/H4 histones is accelerated in radiation-nicked chromatin (1 SSB per loop) at large dye concentrations. This is consistent with cooperative binding of H3/H4 tetramers to supercoiled DNA. Our experimental data show a correlation between the increase/decrease in nucleoid loop size (unfolded/compact chromatin conformation) and the binding/release of Na^+ ions to/from DNA. Polyelectrolyte network theory (e.g.,Grosberg and Khokhlov, *Statistical Physics of Macromolecules*,1994) predicts this correlation as a result of the osmotic pressure of Na^+ ions bound to DNA. In this sense, the loop expansion due to removal of H2A/H2B histones seems to be a cause -- rather a consequence -- of chromatin unfolding. Otherwise, it is not clear why removal of H2A/H2B dimers (which control 20 bp/nucleosome each) has such a large effect on chromatin unfolding in comparison with removal of H3/H4 tetramer (120 bp/nucleosome). Removal of H2A/H2B histones from nucleosomes enhances gene activity (Hansen and Wolffe, *PNAS* 91,2339,1994). Supported by NIH grant GM 43374.

J8-317 CHARACTERIZATION OF THE XENOPUS HOMOLOGS OF THE POLYCOMB AND BMI-1 GENES DURING XENOPUS DEVELOPMENT, Arie P. Otte, Marleen J. Reijen, Roel van Driel, Carien Hamer, and Jan den Blaauwen, E.C. Slater Institute, University of Amsterdam, Plantage Muidergracht 12, 1018 TV Amsterdam, The Netherlands.

In Drosophila genes of the Polycomb family are thought to play an important role in maintenance mechanisms which keep cells in a particular differentiation state. The products of these genes act by stably repressing the expression of in particular homeotic genes, presumably by forming complexes which bind to the chromatin. A role for this type of genes in vertebrate development has not been established. We started to study the function of some of these genes in Xenopus development. We characterized the Xenopus homologues of Polycomb and the mouse proto-oncogene Bmi-1 which is homologous to Posterior Sex Combs, a Polycomb-like gene. We report that both genes are expressed in overlapping patterns during early Xenopus development but Xenopus Bmi-1 has a more restricted expression pattern than the Xenopus Polycomb gene. A functional relationship between both genes is suggested by the finding that the Xenopus Polycomb and Bmi-1 proteins bind to each other.

J8-318 METHYLATION-RELATED CHROMATIN STRUCTURE IS ASSOCIATED WITH EXCLUSION OF TRANSCRIPTION FACTORS FROM THE O-6 METHYLGUANINE DNA METHYLTRANSFERASE GENE IN HUMAN GLIOMA CELL LINES. Russell O. Pieper, Sonal Patel, Dawn M. Graunke, and Joeseph F. Costello. Division of Hematology/Oncology, Loyola University Medical Center, Loyola University of Chicago, Maywood, IL 60153.

MGMT is a major determinant in the response of glioma cells to the chemotherapeutic agent 1,3 bis (2-chloroethyl)-1-nitrosourea. Recently we have shown that MGMT expression is correlated in a direct, graded fashion with methylation in the body of the MGMT gene and in an inverse graded fashion with promoter methylation in human glioma cell lines. To determine if promoter methylation is an important component of MGMT expression, this study addressed the complex interactions between methylation, chromatin structure and in vivo transcription factor occupancy in the MGMT promoter of glioma cell lines using linker-mediated PCR. In the regions -895 to +202 and -955 to -11 (relative to the transcription start site) in the transcribed and non-transcribed strands, respectively, DNA-protein interactions consisted of one double-stranded footprint at a novel binding site, and six single-strand footprints at consensus Sp1 binding sites. These interactions were noted in glioma cell lines expressing MGMT, but not in those not expressing MGMT, despite the presence of Sp1 binding activity in all cell lines examined. In cells expressing the gene, the DNA region encompassing these sites was 100% unmethylated and very accessible to exogenously provided restriction enzymes at all sites tested, suggesting that this region may be nucleosome free. The same region in glioma cells with minimal MGMT expression, however, which was 75% unmethylated, was entirely inaccessible to restriction enzymes. We conclude that Sp1 is an important component of MGMT transcription. These correlations also strongly suggest that methylation and chromatin structure, by determining whether Sp1 and other transcription factors can access the MGMT promoter, set the transcriptional state of the MGMT gene.

J8-319 CONTRIBUTION TO THE FUNCTION OF LYMPHOID ENHANCER FACTOR BY THE HMG DNA BINDING DOMAIN. Mary G. Prieve, Katherine L. Guttridge, and Marian L. Waterman, Department of Microbiology and Molecular Genetics, UC Irvine, Irvine, CA 92717

LEF-1 (for human Lymphoid Enhancer Factor) is a T and pre-B cell transcription factor that belongs to the HMG box family of DNA binding proteins. The LEF-1 protein can be subdivided into two domains; the transcriptional activation domain and the HMG domain which binds and bends DNA. We are interested in understanding the contribution of the HMG DNA binding/bending domain to the overall function of LEF-1. Previous work has established that the activation domain can activate transcription in a T cell- and context-dependent manner when fused to the yeast Gal4 DNA binding domain, a domain that retains none of the DNA binding/bending characteristics of the LEF-1 HMG box. Nevertheless, the amino acid sequence of the HMG box and flanking regions are very highly conserved from mouse to human, suggesting that this region provides an important function to the overall activities of LEF-1. Recent work in our laboratory has shown that wild type LEF-1 can activate gene expression from its site in the TCRα core enhancer that is placed 3' of a luciferase reporter plasmid. In contrast, the activation domain of LEF fused to Gal4 or a heterologous DNA bending domain can only activate gene expression to a level 25% to 40% that of wild type LEF-1. To explain this data, we hypothesize that in addition to DNA binding and bending, the HMG box of LEF-1 is involved in direct protein contacts with other factors. To test this hypothesis, we have employed the yeast two-hybrid system to look for cDNAs that encode proteins which specifically interact with the HMG domain. A yeast strain was created that contained the HMG domain and flanking sequences (aa 297 - aa 399) of LEF-1 fused to the C-terminus of the yeast Gal4 DNA binding domain. A mouse thymocyte cDNA library fused to the transcription activation domain of Gal4 was transformed into the LEF-1/Gal4 yeast strain. We are in the process of identifying and testing positive clones for their specificity and their interaction with the LEF-1 HMG box.

J8-320 DAPI AS A FLUORESCENT PROBE FOR DNA-LINKER HISTONE INTERACTIONS *IN SITU*, Ingemar Rundquist and Helena Loborg, Department of Cell Biology, Faculty of Health Sciences, S-581 85 Linköping, Sweden.

Histones are well known to be the major component of eukaryotic cell nuclei that contributes to the formation of higher order structures of chromatin. Linker histones (H1) are of special interest in the control of gene expression since they regulate the dynamic switching between the 10 nm and 30 nm chromatin fiber. This can be achieved by changing H1 subtype in combination with post-translational modifications, such as phosphorylation, that may change the binding behavior between H1 and DNA. Since histones have structural as well as functional properties it is difficult to study their interaction with DNA using biochemical or ultrastructural methods. We have therefore tried to develop a cytochemical method, using the fluorescent DNA-binding dye 4',6-diamidino-2-phenylindole (DAPI) as a probe for DNA-H1 interactions. Human peripheral lymphocytes and frog erythrocytes were permeabilized with digitonin and exposed to increasing concentrations of sodium chloride in order to remove ionically bound H1 from the nuclei. Cells exposed to HCl served as controls. The cells were stained to equilibrium with 0.05 μM DAPI. Single cell fluorescence was measured by image cytofluorometry. Lymphocytes showed a gradual increase in DAPI fluorescence from about 50% to 65% of control cell fluorescence when the salt concentration was increased from 0.35 to 0.7 M. Frog erythrocytes showed a different response, with a rapid shift from 60% to 70% between 0.35 and 0.45 M salt. Digitonin permeabilization itself had a major influence on frog erythrocytes, DAPI fluorescence increased from 30% to 60%, whereas it remained at 50% for the lymphocytes. We conclude that DAPI may be used as a probe for DNA-linker histone interactions but also that other DNA-protein interactions or conformational changes in DNA topology may affect DAPI fluorescence when bound to chromatin *in situ*.

J8-321 CHROMATIN FIBER STRUCTURE AT LOW AND HIGH IONIC STRENGTH: SCANNING FORCE MICROSCOPY AND MODELING STUDIES, Ken van Holde*, Sanford Leuba@, Guoliang Yang@, Carlos Bustamante@ and Jordanka Zlatanova*, *Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331-7305 and @Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

The three-dimensional organization of the chromatin fiber *in vivo* still remains a matter of controversy. The condensation afforded to the DNA by nucleosomes cannot account for the many-thousand fold compaction in the nucleus. To get some insight into chromatin fiber structure long chromatin fragments from chicken erythrocytes were imaged under the scanning force microscope (SFM) under various ionic conditions. The microscopic observations were supplemented with mathematical modeling in an attempt to better understand the determinants of the 3D structure (1, 2).

At low ionic strength the chromatin fibers exhibit an irregular, loose 3D structure. Extended "beads-on-a-string" fibers are seen only in chromatin depleted of linker histones. The experimental SFM images can be closely simulated by a model in which the entry/exit angle of the DNA in the nucleosome is fixed and linker lengths vary. The model incorporates the effects of fiber deposition on the surface, tip convolution and tip compression during SFM imaging.

Increasing of the ionic strength leads to a gradual compaction of the fiber with concomitant increase in the diameter. The structure is highly irregular and segmented. Further experiments will attempt imaging under solution to reduce potential artefacts due to imaging in air.

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J8-322 HISTONE H1/DNA INTERACTIONS IN VITRO - POSSIBLE PREFERENCE FOR BENT DNA FRAGMENTS, Jordanka Zlatanova, Julia Yaneva, Gary Schroth and Ken van Holde, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331-7305

The interactions of histone H1 with linker DNA in chromatin can be studied in H1/DNA model systems (1). The major binding site of histone H1 in chromatin is at the cross-over of DNA strands exiting and entering the nucleosome. Histone H1 shows preference for binding to four-way-junction (4WJ) DNA (2) probably because of structural similarity between its major binding site in chromatin and the 4WJ. In addition, H1 has been shown to exhibit some preference for binding to specific DNA sequences, both in isolated restriction fragments (1) and in fragments cloned in superhelical plasmids (3). The molecular basis of this apparent "sequence-specificity" is not clear. It may depend on some characteristic structural features of the preferred sequences such as non-B structures, bent or otherwise distorted B-DNA.

In order to further elucidate this issue we initiated a study on the interactions of pBR322 or pUC19 restriction fragments with H1. In both plasmids we identified fragments which were preferentially aggregated by the histone, disappearing from electrophoretic gels at much lower H1/DNA ratios than the rest of the fragments. These fragments, when analysed by a computer program designed to identify bent DNA, were predicted to be significantly bent. However, a cloned oligonucleotide containing the bending center of kinetoplast DNA was not significantly preferred. Further experiments will be performed to exactly map the site of preferred binding of histones H1 to these DNA fragments.

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J8-323 POSITION INDEPENDENT VARIEGATION OF KERATIN-LacZ GENES IN TRANSGENIC MICE.

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Chromosomal position effects often involve the repression of a euchromatic gene when it has been placed into or near heterochromatin as the result of a genetic rearrangement. When the translocated gene escapes inactivation in some cells, a variegated phenotype is observed, characterized by patches of expressing and non expressing cells. Examples of these mosaic expression patterns have been studied in *Drosophila* and in yeast. A related silencing phenomena in mammals is the inactivation of the X-chromosome. Here we report bovine keratin-*E. coli lacZ* hybrid genes that showed mosaic expression patterns in F1 transgenic mice. In particular, we studied mice carrying a construct containing 5.3 kb of 5' flanking and promoter sequences of the stratified epithelial keratin 5 gene fused to the *lacZ* gene. Six lines were generated with this construct, three of which expressed the transgene in the right tissues, but in a mosaic fashion. None of these lines had the transgene inserted in the X-chromosome. The degree of mosaicism varied among the three lines. Because every expressing line showed a variegated phenotype, we think it unlikely that the mosaicism is due to transgene insertion in or near pericentromeric chromatin. Moreover, we found that transgene expression in embryos was little or no mosaic. That is why we have started to address the possibility that the transgene itself rather than the integration site is responsible for the mosaic expression pattern.

Genome Imprinting

J8-400 METHYLATION SENSITIVE DNA-PROTEIN INTERACTIONS IN AN INTRAGENIC REGION OF THE KANGAROO X-LINKED HPRT GENE, Suyinn Chong and Anita A. Piper, School of Biological and Biomedical Sciences, University of Technology, Sydney N.S.W 2065, Australia

Mammalian X chromosome inactivation (XCI) serves to equalise sex differences in the dosage of X-linked gene products and is probably the result of interactions between methylated cytosines, higher order DNA structure and replication timing. Inactive-X specific promoter hypermethylation has been demonstrated for eutherian constitutive genes, but does not seem to occur on metatherian genes. However, there are intragenic sites both in eutherian and metatherian genes that appear to be methylated specifically on the active X. To investigate whether these intragenic sites also contribute to XCI, possibly by interactions with regulatory proteins, we examined DNA-protein interactions in a region in intron 3 of the kangaroo X-linked hypoxanthine phosphoribosyltransferase (HPRT) gene which contains three previously identified differentially methylated sites. Three specific protein binding sites (*in vitro* DNase I footprints) were identified on unmethylated DNA. Footprint 1 (35bp) covers a region that contains two known differentially methylated sites, footprint 2 (22bp) includes a potentially differentially methylated cytosine (a CpG dinucleotide) and footprint 3 (28bp) has no CpGs. Further characterisation (mobility shift assays) of footprint 1 resulted in the identification of several different specific interactions, one of which was abolished by *in vitro* methylation of a single cytosine within the binding site. These DNA-protein interactions, including the methylation sensitive interaction, have been observed with extracts prepared from both kangaroo and Hela nuclei. Future experiments will focus on defining the various binding sites more accurately and examining the effects of this region on the transcription of reporter genes, to test whether it can act as a silencer.

J8-401 PROPAGATION OF DNA METHYLATION AT NON-SYMMETRICAL SITES IN *ASCOBOLUS* AND *NEUROSPORA*, Godeleine Faugeron, *Eric U. Selker, *Brian Margolin, *Silvia Lommel, *Vivian P. W. Miao, Annie Grégoire, and Jean-Luc Rossignol, Institut de Génétique et Microbiologie, Université Paris-Sud, Bât. 400, 91405 Orsay cedex, France, and *Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229

In both of the filamentous fungi, *Ascobolus* and *Neurospora*, DNA methylation can be induced during the sexual cycle by duplication of DNA sequences above a certain length. In *Neurospora*, repeated sequences acquire point mutations prior to meiosis by the "Repeat-Induced Point mutation" process (RIP; 1) and are typically methylated. In *Ascobolus*, the presence of repeats at the same stage of the sexual cycle leads to methylation without mutation by the "Methylation Induced Premeiotically" process (MIP; 2). Genes subject to MIP and RIP are generally transcriptionally repressed. In both fungi methylation is usually maintained in vegetative cells and affects cytosines whether or not they are part of symmetrical sequences (3; 4). This contrasts with the situation in vertebrates in which methylation is only found at CpG's and this implies the existence of one or more novel mechanisms for maintaining methylation. Methylation is often dense with most or all of the C's in a region being methylated. Results of previous work suggested that methylation in *Neurospora* depends on reiterative *de novo* methylation while methylation in *Ascobolus* depends simply on preexisting methylation. We will report the results of direct tests for maintenance and *de novo* methylation in *Ascobolus* and *Neurospora*. Differences and similarities were found.

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J8-402 DNA HYPOMETHYLATION DUE TO ACCUMULATION OF DECARBOXYLATED S-ADENOSYLMETHIONINE ?

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The biosynthesis of the polyamines (PA), putrescine, spermidine and spermine is essential for growth and differentiation of eukaryotic cells. I have shown that depletion of PAs by DFMO (an irreversible inhibitor of ornithine decarboxylase) in F9 teratocarcinoma stem cells (a model system for the early embryonic development of mammals) causes a dramatic accumulation of decarboxylated S-adenosylmethionine (dcAdoMet), the aminopropyl donor in the synthesis of spermidine and spermine. The accumulation of dcAdoMet induces a genome-wide loss of DNA methylation and as a consequence, inducing terminal differentiation into parietal endoderm-like cells. Our current view for the mechanism in action is that dcAdoMet acts by competing with AdoMet as a substrate for DNA methyltransferase, but preliminary data indicate that dcAdoMet directly interfere with the expression of DNA methyltransferase. Presently I am studying the expression and the regulation of DNA methyltransferase in the context of dcAdoMet accumulation to find out at which level dcAdoMet interferes with the mechanism of DNA methylation.

J8-403 CYTOSINE METHYLATION AND INVADING DNA IN *ASCOBOLUS*, Christophe Goyon, Jean-Luc Rossignol, and Godeleine Faugeron. Institut de Génétique et de Microbiologie, Université Paris-Sud, Bât. 400, 91405 Orsay Cedex, France.

In the filamentous fungus *Ascobolus immersus*, DNA fragments duplicated following transgenesis are subject to the epigenetic process of Methylation Induced Premeiotically (MIP)[1,2], acting exclusively during the sexual cycle. MIP affects most of the cytosine residues carried by the duplications[3]. Genes present on the duplicated fragments are transcriptionally repressed[4]. Once established, both methylation and silencing are stably maintained through vegetative and sexual reproduction, even after segregation of the duplicated fragments. One possible role for MIP might be to control the dissemination of transposable elements in the genome. In order to test this hypothesis, we have begun to study the nature of *Ascobolus* natural repeats and their methylation status.

Using a non-selective approach, several natural repeats have been cloned, that display various levels of methylation. The molecular characterization of these repeats is under progress. Preliminary results suggest that retrotransposable elements or remnants of them constitute a large part of the heavily methylated repeats, and are therefore likely to be natural targets for MIP.

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J8-404 **A Study of Matrix-Attachment Regions in an Imprinted Domain.**

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Matrix-attachment regions (MARs) have the potential to influence gene activity in *cis*, as well as acting as origins of replication in eukaryotic cells. We are pursuing the hypothesis that the differential establishment and propagation of MARs on homologous chromosomes could give rise to what we recognise as genetic imprinting, which manifests differences in gene activity and replication timing as constant features. Preliminary evidence supporting this hypothesis comes from the report of derepression of the maternal Igf2 allele following exposure to the drug distamycin,[1] which appears to act by specifically disrupting attachment of DNA to the nuclear matrix.

Our genes of interest, Ins2, Igf2, H19 and Mash2, exist in an imprinted domain on distal mouse chromosome 7. We are using the isotonic LIS technique[2] to identify MARs within a 450 kb YAC which contains the first three of these genes. To date, over 15 kb around the H19 gene have been assayed using this technique. We have excluded the presence of MARs in this first region studied. We have also developed a technique to probe these LIS-extracted nuclei using FISH. Probing with the whole YAC, we find that it contains elements associated with the nuclear matrix, and that the homologous areas decondense differently, one looping extensively, the other remaining more compacted within the matrix. This adds suggestive evidence that the number of MARs within each homologue is different.

We have developed chimaeric mice with embryonic stem cells uniparentally disomic for this region of chromosome 7. If discordant MAR establishment is suspected we will be able to confirm this using cells derived from these mice. The use of distamycin to disrupt identified MARs should then allow us to compare the relative contributions of the MARs with other presumed mechanisms of imprinting, such as cytosine methylation.

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J8-405 FUNCTIONAL ANALYSIS OF THE HUMAN *XIST* PROMOTER, Brian D. Hendrich[1,2] and Huntington F. Willard[1], [1]Department of Genetics and Center for Human Genetics, Case Western Reserve University, Cleveland, OH 44106-4955; and [2]Department of Genetics, Stanford University, Stanford, CA 94305

The human and murine *XIST/Xist* genes both show inactive X chromosome-specific expression and physically map to within the smallest defined region on the X chromosome absolutely required *in cis* for X inactivation to occur, their respective X Inactivation Centers (*XIC/Xic*). These facts together suggest that *XIST/Xist* expression is either a cause of or a direct result of the process of X chromosome inactivation in humans and mice. Studies in early mouse development have shown that *Xist* transcripts are detectable before X inactivation occurs, implying that *Xist* expression is a cause of X inactivation rather than merely a by-product of the inactivation process. In addition, early mouse studies have revealed that the earliest *Xist* expression is from the paternal chromosome only, and is thus subject to genomic imprinting. While imprinted expression persists in the extraembryonic lineages, the embryo shows the random *Xist* expression found in adult mice and in humans. Thus the *Xist* gene is capable of imprinted expression as well as random expression. It is unclear whether human *XIST* gene expression is imprinted in extraembryonic tissues. We have undertaken to characterize the promoter of the human *XIST* gene in order to identify factors responsible for *XIST*'s unique expression pattern. Transient transfection assays of somatic cells using different parts of the human *XIST* promoter driving reporter gene constructs have allowed us to identify a minimal promoter as well as sequences which modulate this promoter activity. Minimal promoter activity has also been identified in the corresponding regions of the lepine and equine *Xist* genes. Saturation site-directed mutagenesis of the human minimal promoter region has identified three distinct, highly conserved sequences which, when mutated, cause a dramatic decrease in promoter activity in transfection assays. One of these regions has been shown to bind the transcription factor Sp1, another binds the TATAA binding protein, and the third binds an as yet unidentified factor in vitro. We are currently precisely mapping regions that modify this minimal promoter activity using promoter constructs and in vivo footprinting. Transgenic mice carrying these promoter constructs have been made in order to analyze certain promoter activities in vivo. Understanding how these promoter activities act in concert to give the *XIST* gene its unique expression pattern will likely provide some insight on the nature of the X inactivation process and its relationship to imprinting.

J8-406 **A POSSIBLE APPROACH TO CLONE GENES HAVING ANTISENSE OVERLAPPING TRANSCRIPTS** **Sergei Kozlov, Vladimir Evtushenko, Laboratory of Genetic Engineering, Research Institute of Roentgenology and Radiology, St.Petersburg 189646, Russia**

A growing number of data suggests that eukaryotic genes encoded on the opposite DNA strand might play an important role in regulation of gene activity. Genes overlapping on the opposite DNA strands may code for transcripts which form RNA-RNA duplexes existing in vivo.
In our lab we have tried several experimental strategies aiming to clone the antisense overlapping genes. The following design of cloning has been used. The rat brain poly(A)$^+$RNA was converted to cDNA and directional library in lambdaZAPII has been constructed according to manufacturer's instructions(Stratagene) with several modifications improving the cloning efficiency. The mass library excision has been done using method developed in our lab (in press) and the resulting single-stranded DNAs were allowed to hybridize under phenol-emulsion reassociation technique (PERT) conditions. The resulting double stranded fraction should contain possible regions of complementary between inserts in the library. The remaining single-stranded fraction was removed by treatment with strand-specific nuclease. The double stranded fraction was cloned using standard procedures into EcoRI site of plasmid vector pBluescriptIISK(+) and resulting ligation mixture was electrotransformed into Sure$^{(TM)}$cells The several thousand clones were obtained. They are currently under analysis. Our preliminary sequencing data shows that some of them contains a region of homology with 5'end of several eukaryotic RNAs. The further experimentation and improving of cloning strategy could help to identify an eukaryotic genes having antisense overlapping transcripts.

J8-407 CONTROL OF EXPRESSION OF THE BOVINE α-LACTALBUMIN GENE IN TRANSGENIC MICE, Peta A. Madgwick and Robert D. Bremel, Department of Dairy Science, University of Wisconsin-Madison, Madison, WI 53703

α-lactalbumin is a milk protein thought to be involved in controlling water movement into milk. Transgenic mice carrying the bovine gene produce the protein at up to 1.5 mg/ml of milk although the average is 0.42 mg/ml, while the endogenous gene is expressed at 0.65 mg/ml. Both proteins are detected using an enzyme linked immunoabsorbent assay in which the different species antibodies are not cross reactive. Expression of the transgene does not decrease expression of the endogenous gene. While the expression of the endogenous gene has low variability even between different strains of mice (σ = 0.08), expression of the bovine gene varies widely (σ = 0.2) both between and within lines of mice, where a line represents a single transgenic insertion event. This variation is not copy number dependent. Within one line which has been maintained as a hemizygote by crossing into an outbred strain of mice, the variation is extreme (σ = 0.27), one group of descendants producing the protein at an average of 0.8 mg/ml and another group at an average of 0.1 mg/ml. Imprinting has been shown to influence expression level of transgenes, and experiments examining its role in controlling expression of this bovine gene will be presented.

J8-408 IDENTIFICATION OF GENES SHOWING DIFFERENTIAL EXPRESSION IN PREIMPLANTATION AND EARLY POSTIMPLANTATION PARTHENOGENETIC EMBRYOS, Mellissa Mann[1], Keith Latham[2] and Sue Varmuza[1], [1]Department of Zoology, University of Toronto, Toronto, Ontario, M5S 1A1 Canada; [2]Temple University School of Medicine, Department of Biochemistry and The Fels Institute for Cancer Research and Molecular Biology, Philadelphia, Pennsylvania 19149 USA

In this study, we set out to identify imprinted genes involved in early embryogenesis. Evidence for the involvement of imprinted genes in early development comes from two sets of studies. The first is from studies involving the generation of uniparental embryos. Parthenogenetic/gynogenetic and androgenetic embryos fail to develop to term. The majority die early in development. We analyzed embryonic death in parthenotes and found that 50% die at or just after implantation. Another 30% die at the egg cylinder stage. The second line of evidence comes from translocation studies. Disomies of chromosome 2, 6, 7 result in early embryonic lethality. The targets of our search were blastocyst stage and day 5.5 embryos.

The rationale behind our approach is that parthenogenetic embryos will be deficient in transcripts expressed from the paternal genome. Differential screens represent a means of identifying candidate genes. We found three genes in a differential screen that showed differences in expression between day 5.5 parthenogenetic and wild type embryos. The maternal allele had significantly lower expression (roughly half) than that from the paternal allele. Similar results comparing day 5.5 embryos were observed for Igf2. These genes represent excellent candidates for imprinted genes. Confirmation of the imprint is being assessed through analysis of parental-allele-specific expression. Finally, two novel genes were identified that are predominantly expressed in trophoblast tissues.

J8-409 CONSERVATION OF *BROWN* GENE *TRANS*-INACTIVATION IN *DROSOPHILA*, Linda Martin-Morris and Steven Henikoff, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA. 98104

The *brown* gene of *Drosophila melanogaster* is necessary for uptake of pteridine (red and yellow) pigments in the adult eye. Many mutant alleles of the *brown* gene have been isolated in which *brown* expression is variably silenced by association with heterochromatin, a phenomenon known as position effect variegation (PEV). Unlike most examples of PEV in *Drosophila*, variegation of *brown* causes silencing of a paired wild-type allele (termed *trans*-inactivation). A model for *trans*-inactivation involves unusual sensitivity of a positive regulatory protein to association with heterochromatin in *trans*. One of the predictions of this model is that sequences responsible for *trans*-inactivation should be conserved over evolutionary time because these sequences are necessary for the correct expression of *brown*. We have tested this prediction by comparing the *brown* gene in *D. melanogaster* with its distant relative in *D. virilis*. By transformation of the *D. virilis* homolog of *brown* into *D. melanogaster*, we have shown that the *D. virilis brown* gene is capable of being expressed in *D. melanogaster*. Induction of a PEV mutation on a *D. virilis* transgene reveals that the *D. virilis* gene is capable of being *trans*-inactivated in *D. melanogaster*. Thus, *trans*-inactivation is preserved over approximately 60 million years evolutionary time, even though PEV has not been subjected to natural selection. This supports the conclusion that sequences responsible for *trans*-inactivation have been conserved to regulate *brown* gene expression, as predicted by the model.

J8-410 DOES ABSENCE OF GENOMIC IMPRINTING DISCREDIT THE ANTICIPATION HYPOTHESIS IN SCHIZOPHRENIA? Arturas Petronis, Tsukasa Sasaki, Anne S. Bassett, William G. Honer, James L. Kennedy, Neurogenetics Section, Clarke Institute of Psychiatry, Toronto, Ontario M5T 1R8.

Recently, unstable trinucleotide repeats have been shown to be the etiologic factor in seven anticipation demonstrating neuropsychiatric diseases. In addition, all these disorders demonstrated some difference between maternal versus paternal transmissions, i.e. genomic imprinting (GI). The presence of GI was proposed as a criterion in separating true genetic anticipation from ascertainment biased anticipation-like statistical artefacts (Ridley et al, 1988).

Although schizophrenia (SCH) studies have consistently shown the presence of increased severity and earlier age of onset in subsequent generations, no clinical evidence for GI has been detected. We postulate that the criterion of GI is of great value to Mendelian disorders only. For complex non-mendelian diseases, both false absence and false presence of GI could be detected. In the first case, evidence for GI could be masked when the interaction of a number of genes predisposes to a disease. Some of them could be maternaly GI while the rest exhibit mostly paternal GI. Both types of genes would cancel each other in the sense of paternal differences, while the effect of anticipation would still remain. In the second case, a false evidence for GI might be caused by male/female differences in susceptibility for the disease. SCH affects males at an earlier age and usually has a more severe course than in females. It is likely that a smaller number of polygenes with an additive effect is required to produce SCH affected males in comparison to SCH affected females. Under this hypothesis, the risk to relatives of a SCH female to be affected is higher than for the relatives of a male SCH patient. Higher risk includes a greater possibility of being affected, an earlier age of onset and a more severe course. In order to test the anticipation hypothesis in SCH, we developed a set of molecular genetic techniques for direct detection of unstable DNA. The results of the screening of the SCH families which showed evidence for anticipation will be presented.

J8-411 INTERACTION BETWEEN *CIS*-ELEMENTS AND THE *TRANS*-FACTORS IN REGULATING THE EPIGENETIC ACTIVITY OF THE MAIZE *SPM* TRANSPOSABLE ELEMENT. Ramesh Raina, Michael Schlappi and Nina Fedoroff, Department of Embryology, Carnegie Institution of Washington, Baltimore, MD 21210.

The transcriptional and transpositional activity of the maize *Suppressor-mutator* transposable element (*Spm*) is subject to epigenetic regulation. There are several distinct epigenetic forms of the *Spm* element which differ in the extent of methylation at the 5'-end of the element. The active elements are unmethylated and the inactive elements are hypermethylated at the 5'-end of the element. The activity of the element is autoregulated and involves the *cis*-element (5'-end of the element) and the *trans*-factors (element encoded protein TnpA; see also accompaning abstract by Schlappi et al.). The 5'-end of the element has two regions: 0.2 kb upstream control region (UCR) which is the promoter of the element and 0.35 kb down control region (DCR) which is G+C-rich untranslated leader sequence. The DCR acts as a 'silencer' for the promoter and its presence results in *de novo* methylation and inactivation of the promoter. TnpA demethylates and activates a methylated and inactive promoter and represses an active and unmethylated promoter.

Results will be presented to show that TnpA binds only to the UCR and not to the DCR but still results in the demethylation of both UCR and DCR. We will present evidences that TnpA and DCR may interact indirectly and the DCR is a very important *cis*-determinant in regulating the methylation state of the element. These results will be discussed in context to our present model of transcription and transposition regulation of the *Spm* element.

J8-412 EPIGENETIC REGULATION OF THE MAIZE *SPM* TRANSPOSABLE ELEMENT: INTERACTION BETWEEN ELEMENT-ENCODED PROTEINS, Michael Schläppi, Ramesh Raina, and Nina Fedoroff, Department of Embryology, Carnegie Institution of Washington, Baltimore, MD 21210

The maize *Suppressor-mutator* transposable element is subject to epigenetic control involving reversible DNA methylation and element-encoded *trans*-acting factors (see also accompanying abstract by Raina et al.). Epigenetic forms of *Spm* differ in their degree of methylation in the element promoter and in the first G+C-rich untranslated exon: methylated elements are transcriptionally and genetically inactive. TnpA, the most abundant element-encoded protein, activates the methylated *Spm* promoter, resulting in demethylation, but represses the unmethylated promoter. TnpD is required for transposition, but not *trans*-activation, and may represent the element-specific endonuclease. Results will be presented which demonstrate a functional *in vivo* interaction between TnpA and TnpD and possibly with other cellular factors. We will discuss a model in which the epigenetic control of *Spm* is linked to the intimate association of two element-encoded proteins, which individually and in concert regulate activation, demethylation, repression, and transposition of the transposable element.

J8-413 **GENE INACTIVATION INDUCED BY A CIS-ACTING DNA METHYLATION CENTER**

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Methylation of gene promoter regions has been correlated with inactivation of both X-linked and autosomal genes. However, the signal(s) for the methylation event is still unknown. In this report we test the hypothesis that a cis-acting element can induce epigenetic gene inactivation by signaling de novo methylation. The cis-acting element used is a 820 base-pair fragment that is shown to provide a de novo methylation signal. This fragment, taken from a region located approximately 1.3 Kbp upstream of the mouse *aprt* (adenine phosphoribosyltransferase) gene, is termed a methylation center. To determine if the methylation center could induce inactivation of the *aprt* gene, a plasmid construct was created in which the methylation center was moved next to the *aprt* promoter. Transfection experiments demonstrated inactivation of the *aprt* gene on the hybrid construct. The inactivation event was shown to correlate with hypermethylation with a Southern blot analysis. Moreover, the inactive construct could be reactivated by treating transfectants with 2-deoxy-5'-azacytidine, a demethylating agent. Interestingly, gene inactivation induced by the methylation center required the removal of 2 of the 4 Sp1 binding sites that comprise the *aprt* promoter. The 2 remaining Sp1 binding sites were sufficient for high level expression of the *aprt* gene when the methylation center was removed. The results demonstrate that methylation associated gene inactivation can be induced by a cis-acting element. They also suggest that the methylation event can be blocked by an excess number of transcription factor binding sites.

Late Abstract

TRANSCRIPTION REGULATION OF *XENOPUS* TRßA GENE BY THYROID HORMONE RECEPTOR AND EFFECT OF CHROMATIN ASSEMBLY. Jiemin Wong, Alan Wolffe, and Yun-Bo Shi Laboratory of Molecular Embryology, NICHD, Building 18, Room 101, Bethesda, MD 20892

Amphibian metamorphosis, the complex transition from a tadpole to a frog, is entirely controlled by thyroid hormone (T3). To understand the mechanism underlining this transition, we have identified from *Xenopus Laevis* a number of genes which are directly regulated by T3. Of particular interest are the two thyroid hormone receptor genes (TRßA and TRßB), the expression of which correlates very well with their potential role in mediating the T3 effect in the process of metamorphosis. We have recently identified a T3 response element (TRE) in the TRßA gene using a tissue culture transfection assay. We now use an *Xenopus* oocyte system to investigate how thyroid hormone receptors (TRs) regulate the transcription from the TRßA promoter and the effect of chromatin assembly. The hormone responsiveness of the oocytes was established by microinjection of mRNAs encoding *Xenopus* TRßA and RXRα receptors. We have demonstrated that the xTRß-RXRα heterodimers repress the transcription from the TRßA promoter in the absence of T3, while activate it in the presence of T3. The repression and the activation observed in the oocytes are dependent on the TRE, since both activities were abolished when the TRE was mutated. Furthermore, by using single stranded reporter plasmid containing the TRßA promoter, which is efficiently replicated and chromatinized when injected into oocytes, we have shown that this replication-associated chromatin assembly greatly represses the basal activity of the promoter. The titration of histones in nuclei with competitor DNA resulted in a higher level of transcription from the single-stranded templates, indicating the replication-coupled chromatin assembly is the causal for the repression. *In vivo* footprinting experiments demonstrated that the TRß-RXRα heterodimers were bound to the TRE regardless of the presence or absence of T3. In addition, the receptors can bind to the DNA templates which were pre-assembled into chromatin. These results strongly suggest that TR-RXR heterodimers can bind to TRE in the chromatinized template and repress the transcription in the absence of T3. In the presence of T3, the heterodimer most likely undergo conformational changes that lead to the transcriptional activation. We are currently studying the possible changes of the chromatin structure induced by the binding of TRß-RXRα complexes.