THE NUCLEAR MATRIX: INVOLVEMENT IN REPLICATION, TRANSCRIPTION, GENE SPLICING AND CELLULAR REGULATION

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The Nuclear Matrix: Involvement in Replication, Transcription, Gene Splicing and Cellular Regulation

Role of the Nuclear Matrix, Cytoskeleton & Extracellular Matrix in Cellular Architecture and Functional Integration

J7-001 THE SYNDECANS, PROTEOGLYCAN MEDIATORS OF MORPHOGENETIC CELL BEHAVIOR, Merton Bernfield, Marilyn Fitzgerald, Richard Gallo, Michael Hinkes, Masato Kato, Karen McAlmon, Huiming Wang, Joint Program in Neonatology, Harvard Medical School, Boston, MA 02115.

During embryogenesis, cell-cell interaction molecules trigger signal transduction pathways that affect cell movements and adhesion, cell growth and death and the accumulation of extracellular matrix (ECM). These cell behaviors result in the mechanical changes recognized as change in tissue form. This morphogenesis is recapitulated, at least in part, during the repair of injured tissues. Both morphogenesis and wound repair are accompanied by the selective induction of members of the syndecan family of transmembrane proteoglycans.

These four proteoglycans in mammals, the predominant source of the heparan sulfate that is ubiquitous at cell surfaces, are ancient molecules that arose by gene duplication and divergent evolution from a single ancestral proteoglycan. The heparan sulfate chains bind a variety of cell-cell interaction molecules (e.g. growth factors (GF), GF-binding proteins, ECM components, chemokines, cell adhesion molecules, degradative enzymes and protease inhibitors). At the cell surface they mediate the action of these ligands by acting directly as receptors or, more often, as coreceptors in concert with ligand-specific receptors. Although binding is widespread, the syndecans show selectivity: different tissues have a distinctive syndecan repertoire (epithelia and plasma cells express predominantly syndecan-1; endothelia, syndecan-2; neural and neural crest, syndecan-3; and nearly all cells, syndecan-4), and syndecans from different cell types vary in the size, fine structure and ligand-binding specificity of their heparan sulfate chains. In addition to the autocrine action of cell surface syndecans, the syndecan extracellular domains (ectodomains) are shed intact from the cell surface and act as paracrine effectors. The syndecan-1 ectodomain stimulates cell proliferation with aFGF and bFGF, but inhibits with HB-EGF, effects due to distinct heparan sulfate regions: the growth stimulation involves highly sulfated regions and the growth inhibition involves less extensively sulfated regions. The syndecans also have intracellular actions; loss of syndecan-1 expression causes epithelia to become mesenchyme-like and to lose anchorage independent growth.

Syndecan expression in embryos follows morphogenetic rather than histologic boundaries. Syndecan-1 appears in the preimplantation embryo, syndecan-3 during neurulation, and syndecan-2 and -4 appear later, during organogenesis. Syndecan-1 is induced during epithelial-mesenchymal interactions, and syndecan-1 and -4 are transiently induced in capillaries and fibroblasts at the edges of repairing dermal wounds. The latter induction can be due to synducin, a 39 amino acid proline and arginine-rich peptide that is identical to PR-39, a previously described antibacterial peptide. Synducin/PR-39 is derived from the wound leukocytes, and its propeptide is nearly identical to that of bac-5 and bac-7, antibacterial peptides from bovine neutrophils. Antibacterial and syndecan inductive concentrations are identical, suggesting that leukocytes deposit into wounds a peptide that both induces adjacent mesenchymal cells to express syndecans as part of the repair process and kills invading bacteria as part of a non-immune host defense mechanism. Thus, by several mechanisms, syndecan expression regulates morphogenetic cell behavior.

J7-002 DEMONSTRATION OF MECHANICAL CONTINUITY BETWEEN CELL SURFACE INTEGRINS, CYTOSKELETAL FILAMENTS, AND NUCLEI IN LIVING CELLS. Donald E. Ingber and Andrew J. Maniotis, Children's Hospital and Harvard Medical School, Boston, MA 02115.

We have previously demonstrated that extracellular matrix (ECM) controls cell growth and differentiation by modulating cell shape. In general, matrix substrata that promote cell spreading stimulate growth whereas those that prevent cell extension suppress growth and induce differentiation. We found that these functional alterations also correlate with alterations in nuclear form: nuclei spread when cells extend on ECM and round when cells retract (e.g., by dislodging ECM contacts). This coupling between cell and nuclear shape could be explained if cell surface ECM receptors, such as integrins, were structurally interconnected with nuclear scaffolds via intervening cytoskeletal filaments. Global and dynamic rearrangements could occur within a structurally interconnected lattice of this type, if cells used tensegrity architecture to organize their internal structure, as we have previously proposed. We reasoned that if structural continuity exists in cells between integrins and nuclear structures, then we should be able to demonstrate mechanical continuity. To explore this hypothesis, cell surface integrin receptors were mechanically stressed by micromanipulating surface-bound microbeads that were coated with integrin ligands or by pulling directly on surface-bound micropipettes that were similarly coated. When integrin receptors were mechanically pulled in either living or membrane-permeabilized cells, cytoskeletal filaments and nuclei immediately reoriented into the lines of force and changes in the distribution of nucleoli and the RNA splicing factor, SC-35, were observed throughout the depth of the nucleus. Structural interconnectedness between different nucleoli could be demonstrated within interphase nuclei and between different chromosomes in mitotic cells that lacked nuclear membranes. These results demonstrate that cell surface integrins, cytoskeletal filaments, and nuclear scaffolds are mechanically coupled in living cells and, hence provide a possible mechanism by which mechanical distortion of living cells might alter nuclear functions.

Dynamics of the Nuclear Matrix and Cellular Function

J7-003 **REPLICATION AND TRANSCRIPTION FACTORIES ATTACHED TO A NUCLEOSKELETON.** Peter R Cook.

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The major nuclear processes of replication, transcription and the repair of DNA damage take place in discrete sub-compartments. These compartments can be labelled by incubating permeabilized HeLa cells with biotin-dUTP (replication/repair sites) or Br-UTP (transcription sites); after immunolabelling with appropriately-tagged antibodies, sites containing incorporated analogues can then be visualized by light or electron microscopy.

Transcription occurs in ~300 discrete sites. As cells enter S-phase, replication begins in ~150 'factories' that are probably assembled around pre-existing transcription sites; replication sites then fuse and grow so that by the end of S-phase all replication occurs in a few large factories that are also transcriptionally active.

Replication factories are attached to a diffuse nucleoskeleton that ramifies throughout nuclei. Individual filaments in this network have the axial repeat characteristic of intermediate filaments and its nodes can be immunolabelled with anti-lamin antibodies; the lamins are misnamed as they are also found internally.

The attachment of active polymerases in large factories has led us to re-evaluate the mechanisms of polymerization. The traditional view involved polymerases that tracked along templates; we now imagine that several templates slide past a number of polymerases fixed within factories. This means that higher-order structure dictates how and when DNA is replicated, transcribed or repaired.

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J7-004 HISTONE MODIFICATIONS, CHROMATIN STRUCTURE AND THE NUCLEAR MATRIX, James R. Davie[1], Jian-Min Sun[1], Hou Yu Chen[1], Wei Li[1], Michael J. Hendzel[1], Debbie Chadee[1], William R. Taylor[2], Jim A. Wright[2], and C. David Allis[3], [1]Department of Biochemistry and Molecular Biology, [2]Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Manitoba, Canada R3E OW3, and [3]Department of Biology, Syracuse University, Syracuse, New York 13244-1270.

Transcriptionally active chromatin has a soluble and insoluble nature, i.e., active DNA is found in chromatin fragments that are soluble in 150 mM NaCl and in chromatin fragments that are bound to the residual nuclear material (nuclear matrix). Active chromatin regions are thought to be immobilized on the nuclear matrix by multiple dynamic attachment sites. Rapidly acetylated and deacetylated histones are primarily associated with transcriptionally active DNA-enriched chromatin fragments. Histone acetylation has multiple functions, including altering the capacity of H1 histones to condense transcriptionally active chromatin and changing nucleosome structure. Reversible histone acetylation is catalyzed by histone acetyltransferase and histone deacetylase. Vertebrate histone acetyltransferase and deacetylase are associated with the nuclear matrix. Two isoforms (HD1 and HD2) of histone deacetylase have been isolated from chicken erythrocytes. Nuclear matrix bound histone deacetylase prefers histone H2B, while the soluble histone deacetylases HD1 (45-65 kDa) and HD2 (200-250 kDa) prefer histones H3 and H4. At high ionic strengths with reducing agents, HD2 dissociates to HD1. These results suggest that the nuclear matrix-bound histone deacetylase exists as a high molecular mass complex. Transcription factor NF1 is also a component of the nuclear matrix (chicken erythrocytes, chicken and trout liver). We propose that histone acetyltransferase, histone deacetylase and NF1 have roles in maintaining the association of active chromatin domains with the nuclear matrix at sites of ongoing transcription. Histone H1 is phosphorylated, with levels of H1 phosphorylation increasing as the cells progress from G1 to S to G2/M phases of the cell cycle. Using an anti-phosphorylated histone H1 antibody, we show that the level of a phosphorylated histone H1 subtype, H1b, is elevated in oncogene-transformed mouse fibroblasts. Indirect immunofluorescence studies with the antibody revealed that most of the interphase oncogene-transformed and parental 10T1/2 mouse fibroblasts had a punctate pattern of nuclear fluorescence. The subnuclear location of phosphorylated histone H1b coincided with that of nuclear matrix antigen B1C8 which is confined to regions of RNA splicing. These observations provide evidence that phosphorylated histone H1b is associated with transcriptionally active chromatin. (Supported by Medical Research Council of Canada (MT-9186, MT-12147, MA-12283) and Public Health Service NIH Grant (GM 40922)).

J7-005 NUCLEAR DOMAINS INVOLVED IN REPLICATION, TRANSCRIPTION AND RNA PROCESSING, Luitzen de Jong, Wouter Schul, Rick Wansink, Bas van Steensel, Krina van der Meulen, Bas Groenhout and Roel van Driel, E.C. Slater Institute, BioCentrum, University of Amsterdam, Plantage Muidergracht 12, 1018 TV Amsterdam, The Netherlands

To obtain a better understanding of the principles of nuclear organization, we have studied by immunofluorescence microscopy the spatial distributions of nascent DNA, nascent RNA synthesized by RNA polymerase II (RP II) and several components involved in transcription and RNA processing in human cells. Among these components are the glucocorticoid receptor (GR), the cleavage stimulation factor (CstF), the poly(A) binding protein II (PAB II), snRNPs and the non-snRNP splicing factor SC-35. Without exception, these components are strikingly non-homogenously distributed, often in a unique pattern. This indicates that the nucleus is highly compartmentalized. Several hundreds of domains containing nascent pre-mRNA are distributed throughout the nucleoplasm outside the nucleolus. Number, size and spatial distribution of nascent DNA depend on the stage of S phase. Domains of transcription and replication tend to exclude each other. This is interpreted to mean that transcription by RP II in a domain is interrupted when DNA synthesis starts, until the DNA in that particular domain has been completely replicated, which lasts 30-60 min. GR molecules are clustered in several hundreds of domains, the spatial distribution of which resembles that of nascent pre-mRNA. Clusters of GR and sites of nascent pre-mRNA do not colocalize to a large extent. This probably means that most GRs are not directly involved in activation of transcription. CstF is distributed in a fibrogranular pattern troughout the nucleoplasm. In addition, CstF is highly concentrated in a novel nuclear domain (CstF bodies), which is closely associated with, and sometimes overlapping coilin- and snRNP-containing coiled bodies. This suggests a functional link between these two nuclear compartments. PAB II is concentrated in a few tens of irregular domains, also called speckles, which are known to be enriched in snRNPs, SC-35 and poly(A) RNA. These compartments correspond to clusters of interchromatin granules that can be recognized by electron microscopy. In addition, PAB II shows a widespread fibrogranular distribution, similarly to most snRNPs. Upon treatment with inhibitors of RP II transcription, CstF bodies fuse with coiled bodies, concomitant with the release of snRNPs from these nuclear organelles. It is well known that snRNPs become almost exclusively associated with the rounded up clusters of interchromatin granules when transcription is inhibited. PAB II follows the redistribution of snRNPs after treatment with inhibitors of transcription or with cordycepin, an inhibitor of polyadenylation. In contrast, ara-A, which inhibits both cleavage and polyadenylation, results in a reversible fragmentation of the clusters of interchromatin granules as monitored by the spatial distributed of PAB II, U2 snRNP and SC-35. CstF bodies disappear in the presence of ara-A. These observations underscore the dynamics of the compartmentalized nuclear organization, which is tightly coupled to nucleic acid metabolism.

Replication on the Nuclear Matrix and Nucleosome Assembly (Joint)

J7-006 *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.

J7-007 **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.

J7-008 **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.

J7-009 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.

Discussion: Imaging Approaches for Addressing Cell Structure-Gene Expression Interrelationships

J7-010 CONFOCAL MICROSCOPY: PROBING GENOMIC ORGANIZATION AND FUNCTION IN 3D, Ping-chin Cheng, Advanced Microscopy and Imaging Laboratory (AMIL), Department of Electrical and Computer Engineering, State University of New York at Buffalo, Buffalo, NY 14260 USA

Recent development in confocal light microscopy and multidimensional image analysis tools has provided the possibility of probing genomic organization and function in three and higher dimensions. Confocal microscopy, characteristic by its optical sectioning capability, can provide stacks of well-aligned serial sections from a specimen labeled with multiple fluorochromes. With the aid of computer data acquisition, these digital volumetric data can then be used for subsequent image analysis to obtain quantitative description about the structure of interest. It is the intent of this presentation to examine the applications of confocal microscopy in the study of 3D nuclear structures along with the physical properties of confocal microscope and its limitations. The transverse (x-y) resolution of a confocal microscope is significantly higher then its axial (z) resolution (approximately 0.1μm for the transverse resolution and 0.7μm for the axial resolution when a NA=1.4 objective lens was used). If improper mounting medium or cover slip was used, the z resolution can be further reduced by degrading the correction of spherical aberration of the objective lens. This unequal x-y and z resolution not only posts many difficulties for subsequent image processing and analysis, but also limits effective resolution in the study of small 3D structures. For instance, a 0.2μm spherical structure will looks like an American football in the 3D data set due to the lower axial resolution. In addition, the proper selection of fluorochromes, dichroic filter sets and excitation wavelengths are important issues in order to minimize problems, such as photo-bleaching and cross-talks between channels, frequently associated with fluorescent imaging. Measures to improve the effective z resolution and to deal with the cross-talks between fluorescent channels will be discussed. Furthermore, physical properties of the confocal imaging system such as field curvature, chromatic aberration and detector sensitivity will be examined. (Supported by a grant from the National Science Council, Republic of China; NSC-83-0211-B-001-001).

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P. C. Cheng, T. H. Lin, W. L. Wu and J. L. Wu, eds (1994): Multidimensional microscopy, Springer-Verlag, New York.

J7-011 THREE-DIMENSIONAL MICROSCOPY AND IMAGING APPROACHES FOR STUDIES OF CHROMOSOME TERRITORIES AND SUBREGIONAL CHROMOSOMAL TARGETS IN HUMAN CELL NUCLEI

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Chromosome territories and subchromosomal regions in three-dimensionally intact female human cell nuclei were visualized by FISH. Optical sectioning was performed using confocal laser scanning fluorescence microscopy. Two approaches were used for volume measurements: 1.) Chromosome territory areas were segmented in each confocal section applying a range of gray value thresholds. For each threshold chromosome territory volumes were computed from all sections using the Cavalieri estimator (1). 2.) A truly three-dimensional segmentation procedure was performed with the stack of serial sections obtained for each nucleus on the basis of a Voronoi-diagram, which consists of convex polyhedra structured in a graph environment (2,3). Volume ratios calculated for a reasonable range of thresholds for several pairs of homologous autosome territories and for the two X-chromosome territories in female human amniotic fluid cell nuclei suggested that the inactive X chromosome territory was not much more condensed than the active one. Differences in shape and surface parameters, however, were much more pronounced for the two X-territories than for autosome territories (see abstract by Dietzel et al., this conference). Approaches to delineate the positioning of subregional chromosome targets within or at the surface of chromosome territories are also presented. Such approaches are still hampered by the limited spatial resolution of present far field light microscopy. New approaches, such as 4Pi-confocal microscopy (4), confocal theta-microscopy (5), and axial tomographic light microscopy (6), are being developed to overcome these limitations.

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J7-012 COMPUTER AIDED STRATEGIES FOR DECIPHERING THE THREE DIMENSIONAL ARCHITECTURE AND FUNCTIONAL DYNAMICS OF THE MAMMALIAN CELL NUCLEUS, Jagath Samarabandu, Department of Biological sciences, State University of New York at Buffalo, Buffalo, NY 14260

A laser scanning confocal microscope (LSCM) provides the ability to capture three dimensional images of microscopic structures. As data from the LSCM is already in digitized form, this lends itself easily to computer assisted processing and analysis of the acquired image. Typical operations carried out in our image analysis system include enhancements, corrections for imperfections in image acquisition, detection of structures, morphometry and visualization. Image corrections are applied to the two channel LSCM images obtained from samples labeled with two color fluorescent dyes (FITC and Texas Red) because of the spill-over observed due to the overlapping emission spectra of these two dyes. The structure detection algorithms range from the simple approaches such as thresholding and boundary tracing to more complex techniques where intensity maxima are used to find the boundary. These boundary detection algorithms operate on the 2D sections of the 3D data set first. The resulting contours are analyzed for connectivity in 3D space to build up the structures. Once boundary of these structures are obtained, another set of programs calculate various parameters such as the volume and center of gravity etc. Higher levels of analysis that are being developed include quantizing various aspects of co-localization for two channel images which shows the DNA replication at different times and elucidating the 3D higher order assembly of individual replication sites. The visualization system consists of projection and rendering algorithms which generate 2D views of the raw and processed 3D data sets. For raw images, simple techniques such as intensity maxima projection combined with pixel shifting yields stereo images with a minimum amount of computation. More advanced techniques such as volume rendering with ray-tracing where different intensity ranges are assigned different colors and opacity are much more computationally intensive and in general provide better results. Visualizing the processed data sets where the boundaries of the structures are obtained using image analysis range from wire-frame views to surface shading techniques. Because of the lesser amount of computations needed, we combined the wire-frame views with interactive exploration of the structures where one can look at the structure at different directions and obtain morphometrical information of specific structures by selecting them with a pointing device such as a mouse cursor. With the introduction of cheaper and faster computer hardware, image analysis and visualization have become a very attractive approach to extract information from multidimensional images. Careful selection of image acquisition, processing, analysis and visualization strategies is needed for optimum utilization of human and computer resources.

The Nuclear Matrix: Involvement in Replication, Transcription, Gene Splicing and Cellular Regulation

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

J7-013 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.

J7-014 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.

J7-015 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.

The Nuclear Matrix: Involvement in Replication, Transcription, Gene Splicing and Cellular Regulation

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

J7-016 REGULATION OF CHICKEN LYSOZYME LOCUS ACTIVATION IN TRANSGENIC MICE, Matthias Huber, Albrecht E. Sippel and Constanze Bonifer, Institut für Biologie III der Universität Freiburg, Schänzlestr. 1, 79104 Freiburg, Germany

The complete chicken lysozyme gene domain is expressed copy number dependently and at a high level in macrophages of transgenic mice. Position independency of expression can only be achieved if the complete set of regulatory elements located on the lysozyme gene domain is present. The DNaseI hypersensitive chromatin sites (DHSs) of the lysozyme gene domain indicating the activity of the various cis regulatory elements are not present in multipotent myeloid progenitor cells. Following commitment towards the myelomonocytic lineage the chromatin of the chicken lysozyme domain is extensively reorganized. However, DHS formation and nucleosomal rearrangements occur in a stepwise fashion, dependening on the differentiation state of the cell. In order to examine the influence of cis regulatory deletions on chromatin structure, we investigated DHS patterns and nucleosomal organization of position independently and position dependently expressed transgenes. Nucleosome phasing patterns on the various transgenic cis regulatory elements in mouse cells are the same as in the endogenous chicken lysozyme gene. The presence of DHSs in the clustered chicken lysozyme transgene domains expressing variable levels of lysozyme mRNA is correlated with transgene expression levels and is dependent on the chromosomal position. We demonstrate that in position independently expressed transgenes of a given transgene cluster all DHSs on all genes are formed with the authentic relative frequency. In contrast, in position dependently expressed transgenes only some genes within each transgene cluster develop an active chromatin configuration and are transcriptionally active. Our results show, that if one essential regulatory element is lacking, the efficiency of active chromatin formation on the chicken lysozyme gene domain during myeloid differentiation is reduced and influenced by the chromosomal position. Hence, no individual regulatory element on the lysozyme domain is capable of organizing the chromatin structure of the whole locus in a dominant fashion. Further experiments will be discussed which investigate the role of chicken lysozyme promoter elements in locus activation and active chromatin formation.

J7-017 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.

Visualizing Domains of Genomic Organization, Transcription and Splicing in the Cell Nucleus

J7-018 **Chromosome territories and the interchromatin domain compartment: a model for the functional compartmentalization of the cell nucleus** T. Cremer[1], P. Lichter[2], C. Cremer[3], [1]Institute of Human Genetics and Anthropology, University of Heidelberg, [2]German Cancer Research Center, Heidelberg, [3]Institute of Applied Physics, University of Heidelberg, Heidelberg, Germany

Chromosomes apparently occupy distinct territories in the cell nucleus and these territories again appear to represent a mosaic of distinct subchromosomal domains, ranging from G-, R- and T-band domains to individual chromatin loop domains. Based on this view of interphase chromosome architecture, we have proposed a model for the functional compartmentalization of the cell nucleus (1,2). This model predicts: (a) Repulsive electric forces between negatively charged chromosome territory and chromatin domains maintain a threedimensional network of nuclear channels, which we call the interchromatin domain (ICD) compartment. This channel network is connected to the nuclear pores and and extended between the surfaces of all chromosome territories. From these surfaces the channels branch out into the territoriy interior where its ramifications end between individual chromatin domains. (b) Within the ICD-compartment a facilitated diffusion of macromolecules (e.g. specific DNA binding factors, RNAs) is possible. Depending on their size and electric net charge macromolecules may become enriched within the ICD-compartment facilitating the preferential formation of macromolecule aggregates with negative electric net charge at chromatin territory and chromatin domain surfaces. Such aggregates comprise the machineries for transcription, splicing, DNA-replication and repair. (c) High salt conditions and/or the removal of fixed negative electric net charges of chromatin domains by DNAse digestion, two treatments often performed in nuclear matrix preparations, reduce the repulsive forces which normally exist between negatively charged macromolecule aggregates and negatively charged chromatin surfaces to a critical extent. This results in the precipitation of the constitutents present in the ICD-compartment. Accordingly, we consider the ICD-compartment as the (partial?) *in vivo* equivalent of *in vitro* nuclear matrix preparations and we predict that functions which have been associated with the nuclear matrix occur in this compartment *in vivo*. (d) Positioning of genes at chromatin domain surfaces is an essential condition of their transcription. Conversely, compaction of genes or their regulatory sequences into the interior of chromatin domains may contribute to gene inactivation. (f) Replication granules may be large enough to become spatially fixed in the ICD-space without resort to a nuclear skeleton. In such a scenario DNA-replication should start at origins located at chromatin domain surfaces in close association with replication granules. While our model does not necessarily exclude the possibility that skeleton like structures form in the ICD-space or elsewhere, such structures do not appear *a priori* indispensable for the functional compartmentalization of nuclear chromatin. The predicitions of this model open avenues for a number of critical experimental tests. We have initiated experiments to test aspects of this model by fluorescence in situ hybridization (FISH) of chromosome territories and subregional chromosome targets, as well as the immunocytochemical detection of specific nuclear antigens in combination with threedimensional digital microscopy and image analysis (see abstracts of C. Cremer et al. and S. Dietzel et al., this conference).

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J7-019 CHROMOSOME STRUCTURE AND FUNCTION IN DEFINED GENETIC DOMAINS, Laura Manuelidis, Yale Medical School, New Haven, CT, 06510.

Although it has not yet been possible to directly examine defined chromosome regions in a living cell, one can visualize such regions at any instant in time using adequate fixation and in-situ hybridization methods. I will present some of our data on discrete regions of the genome that cover a spectrum of functional states, e.g., from transcriptional silence to activation. Several recombinant experimental systems suggest that transcriptionally active domains are more compact than predicted by loop extension models. The biological reasons for this will be discussed in the context of replication and dynamic changes within the interphase nucleus.

J7-020 **PRE-mRNA SPLICING AND RNA TRANSPORT,** David L. Spector, Raymond T. O'Keefe, Shelley Kaurin, and Sui Huang, Cold Spring Harbor Laboratory, P. O. Box 100, Cold Spring Harbor, New York 11724.

Splicing is an essential step in the processing of intron-containing pre-mRNAs prior to their transport out of the nucleus. We have previously shown that in actively transcribing cells splicing factors are localized in a speckled distribution pattern which corresponds to interchromatin granule clusters and perichromatin fibrils. Experiments using antisense DNA probes, which would specifically interfere with pre-mRNA splicing, or drugs which specifically inhibit RNA polymerase II demonstrated that upon inhibition of either of these processes interchromatin granule clusters round up, increase in size, and the connections between clusters are no longer visible. However, upon reinitiation of these functions the typical speckled pattern of splicing factors is reformed. Therefore, interfering with pre-mRNA splicing or RNA polymerase II transcription results in a specific reorganization of splicing factors. These data suggest that pre-mRNA splicing occurs at the site of transcription (perichromatin fibrils) and that factors shuttle between storage and/or assembly sites (interchromatin granule clusters) and sites of active transcription (perichromatin fibrils).

To further understand the functional organization of pre-mRNA processing in the mammalian cell nucleus, we have studied the distribution of poly(A)$^+$ RNA at the electron microscopic level and its transport through nuclear pores. Poly(A)$^+$ RNA was detected in the nucleus at interchromatin granule clusters and perichromatin fibrils. After inhibition of RNA polymerase II transcription for 5-10 hrs., a stable population of poly(A)$^+$ RNA remained in the nucleus and was reorganized into fewer and larger interchromatin granule clusters along with pre-mRNA splicing factors. This stable population of nuclear RNA may play an important role in chromatin regulation and/or nuclear structure. Furthermore, we have observed that, in actively transcribing cells, the regions of poly(A)$^+$ RNA which reached the nuclear pore complexes appeared as narrow concentrations of RNA suggesting a limited or directed pathway of movement. All of the observed nuclear pores contained poly(A)$^+$ RNA suggesting that they are all capable of exporting RNA. In addition, we have visualized, for the first time in mammalian cells, the transport of poly(A)$^+$ RNA through the nuclear pore complexes.

In summary, our studies have shown a direct correlation between RNA polymerase II transcription and the nuclear organization of splicing factors. We propose a recruiting model to account for the dynamic localization of splicing factors. In this model, factors are associated with specific storage and/or reassembly sites (interchromatin granule clusters) in the nucleus. Prior to or at the initiation of transcription, these factors are recruited to the active sites of transcription (perichromatin fibrils). Our model suggests that there are signals generated in the nucleus which regulate the compartmentalization of factors to nuclear regions where they will be functioning. Identification of these signaling mechanisms will be key to understanding the integration of a variety of functional events which occur within the boundaries of the nuclear envelope.

Nuclear Matrix Proteins and Nuclear Architecture

J7-021 NUCLEAR MATRIX PROTEINS WITH BIFUNCTIONAL ROLES IN THE NUCLEUS AND MITOTIC APPARATUS, B. R. Brinkley, B. Scott, D. Turner, C. Zeng* and D. He, Departments of Cell Biology and *Biochemistry, Baylor College of Medicine, Houston, TX, 77030.

Mitosis in mammalian cells is accompanied by the dissolution of the nucleus and formation of a mitotic apparatus (MA) required for the movement, alignment and partitioning of chromosomes into daughter cells. The complex organization of the interphase nucleus is transiently disassembled at mitosis but reassembled in daughter nuclei at telophase. We have identified several nuclear protein complexes that are components of the nuclear matrix in interphase and also become functional assemblies of the MA during mitosis. When mitosis is completed, the same proteins re-enter the nucleus to assume different roles in maintaining organization and spatial arrangements of nuclear domains. Thus, proteins that are associated with the nucleoskeleton in interphase can transiently interact with cytoskeletal components in mitosis. One such bifunctional protein is NuMA, an abundant nucleus-mitotic apparatus protein in HeLa cells. During interphase, NuMA isoforms associate with nuclear core filaments as shown by immunogold EM and form complexes with snRNPs as shown by immunofluorescence and immunoprecipitation. At mitosis, NuMA is translocated to the spindle poles where it associates with the (-) ends of Mts and functions in chromosome partitioning and nuclear reformation. Constitutive centromere proteins of metaphase chromosomes are also intimately associated with the nucleoskeleton in interphase. CENP-B and CENP-C remain bound to core filaments as insoluble components of the centromere after nuclear extraction. Movement and clustering of centromeres into discrete intercentromeric arrays occurs in conjunction with cen-DNA replication in mid to late S-phase in Muntjac cells. These arrays appear to be arranged along filaments of the nucleoskeleton. Other nuclear matrix-associated proteins transiently associate with kinetochores until metaphase but detach at anaphase to remain part of the interzonal microtubule complex. Collectively, our studies indicate that nucleoskeletal protein complexes persist in mitosis and are essential components in spindle function. Later, these same proteins return to the nucleus where they play significant roles in nuclear structure and function.

J7-022 THE DYNAMIC PROPERTIES OF THE NUCLEAR LAMINS, Robert D. Goldman[1], Anne Goldman[2], Satya Khuon[1], Michelle Montag-Lowy[1], Robert D. Moir[1], and Tim Spann[1], [1]Northwestern University Medical School, Department of Cell and Molecular Biology, Chicago.

Nuclear lamins A, B and C are the major proteins comprising the mammalian nuclear lamina which is located in the peripheral region of the nucleoplasm. Until quite recently, the nuclear lamins were thought to be relatively static polymeric constituents of the interphase karyoskeleton due to their resistance to high salt and detergent extraction. It has been proposed that this lamina functions in numerous activities including the maintenance of the shape and integrity of the nucleus during interphase, the processes of nuclear disassembly and reassembly which accompany mitosis, and the regulation of the organization and replication of chromatin. However, there is a paucity of direct evidence available to support these possible functions since little is known about the *in vivo* properties of the lamins, especially in interphase. Based on these considerations we initiated a series of experiments aimed at determining the properties of lamins *in vivo*. Initially, these experiments have involved the microinjection of biotinylated human lamins into live cells. The results demonstrate that microinjected lamins are incorporated into the lamina during interphase. This illustrates that the lamina is a dynamic structure, capable of subunit/polymer exchange. The incorporation process appears to involve discrete lamin rich foci located deep within the nucleoplasm. We now have evidence that these foci may be involved in the post-translational processing of the lamins. Similar nuclear foci are also seen as normal constituents of uninjected cells, and we have been able to link their distribution to different stages of the cell cycle. One of our most intriguing observations is that the DNA replication foci which typify mid-late S phase appear to contain nuclear lamin B, but not lamins A/C, indicating that lamin B may play a role in DNA synthesis. Other studies have been initiated which attempt to determine the dynamic properties of the nuclear lamins in transiently transfected cells. In order to gain insights into the biochemical mechanisms regulating lamin dynamics and function, we have been using two cell free systems. These involve studies of nuclear envelope breakdown in the surf clam oocyte in an attempt to show the relationship between nuclear lamin disassembly and the congression of chromosomes to form the metaphase plate. A hybrid system consisting of Xenopus laevis extracts spiked with bacterially expressed human lamins, is employed to study lamin-lamin interactions during the formation of the lamina. This work has been supported by the NCI.

J7-023 **MOLECULAR AND ARCHITECTURAL STUDIES OF NUCLEAR MATRIX PROTEINS**, Michael J. Mortillaro and Ronald Berezney, Department of Biological Sciences, SUNY at Buffalo, Buffalo, New York 14260

Our understanding of the proteins which compose the internal architecture of the nuclear matrix has lagged behind recent progress defining domains of replication, transcription and premRNA splicing associated with these structures. We are currently focusing our attention on identifying the major proteins which constitute the nuclear matrix. A limited number of interior nuclear matrix proteins, termed nuclear matrins, were previously identified by our lab. In this presentation we report our progress on the analysis of three different nuclear matrins: matrin 3, P-250, and SFA-Cyp. Studies of genomic DNA reveal that the gene for matrin 3, an acidic protein of 125kDa, is a member of a small family of genes. RNA and cDNA analysis suggest that matrin 3 undergoes tissue specific regulation at the posttranscriptional level. To characterize this gene family several matrin 3-like genes have been cloned. Analysis of these clones with implications for regulation of their expression will be discussed. Additionally, we will report on the cloning and characterization of a previously unidentified nuclear matrin termed splicing factor associated-cyclophilin (SFA-Cyp). Antibodies raised against a fusion protein of SFA-Cyp indicate that it is a basic 103kDa protein which is located in the nucleus and enriched in the nuclear matrix. Also, this antibody stains a series of bright foci in mammalian nuclei which colocalize with splicing factors. As suggested by its derived amino acid sequence and immunofluorescent pattern SFA-Cyp may chaperone specific proteins to sites of splicesome assembly where it may then assist these proteins to associate with forming splicesomes. A monoclonal antibody raised against nuclear matrices recognizes a large acidic protein of 250kDa that is enriched in the nuclear matrix and termed P-250. The protein detected by our antibody was shown by antibody cross reactivity studies not to be a NuMA protein. Laser scanning confocal microscopy of P-250 visualizes a complex architectural arrangement in the nucleus consisting of several large bright foci and many small, less intensely staining granules. Some of the large bright foci are also stained by splicing factor specific antibodies. This may indicate an association of P-250 with splicing factors.

Targeting of Nuclear Proteins and Cell Cycle Control of Nuclear Assembly

J7-024 **The Role of B-type Lamins in the Assembly of a Replication Competent Nucleus.** [1]Christopher J. Hutchison, [1]Hazel E. Jenkins, [2]Carl Smythe and [1]William G.F. Whitfield. [1]The Department of Biological Sciences, The University of Dundee, Dundee, DD1 4HN, Scotland. [2]The Department of Biochemistry, The University of Dundee, Dundee, DD1 4HN, Scotland.

The assembly of a replication competent nucleus can be achieved *in vitro* using cell-free extracts of *Xenopus* eggs. In this system nuclear assembly occurrs through a dependent pathway which probably involves chromatin decondensation, nuclear envelope assembly, nuclear pore assembly, lamina assembly and finally the assembly of replication factories. Extracts which are depleted of lamins, using monoclonal antibodies linked to paramagnetic beads, support the first three steps in this pathway but do not support the assembly of replication factories. Thus pronuclei are assembled around sperm chromatin which have continuous membrane structures and well formed nuclear pores. These nuclei exclude IgG but actively transport a range of karyophillic proteins. Nevertheless, the nuclei remain abnormally small and do not initiate semi-conservative DNA synthesis. Since the capacity to assemble a replication competent nucleus in lamin depleted extracts can be reconstituted by the addition of purified B-type lamins it seems likely that this inability is a direct consequence of removal of lamins. The small size ($\sim 10\mu m$) of nuclei assembled in lamin depleted extracts is reminiscent of the size of normal nuclei at a pre-initiation stage. Extracts treated with the protein kinase inhibitor olomoucine assemble nuclei possessing a morphologically normal lamina but with an average diameter of only $10\mu m$ and lacking replication factories. Other protein kinase inhibitors such as staurosporin and cip1 do not have the same effect but instead assemble normal sized nuclei ($\sim 20\mu m$) diameter but possessing non-functional replication factories. These results can be explained if in the stepwise assembly of a replication competent nucleus the formation of a nucleoskeleton is dependent upon and immediately follows the assembly of a lamina. Thus the inability of lamin depleted nuclei to initiate semi-conservative DNA replication may result from an absence of nucleoskeleton filaments in those nuclei. Similarly, the inability of olomoucine treated nuclei both to grow and to assemble replication factories may also result from inhibition of nucleoskeleton assembly.

J7-025 NUCLEAR ARCHITECTURE AND AND THE ASSEMBLY OF VIRAL REPLICATION STRUCTURES. David M. Knipe, Susan Uprichard and Lauren Liptak. Dept. of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA

Herpes simplex virus DNA replication takes place in intranuclear structures called replication compartments. When viral DNA replication is blocked, viral DNA replication proteins, the host cell DNA replication apparatus, and other cellular proteins localize to smaller punctate structures called prereplicative sites. The HSV ssDNA-binding protein, ICP8, has been shown not only to localize to these structures but also to be essential for the formation of these structures. Confocal microscopy with ICP8 immunofluorescence has shown that these structures extend through the interior of the cell nucleus, and examination of binucleate cells has shown that the intranuclear location of these structures is determined by pre-existing nuclear architecture(1). When viral DNA replication is blocked, viral DNA replication proteins localize to punctate structures called prereplicative sites. Localization of ICP8 to these nuclear structures in infected cells requires four other viral DNA replication proteins. Furthermore, ICP8 undergoes a conformational change at or during assembly of the prereplicative sites in the infected cell nucleus. This structural change requires the same four viral proteins and possibly DNA and/or host proteins. The conformational change correlates with the DNA-binding ability of ICP8 and therefore may be induced by DNA-binding or may activate the DNA-binding activity of ICP8. Thus, the assembly of HSV DNA replication structures involves the interaction of specific viral proteins and possibly host proteins accompanied by the conformational rearrangement of ICP8, all occurring at specific intranuclear sites.

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J7-026 TARGETING OF PROTEINS TO FUNCTIONAL DOMAINS WITHIN THE MAMMALIAN NUCLEUS
Heinrich Leonhardt and M. Cristina Cardoso, Harvard Medical School, Boston.

The mammalian nucleus is highly organized into distinct functional domains separating different biochemical processes like transcription, RNA processing, DNA synthesis and ribosome assembly. DNA replication is known to occur in discrete patterns of nuclear foci, which undergo characteristic changes throughout S-phase. By the use of multiple labeling immunofluorescence as well as immunoelectron microscopy, replication factors PCNA [1], DNA polymerase alpha [2] and RPA70 [3] as well as DNA methyltransferase (DNA MTase) [4] have been shown to specifically redistribute within the nucleus and to colocalize with sites of ongoing DNA replication.
We took advantage of the functional organization of mammalian nuclei to analyse regulatory pathways leading to S-phase and to look for a link between cell cycle regulation and DNA replication. We screened for cell cycle proteins present at nuclear replication foci and found cyclin A and cdk2, but not cyclin B1 and cdc2, localized at nuclear replication foci [3]. This colocalisation was observed throughout S-phase suggesting a direct role of cyclin A and cdk2 in the control of DNA replication. This functional organization may explain how cell cycle kinases have similar biochemical properties in vitro but catalyze specific reactions in vivo.
The biochemical principles of the functional organization of the mammalian nucleus are still largely unknown. In case of the DNA MTase we have, however, identified a distinct protein domain controlling the subnuclear localization. This targeting sequence is located within the regulatory domain of the DNA MTase and is dispensable for enzyme activity in vitro, suggesting a regulatory role in the coordination of DNA replication and methylation [4].
These results raised the possibility that such targeting sequences may play a general role in the functional organization of the mammalian nucleus. In support of this hypothesis, we found that human DNA ligase I contains, like DNA MTase, a targeting sequence, which is necessary and sufficient for localization at replication foci. The targeting sequence of the human DNA ligase I is bipartite and has no obvious similarity with the DNA MTase, but is highly conserved between human and mouse. The fact that the targeting sequence is absent in lower eukaryotic and prokaryotic homologues, suggests that 'targeting' is a rather recent development in evolution. Finally, targeting sequences have also been identified in some splicing factors and in viral proteins, which are responsible for their localization to the speckled compartment and to the nucleolus, respectively [5,6].These higher levels of organization are likely to contribute to the regulation and coordination of the complex and interdependent biochemical processes in the mammalian nucleus.

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The Role of the Nuclear Matrix in the Integration of Signal Mechanisms during Cellular Growth and Development

J7-027 **NUCLEAR PROCESSES MODULATED BY WILDTYPE AND MUTANT P53, Wolfgang Deppert, Jörn Mosner, Beate Müller, Torsten Mummenbrauer, and Frank Grosse, Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie, Martinistr. 52, D-20251 Hamburg, Germany.**

The tumor suppressor p53 plays a major role in preserving the integrity of the genome in vertebrate cells. Its main function is thought to mediate growth arrest of cells upon DNA-damage in order, to allow damage to be repaired before it becomes fixed during DNA-replication. The finding that mice made deficient in p53 expression (p53 null-mice) develop normally, but are prone to an early occurence of a variety of tumors led to the conclusion that p53 is not required for normal cell growth and development. However, analysis of primary mouse lymphocytes stimulated into proliferation demonstrated that p53 expression in these cells is tightly regulated, and strictly correlates temporally and structurally with cellular DNA synthesis, arguing for a direct involvement of p53 in processes associated with cellular DNA replication. As p53 is not absolutely required for DNA replication, we postulated an auxiliary function for p53 in cellular DNA replication. In line with a possible involvement of p53 in DNA repair processes during replication, we found that purified p53 exhibits a 3'-to 5'-exonuclease activity. The lack of such an activity in p53 null mice might contribute to the accumulation of DNA alterations leading to tumor development in these animals.

Mutant p53 not simply has lost its tumor suppressor activities, but may act as a dominant oncogene. In analyzing the molecular basis for this "gain of function" of mutant p53, we found that mutant, but not wild-type p53 specifically binds to MAR/SAR elements. Considering the postulated structural and functional role of MAR/SAR elements in controlling the temporal and spatial organization of eukaryotic gene expression and replication, mutant p53, by binding to these elements could modulate the transcription and/or replication of gene clusters encoding genes necessary for the progression of tumors to a more tumorigenic phenotype. Such a role for mutant p53 would fit the observation that mutations in the p53 gene often are secondary events in tumor development.

J7-028 CELL CYCLE DEPENDENT ASSOCIATION OF PROTEINS WITH THE NUCLEAR MATRIX, Peter Loidl, Gerald Brosch, Anton Eberharter, and Thomas Lechner, Department of Microbiology, University of Innsbruck - Medical School, A-6020 Innsbruck, Austria.

The facts that the nuclear matrix represents a structural framework of the cell nucleus and that nuclear processes, such as DNA replication, transcription and DNA repair, are associated with this subnuclear structure, suggest that its components are subject to stringent cell cycle regulation. Little attention has so far been paid to internal nuclear matrix proteins and nuclear matrix-associated proteins with respect to the cell cycle. We set out to study this question during the naturally synchronous cell cycle of *Physarum polycephalum* and found that the action of various proteins is modulated in a cell cycle dependent mode by transient association with the nuclear matrix. Enzymes (shown for thymidine kinase and RNA polymerase) as well as regulatory proteins (shown for proto-oncogene proteins and cell cycle regulatory protein kinases) are only associated with the nuclear matrix at distinct time points during the *Physarum* cell cycle. This cell cycle pattern of matrix association corresponds to the cell cycle schedule of the relevant processes. It is suggested that transient, cell cycle dependent binding to nuclear matrix structures represents a further level of cellular regulation of the function of certain proteins. Furthermore, nuclear matrix associated chromatin is distinguished from bulk nuclear chromatin by an elevated level of highly acetylated core histone subspecies; the acetylation pattern of matrix bound histones fluctuates during the cell cycle, indicating that certain cell cycle dependent properties of this small proportion of chromatin are modulated by posttranslational modification; interestingly, we could get no evidence for association of histone acetyltransferases or histone deacetylases with the nuclear matrix, regardless of the cell cycle stage or of whether we used fungi, plants or vertebrate cells as an experimental system. We suggest that nuclear matrix bound chromatin is more easily accessible to nuclear histone acetyltransferases than bulk chromatin due to a specific conformation of this type of chromatin.

J7-029 A PROGESTERONE RECEPTOR BINDING FACTOR (RBF): A NUCLEAR MATRIX ACCEPTOR PROTEIN WHICH BINDS TO MAR SEQUENCES IN THE C-MYC GENE, Thomas C. Spelsberg, Andrea Lauber, Nicole Sandhu, Mark Schuchard, and M. Subramaniam. Department of Biochemistry and Molecular Biology, Mayo Clinic and Mayo Graduate School of Medicine, Rochester, MN 55905.

Years ago, steroid receptor complexes were found to bind *in vitro* and *in vivo* to specific acceptor sites in the nuclear chromatin (Spelsberg, et al, 1971, J. Biol. Chem. 246:4188-4197) and to the nuclear matrix (Berezney and Coffey, 1977, J. Cell Biol. 73:616-637). Later the chromatin acceptor sites and the nuclear matrix sites for the avian progesterone receptor (PR) were shown to be one and the same (Schuchard, et al, 1991, Biochemistry 30:9516-9522). In this same paper, the purification of a nuclear matrix "acceptor protein" for the avian PR, termed receptor binding factor (RBF) was reported by this laboratory. This factor was isolated based on its ability to generate specific, high affinity PR binding of avian genomic DNA. Immunohistochemistry, utilizing antibodies to RBF, supported the nuclear matrix association of RBF in avian oviduct. A co-localization of RBF and PR in selected cell types was found. The RBF is a 10 kD protein with a unique sequence with some homology to an ATPase subunit and a UV-induced nuclear protein. Interestingly, Southwestern blot analysis has demonstrated that RBF binds to specific DNA sequences in the *c-myc* gene. The binding domain in the *c-myc* gene has an AT-rich sequence flanked by GC-rich domains and has been identified as a MAR-like (AT-rich) domain. The RBF did not bind to a different, but 70% homologous, AT-rich domain found in another gene. Studies are underway to assess the role, if any, that this AT-rich domain has on the activity and steroid regulation of the *c-myc* promoter. The full length cDNA to RBF-1 has been prepared and used to identify a 700 bp mRNA whose levels are regulated by E_2 in avian oviduct. The genomic sequence of RBF has been isolated and shown to contain 4 exons and functional EREs and heat shock elements in the 5′ domain. Studies are underway to assess the effects of expression of the RBF in quail fibroblast (QT-6) cells on reporter gene expression using a construct containing the *c-myc* promoter domains linked to a luciferase reporter. Preliminary studies indicate that the RBF reverses the progesterone down regulation of this reporter gene. The effects on P response elements in these expression constructs will also be assessed. Supported by NIH grant HD9140-P1, Training Grant CA09441, and the Mayo Foundation.

The Nuclear Matrix in Carcinogenesis, Tumor Progression and Diagnosis

J7-030 NUCLEAR MATRIX PROTEINS IN HUMAN BREAST AND PROSTATE CANCER PATIENTS, Kenneth J. Pienta and Tracy S. Replogle, University of Michigan Medical Center, Ann Arbor, MI 48109-0680

The development of cancer from normal epithelium depends on a variety of events including aberrations at the cellular and molecular level. It has been long realized that cancer cells have markedly altered nuclear shapes, patterns of chromatin organization, and gene expression. While alterations in nuclear shape and DNA organization and function have not been well characterized, they appear to be related, at least in part, to alterations of the nuclear matrix, i.e., the dynamic RNA-protein scaffold of the nucleus. The nuclear matrix plays a central role in the control of DNA organization and gene expression. Evidence demonstrates that nuclear matrix protein (NMP) composition is altered in breast and prostate cancer cells as compared to their normal cell counterparts. In breast cancer cells, we have identified two groups of NMPs; a normal cell specific group consisting of two proteins which are found only in normal breast epithelial cells and a cancer specific group consisting of four proteins expressed only in breast cancer cells. The characterization of one of these potentially cancer specific NMPs suggests that it is associated with nucleoli. In the prostate, we have identified three prostate cancer specific NMPs. We suggest that alterations in NMP expression may be a related to altered genes expression observed in human cancers and that the characterization of these NMPs may lead to the identification of new biomarkers and/or intermediate endpoints for epithelial cell transformation.

J7-031 NUCLEAR MATRIX PROTEINS AS TUMOR MARKERS, Ying-Jye Wu, Sarah M. Wiesbrock, Louisa I. Caamano, Robert P. Szaro, Daron S. Forman, David S. Peal , David H. Foehl, and Susan K. Keesee, Matritech Inc., Cambridge, Massachusetts 02138

The nuclear matrix is the non-chromatin structural network remaining in the nucleus following digestion with detergents, nucleases and high ionic strength buffers. We have used two-dimension gel electrophoresis to identify cancer specific nuclear matrix proteins (NMPs) from colon and breast tumors. Tumor and normal tissues were freshly obtained and nuclear matrix proteins were extracted and analyzed by two-dimension gel electrophoresis. The gel patterns were carefully compared to find the cancer-specific nuclear matrix proteins. For the colon study, NMPs from 18 cancer patients were compared with NMPs from 10 normal individuals. Six NMPs were identified which were present in 18/18 tumors but in 0/10 normals. For the breast study, seven NMPs were identified which were present in 13/13 tumors but in 0/10 normals. The cancer-specific NMPs were then excised from two dimension gels and used as the immunogens to raise monoclonal antibodies. All antibodies were characterized by both immunofluorescent staining and two- dimension Western blot. Antibodies raised against one of colon cancer-specific NMPs displayed a variety of nuclear immunofluorescent patterns. At least two antibodies recognize an antigen which is cell cycle-dependent, since the antigen shows a nuclear pattern in interphase cells which disappears in mitotic cells. Two other antibodies localize an antigen in subnuclear domains which remains following chromatin removal and treatment with 2M NaCl. A third group of antibodies show nucleoplasmic staining following chromatin removal. One antibody defines a filamentous network that fills the nucleus, defines the nuclear rim and extends out into the cytoplasmic space following chromatin removal and high salt treatment. The antigen recognized by these antibodies is nuclear matrix protein, since it remains on the nuclear matrix following chromatin removal and high salt treatment. The finding of different nuclear staining patterns may be due to the fact that different antibodies recognize different epitopes being represented differently in the cell, some of which may be partially or completely masked by other cell structures. We are also using these antibodies to detect cancer-specific NMPs in the serum of cancer patients. Preliminary results indicate that some antibody pairs in the ELISA format can distinguish cancer from normal serum.

Late Abstracts

DIFFERENTIAL TRANSLOCATION OF PROTEIN KINASE CK2 TO THE NUCLEAR MATRIX: A SYSTEM FOR INTRANUCLEAR SIGNALLING FOLLOWING A GROWTH STIMULUS, Khalil Ahmed and Sherif Tawfic, Cellular and Molecular Biochemistry Research Laboratory (151), and Department of Laboratory Medicine and Pathology, V.A. Medical Center and University of Minnesota, Minneapolis

Protein kinase CK2 (casein kinase 2) is a ubiquitous messenger-independent protein serine/threonine kinase that has been implicated in growth control. We have been interested in the mechanism of nuclear CK2 signalling, and to that end have employed androgenic regulation of the prostate as an experimental model. Androgen deprivation in the animal leads to prostatic epithelial cell death through apoptosis, whereas androgen administration to castrated rats evokes a rapid induction of genomic activity (via the androgen-receptor system) leading to cellular growth and proliferation. In recent studies, we have established that there is a differential rate of loss of chromatin-associated CK2 (compared with that in the total nucleus) early in the sequence of events leading to apoptosis in the prostatic epithelial cells. Stimulation of growth by administration of a single dose of 5α-dihydrotestosterone to androgen-deprived rats is accompanied by a differential early enhancement of chromatin-associated CK2, with a concomitant decrease in the kinase present in the cytosol (1). Considering that nuclear matrix (NM) is a framework that may contribute to chromatin organization and regulation of gene activity and cell proliferation, we also examined if CK2 is localized in the NM. We have estimated that about 40% of the nuclear CK2 is tightly associated with NM where it retains its kinase activity and appears to be involved in the phosphorylation of several NM proteins (2). We have established that NM associated CK2 is dynamically regulated in response to growth stimulus. Thus, androgen deprivation results in a rapid differential loss of CK2 protein and activity from the NM fraction in the prostate, whereas androgen administration to the castrated rats evokes a differential and rapid (i.e., within 1 hr) increase in CK2 in the NM. The phosphorylation of NM proteins is regulated primarily by the NM-associated rather than the total nuclear CK2 (3). Other studies in progress have suggested that CK2 activity in the NM may be regulated by histones presumably at the matrix attachment regions (MARs), the sites of localization of active genes. We propose that NM serves as an intranuclear anchor/mediator for protein kinase CK2 signalling in response to cell growth and proliferation.

1. Ahmed, K. et al., (1993) Proc. Natl. Acad. Sci., U.S.A. 90: 4426-4430.
2. Tawfic, S., and Ahmed, K. (1994) J. Biol. Chem. 269: 7489-7493.
3. Tawfic, S., and Ahmed, K. (1994) J. Biol. Chem. 269: 24615-24620.

PROBING FUNCTIONAL ORGANIZATION OF GENES AND THEIR RNAs WITHIN THE NUCLEUS, Jeanne B. Lawrence, Department of Cell Biology, University of Massachusetts, Worcester, Massachusetts USA

Several recent studies provide evidence for the higher level organization of pol II gene transcription and RNA splicing in mammalian cells. RNAs from different genes show different characteristic nuclear distributions, ranging from small foci to elongated tracks containing, with some d. By simultaneous hybridization to DNA and RNA using a combination of genomic, intron and exon probes, it was shown that both transcription and splicing of RNA for the fibronectin gene occured within a focal accumulation or "track". Rusults showed the gene was coincident with the RNA "track" or focus, and suggested a polarity to the track with the gene towards one end. THis finding has now been confirmed for the collagen gene, by demonstrating a probe to the full-length of this gene hybridized to a single spot at one end of the RNA focus or track. Simultaneous visualization of fibronectin RNA introns and exons showed that the specific intron studied was present throughout only part of the RNA track defined by the cDNA probe, demonstrating that splicing occurred within the track, possibly in "assembly line" fashion. The transcription/splicing site for the fibronectin gene exhibited a specific higher-level organization, such that in the majority of cells it associated with larger "transcript domains", enriched in ply(A) RNA, snRNPs, and SC-35. It was most frequently positioned just at the periphery of these domains, which themselves show a specific three-dimensional distribution. Ongoing studies concerning the position of other genes and primary transcripts with respect to these regions will be summarized. Of eight active genes studied thus far, most but not all of the genes and/or their RNAs were associated with a domain greatly enriched in splicing components. The detailed spatial arrangement for one gene and its RNA will be described. In other work we have demonstrated a new approach for studying the arrangement of specific DNA sequences with respect to chromatin loop domains, by coupling high resolution hybridization with nuclear fractionation techniques. Results provide direct visual evidence for the differential packaging of replicating DNA and transcriptionally active genes as well as mitotic chromatin. Finally, results will be presented describing the nuclear distribution of XIST RNA, which is implicated as the product of the X-inactivation center and exhibits a nuclear distribution consistent with its stable association with the inactive X chromosome in interphase nuclei.

The Nuclear Matrix: Involvement in Replication, Transcription, Gene Splicing and Cellular Regulation

MARs Elements, Transcriptional Regulation and Transgenic Expression

J7-100 CALCIUM INDUCES DNA SLIDING IN THE DINUCLEOTIDE REPEAT (TG/AC)n Denes v. Agoston and Albert L. Dobi, Molecular Control of Neurodifferentiation, LDN, NICHD, NIH, Bldg. 49, Room 5A-38, Bethesda, MD 20892, USA

Different in vivo imaging techniques have demonstrated rearrangements in intranuclear free and bound calcium as well as suggested direct calcium-DNA interactions during increased transcriptional activity such as cellular differentiation and regeneration suggesting a novel role for intranuclear calcium signaling in gene regulation. The tandem di-nucleotide repeat (TG/AC)n located almost exclusively on 5' or 3' flanking or intronic regions of various genes including the enkephalin gene of rat, mouse and man is known about its potential to form unusual DNA structures such as Z-DNA and four stranded complexes with possible consequences on genomic functions. We reasoned that intranuclear calcium may be able to interact directly with DNA and the (TG/AC)n repeat due to its known structural features has been our prime candidate for such a specific interaction.

Using various techniques to detect structural changes of the DNA, including osmium tetroxide footprinting, we found that Ca^{2+} (or Mg^{2+}) induces a DNA motion specific to (TG/AC)n repeats. This motion is a DNA sliding, significant at 100 µM and maximal at 1 mM of Ca^{2+}, within the physiological range of intranuclear Ca^{2+}. This DNA motion does not require the presence of protein, and it is most likely unidirectional (3'>5'). We propose a new model in which the (TG/AC)n repeats may function as "hinge", an especially flexible part of the DNA molecule for the flanking non-repetitive sequences and Ca^{2+} can "oil" this "hinge". Due to the directionality of the sliding, the Ca^{2+}-induced, (TG/AC)n mediated DNA motion could result *in vivo* in a precisely oriented, concentration-dependent DNA bending affecting far distant DNA-DNA interactions with possible consequences on various genomic functions.

J7-101 **CHROMATIN DOMAIN ORGANIZATION AROUND THE MAIZE *ADH1* GENE; MAPPING OF MARs AND POTENTIAL TRANSCRIPTIONAL UNITS ALONG A CONTINUOUS CHROMOSOMAL REGION,** Zoya Avramova, Elena Georgieva, Alexander Tikhonov and Jeff Bennetzen, Department of Biological Sciences, Purdue University West Lafayette, IN 47907

The studies address a general problem concerning the relationship between genome spatial organization and its functional activity. The chromatin "domain" model offers a unique standpoint from which to investigate this relationship. Functionally competent nuclear matrices were used to screen a region around the maize *Adh*1 gene. Two sites with the capacity to bind to the nuclear matrix were localized 5' and 3' of the gene. The 5' MAR encompasses a site with a prominent DNase I hypersensitivity (Vayda & Freeling, 1986) and is adjacent to all regulatory elements located 5' to the gene (Paul & Ferl, 1991). The 3' MAR is located on a 1.15 kb DNA fragment containing dispersed highly repetitive sequences. The two MARs define a potential loop of approximately 17 kbp containing the gene and a few different classes of dispersed repeats flanking it at the 3'end.

A large chromosomal region around the *Adh*1 gene has been restriction mapped, and characterized with respect to the distribution of repetitive and unique sequences (Springer et al., 1994). A contiguous series of overlaping clones covering nearly 300 kbp have been tested for the presence of regions with the capacity to bind to the nuclear matrix. Several MARs have been located dividing the region into potential loops of heterogeneous size (10 to >80 kbp). Transcribed units are currently being identified within these domains. Such an information is indispensable for relating the structural organization (folding) with the biological activity of a chromosomal region.

J7-102 MATRIX ATTACHMENT REGIONS AS TRANSCRIPTION ENHANCING ELEMENTS IN PLASMIDS DESIGNED FOR *IN VIVO* HUMAN GENE THERAPY, Kerry M. Barnhart, Xiaowu Liang, Jukka Hartikka, and Peter Hobart, Vical, Inc., San Diego, CA 92121

Direct tissue injection of plasmid DNA has proven to be an effective means of expressing antigen proteins at levels sufficient to manifest a protective vaccination against infectious viral, bacterial, and parasitic agents. However, the use of plasmid DNA to deliver therapeutic levels of proteins required to treat congenital and metabolic diseases necessitates the development of new vectors able to express proteins at significantly higher levels. A straightforward approach to generating these new plasmids can involve the modification of current plasmid sequence or the inclusion of novel cis-acting DNA elements known to have expression-increasing potential. Such sequences include transcriptional enhancers (some of which are tissue-specific), mRNA stabilizing elements, sequences encoding heterologous transcription factors, and regions able to promote active chromatin structures. As part of this approach, we have begun to examine the effect of matrix attachment regions (MARs) on levels of gene expression upon transfection of reporter gene plasmids. Multimers of MARs have been shown to enhance the transcriptional activity of associated genes when the multimers are positioned 5' to promoter elements in plasmids stably integrated into the genome of transformed cell lines. We are attempting to extend this finding to plasmids introduced into cells *in vitro* and *in vivo* and carried as episomal elements. Using luciferase reporter gene constructs, we have tested the effect of inserting multimerized MARs sequences at several sites relative to the eukaryotic promoter (5' and adjacent, 5' and distant, and 3' to all coding sequences), and will present the results of our analysis.

J7-103 **USE OF RETROVIRAL VECTORS FOR THE STUDY OF NUCLEAR MATRIX FUNCTIONS**

Jürgen Bode*, Christian Mielke and Dirk Schübeler

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Retroviruses are known for their propensity to recognize and integrate into chromatin domains with a high transcriptional potential, usually close to DNAse I hypersensitive sites. By inverse PCR techniques, we isolated the genomic sequences flanking a number of integration sites. Although the recovered sequences were only moderately AT-rich, they showed a distinct propensity of associating with nuclear scaffolds. Since, as reported by others, there are no sequence homologies, the flanks were further investigated for common structural properties as bending or unwinding. Bending turned out to be a prominent feature. However, in contrast to recent in vitro data, we could demonstrate by a PCR-based reconstitution of the original cellular target site that integration mostly occured adjacent to and not centrally at DNA bents.

The evaluation of SAR sequences requires that single copies of a transgene are integrated in the genome of a host cell: a head-to tail integration as it is common for transfection protocols does not permit conclusions about the activity of single SARs or SAR-SAR interactions, resp.. Along these lines we provided retroviral vectors with SARs, positioned at various sites within and adjacent to a bicistronic transcription unit. While internal SARs represented an impediment to transcription, transcriptional rates could be increased by flanking elements, especially in case they were part of the LTRs.

The apparent activity of prototype SARs, even at chromosomal sites which by themselves favor transcription, led us to re-use a number of these loci for the integration of SAR-constructs and SAR-free controls. This approach was enabled by the incorporation of an FLP-recognition target (FRT-) site into the LTRs. FLP recombinase excises the sequences intervening the FRT-sites of integrated proviral constructs enabling the re-use of the remaining FRT-site for re-integration.

J7-104 MARS AND Dam METHYLASE IN MAIZE, Cory Brouwer[1], Zoya Avramova[2], Jeff Bennetzen[2], Loralee Logan[3] and Ben Bowen[1,3]: [1]Dept. of Zoology and Genetics, Iowa State University, Ames, IA 50011, [2]Dept. of Biol. Sci., Purdue University, W. Lafayette, IN 47906 & [3]Pioneer Hi-Bred Int'l, Inc., PO Box 1004, Johnston, IA 50131.

Gene expression levels among maize cell-lines transformed via micro-projectile bombardment are bimodally distributed and can vary by up to 1000-fold. We have investigated the effects of flanking transgenes with three sequences that bind maize nuclear matrix proteins *in vitro* (yeast ARS1, and regions upstream of maize *Adh1* and *Mha1*). Two genes (BAR and LUCI) were introduced in *trans*- on circular plasmids at a DNA dose that leads to a median copy number of one per host genome. Each gene was either flanked by plasmid vector sequences alone or by one of the three MARs. BAR-expressing transformants that were first selected on herbicide-containing media were then screened for LUCI expression. Although the *Mha1* 5' MAR bound maize matrix proteins about ten times more strongly than the *Adh1* 5' MAR, it had negligible effect on LUCI expression levels in any configuration. In contrast, when both BAR and LUCI were flanked by the *Adh1* 5' MAR, the frequency of high level LUCI expressors was increased 3-fold at the expense of transformants that would normally not express LUCI without the flanking MARs. Because the frequency of low level LUCI expressors among the BAR expressing transformants was not affected, the most significant effect of the flanking *Adh1* 5' MARs was to change the proportions of non-expressors and high level LUCI expressors, not the overall variance. A similar but more marginal effect was observed when both BAR and LUCI were flanked by yeast ARS1. In contrast to the other MARs, however, when BAR alone was flanked by ARS1, very few transformants expressed LUCI at all. Similarly, when LUCI flanked by ARS1 was introduced with BAR, no herbicide-resistant colonies were recovered. Experiments are now underway to test if this suppression effect is seen in *cis*-.

Because we suspect that transgene expression levels are modulated by chromatin effects, we are also attempting to probe the accessibility of transcribed regions and MARs to Dam methylase modification *in vivo*. Results from initial experiments with maize cell-lines that express Dam methylase under inducible control will be presented.

J7-105 EFFICIENT GENE TARGETING OF THE HUMAN BETA GLOBIN LOCUS IN RECOMBINATION-PROFICIENT CHICKEN/HUMAN MICROCELL HYBRIDS, Ellen S. Dieken, Elliot Epner, Mark Groudine and R.E. Keith Fournier, Molecular Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

The ability to introduce predetermined mutations into the genome of a living cell by homologous recombination provides valuable insight into gene function and regulation. Gene targeting in mammalian cells is difficult because random integration of the exogenous DNA by nonhomologous recombination occurs at a high frequency. The ratio of targeted to random integration events in mammalian cells is generally 10^{-2}-10^{-4}. DT40 cells, an avian leukosis virus-induced chicken B cell line, exhibit relative frequencies of targeted integrations orders of magnitude higher than mammalian cells. To determine whether these cells could be used to modify human chromosomal alleles by homologous recombination (HR), we transferred a HyTk-tagged human chromosome 11 into DT40 cells by microcell fusion. Transfection of a DT40 microcell hybrid containing an intact human chromosome 11 with a construct derived from the chicken ovalbumin locus resulted in HR frequencies of >90%, similar to that exhibited by the parental DT40 cells. Transfection with a construct derived from the human beta globin locus yielded an HR frequency of 15%. Furthermore, a targeting vector derived from the human Hras locus displayed similar HR frequencies (13%). Transfer of the HyTk-tagged human chromosome 11 from the DT40 microcell hybrid into mouse erythroleukemia cells, which are capable of expressing the human beta globin gene, is also demonstrated. This hybrid system allows not only the rapid and efficient isolation of gene targeting events in the human beta globin locus but also a direct phenotypic analysis of the introduced mutations.

J7-106 YAC BASED DESIGN OF AN IGκ MINI-LOCUS Julia B. George, Dan Li and William T. Garrard University of Texas Southwestern Medical Center at Dallas, Department of Biochemistry, Dallas, TX 75235-9038

The mouse immunoglobulin kappa gene locus (Igκ) is a classic example of a genetic domain which displays tissue specific patterns of transcription, replication, and methylation as well as rearrangement and point mutation. Although well studied, no κ-gene construct expressed in mice to date has displayed position independent, copy-number dependent expression suggesting that important regulatory or structural elements remain to be discovered. In order to identify regions that contain potential regulatory elements, we have isolated 29 yeast artificial chromosome clones (YACs) which span Igκ and constitute more than 3.0 megabases in two non-overlapping contigs. Analysis of the variable region (Vκ) content of the YACs and comparison with genetic mapping data has allowed us to refine the order of the Vκ genes in the locus. From this set of YACs we have selected one 3' clone that contains all known regulatory elements including both enhancers and the matrix associated region. We have also selected a YAC from from the 5' end of the locus that contains the terminal Vκ gene family. By exploiting yeast genetics we are fusing the two ends of the locus into a mini-locus that contains both upstream and downstream flanking material in cis. The mini-locus and control constructs will be introduced into κ-gene knock out mice and pre-B cell lines. With this approach we hope to identify regulatory and/or structural elements that are involved in the control of this exceptionally large locus.

J7-107 SOMATIC HYPERMUTATION OF IMMUNOGLOBULIN LIGHT CHAIN GENES.

Africa González-Fernández, Satish K. Gupta, Richard Pannell, Michael S. Neuberger and Cesar Milstein. MRC, Lab. of Molecular Biology, Cambridge, England.

Somatic hypermutation is a key element in antibody diversification and in the maturation of the immune response. The mechanism responsible for the hypermutation is still unknown, although cis elements originally implicated in the control of κ light chain transcription, seem to play also a role in the process of hypermutation, as indicated by deletion analysis in transgenic mice of the intron enhancer/MAR region and the 3' enhancer in κ light chain transgenes. Although both κ and λ genes have enhancer elements in their 3' region, no evidence of MAR/intron region have been found in λ genes. To investigate the implications of such differences between κ and λ genes we have analyzed the pattern and rate of mutations in the λ 1 gene in an enriched population of λ^+ B cells from mouse Peyer's patches. The results indicate that the λ1 gene is able to mutate at a similar rate comparable to that previously found for a κ transgene carrying both enhancer elements.

J7-108 IDENTIFICATION OF NUCLEAR MATRIX ATTACHMENT REGION AT THE ILLEGITIMATE RECOMBINATION JUNCTION IN THE HUMAN DYSTROPHIN GENE, Xiuyuan Hu and Ronald G. Worton, Genetics Department, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8; and the Department of Molecular and Medical Genetics, University of Toronto.

We have previously identified three tandem duplications in patients with Duchenne muscular dystrophy and showed that in each case a subset of dystrophin gene exons were duplicated. The origin of these duplications was traced to the single X chromosome of the maternal grandfathers, suggesting that unequal sister chromatid exchange was involved in the formation of these duplications. Sequence analysis of the duplication junctions revealed that one duplication was due to homologous recombination between two Alu repetitive elements and the other two were due to recombination between unrelated nonhomologous sequences. In the latter cases, the junction fragments were found to be A/T rich (>70%) and contain topoisomerase II (topo II) binding and cleavage consensus sequence and other A/T-rich motifs commonly seen in matrix attachment regions (MARs). In the present study, Hela nuclei were extracted with lithium diiodosalicylate (LIS) to deplete histones and the resulting nuclear scaffold was digested with the enzymes EcoRI, HindIII and BamHI. The DNA of the scaffold and the released fractions were purified, fractionated on an agarose gel, blotted, and probed with the DNA fragments spanning the junctions of the two duplications produced by illegitimate recombination. In both cases the junction fragment hybridized with DNA from the pellet enriched for MARs. As a control, the 3' MAR of the human apolipoprotein B gene was used as a probe and the expected result was obtained in which the subfragment containing the MAR hybridized with DNA from the pellet and an ajecent fragment that does not contain the MAR hybridized with DNA from the supernatant. Further studies are underway to confirm the observation by using the in vitro binding assay for MAR detection and to test larger DNA segments spanning the duplication junctions.

J7-109 SPECIFIC TARGETING OF TRANSFECTED rDNA SIGNALS TO THE NUCLEOLUS AND NUCLEOLAR TRANSCRIPTION OF TRANSFECTED rRNA GENES, Inara B. Lazdins and Barbara Sollner-Webb, Department of Biological Chemistry, The Johns Hopkins School of Medicine, Baltimore, MD 21205

Despite a great deal of study on the nucleolus and its function, very little is known about the mechanisms of nucleolar formation and biologically relevant nucleolar targeting. To characterize the processes involved in nucleolar formation, we have determined which segments of rDNA are required for nucleolar transcription and whether these sequences target genes to the nucleolus.

We electroporated mouse rDNA-containing plasmids into CHO cells and localized this DNA and its transcripts by in situ hybridization and fluorescence microscopy. The location was compared to that of endogenous rRNA genes. The analyzed polymerase I transcripts have a half-life of only 2 minutes and therefore, remain as nascent transcripts that do not move away from their site of synthesis to another cellular location. Thus, the RNA detection identifies its site of transcription. Furthermore, transiently transfected mouse rRNA genes do not appreciably recombine into the cellular rDNA, so their presence in the nucleolus would not be adventitious.

We find that only the minimal RNA polymerase I promoter sequences are required for nucleolar transcription. Conversely, when transcription of the same rDNA region was directed by a RNA polymerase II promoter, the transcripts were found in nucleoplasmic speckles, akin to those associated with mRNA splicing. Double-label in situ hybridization and fluorescence microscopy showed the RNA polymerase I plasmid transcripts localized within the region of the nascent endogenous rRNA as a few bright foci. Transfection with two different rRNA gene plasmids, followed by double-label fluorescence microscopy and quantitative analysis, demonstrates that these foci each represent the transcripts of one or a few plasmid molecules.

We also examined the localization of the total population of transfected plasmid molecules. Amazingly, plasmids bearing only a minimal RNA polymerase I promoter are specifically sequestered in the nucleoli and are absent in the nucleoplasm. Notably, many thousands of transfected polymerase I promoter-containing plasmids become localized to the nucleoli, even though only a few of these molecules are transcriptionally active. In contrast, a number of transfected control plasmids all localized to the nonnucleolar nucleoplasm. Two additional regions of the rDNA repeat exhibited nucleolar targeting - the rDNA enhancer and the rDNA 3' transcriptional terminators. Interestingly, these regions have previously been implicated in nucleolar matrix attachment. These data suggest a specific nucleolar recruiting mechanism for transfected rDNA that is independent of transcription, possibly due to the binding of RNA polymerase I transcription factors and their association with the nucleolar matrix.

J7-110 MAPPING OF MATRIX ASSOCIATED REGIONS (MARs) IN A *Sesbania rostrata* LEGHEMOGLOBIN LOCUS (*Srglb3*), Alexander G. Pinaev[1], Andrei O. Zalensky[3] and Frans J. de Bruijn[1,2], MSU-DOE Plant Research Laboratory, [2]Department of Microbiology, Michigan State University, East Lansing, MI, 48824 and [3]Department of Biological Chemistry, University of California School of Medicine, Davis, CA.

The infection of legume plants with rhizobia produces symbiotic nitrogen-fixing organs, termed nodules. Leghemoglobins, oxygen carrier proteins, are specifically induced during the late stages of nodule development. We are studying the involvement of chromatin structure in the infected-cell-specific transcriptional regulation of the *S.rostrata* leghemoglobin *glb3* gene. MARs very often co-localize with important regulatory sequences. It has been shown that the 5' upstream region of the *glb3* gene has a high A/T content (77%) and contains several regulatory sequences responsible for tissue-specific expression. Computer analysis of the sequenced DNA fragment (3.6 Kb) containing the *glb3* locus, revealed the presence of multiple A-box, T-box and MAR motifs in the *glb3* flanking regions and in the introns, which are typical for MARs. In order to localize MARs experimentally, a mixture of *EcoRI/HindIII* restriction fragments of a cloned 17kb region carrying the *Srglb3* locus, was radiolabeled and used in the "exogenous MAR assay" with matrices prepared from *S. rostrata* leaves and tobacco cell culture nuclei. Two distinct restriction fragments of 2.65 kb containing the *glb3* gene with immediate 5' and 3' surrounding regions, and of 2.3 kb of *Srglb3* locus 5' upstream sequences were found to interact specifically with nuclear matrices from both origins, in the presence of a 10,000 fold excess of competitor genomic *E.coli* DNA. In addition, two *EcoRI/HindIII* fragments, one further downstream and the other more then 5 kb upstream from the *glb3* gene, showed weaker binding to the matrix. Binding of any *Srglb3* restriction fragments with *S. rostrata* nuclear matrix was abolished in the presence of Distamycin A. This indicates that the interaction of those restriction fragments with the matrix occurs via A/T-rich DNA sequence tracts. To further delimitate the MARs, subfragments of the 2.65 and 2.3 kb *Srglb3* locus fragments were examined as above. The highest affinity for the nuclear matrix was found with a 426 bp fragment located immediately 5' upstream of the *glb3* gene which contain sequences essential for its transcriptional regulation.

J7-111 SARS STIMULATE BUT DO NOT CONFER POSITION INDEPENDENT GENE EXPRESSION, Leonora G. Poljak*, Carole Seum, Tiziana Mattioni and Ulrich K. Laemmli, Department of Molecular Biology and Biochemistry, University of Geneva, Geneva, CH 1211, Switzerland, *present address: Laboratoire de Biologie Moléculaire Eucaryote, CNRS UPR 9006, 31062 Toulouse, France

Two minimal scaffold-associated regions (SARs) from *Drosophila* , the 657 bp histone gene spacer fragment and a 960 bp intergenic hsp70 fragment, were tested in stably transformed tissue culture cells for their effects on the expression of reporter genes. In pools of stably transformed HeLa and mouse L cells, the expression of genes bounded by two SARs is consistently stimulated by an average of 20- to 40-fold. However, analysis of expression versus gene copy number in individual cell transformants demonstrates that flanking SAR elements stimulate but do not confer position-independent expression on the reporter gene. Thus, the extent of position-dependent variegation is similarly large with or without the flanking SAR elements. The SAR stimulation of expression is observed in stable but not in transiently transfected cell lines. The *Drosophila* scs and scs' boundary elements, which neither bind to the nuclear matrix *in vitro*, nor exhibit enhancer activity in transiently transfected *Drosophila* cells, are only about one-tenth as active as SARs in stimulating expression in stable HeLa or L cell transformants. Interestingly, the SAR stimulatory effect can be blocked by a fragment containing CpG islands (~70% GC) positioned between the SAR and the enhancer. In contrast, when inserted in the same position, control fragments, such as the scs/scs' elements, do not interfere with SAR function.

These results contribute to mounting evidence that SARs function as general facilitators of transcription in a variety of heterologous systems, in a manner that does not appear to be tissue- or gene-specific. Our results are consistent with a putative role for SARs as long-range modulators of chromatin structure. In this model, SAR sequences serve as switch points for the entry of both histone H1 and competitor proteins such as HMG-I/Y, which are also capable of displacing H1. The ratios of active displacement factors, such as HMG-I/Y, to histone H1 in a particular cell would thus help to control the accessibility of sequences flanking SARs to other regulatory proteins, such as transcription factors.

J7-112 **TRANSCRIPTION OF THE EM GENE FROM WHEAT IN HELA NUCLEAR EXTRACT**

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The Em gene encodes an abundant protein that is found in mature embryos of developing cereal grains. The phytohormone Abscisic acid (ABA) and the regulatory protein Viviparous-1 (Vp1) are required for expression of this gene in *vivo*. We have transcribed the Em gene in Hela nuclear extracts to better understand its mechanism of regulation. We have found that the Human Upstream Stimulatory Factor (USF) in HeLa extracts specifically binds an Em1 element (CACGTG) on the Em promotor, and is essential for the transcription of the Em gene in this *in vitro* assay. Antibodies made to sea urchin USF prevent transcription in HeLa extracts and recognize a protein in wheat nuclear extract. Two transcription start sites on the Em promotor were observed and precisely mapped. Addition of the wheat bZip transcription factor EmBP1 to the HeLa nuclear extract specifies transcription from only one of the start sites. When the transcriptional activator protein Vp1 is added to the HeLa nuclear extract, it stimulates transcription from both sites.

J7-113 INTRON-MEDIATED ENHANCEMENT OF TRANSGENE EXPRESSION IN MAIZE, Nikki Rethmeier[1], Jef Seurinck[1], Marc van Montagu[2] and Marc Cornelissen[1], [1] Plant Genetic Systems, Jozef Plateaustraat 22, B-9000 Ghent, Belgium, [2] Department of Genetics, University of Ghent, Belgium.

In monocot plants transgene expression can be stimulated by including an intron in the 5' untranslated region (UTR). The underlying mechanism is presently unknown. Our research focused on identifying determinants of the phenomenon. To this end, chimaeric genes were designed with the *Adh1* and *SalT* introns and *bar* and *cat* coding regions as only variables. In addition, intronless control genes were made that encode transcripts identical to the spliced mRNAs. The relative performance of the genes was tested in transiently transformed maize suspension cells.
Expression of the *cat* gene was higher both at the RNA and protein level when the *SalT* intron was included. The *Adh1* intron did not affect *cat* expression. The observed stimulation of *cat* expression was ~ 50 fold at the protein level, with a maximum of 0.005% of the soluble protein fraction. The intronless *bar* gene directed the synthesis of a 5-fold higher mRNA and a 46-fold higher protein level than the *SalT* containing *cat* gene. Inclusion of either the *Adh1* or the *SalT* intron correlated with reduced steady state *bar* mRNA and protein levels. Typically, more than 50% of the *Adh1* or *SalT* intron carrying *bar* transcripts were unspliced. Taken together, these data indicate that intron-mediated expression stimulation depends on the nature of both the intron and the coding region. Furthermore, we speculate that intron-mediated enhancement might be restricted to genes with a low basal level of expression.

J7-114 A SPECIFIC DNA SEQUENCE IS LOCATED IN THE LOCI OF PROXIMAL β-HETEROCHROMATIN, WHICH ARE ASSOCIATED WITH THE NUCLEAR ENVELOPE IN DROSOPHILA MELANOGASTER, Igor V.Sharakhov(1),Elina M. Baricheva(2), Sergei S.Bogachev(2), Paul A. Fisher(3), Evgeniya R.Lapik(2). 1.Research Institute of Biology and Biophysics, Tomsk State University Lenin Pr 36, Tomsk 634050, Russia; 2. Institute of Cytology and Genetics, Russian Academy of Sciences, Siberian Branch, Novosibirsk 630090, Russia; 3. Department of Pharmacological Sciences University Medical Center at Stony Brook, State University of New York at Stony Brook, Stony Brook, NY 11794-8651
The nuclei of ovarian pseudonurse cells of the mutant otu11 strain of D. melanogaster lacking the chromocenter are suitable for mapping the specific loci of the invariant attachment of the polytene chromosomes to the nuclear envelope. The contact loci were mapped to the 20CD region of the X chromosome, the 41 region of chromosome 2 and the proximal end of the 81 region of chromosome 3. In situ hybridization of λ20p1.4 clone with the otu11 pseudonurse cell polytene chromosomes was performed to determine if the above contact loci contain specfic DNA involved in chromosome-nuclear envelope associations. λ20p1.4 capable of binding to the nuclear lamina was located in the 20CD region, the proximal part of the 41 region, the proximal end of the 81 region and also in the 101 region. The results suggest that there may be a specific DNA presumably involved in attachment of the chromosomes to the nuclear periphery in vivo.

J7-115 PROGRESSIVE MATURATION OF CHROMATIN REGULATES PREIMPLANTATION GENE EXPRESSION IN MOUSE EMBRYOS, Eric M. Thompson, Edith Legouy, and Jean-Paul Renard, Unité de Biologie du Développement, INRA, 78352 Jouy-en Josas, France.
In early embryo development, switching of histone H1 subtypes is often observed. In *Drosophila* and *Xenopus*, the arrival of somatic histone H1 coincides with the end of a series of rapid cleavage cycles and the major activation of the zygotic genome. An essential difference in early mammalian development compared to the two species above, is the absence of an extended series of nuclear divisions prior to activation of the zygotic genome at the 2-cell stage. In the mouse, somatic histone H1 is not detected by immunofluorescence until S-phase of the 4-cell stage.
Scaffold attachment regions (SARs) are known to flank developmentally regulated genes (*Adh, ftz, Sgs-4* in *Drosophila*) and to interact preferentially with histone H1 via their AT-rich nucleotide stretches. They may be implicated in regulating chromatin opening and closing depending on local equilibria between histone H1 and proteins capable of displacing it from AT-tracts, such as the high mobility group protein HMG-I/Y. We have examined the effect of flanking SAR sequences on the expression of an HSP70.1-luciferase transgene in mouse preimplantation embryos. The HSP70.1 endogene is expressed constitutively in 2-cell embryos, repressed at the 4-cell stage and can be induced by thermal stress at the blastocyst stage. The differential preimplantation expression profiles of SAR+ and SAR- transgenic lines have been correlated with alterations in histone H4 acetylation patterns, and cellular levels and localisation of HMG-I/Y in the presence and absence of modifiers of chromatin structure. The results indicate that a progressive maturation of chromatin structure during the 2-, to 4-, to 8-cell transition plays an important role in regulating early HSP70.1 expression in the mouse embryo, and are consistent with models proposing that synergistic interactions between enhancers and SAR elements lead to open chromatin conformations which stimulate transcriptional activity.

J7-116 THE β-PHASEOLIN GENE IS FLANKED BY MATRIX ATTACHMENT REGIONS. Apolonia H.M. van der Geest and Timothy C. Hall, Institute of Developmental and Molecular Biology and Department of Biology, Texas A&M University, College Station, TX 77843-3155.

The β-phaseolin gene, encoding a protein that accumulates to high levels in *Phaseolus vulgaris* seeds, is flanked by matrix attachment regions (MARs). Both 5' (1.1 kb) and 3' (1.2 kb) MAR fragments were found to bind completely to nuclear scaffolds isolated from a tobacco suspension culture (van der Geest et al., 1994, Plant J. 6:413-423). Constructs containing the phaseolin promoter fused to a reporter gene that included the 5' and 3' MAR elements retained their spatial and temporal regulation in stably transformed tobacco plants and exhibited higher expression levels and lower plant-to-plant variability than did similar constructs lacking the MARs. Expression of chimeric constructs in which phaseolin MARs flank a reporter gene driven by the heterologous CaMV 35S promoter (which is active in all plant tissues) is being analyzed in transgenic tobacco plants. This will resolve whether phaseolin MARs can function to yield high, position independent, expression in the context of different promoters and in plant tissues other than the seed.

J7-117 **HEAT-INDUCED MODIFICATIONS IN THE ASSOCIATION OF SPECIFIC PROTEINS WITH THE NUCLEAR MATRIX**, R. Vander Waal, G. Thampy, W.D. Wright, and J.L.Roti Roti, Radiation Oncology Center, Washington University School of Medicine, St. Louis, MO 63108
We have observed that the heat-induced increase in nuclear protein reflects binding of proteins to nuclear structures (Radiat. Res. **81**, 535-552, 1980) and has been correlated with cell survival (Radiat. Res. **138**, 286-290, 1994). Evidence is accumulating which implicates the nuclear matrix (NM) as the critical target for the cellular effects of heat. Most studies addressing the role of excess nuclear protein in the disruption of cellular functions after heat shock focus on the intact nucleus. The present study was conducted to determine if it is sufficient to make measurements at the nuclear level and infer that these changes occur at the NM. We compared the amounts of heat-induced protein associated with HeLa nuclei and NMs by densometric scans of Coomassie blue stained SDS-PAGE and by immunoblotting with commercially available antibodies to nuclear proteins and with antisera raised against total heated HeLa NM proteins. These studies revealed increased levels of many heat-induced NM proteins, including topoisomerase I and II, hsp 27 and hsp 70. Topoisomerase I and II showed a greater relative increase in NM than was measured in intact nuclei, while hsp 27 and hsp 70 showed similar increases in whole nuclei and NM. A large number of proteins increase their NM association in a heat dose-dependent manner to greater extent than could be observed at the nuclear level. These results indicate that a study of increased associations of specified proteins with nuclear structures after heat shock should be observed in the NM to minimize the possibility of failing to detect changes which may be critical. This work was supported by NCI grant CA43198.

J7-118 A FORMAL DESCRIPTION OF BIOLOGICAL SYSTEMS: 2. GENE STATE FUNCTION FOR A VIRTUAL CELL LINE, YAMAMOTO, S. Laboratory of Cell Biology, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya 464, JAPAN.

Computer simulation of biological system has been proved useful tools for the study of protein structure, nucleic acids and drug interactions, and so on. Recent progress in parallel computing gives us a way of simulating multiple parallel processes such as cell replication. The first step of the study must be to devise the description system for the target systems. As the first step, I proposed "Cell State Function (CSF)", denoted by Z( ), a formal expression system for the cell replication processes as a function of time, with "Environment State Function (ENVSF)" for the external field. By Cell State Function, we can describe the cell replication processes as formal expressions.

Here, I present "Gene State Function", a parts of the "Cell State Function", which expressing the state of stored and working genetic informations. DNAs are included in the separate chromosomes with different numbers for different species. We assume the virtual cell line's genomic entities are conserved for ever. The general form of Gene State Function (GSF) is given as follows:

$$G\text{-}1 \quad \prod_{j=1,\,k=a}^{n,\,z} G\left(\sum CHR^{Tri}{}_{i,ph}{}^{tx}\right)^{T}$$

where j means chromosome number defined by karyo-type data base (from j=1 to n), k means chromosome identified by the character (k=a to z), CHR_{ph} means chromosome state at defined cell cycle phase, and CHR^{Tri} means active chromosome in a three state as transistor circuit (explicitly expressed by Tri +, Tri -, TriH, TriL).
Each chromosome is described by the expression G-2;

$$G\text{-}2 \quad {}^{i}{}_{pg}\,CHR^{Tri}{}_{pn}\,(\Sigma STR),$$

here, STR means primary DNA sequences of given chromosome.

S. Yamamoto, J. Cell. Biochem. Suppl. 18c, 92, 1994.

MAR Binding Proteins; DNA Replication and Repair Sites

J7-200 GENERATION OF SATB1-DEFICIENT LYMPHOCYTES: CHIMERIC ANALYSIS, J. D. Alvarez, Terumi Kohwi-Shigematsu* and Dennis Y. Loh, La Jolla Cancer Research Foundation, La Jolla, CA 92037* and Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO 63110

SATB1 (Special AT-rich binding protein 1) is a matrix attachment region (MAR)-binding protein that is expressed specifically in the thymus. SATB1 was cloned by screening an expression library with a probe composed of concatamers of sequences from the MAR located 3' of the immunoglobulin heavy (IgH) chain intronic enhancer. The function of SATB1 is unknown, but the IgH intronic enhancer MARs are necessary for proper immunoglobulin gene expression and for demethylation of the IgH chain locus. Because of the possibility that SATB1 binds the IgH intronic enhancer MARs *in vivo*, we are trying to elucidate its function in lymphocyte development. Our experimental method is to generate a SATB1-null mutant mouse line through homologous recombination in embryonic stem (ES) cells derived from mouse strain 129. We have isolated and mapped genomic clones of SATB1. Using these clones, we have constructed gene-targeting vectors that should abrogate SATB1 expression when homologously recombined in the mouse genome. We are currently selecting for an ES cell clone that has homologously recombined a gene-targeting vector at both alleles of SATB1. This SATB1-deficient ES cell clone will be used to generate chimeric mice by injection into three day old blastocysts from mouse strain C57BL/6. Lymphocytes from these mice will be isolated for study. Those lymphocytes derived from the SATB1-deficient ES cells can be differentiated from those derived from the blastocyst by the expression of Ly9.1, a lymphocyte cell surface protein that is found in 129, but not C57BL/6, mice. We specifically plan to study the effect of SATB1 deficiency upon the expression of lymphocyte-specific proteins and upon the rearrangement of antigen receptor genes.

J7-201 ADENOVIRUS pTP ASSOCIATES WITH A NUCLEAR MATRIX COMPONENT, AND THE COMPLEX CAN BE SOLUBILIZED BY PHOSPHORYLATION, Peter C. Angeletti and Jeffrey A. Engler, Department of Biochemistry and Molecular Genetics, UAB, Birmingham, AL 35294-0005.

The adenovirus precursor to the terminal protein (pTP) binds tightly to HeLa cell nuclear matrix (NM). We have identified conditions under which pTP can be released from the NM. Incubation of pTP-NM with 5 or 10 μCi of ^{32}P-γ-ATP caused multiple nuclear matrix proteins to be phosphorylated and solubilized. pTP was also solubilized but was not phosphorylated. Phosphorylation and pTP release were decreased or abolished by heating of pTP-NM at 70°C for 10 minutes or by the addition of quercetin, a specific inhibitor of tyrosine phosphorylation. A 220-230 kD complex containing pTP and at least one NM protein was released by ATP treatment. This phosphorylation dependent pTP release may be a probe for the biological process of NM disassembly.

Soluble extracts of uninfected nuclear matrix (NME) were prepared by extraction with 8M urea and step-dialysis or by ATP incubation. These extracts were able to reconstitute a pTP-NM complex in vitro. Gelshift assays were performed with pTP using a dsDNA oligonucleotide spanning bases 1-18 of the origin of replication. When NME was added to the pTP gelshift reaction, there was a 3-4 fold enhancement in two supershifted complexes and a corresponding decrease in a complex involving pTP and DNA alone.

We have demonstrated ATP dependent release of pTP, complex formation with an NM protein, and that a pTP-NM complex can bind to an Ad origin. Supported by NIH grant AI20408 (to J.A.E.); P.A. was supported by NIH T32 AI107150.

J7-202 AN M/SAR-CONTAINING ELEMENT FROM DROSOPHILA β-HETEROCHROMATIN BINDS SPECIFICALLY TO NUCLEAR LAMINS IN VITRO AND THE NUCLEAR ENVELOPE IN SITU, Sergei S.Bogachev, Elina M. Baricheva, Meguel Berrios, Igor V.Borisevich, Paul A.Fisher, Igor V. Sharakhov, Nico Stuurman. Institute of Cytology and Genetics, Russian Academy of Sciences, Siberian Branch, Novosibirsk 630090 Russia

A DNA fragment designated λ20p1.4 binds in vitro to polymerized Drosophila lamin Dm. In situ hybridization of λ20p1.4 to isolated polytene chromosomes revealed localization at both the chromocenter and at or near position 49CD on the right arm of chromosome 2. Sequences homologous to λ20p1.4 are present in about 120 copies per haploid Drosophila genome, reside in two different contexts and copurify with karyoskeletal protein -enriched (nuclear matrix) fractions. This last observation demonstrates that λ20p1.4 contains a matrix/scaffold attached region (M/SAR). Nucleotide sequence analysis of λ20p1.4 revealed an A+T rich, 1327-base pair long fragment containing four repeated units between nucleotides 595 and 919. The λ20p1.4 was distinct from previously characterized M/SAR sequences. Results suggest that lamin interacts with a region of λ20p1.4 between nucleotides 350 and 1010. Confocal colocalization studies demonstrated that in situ, the major locus of λ20p1.4 hybridisation, presumably the chromocenter, was found juxtaposed to the nuclear envelope (lamina). A second locus of λ20p1.4 hybridization, presumably at position 49CD, was located internally. This is the first demotstration that DNA sequence that binds specifically to nuclear lamins in vitro, is located at or near the nuclear envelope in situ and presumably, in vivo. This is also the first demonstration of a multicopy M/SAR apparently not associated with a specific gene but rather acting structurally to influence nuclear organization.

J7-203 A HUMAN REGULATORY REGION WITH MATRIX ATTACHMENT, ORIGIN OF REPLICATION , AND ENHANCER ACTIVITIES

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Random cloning of the attachment points of the various chromatin domains in human cells in culture representing 1-2% of total DNA is being used for the isolation of origins of replication (ORIs). In addition this method is allowing us to fish out transcriptional enhancers and to have a statistically significant look at the DNA sequences cells use to set the borders between neighboring domains. The method has been refined to exclude genomic DNA sequences that would adventitiously attach to the nuclear matrix during elongation of DNA replication, transcription, and repair. Most of the MAR fragments sequenced do not fall into the AT-rich class of MARs. Searches through the Genbank have linked some of the cloned MAR fragments to known genes. DpnI-resistance assays have identified a very strong origin of replication activity in a 3.6 kb fragment from the immediate 5' flanking region of the human choline acetyltransferase gene. A 0.8 kb and a 0.65 kb fragments with DNA sequence motifs characteristic of viral and other known origins of replication have the strongest ORI activity separated by a 1.2 kb replication silencer. The same fragments have also a notable enhancer activity in CAT assays. The 0.8 kb fragment includes a 200-bp GA-rich motif, an AT-tract with protein footprints in the GA-tract, the AT-tract and in the AT-tract flanking regions. Inverted repeats, thought to be converted into cruciforms during initiation of DNA replication are also present in the MAR/ORI/Enhancer fragment. A single stranded DNA-binding activity, which is a candidate of a mammalian replication initiator protein, interacts only with one strand of a stretch of this fragment which is a potential cruciform but not with the other strand, or with the double-stranded oligonucleotide in band-shift assays. We believe that we have isolated the first human regulatory sequence possessing simultaneously a MAR, an ORI, and a transcriptional enhancer activity.

J7-204 **STRUCTURAL AND FUNCTIONAL INTERACTIONS BETWEEN EPSTEIN BARR VIRUS DNA SEQUENCES AND THE NUCLEAR MATRIX IN RAJI CELLS** D'Erme M.*, Ceridono M.^, Guidobaldi L.*, Mattia E.^ *Dept. of Biochemical Sciences and ^Microbiology Inst., Univ. "La Sapienza", Rome, Italy.

Specific regions of cellular DNA that are attached to the nuclear matrix are called Matrix Attachment Regions (MARs). These regions have been shown to play an important structural and functional role in nuclear DNA organization and expression.

Similarly, viral DNA replication and transcription takes place in a close association with the nuclear matrix. The hypothesis has been made that the interactions between specific viral DNA sequences and the nuclear matrix, mediated or not by viral proteins, are instrumental for efficient replication and transcription of viral genes. It has been reported that Epstein Barr virus (EBV) latent origin of replication (oriP) is associated with the nuclear matrix .via a matrix attachment region located in the BamH1 C fragment of the viral genome(1). To investigate the functional role of this association, we are studying oriP-nuclear matrix interaction in the latent as well as in the lytic phase of EBV infection. In addition we want to investigate whether during the lytic cycle, other regions of the viral genome, in particular the origin of replication for the lytic cycle (oriLyt), would specifically bind to the nuclear matrix.

To induce the lytic cycle, latently infected Raji cells are treated for three days, with sodium butyrate and 12-O-tetradecanoylphorbol-13-acetate (TPA) at a concentration of 20 ng/ml and 2 mM, respectively (2). Under these conditions, the synthesis of early antigens is induced in about 50% of the cells. Nuclei from induced and non induced Raji cells are digested in situ with BamH1. The nuclear matrix and soluble fractions are isolated, the DNA purified, resolved by agarose gel electrophoresis and transferred to nitrocellulose. Blots are probed with cloned BamH1 C and BamH1 F fragments containing oriP and oriLyt, respectively as well as other BamH1 fragments of EBV genome. The results of such experiments will be presented.

These studies will contribute to understand the role of nuclear matrix-DNA interactions in modulating gene expression and in particular, in the regulation the latent and lytic cycle of viral infection.

1.Jankelevich, S. et al. (1992) Embo J. 11, 1165-1176.
2. Ooka et al. (1983) J. Virol. 46, 187-195.

J7-205 THE BINDING OF DNA TO THE NUCLEAR MATRIX STUDIED BY CROSS-LINKING REACTIONS.

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Intact cells or nuclei from chicken liver and erythrocytes were treated with cis-diamminedichloroplatinum,in order to form stable cross-linkages between DNA and non-histone proteins. The proteins present in the cross-linked complexes with DNA were isolated and analyzed by mono- and two-dimensional electrophoresis. Their capability to recognize specifically DNA sequences was tested by South-Western blotting in the presence of an excess competitor DNA, using as probes either the whole of DNA fragments isolated from the cross-linked complexes, or the SAR fragments prepared from chicken liver nuclei according to Mirkovitch et al. (Cell, 39, 223, 1984), or the double stranded poly dA-poly dT. Most of the proteins isolated from the cross-linked complexes appeared to derive from the nuclear matrix, either from the internal (in the case of liver) or from the peripheral fraction. The few cross-linked species derived from erythrocytes were generally different from those, more heterogeneous, derived from liver. Lamin B, however, was found to be cross-linked in both types of nuclei. Five to seven main cross-linked proteins (those from liver differing from those from erythrocytes), were shown by South-Western blotting to be capable of specific DNA recognition. Essentially the same results were obtained when using as probes the DNA fragments prepared from the cross-linked complexes or the SAR fragments, while a different recognition pattern was found when poly dA-poly dT was used. This approach appears to be useful in studying the DNA-nuclear matrix interactions existing before any disruption of the nuclear structure is carried out, or even existing *in vivo* in intact, viable cells. The results obtained confirm that the SARs prepared in the classical way are indeed the anchorage regions of DNA in the intact nucleus; furthermore new SAR-binding proteins have been detected.The results also suggest that, although the same SARs are present in different tissues, different SAR-binding proteins are involved in transcriptionally active cells and in inactive ones.

J7-206 **DIFFERENTIAL INTERACTION OF NUCLEAR MATRIX PROTEINS WITH REGULATORY SEQUENCES UPSTREAM OF IMMUNOGLOBULIN HEAVY CHAIN PROMOTERS.** Stuart I. Hodgetts , Kent L. Buchanan, Jilanne B. Byrnes, Carol F. Webb. Oklahoma Medical Research Foundation, Oklahoma City, OK, 73104.

DNA-binding proteins involved in the regulation of gene transcription have been detected in both the non-matrix and matrix-specific fractions of nuclear extracts. The presence of such transcription factors in the nuclear matrix (NM) lends itself to the proposed involvement of the NM in transcriptional regulation. We previously identified a matrix-associated region (MAR) 5' of an immunoglobulin variable heavy chain (IgV_H) promoter. While characterizing the proteins that bound to this MAR, we observed that solubilized matrix proteins from B lymphocytes also bound to other 5' regulatory promoter sequences in mobility shift assays. This suggests that some NM proteins may interact with other basal transcriptional regulatory factors and may not be directly associated with the structural function of MARS. Stimulation of B cells with lipopolysaccharide (LPS) causes increased transcription of immunoglobulin that correlates directly with an increase in binding of the octamer-binding protein OTF-2. Although OTF-2 levels increased in the non-matrix fractions, no increase was seen in the corresponding band in matrix fractions after LPS treatment. Furthermore, B cell matrix contains a unique mobility-shifted complex that binds to a putative, regulatory pyrimidine-rich region of DNA that is conserved in all IgV_H promoters. After LPS treatment, this complex is no longer present. Preliminary *in vitro* transcription studies also indicated that the addition of soluble NM proteins to conventional nuclear extracts affects transcription from IgV_H promoters. Thus the NM fraction may be an important source of previously unidentified regulatory proteins and co-activators required for the cell type-specific expression of Ig genes.

J7-207 A MULTIPROTEIN COMPLEX MEDIATES MAMMALIAN DNA REPLICATION, Linda H. Malkas[1] and Robert J. Hickey[2], [1]Dept. of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, [2] Deptartment of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201

Evidence for multiprotein complexes playing a role in DNA replication has been growing over the years. We have previously reported on a replication-competent multiprotein form of DNA polymerase isolated from human (HeLa) cell extracts that was observed to sediment at 18-21S during velocity sedimentation analyses. The proteins that were found at that time to co-purify with the human cell multiprotein form of DNA polymerase included: DNA polymerase α, DNA primase and PCNA. The multiprotein form of the human cell DNA polymerase was further purified by Q-Sepharose chromatography and shown to be fully competent to support origin-specific and large-T-antigen-dependent simian virus 40 (SV40) DNA replication *in vitro*.

We have now further characterized the human cell replication-competent multiprotein form of DNA polymerase. Several additional DNA replication proteins were identified that co-purify with the multiprotein form of DNA polymerase. The replication requirements, replication initiation kinetics, and the ability of the multiprotein form of DNA polymerase to utilize minichromosome structures for DNA synthesis have also been determined. Experiments were done to determine whether nucleotide metabolism enzymes co-purify with the replication-competent multiprotein form of DNA polymerase.

The model proposed to represent the replication-competent multiprotein form of DNA polymerase that is found in murine cells (Wu et al., J. Cell. Biochem. 54, 1994) can now be extended to include the human cell multiprotein form of DNA polymerase, based on the fractionation and chromatographic behavior of the human cell DNA replication proteins. These results further the evidence that DNA synthesis is mediated by a multiprotein replication complex (MRC) in mammalian cells.

J7-208 **DNA LOOP ORGANIZATION AND NUCLEAR MATRIX PROTEINS IN ONCOGENE-MEDIATED RADIORESISTANCE IN RAT EMBRYONAL CELLS,** R.S.Malyapa, W.D.Wright, Y.C.Taylor and J.L.Roti Roti, Radiation Oncology Center, Washington University School of Medicine, St.Louis, MO.

Transfection with either H-ras or H-ras and c-myc has been shown to confer radioresistance in rat embryonal cells (REC), (*Radiat. Res.* **120,** 267-279, 1989). We used REC primary, transfected with either c-myc or H-ras or cotransfected with c-myc and H-ras as a model system for oncogene-induced radioresistance to study if higher order DNA organization contribute to oncogene-mediated radioresistance. Analysis of the ability of DNA loop domains to undergo supercoiling changes in the presence of radiation-induced damage by the fluorescent halo assay (FHA) showed that the degree of inhibition of DNA supercoil rewinding decreased as the cells became more radioresistant and was inversely correlated with survival. This is consistent with our findings in double-strand break repair deficient mutants (*Radiat. Res.* **140,** 312-320,1994). Since DNA damage induction and repair rates of single strand breaks in all these cell lines were equal, it is possible that oncogene-conferred radioresistance is due to differences in chromatin conformation. As DNA loops are organized by the nuclear matrix (NM), a study of NM protein composition was conducted for these cell lines. Analysis of 2D-PAGE did not reveal any specific protein changes that could be directly associated with altered radiosensitivity. However, a progressive loss of individual proteins were observed to correlate with increasing tumorigenicity in these cell lines and alterations in NM proteins have been observed to correlate to with the development of numerous tumors. These results suggesting that DNA conformational changes and differences in NM protein composition contribute to radioresistance suggest that increased radioresistance associated with increasing tumorigenicity could be due to organization of DNA mediated by NM changes that are known to be altered with tumor progression (*Cancer Res.* **53,** 744-746, 1993). This work was supported by NCI grant CA51116. REC lines courtesy of Dr.C.C.Ling.

J7-209 POSSIBLE ROLES OF INTRANUCLEAR STRUCTURES FOR THE DYNAMIC MOVEMENT OF HUMAN REPAIR PROTEIN XPG, Min S. Park, Mark A.MacInnes and G. F. Strniste, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM87545

Xeroderma pigmentosum Group G (XPG) is one of the seven genetic complementation groups (A through G) whose defects cause individuals to exhibit a variety of pathophysiological symptoms, including a deficiency in nucleotide excision repair, sun-sensitivity, growth and mental retardation, and neurodegeneracy. The XPG gene encodes a 134 kDa nuclear protein with a structure-specific DNA endonuclease activity that is essential for removal of various DNA damages. Here we report dynamic movement of XPG in human skin fibroblast cells under UV irradiation. Indirect immunofluorescence and confocal laser scanning microscopy suggested presence of XPG protein as foci throughout the nucleus. Detailed biochemical fractionation and immunoprecipitation studies indicated a tight but reversible association of XPG protein with intranuclear structure(s), possibly the nuclear matrix. When human fibroblast cells were irradiated with UV light (5 J/m^2), XPG protein redistributed from the limited number of foci to the entire nucleus. By using a heterologous β-galactosidase reporter system, we further identified that the evolutionarily conserved C-terminus (aa 1146-1186) is responsible for the nuclear transport and association of XPG with intranuclear structures, including the nucleoli. This β-galactosidase fusion protein also rapidly dissociated from the perinucleolar regions upon UV irradiation, and it completely reassociated with the structure within 24 hours post-irradiation. These observations indicate that the intranuclear structures are important for the retention and redistribution of the active XPG protein from the intranuclear storage pool to other nuclear regions upon UV irradiation. They suggest the functional significance of the compartmentalization of DNA repair proteins in the nucleus and the reversible nature of the matrix-association of repair machinery. This nuclear matrix-mediated, localized distribution and redistribution of DNA repair enzymes, e.g., XPG, may be a previously unrecognized controlling mechanisms of DNA repair. This research is supported by the US DOE under contract #KP0204000.

J7-210 **DNA LOOP ORGANIZATION AND NUCLEAR MATRIX PROTEINS INFLUENCE REPAIR OR FIXATION OF RADIATION-INDUCED DAMAGE,** J.L.Roti Roti, R.S.Malyapa and W.D.Wright, Radiation Oncology Center, Washington University School of Medicine, St.Louis, MO.

We have studied the DNA supercoiling characteristics by the fluorescent halo assay (FHA) and the NM protein composition by high resolution 2D-PAGE in cell lines expressing altered radiosensitivity to investigate the possibility that organization of eukaryotic supercoiled nuclear DNA into loop domains anchored to the nuclear matrix (NM) by nucleoprotein interactions plays a role in the repair or fixation of radiation-induced damage. Two groups of cell lines, Chinese hamster fibroblasts (radioresistant - 4364, XR-122 and CHO.K1), (radiosensitive - XR-1 and xrs-5) and murine lymphoid cells (radioresistant - L5178Y/R, /SR, /S35 and M10(neo5)-1), (radiosensitive - L5178Y/S, M10 and LX830) were used as a model system for radiosensitivity due to defective repair of DNA double-stand breaks (DSBs). Analysis of the ability of DNA loop domains to undergo supercoiling changes in the presence of radiation-induced damage revealed that in all cases DNA loop rewindability was reduced as cells became increasingly radiosensitive. A study of the NM protein composition revealed an absence of 13 proteins in the radiosensitive XR-1, 5 of which were restored in XR-122 (a radiosresistant variant of XR-1 with human chromosome 5). Extension of these studies to xrs-5 revealed 7 of the 13 proteins were absent compared to the radioresistant cell lines. In murine lymphoid cells, 4 proteins were absent between radioresistant L5178Y/R and radiosensitive /S, M10 and LX830. In M10(neo5)-1, a radioresistant variant of M10 with human chromosome 5, 3 of the 4 proteins reappeared. The differences in the DNA supercoil rewinding characteristics following radiation and the presence or absence of proteins between radioresistant and radiosensitive cells suggest the involvement of DNA-matrix attachment points in the ability of the cells to either repair or fix radiation-induced DSBs. This work was supported by NCI grant CA51116.

J7-211 UV-INDUCED NUCLEOTIDE EXCISION REPAIR IN INTACT CHROMATIN; Anneke van Hoffen, A.S. Balajee, Khalil Bouayadi, Bert van Zeeland, Leon Mullenders; MGC - Dept. of Radiation Genetics and Chemical Mutagenesis, Leiden University, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands.

In human cells UV-induced photolesions are preferentially removed from transcriptionally active genes by nucleotide excision repair (NER). Several factors may account for this heterogeneity of repair. RNA polymerase II activity is essential for the accelerated repair of the transcribed strand of active genes (i.e. transcription coupled repair). Moreover, the preferential localization of repair events in the matrix attachment regions of chromatin loops and hyperacetylated chromatin, suggest that also chromatin structure plays a role in the process of preferential repair. Detailed analysis of the role of chromatin structure and transcription in NER and the identification of factors involved requires an in vitro system with intact chromatin, capable to transcribe. The model system developed by D.A. Jackson and P.R.Cook (J. Cell Science 1988, 90:365-378) meets these requirements. Cells are encapsulated in agarose microbeads and 80% of the cellular proteins is extracted using physiologic salt concentrations. This results in chromatin containing intact DNA, with the ability to perform efficient replication (S-phase dependent) and transcription (measured as incorporation of ^{32}P labelled nucleotides) without added cell extracts or purified proteins. We show that chromatin from normal cells is able to perform UV-induced DNA repair. This repair activity is firmly attached to the nucleoskeleton. It needs to be initiated before extraction, since cells which are lysed immediately after UV-irradiation do not show UV-stimulated incorporation of ^{32}P-dAMP. Experiments were performed also with excision repair deficient cell lines. XP-A cells (completely excision repair deficient) do not show any UV-stimulated incorporation. Mildy permeabilized XP-C cells (only capable of performing transcription coupled repair) show 20% UV-stimulated incorporation compared to normal cells, which is completely inhibited by α-amanitin, whereas in normal cells no inhibition of repair by α-amanitin could be detected. Cockayne's syndrome group B cells (deficient in transcription coupled repair) show partial impairment of repair activity in vitro. With help of specific inhibitors and antibodies and the application of cell extracts or purified proteins this model system may be an important tool to unravel the menchanism of preferential repair and the role of chromatin strucure.

J7-212 EXPRESSION AND DNA-BINDING ACTIVITY OF THE CONSERVED DOMAIN OF MODULATOR RECOGNITION FACTOR-2, A HUMAN MATRIX ATTACHMENT REGION BINDING PROTEIN, Robert H. Whitson and Keiichi Itakura, Department of Molecular Genetics, Beckman Res. Inst., City of Hope, Duarte, California 91010.

Modulator Recognition Factors 1 and 2 (MRF-1, MRF-2) were cloned by virtue of their ability to bind DNA sequences in or near the matrix attachment region (MAR) in the transcriptional modulator of the major immediate-early gene of human cytomegalovirus. These two proteins have highly-homologous domains of 108 amino acids that relate them to four other proteins. Four of the six members of this protein family have been demonstrated to bind to DNA, and the other two have been shown to be associated with large DNA-protein complexes. The amino acid sequences of the conserved regions do not correspond to any known DNA binding motif, however. cDNA encoding the conserved region of MRF-2 was amplified using the polymerase chain reaction and cloned into an expression vector. The resulting plasmid expressed the conserved domain peptide as part of a 120 amino acid fusion peptide carrying a His_6 affinity tag. The fusion peptide was extracted from *E coli* under strongly denaturing conditions (6 M guanidine HCl) and purified to greater than 95% homogeneity in a single step using a Ni^+-chelate column. After renaturation by a simple dialysis protocol, the purified fusion peptide bound to both synthetic oligonucleotides and restriction fragments carrying its A-plus-T rich recognition sequence. The dissociation constant for binding to the target oligonucleotide was less than 10 nM. Oligonucleotides carrying unrelated sequences or subtle mutations of the MRF-2 recognition sequence did not compete with the native target oligonucleotide for binding to the conserved domain peptide. These results demonstrate that the conserved MRF domain encompasses a novel independently-folding structural motif that is capable of high-affinity sequence-specific DNA binding. This motif may be a common feature of many MAR-binding proteins.

J7-213 CONTROL OF PROXIMAL PROMOTER ASSEMBLY - ROLES FOR THE CCAAT BOX FACTOR, NF-Y AND THE TATA BOX. Kenneth L. Wright, Terry L. Moore, Cheryl Skinner, Michael Linhoff and Jenny P.-Y. Ting. Lineberger Comprehensive Cancer, Department of Microbiology-Immunology, University of North Carolina, Chapel Hill, NC 27599-7295.

NF-Y binds a CCAAT motif found in many eukaryotic polymerase II-dependent promoters. NF-Y is required for expression of the MHC class II DRA gene. We have recently demonstrated by *in vivo* footprinting of DRA promoter-mutant cell lines that NF-Y is the key event in establishing a stable proximal promoter complex. Furthermore, NF-Y and the neighboring X box factor cooperate to stabilize their DNA binding activities. We have now expanded these studies to include two NF-Y binding sites in the MHC class II-associated invariant chain (*Ii*) gene. The proximal NF-Y site lies between the TATA box and a GC box, while the distal site is associated with an X box. *In vivo* footprinting of the proximal region identified an occupied, imperfect NF-Y/CCAAT box and an adjacent GC box between -42 and -74 base pairs upstream of the transcription initiation site. Together these two sites synergize to stimulate transcription, however, neither site alone was functional. Independently, NF-Y binds poorly to the imperfect CCAAT box with a rapid off rate, while Sp1 binds to the GC box. Stabilization of both NF-Y and Sp1 binding occurs upon adjacent binding of both factors to the DNA. These findings suggest a mechanism for the complete functional synergy of the GC and CCAAT elements observed in Ii transcription. Collectively, the studies of DRA and Ii suggest NF-Y may interact with multiple transcription factors. Furthermore, our results would suggest that NF-Y may initiate protein complex formation among upstream activating elements analogous to the role of the TATA box in basal promoter assembly. We are now investigating the role of TATA box binding *in vivo* on the assembly of these upstream proximal promoter complexes. Considering the prevalence of NF-Y/CCAAT sites in multiple promoters, interactions between NF-Y and other factors are likely pivotal steps in transcriptional regulation.

J7-214 Cloning and characterization of the gene *cramped of D.melamogaster* ; a unique Polycomb-groupe gene, Yutaka Yamamoto, Markus Affolter & Walter J. Gehring, Department of Cellbiology, Biozentrum, Basel university, 4056 Basel, Switzerland

In Drosophila a class of genes called Polycomb-group (Pc-G) is required to keep homeotic genes repressed. Pc-G genes were identified by their specific mutant phenotype: additional sex comb teeth on the 2nd and 3rd legs of mutant male flies. This phenotype reflects posterior to anterior transformations. In addition to this typical *polycomb* phenotype *cramped(crm)* mutant males show distal to proximal transformation : additional sex comb teeth on the 2nd tarsal segment of 1st legs. Only the posterior to anterior transformations are enhanced in *Polycomb (Pc)*; *crm* mutant background whereas distal to proximal transformation remain unaffected. We have cloned a 15kb genomic fragment which contains a single transcription unit and completely rescues the mutant crm phenotypes. The transcript encodes a protein of 694 amino acids. No strong similality to other known proteins were found in the database. Although the enhancement of phenotypes between *crm* and *Pc* mutant suggest synergystic interactions (as has been observed among other Pc-G genes), we found some interesting differences in the localization of these two gene products. In embryos crm protein is associating with interphase nuclei and dissociates from nuclei immediately after the chromosomes start to condense. Furthermore in salivaly gland nucleus the crm antigen is localizing on intranuclear fibro-granular structures. In contrast, the Pc gene product remains detectable on the chromosomes during cell division in Drosophila tissue culture cells and distributed in a banded pattern on chromosomes in salivaly gland nuclei (Messmer et al.,Genes&Dev.6,1992). We also found that PCNA is associating with nucleus in similar way, and co-localizing with crm protein in salivaly gland nucleus. The temperature-sensitive mutant male flies of the gene encoding PCNA (*mus(mutagen-sensitive) 209*) (Henderson et al.,EMBO.J,13,6,1994) also have additional sex comb tooth on the 2nd tarsal segment of the 1st legs. These observations suggest a unique function of the gene *cramped* through DNA replication. We are in the process to examine genetic interactions between *cramped* and *mus(209)* and trying to identify putative target genes of the crm gene product by immunostaining of the squashed salivaly gland polytene chromosomes.

J7-215 **PULSE-CHASE STUDIES OF DNA REPLICATION SITES IN MOUSE 3T3 FIBROBLASTS,** H. Ma, C. Meng, J. Samarabandu, P.C. Cheng and R. Berezney, Department of Biological Sciences, SUNY at Buffalo, Buffalo, NY 14260

Fluorescence microscopic analysis of newly replicated DNA has revealed discrete granular sites of replication (RS). The average size and number of RS from early to mid-S phase suggests that each RS contains numerous replicons clustered together. We are using fluorescence laser scanning confocal microscopy in conjunction with multi-dimensional image analysis to gain more precise information about RS and their spatio-temporal relationships. Using newly improved imaging techniques, we report an average of about $1x10^3$ RS following a 2 min pulse of cells synchronized in early S phase. "Pulse-chase-pulse" double labeling experiments revealed that the average RS took about 1 hr to complete replication. Assuming approximately $4\text{-}8x10^4$ replicons per nucleus, this suggests that each RS contains an average of 5-10 replicons which are replicated in a relatively synchronous wave. Previous studies from our laboratory revealed that the arrangement of replicated DNA into "RS-like" structures persists throughout the cell cycle and subsequent daughter cells. These results may be a consequence of the three dimensional arrangement of chromatin into precise domains of clustered loops. We have designed "double pulse - double chase" experiments to determine whether the DNA sequences replicated at individual RS are precisely maintained as the cell progresses through the cell cycle. RS were labeled in early S (green probe) and two hours later (red probe). Our results demonstrate the maintenance of the temporally distinct replicated DNA into spatially distinct sites (separate green and red labeled sites) throughout the 8 hr S-phase. The G_2-phase, however, was characterized by a significant mixing of DNA between the two temporally distinct populations of sites (~30% yellow sites). The percent of "DNA mixing" between the two populations of sites increased to >50% in subsequent cell generations. In future experiments we will determine the specific genomic sequences (genes) at individual RS and the cell cycle dynamics of these associations. (Supported by NIH grant GM-23922)

J7-216 COMPUTER AIDED ANALYSIS OF DNA REPLICATION SITES IN THE MAMMALIAN CELL NUCLEUS, Jagath Samarabandu, Hong Ma, Ping-ching Cheng and Ronald Berezney, Department of Biological sciences, State University of New York at Buffalo, Buffalo, NY 14260

Apart from the ability to capture 3D images of microscopic structures, a confocal laser scanning microscope (CLSM) equipped with multiple detectors allows one to add an extra dimension to the data acquisition process. The two channel CLSM images were obtained from double labeling of DNA replication sites where mouse 3T3 cells exponentially grown on cover slips or synchronized at specific times in S-phase were pulsed for brief times (2-5 min) with CldU (chlorodeoxyuridine) followed by a chase period and a second labeling with IdU (iododeoxyuridine). The cells were then fixed and processed for fluorescence microscopy using monoclonal antibodies, appropriate extraction conditions and fluorochrome-conjugated secondary antibodies which enabled differential recognition of sites of CldU versus IdU incorporation into newly replicated DNA. Because of the overlapping emission spectra of the two dyes, some spill-over was observed from the green (FITC) to the red (Texas red) channel. Thus the acquired images were corrected using a leakage factor computed from a calibration image which was obtained from a sample which is identically labeled in both color dyes. The corrected images were analyzed using a new segmentation algorithm developed at our laboratory for improved detection of clustered replication sites to obtain the boundary of replication sites in individual channels. This boundary data is then used to detect the overlapping particles between channels and the overlap area is calculated for each overlapping particle. The results are presented in several forms including a two dimensional histogram of particle volume and overlap percentage. We are currently applying pattern recognition techniques to further elucidate the 3D higher order assembly of individual replication sites. (Supported by NIH grant GM-23922).

J7-217 ELUCIDATING THE HIGHER ORDER ASSEMBLY OF REPLICATION SITES IN MOUSE 3T3 CELLS, Ronald Berezney, Hong Ma, Xiangyun Wei, Chunling Meng, Jagath Samarabandu and Ping-chin Cheng, Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14260

We are using fluorescence laser scanning confocal microscopy coupled to multi-dimensional image analysis to investigate the three-dimensional arrangement of individual replication sites (RS) in synchronized mouse 3T3 fibroblasts. Individual RS are optimally visualized following short pulses with BrdU (2 min). Increasing pulse times leads to progressively more elongated structures and to increasing difficulty in resolving specific sites. Double labeling experiments performed at two different times (pulse-chase-pulse) enabled us to examine the relationship of early versus later replicated DNA at individual sites. Cells in early S phase were labeled for 2 min with CldU (FITC secondary antibody), chased for 15 min and pulsed again for 5 min with IdU (Texas Red secondary antibody). Over 50% of the total RS were co-localized and decorated granular RS similar to those observed after a single 2 min pulse. Later pulsed replication sites (red sites) were consistently observed in juxtaposition to early ones (green or yellow sites). Increasing the chase time between pulses to 1-2 hr resulted in an increasing spatial separation between early and later RS. These results led us to consider the existence of higher order spatial domains of neighboring RS whose replication may be temporally regulated. To investigate this further, we performed long term double labeling (pulse-chase-pulse) experiments. 3T3 cells in early S-phase were pulsed for 1-5 hr with CldU, chased for 0-4 hr and pulsed a second time for 3 hr with IdU. Initial results suggest that a significant portion of the temporally distinct RS occupy separate spatial domains within the nucleus. Arrays of RS in close proximity were often observed within these domains. We are currently applying computer imaging approaches to further elucidate the 3-D higher order assembly of individual replication sites. (Supported by NIH grant GM-23922).

Nuclear Domains, Transcriptional and RNA Splicing Factor Domains

J7-300 NUCLEAR MATRIX ANTIGENS WITH PROPERTIES COMMON TO MEMBERS OF THE SR FAMILY OF SPLICING FACTORS

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Monoclonal antibodies raised against the human nuclear matrix (anti-NM mAbs) have been used to investigate the role of nuclear matrix antigens in pre-mRNA processing. The three anti-NM mAbs characterized in this study recognize antigens that are highly localized to nuclear matrix structures which correspond to speckles detected by immunofluorescence microscopy. These mAbs preferentially immunoprecipitate splicing complexes containing exon sequences. The anti-NM mAbs preferentially immunoprecipitate the exon product complex but not complexes containing the lariat product after the second step of splicing. Two of the anti-NM mAbs completely inhibit pre-mRNA splicing in vitro. However, none of the anti-NM mAbs appear to recognize factors stably associated with splicing snRNPs.

The three anti-NM mAbs predominantly react with distinct high molecular weight antigens, which belong to a class of nuclear phosphoproteins that selectively precipitate with Ser-Arg (SR) protein-splicing factors in the presence of high Mg^{2+} concentrations. Immunological, biochemical and cell biological data indicate that two of the NM antigens are related to the defined set of SR proteins. Antigens recognized by one of these mAbs (B1C8) specifically bind to pre-mRNA at a very early step in the formation of spliceosomes, which precedes the stable binding of snRNPs. Work is in progress to determine the primary structure of the NM antigens and also to elucidate their role(s) as nuclear matrix components in the biogenesis of pre-mRNA.

J7-301 NUCLEAR CHANGES IN MAIZE CELLS TRANSFORMED BY DNA-COATED MICROPROJECTILES, Mark A. Chamberlin and Ben Bowen, Pioneer Hi-Bred International, Inc., PO Box 1004, Johnston, IA 50131-1004.

Microprojectile bombardment is the method of choice for transforming maize cells with DNA. To examine more closely the fate of DNA and cells immediately following bombardment, we introduce marker genes (R/C1) that induce accumulation of pigmented anthocyanins in vacuolar bodies within transfected cells. Bombarded cells can also be identified by staining for callose plugs in the cell walls with aniline blue. Microprojectiles (1.8μm tungsten particles) are easily visible using DIC optics. In anthocyanin-containing cells, particles are generally found in healthy-looking nuclei that are small and have a dense nucleoplasm. Not all cells that contain particles in their nuclei accumulate anthocyanins, however, and the nuclei in these cells undergo a series of morphological changes somewhat reminiscent of mammalian cell apoptosis. First, the nuclei become highly dilated and increase in volume by up to 8-fold. Coincident with this enlargement, a large number of small Hoechst-staining vesicles bleb off from the nuclear envelope into the cytoplasm where they eventually degenerate. The nuclei then contract as the chromatin becomes highly condensed, and, at this stage, the nuclear envelope appears thin and reticulate. Eventually, the intensity of Hoechst-staining within the nuclei becomes progressively weaker until the nucleus disappears altogether, at which time only a very diffuse staining is seen within the cytoplasm. Perhaps the most striking observation is that nuclei that undergo these dramatic changes are only seen in cells bombarded with DNA-coated microprojectiles and not in cells shot with tungsten particles alone. Experiments are underway to determine if these nuclear alterations involve DNA fragmentation and/or changes in the nuclear matrix. We anticipate that such information could lead to a better understanding of factors that might allow recovery of transformed maize cells at higher frequency.

J7-302 EXPRESSION AND LOCALIZATION OF EPITOPE-TAGGED HUMAN RNA POLYMERASE II LARGE SUBUNIT. Lei Du[1], David Bregman[2], and Stephen Warren[2], Departments of Genetics[1] and Pathology[2], Yale University School of Medicine, New Haven, CT 06510.

Abstract. Recent observation that a subpopulation of human RNA polymerase II large subunit (HRPIILS) is distributed in discrete nuclear speckle domains where splicing proteins are stored led us to study the localization of HRPIILS in more detail. In vitro results show that monoclonal antibody H14 binds to both hyper- and hypophosphorylated form of HRPIILS (IIo and IIa respectively). H5 only binds to Pol IIo. MAb 8WG16 preferentially binds to Pol IIa. It has been shown that phosphorylation occurs predominantly on the C-terminal domain (CTD) of HRPIILS. Therefore it can be postulated that H14 binds to multiple reiterated epitopes in the CTD, H5 may bind to one or a few limited epitopes, and 8WG16 may bind to multiple hypophosphorylated epitopes. Evidences showing that Pol IIo is tightly bound to speckle domains suggest that the CTD may be responsible for HRPIILS's localization to the speckles. To detect CTD epitopes irrespective of its phosphorylation state, we tagged HRPIILS cDNA on the C-terminus with the FLAG peptide, DYKDDDDK, and expressed it in E. coli as GST fusion protein as well as in MDCK and HeLa cells. Recombinant peptides were blotted with various antibodies against HRPIILS and the phosphorylation dependency of epitopes was studied. The localization of tagged HRPIILS in mammalian cells was also examined. The results of epitope mapping may help to explain why certain antibodies recognize HRPIILS in the speckle domains, whereas other published antibodies only recognize the diffuse population.

J7-303 REPROGRAMMATION AFTER NUCLEAR TRANSFER IN RABBIT ENUCLEATED OOCYTES.

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After fertilization, RNA synthesis, as determined by α-amanitin-sensitive ^{3}H-uridine incorporation (autoradiography) and ^{3}H-poly (U) labeling (*in situ* hybridization) increases slowly from the 2- to 4-cell stage and more intensely from the 8- to 16-cell stage. Nucleologenesis observed by transmission electron microscopy reveals that nucleoli active in RNA synthesis are formed at the 8-cell stage. When 32-cell blastomeres are fused with oocytes matured *in vitro* and enucleated, the nuclear incorporation of ^{3}H-uridine is reduced 4h later and completely inhibited after 12h. From the 2-cell stage, the incorporation in reconstructed embryos parallels that of control embryos. Active nucleoli of the transplanted nuclei are desaggregating 6h after fusion and return to a state of compact "nucleolar precursors". From the 2-cell stage on, nucleogenesis proceeds as in control embryos, except that *nucleoli* at the 32-cell stage are depleted in the granular component. We conclude that factors present in the one-cell stage embryo repress both mRNA and rRNA synthesis and that reinitiation of gene expression seems to occur almost as in normal embryos. In fact the rate of blastocyst obtention is > 30%. We are starting to analyse the distribution of nuclear and nucleolar proteins such as lamins, nucleolin and fibrillarin in normal and reconstructed embryos.

J7-304 THE NUCLEOSKELETON AND CHROMATIN ORGANIZATION: MOVEMENTS, ALIGNMENT AND ASSOCIATION OF CENTROMERES IN INTERPHASE INVOLVES THE NUCLEAR MATRIX CORE FILAMENTS, D. He, D. Turner, B. Scott and B.R. Brinkley, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

The centromeres represent a large component of unique nuclear chromatin and account for as much as 5% of genomic DNA in human cells. Most studies have concentrated on the structure and function of centromeres on mitotic chromosomes and relatively little is known of the structure, molecular organization and behavior of centromeres (pre-kinetochores) in the interphase nucleus. Using CREST antikinetochore and anti-BrdU antibodies, confocal microscopy and immunogold label EM, we have analyzed structure, replication and distribution of pre-kinetochores and their association with the nuclear matrix in several mammalian cell lines. When CREST is used in Western blots of chromatin from interphase nuclei and mitotic chromatin, three bands representing CENP-A (17kD), B (80kD) and C (140kD) are detected. After extraction in DNase, ammonium sulfate and high salts, to produce core filaments, CENP-B and C were detected. However, examination by both immunofluorescence and immunogold EM revealed centromere staining patterns very much like those seen by standard procedures. Of particular interest to interphase chromatin organization, centromeres of Indian muntjac (IM:2N=6,7) and Chinese muntjac chromosomes (CM:2n=46) undergo dramatic movements and reorganization throughout interphase. In CM cells, 46 centromeres align into 8-10 linear arrays arranged perpendicular to the long axis of the nucleus prior to replication. Likewise, IM centromeres unfold and elongate into 7 linear arrays representing a 10-50 fold elongation of each centromere prior to Cen-DNA replication. Immunogold EM staining of the extracted nucleoskeleton revealed centromere association with 11nm core filaments. Moreover, the pre-replication patterns of centromere association were retained along core filaments. This result provides strong evidence for the attachment of centromeres to core filaments and their role in interchromosomal arrangements associated with centromere interaction and replication. We have also investigated centromere replication in rat mammary glands in vivo. It is possible to determine whether or not a mammary gland cell is in G_0/G_1, G_2 or M on the basis of pre-kinetochore staining patterns. We propose that the nucleoskeleton may function in internuclear centromere movements and may have facilitated non-random centromeric fusions in the evolution of IM chromosomes.

J7-305 ELECTRON SPECTROSCOPIC IMAGING (ESI)-A NEW TOOL FOR THE STUDY OF GENE EXPRESSION *IN SITU*, Michael J. Hendzel and David P. Bazett-Jones, Department of Medical Biochemistry, University of Calgary, Calgary, Alberta, Canada T2N 4N1

ESI is an electron microscopy technique that images electrons that have interacted inelastically with the specimen and thereby loss energy. The ability of ESI to map phosphorous within nucleoprotein structures is a well established application of ESI for *in vitro* analysis of purified nucleoprotein complexes. The ability to spatially map phosphorous within nucleoprotein structures as well as the exceptional contrast and spatial resolution of energy loss images makes ESI ideal for the investigation of nucleoprotein structures of the nucleus *in situ*. Removal of nucleoplasm from nuclei by lysing cells in an isotonic buffer containing 0.25% v/v Triton X-100 facilitates the visualization of nucleoprotein structures within the nucleus. Using nucleoplasm-depleted nuclei and ESI, we have been able to visualize size gradients of hnRNPs connected by intervening DNA. These structures are related to the previously characterized perichromatin fibrils and we believe that they represent RNA pol II transcription units. We have observed RNA pol II transcription units extending into interchromatin granule clusters (snRNP "speckled domains"). We believe that these correspond to "tracks" of primary transcripts that extend into "transcript domains". We have also observed that hnRNPs accumulate at the surface of interchromatin granule clusters. This likely corresponds to the accumulation of poly(A)RNA around "snRNP speckles" characterized by fluorescence microscopy. Interconnections between indivual granules within the interchromatin granule cluster support a structural model for the retention of snRNPs within interchromatin granule clusters. These studies serve as a basis for the study of structure/function relationships in the regulation of RNA pol II-mediated gene

J7-306 A NOVEL ZINC FINGER PROTEIN LOCALIZED IN DISCRETE NUCLEAR DOMAINS IN DEVELOPING OLIGODENDROCYTES, Lynn D. Hudson, J.G. Kim and Regina C. Armstrong*, Laboratory of Developmental Neurogenetics, NINDS, NIH and *USUHS, Bethesda, MD 20892

Oligodendrocytes coordinately synthesize massive amounts of a limited array of proteins and lipids that comprise the myelin sheath, a program that is dependent on transcriptional controls. One of the DNA-binding proteins that recognizes the myelin PLP gene is MyTI (Myelin Transcription factor I), a novel member of the zinc finger superfamily (Kim and Hudson, Mol. Cell Biol. 12:5632, 1992). MyTI has two clusters of the Cys-Cys-His-Cys class of zinc fingers which, by a random site selection assay, bind a G-rich consensus sequence (GGTGGGGPuPuPu) that is present in a number of myelin genes. Expression of MyTI is localized to the nucleus of early oligodendrocyte lineage cells, where it appears in a speckled pattern. MyTI immunoreactivity is excluded from nucleoli, visualized by phase contrast. The nuclear pattern of MyTI immunoreactivity is distinct from, but partially overlapping with, that obtained with several monoclonal antibodies (mAb) against proteins associated with compartmentalized nuclear functions, including proliferating cell nuclear antigen (mAb PC10; DNA replication), hnRNP (mAb 4D11; preparation of pre-mRNA for processing), and snRNP and non-snRNP splicing factors (mABs Y12 and αSC-35; pre-mRNA splicing). However, the MyTI nuclear pattern is quite similar to that obtained with an antibody against thyroid hormone receptor β1, a regulator of transcription in oligodendrocytes. In addition, MyTI nuclear immunoreactivity is markedly reduced by treatment with DNAase I. These findings are consistent with the localization of MyTI in nuclear domains associated with transcription. In view of other features of MyTI structure and expression, we hypothesize that MyTI may play an architectural role in arranging gene loci for subsequent active transcription.

J7-307 DYNAMIC REDISTRIBUTION DURING MITOSIS OF A NOVEL NUCLEAR MATRIX ASSOCIATED PROTEIN WITH *raf*-KINASE HOMOLOGY, K.M. Johansen and J. Johansen. Department of Zoology and Genetics, Iowa State University, Ames, IA 50011.

We have cloned a novel nuclear kinase p2Ab17 in *Drosophila* which by antibody labeling shows a dynamic cell cycle-specific pattern of distribution within the nucleus. Using enhanced video and confocal microscopy of triple-labeled preparations with probes for p2Ab17, α-tubulin, and DNA/histone we show that during interphase the p2Ab17 labeling is localized to the chromatin associated nuclear matrix and the nuclear envelope. However, during prophase and early metaphase the chromatin condenses and is disengaged from p2Ab17 which is realigned into a structure which becomes coextensive with the mitotic spindle apparatus as defined by tubulin immunoreactivity. Interestingly, the p2Ab17 spindle-like structures are formed before the assembly of the tubulin spindles. Thus, this data suggests that p2Ab17 may be a necessary structural component for assembly of the mitotic spindle. At telophase p2Ab17 spindles disintegrate and the protein redistributes to the decondensing chromatin and the reforming nuclear matrix. We have biochemically and molecularly characterized p2Ab17 and show that on Western blots a triplet of protein bands ranging from 170-180 kD are detected. These proteins are found only in nuclear fractions and not in the cytoplasm. The size of the proteins is consistent with data from Northern analysis which shows that p2Ab17 encodes a single transcript of approximately 6.5 kb. Sequencing of a partial p2Ab17 cDNA clone has currently yielded a unique C-terminal domain as well as sequence comprising the structure of an entire catalytic kinase domain. Searches of the sequence data bases with the kinase domain show that p2Ab17 is most homologous to serine-threonine kinases related to the *raf*-family of protooncogenes. However, p2Ab17 is only about 40% homologous to members of the *raf*-family, whereas homology between *raf*-family members is greater than 80%. In addition, p2Ab17 shows intriguing lower level homologies to other kinases which appear to be directly involved in cell cycle control, such as *nimA*. Thus, p2Ab17 may define a new class of nuclear matrix associated kinases related to the *raf*-family which may play a structural and/or signal transduction role in mitosis and cell division. Supported by NIH grant GM 50906.

J7-308 THE NUCLEAR BODY ASSOCIATED-PML PROTEIN IS AN INTERFERON INDUCED ANTI-ONCOGENE WHICH HAS AN ALTERED EXPRESSION DURING ONCOGENESIS M.H.M.Koken, G.Linares-Cruz, F. Puvion-Dutilleul[1], E.Puvion[1], F.Quignon, M.Stadler, M.K.Chelbi-Alix, M.C.Guillemin, J.Sobzack[2], F.Calvo, L.Degos, and H. de Thé. Hôpital St Louis, CNRS UPR43, 1, Av. Claude Vellefaux 75010 Paris, France. Tel.33.1.42063153/ Fax. 42064857, [1]IRSC, Villejuif, France. [2]Hôpital Necker, Paris, France.

The t(15;17) translocation, specific for acute promyelocytic leukaemia (APL), links the *PML* to the retinoic acid receptor α (*RARα*) gene. APL is an important model system of differentiation therapy as retinoïc acid (RA) induces specifically the differentiation of blast cells and complete remissions in patients. PML is a member of the C3HC4/RING-finger protein family. Several members of this family (PML, T18, RFP) are implicated in oncogenic translocations, which suggests that structural alterations of this group of proteins may be involved in tumourogenesis.

We show that PML co-fractionates with the nuclear matrix (like RFP) and displays a speckled nuclear pattern. Immuno-electron microscopy revealed that PML is specifically associated to the periphery of a nuclear body (NB). By co-localisation studies it was found that the same type of NB is also detectable with antibodies against Sp100, an autoantigen recognised by sera of primary biliary cirrhosis (PBC) patients.

PML/RARα dominantly delocalises wild type PML protein as well as the PBC antigens to much smaller nuclear dots than PML. Importantly, RA-treatment of APL cells *in vitro* or *in vivo*, lead to a re-localisation of the PML and PBC proteins to the larger PML dots. This nuclear shift of the APL-specific fusion protein could be one of the molecular bases of RA-induced remission.

Recently we have shown that PML is transcriptionally induced by interferons α, β and γ. The protein displays anti-oncogenic properties, as overexpression causes a growth decline *in vitro*, and a gross reduction in tumour size and time of appearance in nude mice. The protein is abundantly expressed in all human cell lines tested, although strictly regulated during the cell cycle. *In vivo*, however, most tissues express PML in a highly restricted manner. During tumour progression a drastic increase in protein quantities is found, which however decreases at the moment the tumour turns invasive. These data provide a first link between nuclear matrix, interferons and oncogenesis, and supply the tools to study composition and genesis of this type of nuclear bodies.

J7-309 PROTEIN 4.1 IN THE NUCLEAR MATRIX OF HUMAN FIBROBASTS, Sharon W. Krauss, Joel A. Chassis, Carolyn A. Larabell, Steve Lockett, Rudiger Blaschke and Narla Mohandas, Life Sciences Division, University of California, Lawrence Berkeley Laboratory, Berkeley, CA 94720

The protein 4.1 family of structural proteins are generated from a single gene by complex alternative mRNA splicing which is differentiation and tissue specific. Although protein 4.1 is ubiquitous, only in one case has a role been determined for an isoform: In human red blood cells, an 80kD 4.1 is a multifunctional structural component of the membrane skeleton participating in critical interactions with spectrin and actin within the cytoskeleton and serving as a linking protein between the cytoskeleton and integral proteins of the lipid bilayer. We are exploring the structure/function of protein 4.1 in diploid human fibroblasts which contain 4.1 isoforms ranging from 45kD to 230kD detected by Western blotting. Using IgG's against 80kD 4.1 and against peptides from several 4.1 domains, immunofluorescent "speckles" are observed in fibroblast nuclei, sometimes along with cytoplasmic staining. By microscopic optical sectioning of the nucleus, 4.1 immunofluorescent foci are intranuclear. The nuclear signals persist after cell permeabilization, after salt extraction of nuclear and cytoplasmic proteins from permeabilized cells and in nuclear matrix following DNase/RNase digestion and salt re-extraction. Thus some isoform(s) of protein 4.1 behave as nuclear matrix components in fibroblasts. In double-label immunofluorescent imaging by confocal microscopy, nuclear 4.1 signals are within the nuclear area bounded by nuclear pores and the underlying ring of lamin B. The 4.1 epitopes reside in an interior nuclear region stained by the nuclear matrix protein NUMA. In additional double-label studies, SC35 antibody was used to localize spliceosomes and anti-PCNA to image the accessory protein of delta DNA polymerase involved in DNA synthetic complexes. Preliminary experiments indicate that PCNA epitopes and 4.1 signals appear to be non-randomly associated. This observation suggests that nuclear protein 4.1 may contribute to the organization of replicating chromatin.

J7-310 **SUBNUCLEAR PARTITIONING OF PIT-1 TRANSCRIPTION FACTOR IN RAT PITUITARY-DERIVED GH$_3$ CELLS.** **Michael Mancini, Maureen Mancini, Bing Liu, Debbie Munoz-Medellin, and Z. Dave Sharp Center for Molecular Medicine, University of Texas Institute of Biotechnology, San Antonio, Texas 78245.**

The differentiation of cells in the mammalian anterior pituitary that synthesize and secrete prolactin, growth hormone and thyroid stimulating hormone is dependent on a POU-protein transcription initiation factor named Pit-1. Its mechanism of action in cell differentiation and selective activation of target genes [prolactin, growth hormone, TSHb and *pit*-1] is unknown.

In GH3 pituitary-derived tumor cells that synthesize and secrete PRL and GH, immunocytochemistry with monoclonal antibodies against Pit-1 show a low level of diffuse labeling over the entire nucleus with discreet areas of increased concentration. Nuclear staining and Western assays of biochemically fractionated GH3 cells show that Pit-1 is partitioned between a soluble fraction and a portion tightly associated with the core nuclear matrix.

GH3 cells that transiently expressed epitope-tagged full-length and truncated Pit-1 polypeptides were analyzed for protein expression and nuclear labeling patterns. Under conditions of overexpression, full-length T7-tagged Pit-1 has a staining pattern similar to endogenous Pit-1. Using the tetracycline transcription control system, cells that expressed lower levels of tagged Pit-1 show a limited number [20-50] of foci that suggest a saturable targeting system for Pit-1 in the nucleus. These punctate regions of staining do not overlap with speckles associated with RNA processing.

Similar analyses of eight truncated variants of T7-tagged Pit-1 polypeptides show that the POU-specific domain alone is retained in the nucleus in a pattern most similar to Pit-1. The POU-specific domain has no known nuclear transport signal and, alone, cannot bind DNA. Other truncated polypeptides containing the POU-specific domain routinely demonstrate dramatic nuclear aggregation. The data suggest that this domain may promote Pit-1 self-association in addition to sub-nuclear localization through interactions with other proteins, possibly in the core nuclear matrix.

The working hypothesis is that efficient function of Pit-1 is dependent on placement of the polypeptide in appropriate nuclear compartments and that the POU-specific domain has a role in sub-nuclear targeting.

J7-311 NUCLEAR DOMAIN 10 (ND10) STORED NUCLEAR MATRIX PROTEINS ARE REDISTRIBUTED DURING STRESS AND RECRUITED TO ADENOVIRUS TYPE 5 REPLICATION DOMAINS, Gerd G. Maul, The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104

We have described a specific, precisely circumscribed nuclear domain that is present at a frequency of approximately 10 (ND10). These domains do not correlate with any of the other known domains, such as centromeres, telomeres, and nucleolar organizers or coiled bodies, nor do they colocalize with heterochromatin or splicing domains. There are at least four characterized proteins associated with these domains, among them the potential transcription factor PML. Upon high salt extraction, three are retained on the nuclear matrix. No functions are known for these proteins nor for the ND10 structure, which is in part coincident with nuclear bodies.

As a means to target potential functions, we embarked on a search for environmental conditions that would change the distribution of these proteins. We found that stress in the form of heat shock distributes three of the four proteins to hundreds of very small sites within 15 min in the absence of protein synthesis. Also, interferon upregulates the three proteins tested which proves that they belong to the small group of proteins stimulated by this cytokine. These findings prompted an investigation of the effect of virus on ND10. ND10 are eliminated by Herpes virus, and the viral immediate early protein ICP0 is responsible for this elimination. Its ring finger domain is essential for the effect, but a COOH terminal region is necessary for binding ICP0 to ND10. AD5 also has a profound effect on ND10. Before replication starts, it segregates ND10-associated proteins to tracks. Three of the four proteins are then recruited from those tracks to the Ad5 replication domains.

From our observations, we speculate that ND10 are storage domains for various nuclear matrix proteins that can be activated quickly during global changes like those induced by heat shock, hormone exposure, or viral infection. DNA viruses may differentially utilize some of the matrix proteins as substrate, on which replication and transcription takes place. Other ND10-associated proteins, like PML, a potential suppressor, may be specifically eliminated from these domains. The characterization of the viral action on those matrix components will increase our understanding of a very specific set of nuclear matrix proteins.

J7-312 CELL TYPE-SPECIFIC HIS55 NUCLEAR ANTIGEN IS A NOVEL COMPONENT OF THE PML DOMAIN, Davina Opstelten and Yun W. Lam, University of Hong Kong, Hong Kong (Supported by the Croucher Foundation grant no. 394/032/1236 and CRCG grant no. 335/032/0050)

Mouse monoclonal antibody (mAb) HIS55 labels discrete foci in the nuclei of cells of various mammals, including man. The size and number of HIS55 foci is highly cell type-dependent. We hypothesized that HIS55 antigen (ag) plays a role in the regulation of gene expression. Double immunofluorescence showed that the nuclear foci labelled by HIS55 mAb did not colocalize with splicing domains (interchromatin granules and coiled bodies). We then studied the relationship between HIS55 ag and PML domains, multimolecular complexes of unknown function containing the product of the PML gene and at least 2 other components. In acute promyelocytic leukaemia, the PML gene is fused with the retinoic acid receptor α gene by chromosomal translocation, and PML domains are abnormal. We double-labelled H35B rat hepatoma cell line with 5E10[1] (kindly provided by Dr. L. de Jong, University of Amsterdam) which recognizes *in vitro*-translated human PML, and HIS55. In conventional fluorescence microscopy, the foci labelled by the two mAbs colocalized. The same was found for nuclei isolated from normal rat liver. The tissue distribution of 5E10 ag studied by immunohistology in various organs in rat was identical to that of HIS55 ag. However, Western analysis of isolated rat liver nuclei showed that HIS55 mAb bound to a set of proteins with apparent MW (125, 100 (doublet), 78, 71, 48 (doublet), 33 and 28kD) different from that of 5E10 (in our hands: 115, 100, 85 and 73kD; published: 149, 126, 95 and 63kD). As the MW pattern of HIS55 ag also differed from the reported MW of other known components (Sp100 and NDP55) of PML domain, HIS55 ag may be a novel component of this complex. Further studies of HIS55 ag will give important insight in the function of PML domains.

[1]Stuurman et al. J Cell Sci 101: 773-784 (1992)

J7-313 **Targetting of the Adenovirus E1A and E4-ORF3 Proteins to Nuclear Matrix-Associated PML Bodies**, Jacob -S. Seeler[1], Teresa Carvalho[2], Peter Jordan[2], Karin Öhman[3], Göran Akusjärvi[3], Maria Carmo-Fonseca[2] and Anne Dejean[1], [1]Institut Pasteur, INSERM U163, 75724 Paris Cedex 15, France, [2]Instituto de Histologia, Av. Prof. Egas Moniz, Faculdade de Medicina, 1699 Lisboa Codex, Portugal, [3]Laboratory of Microbial Genetics, Karolinska Institutet, S-17177 Stockholm, Sweden.
Early electronmicroscopic analyses and the systematic characterization of autoimmune antibodies that selectively label individual subnuclear domains has identified a novel type of nuclear structure, the PML nuclear bodies. The PML protein was first identified as part of a fusion product with the retinoic acid receptor α (RARα), PML-RARα, resulting from the t(15;17) chromosomal translocation associated with acute promyelocytic leukaemia (APL). This leukaemia is characterized by a block in differentiation at the promyelocyte stage and a particular sensitivity to retinoic acid. We and others have shown previously that in APL cells, most, if not all of the PML nuclear bodies are disorganized into numerous and aberrant microstructures containing both PML and PML-RARα. The cellular function of PML, and of the three other known components of these nuclear bodies (the 65 kDa protein, NDP55, and Sp100) remains poorly understood. Since viral infection provides an amenable model system for the functional study of host cell subnuclear structures, we wished to investigate the effects of small DNA tumor virus infection on the integrity of the PML nuclear bodies. Here we show that adenovirus infection causes a dramatic redistribution of PML from doughnut-shaped nuclear bodies into fibrous structures. This redistribution is also evident for another component of these nuclear bodies, the Sp100 protein. Using a series of adenovirus early region 4 (E4) mutant viruses and plasmid vectors expressing single E4-ORFs, we demonstrate that the ORF3 product is both necessary and sufficient for the disorganization of the PML subnuclear structures. Unexpectedly, the E1A oncoproteins are also found to concentrate within the PML domains, both in the nuclei of cells that were infected and those transiently transfected. This association requires a region of E1A that overlaps, but is not identical to, the conserved region 2 (CR2), containing the (D)LXCXE amino acid motif common to all viral oncoproteins that bind pRB. Finally, the SV40 large T protein is shown to accumulate in close proximity to the PML nuclear bodies.
Taken together, the present data indicate that the subnuclear domains containing PML may represent a preferential target for DNA tumor viruses, and therefore suggest a more general involvement of the PML nuclear bodies in oncogenic processes.

J7-314 STEROID RECEPTORS, TRANSCRIPTION AND NUCLEAR ARCHITECTURE, [1]Bas van Steensel, [1]Erica van Binnendijk, [1]Krina van der Meulen, [3]Guido Jenster, [4]Klaus Damm, [2]C. Diane Hornsby, [1]Derick G. Wansink, [2]E. Ronald de Kloet and [1]Roel van Driel. *[1]E.C.Slater Institute, University of Amsterdam, Plantage Muidergracht 12, 1018 TV Amsterdam, The Netherlands; [2]Leiden University, Leiden, The Netherlands; [3]Erasmus University, Rotterdam, the Netherlands; [4]Max-Planck-Institute for Psychology, München, Germany.*

Steroid receptors are hormone-controlled transcription factors. To gain insight in the relationship between transcriptional control and nuclear organization, we investigated: (i) the interaction between steroid receptors and the nuclear matrix and (ii) the spatial distribution of steroid receptors in the nucleus.

Steroid receptors bind to the nuclear matrix in a hormone-dependent fashion. We studied nuclear matrix binding of various deletion mutants of the glucocorticoid receptor (GR) and the androgen receptor (AR). We identified specific protein domains in both receptors that are involved in matrix binding. Moreover, using different nuclear matrix extraction methods we show that the GR and AR bind to the nuclear matrix through different mechanisms.

By immunofluorescent labeling and confocal microscopy we show that the GR is concentrated in about 1,000 discrete clusters in the interphase nucleus. These GR clusters are associated with the nuclear matrix. Somewhat surprisingly, dual-labeling experiments show that the majority of GR-clusters does not colocalize with sites of pre-mRNA synthesis (labelled in vivo with BrUTP) or with domains enriched in RNA polymerase II. These data suggest that a large fraction of the GR molecules is concentrated in specific nuclear domains that are not directly involved in activation of transcription.

In rat hippocampus neurons the GR and the mineralocorticoid receptor (MR) regulate neuronal excitability in a coordinated fashion. By dual-labeling confocal microscopy we found that both receptors are concentrated in clusters in the nucleoplasm. Using a novel image analysis technique we show that these GR and MR clusters colocalize partially, in a non-random fashion. This colocalization suggests a close interaction of the GR and MR in specific nuclear domains.

J7-315 IMMUNOCHEMICAL STUDY OF THE PROTEIN COMPONENTS OF THE BALBIANI RING RIBO-NUCLEOPROTEIN PARTICLES, Neus Visa, Alla T. Alzhanova Ericsson, Xin Sun, Elena Kiseleva, Tilmann Wurtz, Birgitta Björkroth and Bertil Daneholt, Department of Cell and Molecular Biology, Karolinska Institute, S-17177 Stockholm, Sweden

Nascent RNA polymerase II transcripts are associated with proteins to form heterogeneous nuclear ribonucleoprotein (hnRNP) complexes. The events required to transform pre-mRNA into mature mRNA take place at the RNP level and, therefore, the structure and the composition of hnRNPs are probably essential for mRNA biogenesis. Also the possible interactions of mRNA with nuclear matrix and cytoskeleton are likely to be mediated by RNA-binding proteins. For these reasons, we are interested in the characterization of the protein components of the hnRNP complexes. We have chosen the Balbiani rings (BR) of *Chironomus tentans* as a model system because the pre-mRNP complexes synthesized in the BR puffs -the BR particles- can be easily identified under the electron microscope. In this way, we can study the assembly, transport and disassembly of a specific pre-mRNP particle *in situ*. Moreover, by using immuno-electron micoscopy, we can study the presence of different proteins in BR particles at different stages of synthesis, processing and nucleocytoplasmic transport.

Following an immunochemical approach, we have been able to identify some of the major protein components of the BR particles. One of them, hrp45, shares high sequence similarity with the SR-family of splicing factors and appears to be localized exclusively in the cell nucleus. In cultured cells, this protein shows a nuclear speckled distribution. Another of the proteins analyzed, hrp36, is similar to the A/B group of hnRNP proteins and displays a more complex intracellular distribution. In the salivary gland cells, hrp36 binds to nascent BR-RNA, remains in the BR particles during intranuclear transport and is exported to the cytoplasm as a complex with the mRNA. In the cytoplasm, hrp36 is found in mRNP complexes engaged in translation. These results suggest that hrp36 is involved in multiple steps of RNA metabolism, in both the nucleus and the cytoplasm, and therefore it might mediate functional relations between events that take place in different cell compartments.

J7-316 COORDINATED, TRANSCRIPTION-DEPENDENT REDISTRIBUTION OF RNA POLYMERASE II AND SPLICING PROTEINS. Stephen Warren†, David Bregman†, Lei Du* and Sarina van der Zee†, Departments of Pathology† and Genetics,* Yale University School of Medicine, New Haven, CT 06510

Abstract. A fraction of the largest subunit of RNA polymerase II (Pol II LS) is diffusely distributed in the nucleoplasm, and is hypophosphorylated to various degrees on the the COOH terminal domain (CTD), an unusual structure comprised of 52 heptapeptide repeats similar or identical to TyrSerProThrSerProSer. Another fraction of Pol II LS is tightly associated with 20-50 "speckle domains," which store splicing proteins. This fraction of Pol II LS is hyperphosphorylated on the CTD (i.e. Pol IIO) and is resistant to detergent and nuclease extraction. Pol IIO and Ser/Arg splicing proteins undergo coordinated, transcription dependent intranuclear redistribution: in transcriptionally active nuclei, Pol IIO and splicing proteins are located in irregularly shaped speckle domains, which appear to be interconnected via a network. During states of transcriptional inhibition, SC35 and Pol IIO accumulate in speckle domains, which are transformed into enlarged, round structures lacking interconnections. When the cells are released from transcriptional inhibition, Pol IIO and SC35 redistribute back to the interconnected speckle pattern. The transcription dependent intranuclear redistribution cycle of Pol II LS and splicing proteins is reversible and temperature dependent. These results imply that: (i) Pol IIO and multiple spliceosomal proteins are stored in the enlarged speckle domains when they are not engaged in pre-mRNA biogenesis; and (ii) hyperphosphorylation of Pol II LS's CTD is not necessarily an indicator of actively transcribing Pol II complexes *in vivo*. Pol II LS and spliceosomal proteins may be coordinately recruited from discrete storage/reassembly sites to Pol II transcription sites, thereby allowing the splicing machinery to gain direct and immediate access to nascent pre-mRNA transcripts.

J7-317 P-250 IS A NOVEL NUCLEAR MATRIX PROTEIN THAT COLOCALIZES WITH SPLICING FACTORS, Xiangyun Wei, Michael J. Mortillaro, Steve Kim, Lee Frego, Linda A. Buchholtz, Hiroshi Nakayasu and Ronald Berezney, Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York 14260

A monoclonal antibody raised against rat liver nuclear matrix proteins recognizes a single protein of 250kDa from mammalian cells. Immunoblot analysis revealed that this protein (P-250) was a nuclear protein enriched in the nuclear matrix. Similarly, P-250 decorated an elaborate fibrogranular structure in mammalian cell nuclei, which was maintained following extraction for in situ nuclear matrix. During mitosis P-250 reorganizes to associate with chromosomes and the mitotic spindle similar to the immunofluorescent pattern observed for the 240kDa nuclear mitotic apparatus associated protein (NuMA). However, immunoblot analysis indicated that P-250 was not NuMA, but a significantly slower migrating protein. Laser scanning confocal microscopy of P-250 showed that most interphase nuclei contained between thirty and fifty brightly staining spots and a much larger number of less intensely staining small granules. Three dimensional analysis revealed that some of the brightly staining spots colocalize with splicing factor specific antibodies. The other brightly staining foci appear to be a novel nuclear domain. The apparent association of P-250 with the mitotic spindle apparatus, mitotic chromosomes, and splicing factors suggests that this protein may be involved in several different nuclear processes. Additional studies to determine the structural and functional relationship of P-250 to splicing, replication, and transcription, are being investigated. Nonequilibrium two dimensional electrophoretic separation of rat liver nuclear matrix proteins, followed by immunoblot analysis has identified P-250 at the acidic side of the gel. Amino acid microsequencing of this 2-D protein spot will be used to create DNA oligomers for screening a cDNA library. (Supported by NIH grant GM-23922).

J7-318 ASSOCIATION OF A NUCLEAR MATRIX CYCLOPHILIN WITH SPLICING FACTORS, Michael J. Mortillaro and Ronald Berezney, Department of Biological Sciences, SUNY at Buffalo, Buffalo, New York 14260

A novel nuclear matrix protein termed splicing factor associated cyclophilin (SFA-Cyp) has been identified from a rat cDNA library. The 4.0 kb full-length cDNA contains a 0.3 kb 5' untranslated region, a 2.3 kb open reading frame, and a 1.4kb 3' untranslated region. The open reading frame encodes a basic protein with a predicted molecular weight of 87 kDa. Analysis of the predicted amino acid sequence revealed three domains which show significant homology to other proteins. Amino acids 6-178 form a complete cyclophilin domain with 66% identity to known cyclophilin proteins. This domain is currently being assayed for prolyl peptidyl cis-trans isomerase activity in a glutathione-S-transferase fusion protein. Amino acids 193-251 form an acidic-serine region followed by a polybasic stretch of amino acids remarkably similar to nuclear signal sequence binding domains originally described for Nopp140. The third domain, a series of serine-arginine repeats, covers most of the carboxy half of the protein and may function to allow SFA-Cyp to associate with other SR proteins. An antibody raised against the carboxy-tail of SFA-Cyp recognizes a protein of 103kDa in rat liver cells. Immunoblot subcellular fractionation indicates that SFA-Cyp is located in the nucleus and is enriched in the nuclear matrix. Consistent with this, the antibody stains from twenty to forty prominent bright foci located in the nucleus. Double staining using laser scanning confocal microscopy clearly indicates that SFA-Cyp colocalizes to speckles stained by splicing factor specific antibodies Y12 and anti-U1-70kDa. Our working hypothesis based upon this early data is that SFA-Cyp is a molecular chaperone involved in targeting splicing factors to the brightly staining nuclear foci. At these foci SFA-Cyp would then assist these proteins to properly associate with forming splicesomes. We are currently testing the different aspects of this model. (Supported by NIH grant GM-23922).

Nuclear Matrix Proteins: Characterization; Cell Specificity; Cellular Regulation, Differentiation and Tumorigenesis

J7-400 INTERNAL MATRIX PROTEINS FROM CHICKEN LIVER NUCLEI.

Fabio Altieri, Bruno Maras, Rodolfo Ippoliti and CarloTurano. Department of Biochemical Sciences "A.Rossi Fanelli" and CNR Center of Molecular Biology, University "La Sapienza", Rome, Italy

Proteins from internal nuclear matrix, a more soluble portion of the nuclear matrix (Kaufmann, S.H. and Shaper, J.M. 1984 Exp. Cell Res. 155, 477), have been subjected to a purification procedure that by means of standard chromatography techniques enabled us to purify them in non denaturing conditions and in a simultaneous way. Several proteins have been purified and/or identified like protein disulfide isomerase, calreticulin and the chicken HSP105 homologous to glucose regulated protein GRP94. Three proteins with molecular weights of 60k, 57k and 32k Daltons were further characterized by two-dimensional electrophoresis, subjected to micro sequencing, analysed for enzymatic activity and their nuclear distribution was confirmed by immunofluorescence studies done on several cell types. The first protein isolated, called M57k and already partially characterized (Altieri, F. et al. 1993 Biochem. Biophys. Res. Commun. 194, 992), showed a sequence almost identical to a previously characterised protein initially identified as phospholipase C and then as a thiol-oxidoreductase. However we isolated this protein from a nuclear matrix fraction suggesting a role in maintaining a particular redeox state of cystein containing nucleoproteins. The protein with a molecular weights of 60kDa, appear to be glycosylated, and its N-terminal sequence showed no homologies with other proteins except for an internal region of the carboxylesterase. Moreover the sequence of a peptide obtained with mild acidic digestion is almost identical to active site region of the same protein and the 60kDa protein has been positively tested for an esterase activity leading us to suppose it may represent a new kind of esterase. This can be considered the first evidence of an esterase activity associated with the nuclear matrix and its hypothetical substrates may be biological active esters like the nuclear diacylglycerols coming from the phosphoinositides cycle. The protein with a molecular weight of 32kDa has the N-terminal blocked, by immunofluorescence showed a particular nuclear distribution and it is at present under further characterization.

J7-401 DYNAMIC PHOSPHORYLATION OF AN ESSENTIAL BACULOVIRUS PROTEIN.

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The *Autographa californica* nuclear polyhedrosis virus (AcMNPV) *39k* gene product, pp31, is a nuclear protein that appears to be essential for viral replication. We have previously shown that pp31 is localized to the chromatin and nuclear matrix subfractions of infected cell nuclei. Southwestern blotting revealed that pp31 is a non-sequence specific DNA binding protein. Metabolic labeling of cells with carrier-free phosphate at a single time after infection showed that pp31 is phosphorylated. Now we are using biochemical and genetic approaches to further characterize this protein and to investigate its function and localization, focusing on phosphorylation as a possible control mechanism.

Phosphorylation of both the nuclear and cytosolic forms of pp31 were detected from 4-30 hpi. Phosphoamino acid analysis of infected cell lysates demonstrated that pp31 is phosphorylated on both serine and threonine residues. By contrast, pp31 expressed in uninfected cells was only phosphorylated on serine residues, indicating that pp31 is phosphorylated by both cellular and virally-encoded or -induced kinases. Aphidicolin treatment blocked threonine phosphorylation of pp31, indicating that a late viral protein is required for this modification. When extracted in the absence of phosphatase inhibitors, cytosolic pp31 was completely dephosphorylated, indicating that it is a substrate for phosphatase activity. However, only the phosphothreonine(s) were removed from nuclear pp31. pp31 expressed in uninfected cells was not dephosphorylated, indicating that the phosphatase(s) is virally-encoded or -induced. Together, these observations suggest that phosphorylation and dephosphorylation of pp31 is a dynamic, virally regulated process, which could influence its intracellular distribution and, perhaps, its function.

Based on this preliminary conclusion, we are proceeding with biochemical and genetic experiments designed to map and mutate phosphorylation sites in pp31. Simultaneously, we are investigating the mechanism of pp31 nuclear targeting and accumulation.

J7-402 **Involvement of Protein Tyrosine Phosphorylation in the Regulation of Expression of p53 and WAF1 in Hela Cells**

Zhen Ping CHEN and Desmond Chak-Yew YEUNG
Department of Biochemistry, University of Hong Kong

It has been shown that p53 stimulates the production of WAF1/cip1/p21/sdi1, a 21 kD protein, which blocks cyclin/cyclin dependent kinases thereby arresting cell growth. Despite recent studies on this growth regulatory cascade, reports on the exact mechanism governing the expression of these genes are still scanty. Since protein tyrosine phosphorylation is implicated in growth control processes, we have studied the effects of insulin, sodium orthovanadate and genistein on p53 and WAF1 gene expression in Hela cells. Our results showed that vanadate dramatically decreased the transcripts of p53 and WAF1, and insulin significantly inhibited p53 gene transcription following 6 hours exposure of the cells to the hormone. Contrary to expectation, the hormone exerted a biphasic effect on WAF1 gene expression. It dramatically stimulated WAF1 gene expression within the first 4 hours of treatment, but at later time points, when a suppressive effect of insulin on p53 expression was elicited, the hormone inhibited WAF1 expression. When cells were treated with genistein, transcription of both p53 and WAF1 genes was enhanced.

The steady state of cellular protein phosphorylation level is determined by both phosphorylation and dephosphorylation. As insulin receptor possesses a tyrosine kinase, and vanadate is an inhibitor of cellular protein tyrosine phosphatases, their presence may increase protein tyrosine phosphorylation. Whereas genistein is a tyrosine kinase inhibitor, it is likely that it decreases the protein tyrosine phosphorylation level. The earlier inductive effect of insulin on WAF1 may be the result of enhanced phosphorylation of the p53 protein, as hyperphosphorylation of p53 may result in higher transactivating activity. Our results clearly suggest that protein tyrosine phosphorylation is intimately involved in the regulation of expression of p53 and WAF1.

J7-403 ESTROGEN REGULATION OF A NUCLEAR MATRIX PROTEIN(S) IN ER+ HUMAN BREAST CANCER CELLS.

AS Coutts, JR Davie, and LC Murphy, Department of Biochemistry and Molecular Biology, University of Manitoba, Winnipeg, Manitoba, Canada, R3E 0W3.

The nuclear matrix (NM) is the structural framework of the nucleus and increasing evidence suggests that the NM is involved in regulation of cell function including DNA organization, gene transcription and processing, replication and steroid hormone action. We have studied estrogen regulation of nuclear matrix proteins in T-47D5 human breast cancer cells. Three proteins (45-60kD) present in the NM of cells grown in estrogen replete conditions were dramatically reduced when the cells were grown in acute (1 week) estrogen-deplete conditions. Replacing estrogen in the medium of acute estrogen-depleted cells restored expression of these proteins. T-47D5 cells that are chronically depleted of estrogen and are estrogen-independent in culture have levels of these proteins comparable to parent cells grown in the presence of estrogen and no further upregulation of these proteins by estrogen can be seen. Treating T-47D5 cells in estrogen replete conditions with the antiestrogen 4-hydroxytamoxifen (100nM, 3 days) resulted in a significant reduction in these proteins, while no effect was seen in long-term chronic estrogen-depleted T-47D5 cells. In conclusion we have identified NM proteins in human breast cancer cells that are estrogen regulated and may play a role in estrogen action in human breast cancer cells. (Funded by: MRC (Canada), Canadian Breast Cancer Research Initiative, and the Manitoba Health Research Council.)

J7-404 IDENTIFICATION OF INTERNAL NUCLEAR MATRIX PROTEINS OF HeLa S3.

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Although it is 20 years ago that the nuclear matrix was first described, the molecular basis of the internal nuclear fibro-granular network is not known yet. The nuclear matrix is still operationally defined as the structure that remains after extraction of nuclei with detergents, nucleases, and high salt. The nuclear matrix can be divided into: (1) the peripheral matrix, consisting of the lamina holding the nuclear pore complexes, and (2) the internal matrix, consisting of a fibro-granular structure and residual nucleolar structures. Detailed information exists about the structure and proteins of the lamina-pore complex, but little is known about the components that constitute the internal matrix.

Our aim is to identify the major proteins of the internal nuclear matrix of HeLa S3 cells. To this end the protein composition of two structures was investigated: complete nuclear matrices, containing both peripheral and internal matrix, and nuclear shells, containing only the peripheral matrix. By quantitative analysis of two-dimensional gels of both preparations a distinction could be made between peripheral proteins and those of the internal matrix. Some of the nuclear matrix proteins were characterized by immunoblotting. Among these, hnRNP C was identified to be a major protein of the internal nuclear matrix. Microsequencing of various matrix proteins is in progress.

J7-405 **DIFFERENTIATION-DEPENDENT NUCLEAR MATRIX PROTEINS CROSS-LINKED TO DNA BY CIS-DIAMMINEDICHLOROPLATINUM**

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The process of differentiation is characterized by specific changes in the pattern of gene expression.
The protein composition of the nuclear matrix is differentiation-stage dependent (1). We and others have hypothesized that nuclear matrix proteins interact with chromatin to coordinate gene expression.
Previous reports have shown that cis-diamminedichloroplatinum (cis-DDP) is able to diffuse to the nucleus of intact cells and to cross link DNA to nearby proteins (2). In this study we meant to characterize differentiation specific nuclear matrix proteins cross-linked to DNA by cis-DDP argueing that these proteins most likely play a role in the structural and/or functional organization of chromatin.
To this end, HL60 cells were induced to differentiate with DMSO and retinoic acid and subsequently incubated with cis-DDP. Nuclear matrices were prepared from control and differentiated HL60 cells according to Berezney (3). DNA cross-linked nuclear matrix proteins were selected by hydroxyapatate/ thiourea method (2). Total nuclear matrix proteins as well as DNA cross-linked nuclear matrix proteins were analyzed by bi-dimensional gel electrophoresis.
Marked changes in total nuclear matrix proteins were observed between undifferentiated and differentiated HL60 cells with several new components that are related to the differentiation state of the cells. The comparison of the electrophoretic patterns of total nuclear matrices and DNA-cross linked nuclear matrix proteins reveals that a number of the new species induced by differentiation are also cross-linked to DNA by cis-DDP. It is conceivable that these proteins are instrumental in the overall control and coordination of gene expression during differentiation.
Experiments are underway to evaluate the binding of these proteins to DNA specific sequences.

1.Stuurman et al.(1989) Exp. Cell Res. 180, 460-466.
2. Ferraro et al. (1991) Biochem. Biophys. Res. Commun. 178, 1365- 1370.
3. Berezney et al. (1977) J. Cell Biol.73, 616-637.

J7-406 EXPORT OF PROTEINS FROM THE NUCLEUS REQUIRES THE GTP-BINDING PROTEIN RAN/TC4

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Nuclear protein export has been investigated in digitonin-permeabilized cells that had imported a nuclear localization signal-bearing conjugate for 15 min and then were incubated with exogenous Xenopus oocytes cytosolic fractions for different periods of time. Fraction B is sufficient to support the export of the conjugate from the nucleus. Protein export from the nucleus is inhibited by the lectin WGA, is ATP independent and GTP dependent. The active component in fraction B is the GTP-binding protein Ran/TC4. GTP increased the export activity of Ran/TC4 whereas GTPγs and GMP-PNP were inhibitors. Moreover, a GTPase-defective mutant Ran/TC4 is unable to support nuclear protein export. This indicate that Ran/TC4 must be in the active GTP-bound state to mediate protein nuclear export and that GTP hydrolysis is involved in the process. Fraction B and Ran/TC4 have been previously found to be required for nuclear import. The present results suggest that Ran/TC4 functions in both directions of nucleocytoplasmic transport.

J7-407 STRUCTURAL AND MOLECULAR STUDIES OF A LIKELY DROSOPHILA NUCLEAR SKELETAL COMPONENT SHOWING BROAD SEQUENCE HOMOLOGY TO HUMAN TPR

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We have previously described an ~180 kD antigen in Drosophila melanogaster which is localized to the nuclear periphery in interphase, remains concentrated in the chromosomal region during metaphase and early anaphase, and isolates biochemically in the nuclear pore complex-lamina fraction[1]. We have recently extended these observations to include 3-D immunofluorescence localizations at higher resolution and cloning and sequencing of Bx34 cDNA. CCD-based deconvolution fluorescence microscopy of Drosophila embryonic and salivary gland nuclei clearly shows a nuclear interior localization for Bx34 in addition to the nuclear periphery. We have cloned and sequenced 5.5 kb of Bx34 cDNA and have identified an open reading frame that spans the entire 5.5 kb. The 1800 amino acids encoded by this stretch constitute a biphasic protein with the N-terminal 60% containing extensive regions of heptad repeats characteristic of coiled-coil forming proteins. The C-terminal 40% is markedly less structured and shows low to moderate homology with a number of nuclear proteins. Most of the Bx34 sequence (1500 of 1800 amino acids) shows significant homology to the human tpr protein. The Bx34 predicted protein secondary structure, interphase and cell cycle localization, and sub-nuclear biochemical fractionation pattern are consistent with a nuclear skeletal protein. We are currently pursuing in vivo fluorescence imaging, molecular genetic, and biochemical approaches to Bx34 in Drosophila.

[1] Frasch, Paddy, and Saumweber (1988): J. Cell Science 90, 247-263.

J7-408 BIOCHEMICAL AND GENETIC STUDIES ON THE HIGH MOBILITY GROUP 1 PROTEIN (HMG1)

Andrea Pontiggia, Luca Falciola, Simona Ferrari, Sabina Calogero, Lorenza Ronfani and Marco E. Bianchi, DIBIT, San Raffaele Scientific Institute, Milano, Italy, and Dept. Genetics & Microbiology, University of Milano, Italy

HMG1 is an abundant an highly conserved component of chromatin in vertebrates; homologues exist in all eukaryotes. HMG1 and its DNA binding domains bind stably to kinked DNA structures, and transiently to linear DNA, causing it to bend. Mutational analysis of the N-terminal DNA binding domain, molecular modelling and comparisons with HMG-I (an unrelated chromatin protein) suggest that HMG1 buries an extended segment in the minor groove of DNA and causes its widening. We have also shown that HMG1 associates stably with nucleosomes.
We have recently cloned and mapped the active genes encoding mouse and human HMG1, among a multitude of retrotransposed pseudogenes. The mouse *Hmg1* gene contains five exons, its promoter coincides with a CpG island and is TATA-less. The inactivation of the gene in the mouse is in progress.

J7-409 NUCLEAR LAMINA-CHROMATIN INTERACTIONS: THE C-TERMINAL DOMAIN OF LAMIN A BINDS TO THE N-TERMINAL DOMAIN OF CORE HISTONES AND TO HISTONE H1, Yves Raymond, A. Marie-Josée Sasseville and Isabelle Clément, Institut du cancer de Montréal, Centre de Recherche L.C. Simard, Montréal, Québec, Canada H2L 4M1.

The nuclear lamina is composed of type A and B lamin polypeptides. Type B lamins are constitutively expressed whereas type A lamins are generally expressed only in differentiated cells. Type A lamins are thought to contribute to the three-dimensional organization of chromatin that is characteristic of differentiated cells. There are two type A lamin isoforms in mammalian cells, lamins A and C, that share the first 566 amino acids and differ only by their unique C-terminal domains of 98 and 6 amino acids, respectively. Indirect evidence supports the suggestion that lamin A, but not lamin C, plays a role during the early steps of some differentiation pathways. In vitro experiments have also shown direct binding of type A lamins to chromatin. The chromatin component involved has not yet been identified. Using a soluble recombinant form of the C-terminal domain of lamin A that is absent from lamin C, we show direct interaction of this fragment with calf thymus histones immobilized on nitrocellulose. Histone H1 and core histones H3 and H4 appear to be the major targets of this interaction. Removal of the N-terminal tails of assembled core histones by tryptic digestion abolishes the reaction on H3 and H4. These results support the hypothesis that lamin A may intervene in the process of chromatin folding in which histone H1 and the N-terminal tails of core histones play a major role. Taken together with recent results showing the presence of lamins in the nucleoplasm and not exclusively in the lamina, these findings suggest a more dynamic role for lamin A than hitherto envisaged.

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J7-410 ESTROGEN RECEPTOR BINDING FACTORS ARE NUCLEAR MATRIX ASSOCIATED PROTEINS., Thomas S. Ruh, Linda K. Burroughs and Mary F. Ruh, Department of Pharmacological and Physiological Science, St. Louis University Health Sciences Center, St. Louis, Mo 63104

Chromosomal nonhistone proteins that impart high affinity and specificity to the binding of the estrogen receptor (ER) to genomic DNA or an oligonucleotide containing a consensus ERE are termed estrogen receptor binding factors (ERBFs). Several ERBFs have been purified after large scale isolation from rabbit uterus by DNA affinity chromatography, molecular sieve chromatography, preparative isoelectric focusing and preparative SDS-PAGE. A monoclonal antibody against a 13 kDa ERBF recognized the 12 kDa, 13 kDa and 17 kDa ERBFs which interact with ER bound by estrogen, but not triphenylethylene antiestrogens. However, this anti-ERBF (B511) did not recognize a 9 kDa ERBF that interacts with ER bound by estrogen or antiestrogen. A monoclonal anti-phosphotyrosine antibody reacted with all the above ERBFs and this reaction was inhibited with addition of excess phosphotyrosine. A nuclear matrix preparation enriched for low molecular weight proteins was shown to contain ERBFs by Western immunoblotting with either anti-ERBF (12,13,17 kDa ERBFs) or anti-phosphotyrosine (all four ERBFs). An immunoblot with anti-ERBF of nuclear matrix preparations from rabbit spleen or liver demonstrated that these tissues did not contain these ERBFs, indicating tissue specificity of the nuclear matrix proteins. Although the N-terminus of the 12 and 13 kDa ERBFs was blocked, tryptic digests have yielded several peptides which have been sequenced. These peptides are unique but share some homology with other known nuclear proteins. Supported by NIH HD13425.

J7-411 CHARACTERISATION OF NUCLEAR TRANSPORT SYSTEM AND MOLECULES INVOLVED IN NUCLEAR PROTEIN LOCALISATION IN HUMAN MONONUCLEAR PHAGOCYTE CELL LINES, Ryan, G.J., Mulcahy, K.A., Morton, K. and Partridge, L.J., Department of Molecular Biology & Biotechnology, The University of Sheffield, S10 2UH, England.

Our laboratory is using the cell lines U937 and THP-1 to investigate the process of nuclear protein localisation with monoclonal antibodies as specific probes. The advantage of these cell lines is that they can be induced to differentiate to a more mature phenotype by stimulation with certain agents e.g. dbcAMP and phorbol ester. This allows nuclear localisation to be studied in differentiated/undifferentiated and dividing/non-dividing cells.

Monoclonal antibodies were raised against isolated whole nuclei from U937 cells. The cellular location of the antigens recognised and their levels of expression were determined by immunofluorescence microscopy. Conjugates of BSA and NLSs of known karyophilic proteins were used to characterise our human nuclear transport permeabilisation system which utilises lysolethicin to give selective permeabilisation of the plasma membrane. This system is temperature dependent, ATP dependent and NLS-specific. Four antibodies (recognising antigens of size 235, 206, 90 and 30kDa) which bind to the nuclear membrane inhibit nuclear localisation of karyophilic proteins and our BSA conjugates. The intracellular location of the 90kd antigen alters dramatically when THP-1 (but not U937) cells are induced to differentiate using phorbol ester, becoming mainly intranuclear. Further characterisation of these antigens is in progress.

J7-412 CELL AND TISSUE SPECIFICITY OF NUCLEAR MATRIX PROTEINS, Georg O. Sauermann, Thomas H. Korosec and Christopher A. Gerner, Institute of Tumorbiology-Cancer Research, University of Vienna, A-1090 Vienna, Austria

Although the existence of cell specific nuclear matrix proteins has been described by other authors, data on this topic are scarce. In the present study, nuclear matrix proteins from human and rat cells and tissues were investigated. The nuclear matrices were isolated both from whole cells as described by Penman and coworkers, and from isolated nuclei. The two-dimensional protein patterns obtained by electrophoresis and silver staining were scanned, matched and analyzed, using the Millipore BioImage System.
Data will be presented on technical aspects as on the finding of common and cell specific proteins.

J7-413 **IDENTIFYING MEMBERS OF THE MATRIN 3 GENE FAMILY**, Suryanarayan Somanathan, Michael J. Mortillaro and Ronald Berezney, Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York 14260

We reported earlier that the cDNA encoding matrin 3, a major nuclear matrix protein, was likely to be a member of a complex gene family. Further progress has been made in elucidating the genomic structure of matrin 3 and the matrin 3-like genes. Southern blot analysis of inbred rats shows that there are three separate regions in the rat genome that show high sequence identity throughout the entire rat matrin 3 cDNA. Attempts to isolate partial gene sequences by low stringency PCR of rat genomic DNA has successfully identified a region from two separate genes that belong to the matrin 3 gene family. The first of the fragments, termed matrin 3A, encodes a sequence identical to base pairs 517-956 in the open reading frame of the matrin 3 cDNA. The second fragment, matrin 3B, is a novel 438bp fragment that has 86% nucleotide identity to base pairs 517-956 of the matrin 3 cDNA. Examination of the three reading frames of matrin 3B reveals that one shows a 54% identity to the predicted amino acid sequence of matrin 3A. However, ten stop codons appear in this reading frame of matrin 3B. This may indicate that matrin 3B is a psuedogene or may encode a truncated matrin 3-like protein. Additionally, DNA clones have been isolated from a rat genomic DNA library which are related to the matrin 3 cDNA. Analysis of these clones will further characterize the relationship of matrin 3A and matrin 3B, as well as help define alternative splicing possibilities in matrin 3A previously uncovered via cDNA sequencing. (Supported by NIH grant GM-23922).

Late Abstracts

IMMUNOLOGICAL RELATIONSHIP OF NUCLEAR MATRIX BETWEEN HUMAN EMBRYONIC LIVER AND HEPATIC CARCINOMA, Wen Bo-gui, Qiu Jun, Zhong Shu-ping, Department of Biochem, Shantou University Medical College, Shantou, GD 515031, P.R. China

The nuclear matrix was extracted from nuclei in human embryonic liver and hepatic carcinoma by high salt solution. Electron microscopic studies revealed that nucleic matrix of human embryonic liver at 24 weeks present essential characters of structure such as the pore-complex-lamine, residual nucleoli, and internal ribonucleoprotein particles attached to a dynamic fibrous network of proteins. BALB/C mice were immunized by the nuclear matrix of human embryonic liver or hepatoma. The sera from immunized animals were used as probe to detect the immunological relation between human embryonic liver and hepatic carcinoma by means of immuno-histochemistry. The result showed that antisera of nuclear matrix of human embryonic liver or hepatic carcinoma reacted specifically to their targed tissues, but not to tissues from normal human liver, rat liver, human colon cancer and breast tumor. Moreover, there are cross-reaction between the antisera of human embryonic liver and hepatic carcinoma, suggesting there must be certain relation between both of them in histogenesis and regulation of gene expression.

IDENTIFICATION OF A LOCUS CONTROL REGION FOR THE HUMAN GROWTH HORMONE GENE CLUSTER, Nancy E. Cooke[1,2], Beverly Jones[1,2], Bob R. Monks[2,3], and Stephen A. Liebhaber[1,2,3], 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*.

The Nuclear Matrix: Involvement in Replication, Transcription, Gene Splicing and Cellular Regulation

ACTIVE AND INACTIVE X-CHROMOSOME TERRITORIES CAN BE DISCRIMINATED BY SURFACE AND SHAPE BUT NOT BY VOLUME S. Dietzel[1], R. Eils[2*], E. Schröck[1#], T. Ried[1#], M.R. Speicher[1$], E. Bertin[3], M. Robert-Nicoud[3], C. Cremer[4,2], T. Cremer[1,2]. 1: Institute of Human Genetics and Anthropology, University of Heidelberg, Heidelberg, Germany, 2: Interdisciplinary Centre of Scientific Computing (IWR) and *Graduate College "Modelling and Scientific Computing in Mathematics and Science", University of Heidelberg, 3: Equipe DyoGen and Lab. TIMC (CNRS URA 1618), Université Joseph Fourier, Grenoble, France, 4: Institute of Applied Physics, University of Heidelberg, #: present address: National Center for Human Genome Research, National Institute of Health, Bethesda, Maryland, $: present address: Dept. of Genetics, Yale University, New Haven, Connecticut

According to a widely held view the difference in the overall genetic activity of the active (Xa) and9 inactive X-chromosome (Xi) is closely associated with or even causally related to a difference in chromatin compaction, i.e. Xi should be strongly condensed throughout the whole cell cycle, while Xa should attain a decondensed structure during interphase. To put this view to an experimental test, fluorescence in situ hybridization with composite probes established from flow sorted human chromosomes was performed to visualize chromosome X- and 7-territories, respectively, in female human amniotic fluid cell nuclei. These composite probes delineate entire mitotic human chromosomes X or 7. Multicolor FISH experiments show that subregional targets, e.g. subtelomeric sequences, co-localize with chromosome territories. Pericentromeric repetitive sequences appear to be located at the chromosome territory surface. These results indicate the suitability of present FISH technology for structural studies not only of mitotic chromosomes but also of entire interphase chromosomes. Estimates of volume, surface and a roundness factor were computed after 3D-segmentation of light optical confocal serial sections (see abstract C. Cremer et al., this conference). Xi-territories were independently identified by Barr body staining. While the volumes of the two X-territories were similar in most nuclei, the Xi-territory generally had a rounder shape and a smaller surface. Surface and shape differences were much more pronounced for the two X-territories than for simultaneously painted chromosome 7-territories. These data are conistent with a model predicting that the overall genetic activity of a chromosome territory is related to its surface (see abstract T. Cremer et al., this conference).

ANALYSIS OF CHROMATIN DOMAINS USING VM-26 INHIBITION OF TOPOISOMERASE II, Michael A. Goldman, Chun Tsai and Donald P. Wigington, Department of Biology, San Francisco State University, San Francisco, CA 94132.

The concept of the chromatin domain as a unit of gene regulation suggests that genes or clusters of genes are found in chromosomal loops bounded by matrix attachment regions (MARs or SARs), and that these domains are assembled as units into active or inactive chromatin. The nuclear matrix is rich in topoisomerase II, and MARs/SARs are often associated with consensus sequences for topoisomerase II cleavage. We are attempting to analyze X-linked transgenes and genes subject to genomic imprinting in order to determine whether the domain is in fact the functional unit for X inactivation and imprinting. We have used the dihydrofolate reductase gene in Chinese hamster ovary cells in order to calibrate this technique using a well-characterized domain in which the MAR/SAR positions are known. Results suggest that the MAR/SARs are sites for topoisomerase II cleavage, and that cleavage occurs in about 50% of cells under conditions used in our laboratory. (This work was supported in part by a grants from the National Institute Child Health and Development/NIH and by the Office of Research and Sponsored Programs at SFSU. We are indebted to Dr. Phil Hanawalt's laboratory for use of DHFR probes.)

ANALYSIS OF A PLANT NUCLEAR SCAFFOLD ATTACHMENT REGION: *IN VITRO* BINDING STUDIES AND EFFECTS ON GENE EXPRESSION IN TRANSGENIC PLANT CELLS. Gerald E. Hall Jr.[1], George C. Allen[2], Susan Michalowski[4], Winnell Newman[4], Arthur K. Weissinger[3], William F. Thompson[2,4], Steven Spiker[4]. [1]Mycogen Plant Sciences, 5649 East Buckeye Rd., Madison WI 53716; Departments of [2]Botany, [3]Crop Sciences, and [4]Genetics, North Carolina State University, Raleigh, NC 27695.

We have previously reported the identification of a nuclear scaffold attachment region (SAR) in the 3' flanking region of the tobacco RB7 root-specific gene (Hall et al. 1991. Proc. Nat. Acad. Sci. 88: 9320). This SAR is located approximately 8 kilobases 3' of the coding region, shares many characteristics of previously reported SARs from other organisms and has a dramatic effect on gene expression when flanking a transgene. In this report, we analyze the nuclear scaffold binding characteristics of the RB7 3' SAR, including: the localization of the "core SAR element", competitive binding assays, and binding of double and single-stranded DNA probes. Using synthetic polydeoxyribonucleic acids we show that the binding specificity resides in the physical characteristics (conformation) of the DNA rather than the nucleotide sequence. Although single-stranded DNA has a much higher affinity for the nuclear scaffold it does not appear to account for the binding specificity that SARs display. We also show that a GUS reporter construct flanked both 5' and 3' by the RB7 3' SAR increases the number of GUS positive transformants, as well as, the level of GUS expression in transformed plant cells. These data are compared to a similar experiment using a yeast SAR (ARS1) (Allen et al. 1993. Plant Cell 5:603). The RB7 3' SAR binds more strongly to plant nuclear scaffolds than the yeast ARS1 and shows a substantially greater effect on GUS expression in stably transformed plant cells.

CELLULAR FACTOR YY1 DOWNREGULATES THE HUMAN PAPILLOMAVIRUS-16 E6-E7 PROMOTER, P97, IN VIVO AND IN VITRO FROM A NEGATIVE ELEMENT OVERLAPPING P97 "INITIATOR" SITE AND MULTIPLE 5' AND 3' SITES, L.P. Turek[1], M.J. Lace[1], Y. Yamakawa[1], M. Ushikai[1], J. Anson[1], S. Parkkinen[2], T.H. Haugen[1], and I. Davidson[3] ; [1]Department of Pathology, VAMC and University of Iowa, Iowa City, Iowa, [2]University of Kuopio, Kuopio, Finland, and [3]Laboratoire de Génétique Moléculaire des Eucaryotes du DNRS, Strasbourg, France

Promoter-proximal and distal cis sequences in the HPV-16 upstream regulatory region (URR) positively and negatively regulate the viral E6 and E7 oncogene promoter, P97. By deletional analysis, we have identified two negative ("silencer") *cis* elements flanking a TEF-1 dependent enhancer upstream in the URR. Mutations within these elements abolished their negative function *in vivo* and the binding of a cellular complex *in vitro*. This factor was identified as YY1 by complex mobility in comparison to vaccinia virus-expressed, purified recombinant YY1 protein, by binding to different YY1 sites, and by antigenic reactivity with YY1 antisera. YY1 was also shows to bind to a site overlapping the transcription initiation ("initiator") site of the P97 promoter, and an additional 3' motif. Simultaneous mutation of the silencer and promoter YY1 sites relieved repression of P97 transcription initiation at the P97 promoter, but found to be required for P97 repression in vivo and in vitro. In contrast to other viral and cellular promoters, such as the adeno-associated virus (AAV) P5 promoter, where YY1 is thought to function as a positive transcription "initiator", YY1 downregulates HPV-16 P97 transcription from a critical motif near the transcription start site in cooperation with multiple additional YY1 motifs in the viral genome.

Chromatin Remodeling Occurs Independent of Transcription Factor Binding During 5-azacytidine Reactivation of the Human HPRT Gene. T. P. Yang[1], I. K. Hornstra[1], M. D. Litt[1], R. S. Hansen[2], S. M. Gartler[2]. [1]University of Florida, Gainesville, Florida, and [2]University of Washington, Seattle, WA.

A novel system of differential gene expression in mammals is established during normal female embryogensis by X chromosome inactivation. Studies of 5-aza-2'-deoxycytidine (5aCdr)-induced reactivation of genes on the inactive human X chromosome strongly implicate DNA methylation in maintaining the transcriptional repression of discrete loci on the inactive X. During the process of 5aCdr-induced reactivation of the human hypoxanthine phosphoribosyltransferase (HPRT) gene on the inactive X chromosome, changes in nuclease sensitivity of chromatin in the 5' region of the HPRT gene and HPRT mRNA levels have been analyzed after 5aCdr exposure. Increased nuclease sensitivity is first detectable at 6 hrs. and reaches a maximum at 24 hrs. after initial exposure to 5aCdr, while the appearance of HPRT mRNA levels is first detectable by RT-PCR at 24 hrs. and reaches a maximum at 48 hrs. after 5aCdr exposure. Thus, the change in chromatin structure of the 5' region as a result of 5aCdr treatment appears to occur prior to active transcription of the gene. However, it is unclear if the remodeling of chromatin requires the binding of transcription factors to the 5' region, or if the binding of transcription factors is only required for transcription of the HPRT gene. We now have assayed the binding of transcription factors to the 5' region of the HPRT gene on the inactive X chromosome during 5aCdr reactivation by ligation-mediated PCR (LMPCR) *in vivo* footprinting. We find that the 5aCdr-induced change in chromatin structure does not require transcription factor binding, and that the binding of factors is correlated with active transcription of the gene rather than remodeling of chromatin structure. These data suggest that the differential binding of transcriptional activators (and differential expression of the HPRT gene) to the active and inactive HPRT genes is modulated by the accessibility of their binding sites due to chromatin structure.