NUCLEAR ENVELOPE STRUCTURE AND RNA MATURATION

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Nuclear Structure

0000 NUCLEOCYTOPLASMIC COMMUNICATION. E. A. Smuckler and G. A. Clawson, Department of Pathology, University of California, San Francisco.

Prokaryotes and eukaryotes have remarkable differences in the structure and localization of their genetic information, and in the mechanisms involved in its expression. The DNA of prokaryotes is naked, its transcription produces RNA transcripts whose processing is simpler, and transport across membranes is not required. On the other hand, eukaryotes have segregated the genetic information in a phospholipid bilayer with specialized perforations, the initial transcripts are processed/spliced and an active transport process is required for their egress to the cytoplasm where they become functional. Additionally, a flow of protein and RNA's from the cytoplasm to the nucleus occurs and may participate in regulation. This conference will explore some of these biological phenomena.

0001 THE NUCLEAR ENVELOPE AND RELATED STRUCTURES, Gerd Maul, The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, PA, 19104

The nuclear envelope represents the interface between the two major compartments of the cell, the nucleus and the cytoplasm. Structural analysis has progressed from light-microscopic guessing of its reality (1) to analyzing the molecular structure of its components and their interactions. Besides the double membrane, the term nuclear envelope began to encompass the pore complexes and associated fibrous lamina (2). The major specialization of the nuclear envelope is the pore complex (3), the obvious gate through which macromolecules are exchanged. Central for building models that explain active transport is the ultrastructure of the 80 nm membrane-rimmed pore complex. It has at its waist and parallel to the nuclear membranes radial fibrils and fibrous rings, interpreted as the sieving structure that restricts diffusion. Perpendicular to the pore complex, eight transverse fibrils pass the membranous hole for a variable distance into the nucleus and cytoplasm. This material either carries the product to be transported (rail car hypothesis) or is itself transported with the product attached (cable car hypothesis) (4). Signals for selective transport into the nucleus are presently being identified in several laboratories. The lamins (5) form, if isolated, a strongly crosslinked structure which may not necessarily exist as such in vivo. During mitosis, chromosomes condense, but the nuclear envelope with the lamins stay intact. Another set of three proteins loses its nuclear envelope localization and envelopes the condensing chromosomes before the nuclear envelope disintegrates. The concept evolving is that these proteins are essential for the attachment of chromatin in interphase and the orderly release of chromosomes during mitosis. Such a concept was challenged by the report (6) that the lamins are absent during spermatogenesis. However, both lamin B and lamin A C are present as detected by monoclonal and human autoimmune antibodies. It was found that certain antisera or monoclonal antibodies recognize lamins in somatic cells, but do not necessarily recognize them in the germ cells. The structural differences of nuclear envelopes, germline cells, embryonic or somatic cells will be elucidated eventually by crosslinking studies and sequence analysis of the proteins involved. These studies should yield the information to understand the interaction of chromatin with the nuclear envelope as well as the transport process through the pore complex.

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0002 EXTRACHROMATIN NUCLEAR COMPONENTS AND STRUCTURAL CHANGES IN NUCLEI, George Setterfield, T. Bladon, N. Chaly, R. Hall, K. Brasch, E. Jones-Villeneuve, and D.L. Brown. Departments of Biology, Carleton University and University of Ottawa, Ottawa, Canada, K1S 5B6 and University of Tulsa, Tulsa, OK.

Within 24 h of treatment with mitogens, prior to DNA replication, lymphocytes show striking changes in nuclear morphology. Nuclear volume increases several-fold, large chromatin clumps partially disaggregate and redistribute and considerable amounts of interchromatinic material accumulate. These changes are inhibited by cycloheximide and are accompanied by appearance of newly synthesized stable proteins in the interchromatinic regions of the nucleus (1). Nuclear matrices isolated from stimulated mouse and bovine lymphocytes show morphological changes which parallel those of the in situ interchromatinic regions. Two-dimensional gel analysis of isolated matrix fractions (2) shows quantitative changes in a number of major and minor proteins during stimulation but no major qualitative shift in composition. Nuclear matrices from unstimulated lymphocytes were used as immunogens to produce monoclonal antibodies which stain non-chromatin regions of nuclei of mouse 3T3 cells (3). One antibody stains the nuclear periphery (P_1), two stain the nuclear interior of the nucleus (I_1 and I_2) and two stain both periphery and interior (PI_1 and PI_2) to varying degrees. The antigens to P_1 and I_1 associate with chromosomes during mitosis and could be involved in ordered arrangements of chromatin. P_1 antibody stains only the periphery of the entire chromosome mass while I_1 coats the surface of all individual chromosomes. Both PI antigens disperse to the cytoplasm during mitosis with PI_2 showing association with the spindle poles. All antibodies gave increased staining of lymphocytes as nuclear size increased following mitogenenic stimulation. P_1, I_1 and both PI antibodies stained insect nuclei while the P_1 and I_1 antibodies react positively with plants and green algae. Little change in staining with the antibodies was seen in several differentiating cell systems. Considerable diversity in staining reaction was seen however, with different lymphoma samples. The non-chromatin components of the nucleus show complex number, distribution and function which must be elaborated before gross structure and integrated functions of the nucleus can be understood. This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Medical Research Council of Canada.

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0003 The NUCLEAR LAMINA IN GERM CELLS AND EARLY DEVELOPMENT, Reimer Stick, Max-Planck-Institut für Entwicklungsbiologie, Tübingen, F.R.G.

The nuclear lamina together with the nuclear membranes and the pore-complexes is a constituent of the nuclear envelope of eucariotic cells. It is a protein sceleton, which is in close contact with both the inner nuclear membrane and the peripheral chromatin. In our attempt to elucidate the function of the nuclear lamina we have followed the fate of the lamina structure and its protein components, the lamins, in gametogenesis and early development, because extensive variation in structure and function of nuclei is found in these developmental stages.

A lamina structure is absent in specific stages during the meiotic prophase (pachytene) in oocytes of amphibians and birds (1) as well as in primary and secondary spermatocytes and in sperm nuclei (2).

In XENOPUS cell-type-specific lamins have been described (3): The L_{GV} lamin as the only lamina constituent of the giant oocyte nucleus (germinal vesicle) and lamins L_I and L_{II} of erythrocyte nuclei. The participation of these three lamins in the composition of the lamina structure changes in the course of development. L_{GV} is found in pronuclei and embryonic nuclei up to tailbud stages but decreases in amount from neurula onwards. It is the only lamin constituent in early cleavage nuclei up to mid-blastula. The L_{GV} of the oocyte lamina structure serves as a lamin pool in these stages of development. The lamina is desaggregated in the course of the germinal vesicle breakdown during egg maturation and the lamin protein is reutilized for the formation of embryonic nuclei. L_I appears in nuclei from mid-blastula onwards, it increases in amount relative to L_{GV} and reaches normal levels in gastrula. The third lamin, L_{II}, does not appear before gastrula. From gastrula to tailbud stages all three lamins are found in the same nuclei.

The appearance of L_I is due to de novo synthesis. The time of its appearance coincides with the onset of the mid-blastula transition (MBT), but its synthesis as well as the synthesis of L_{GV} at that time is independent of transcription, which starts at MBT. The correlation in time of the appearance of L_I as a component of the nuclear sceleton with the changes of nuclear structure and function such as the change in DNA loop domain organisation and the onset of transcription will be discussed.

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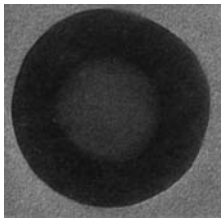
Nuclear Structure and Nuclear Envelope

0004 THE NUCLEAR RETICULE, Gerard Andlauer, Molec Bio Research, Mundelheim, France

The nuclear envelope is thought to be a pavement of polygonal subunits that include polyribosomal rosettes specific of the NPCs - nuclear pore complexes. These are the sites of protein synthesis and plentiful in the active cells. They regulate the exchanges between the nuclear sap and the cytoplasm.

0005 NUCLEAR RINGS:A NOVEL CELL ORGANELLE AT THE NUCLEAR CORTEX,Juan Arechaga and Gunter Bahr,University of Granada (SPAIN) and AFIP,Washington,D.C.20306 (USA)



During the last thirty years speculation about the structure of the nuclear cell periphery has developed a series of proposed architectural elements like nuclear-pores,nuclear-lamina,nuclear-matrix and multiple synonims of each one.A new assessment of the nuclear cortex utilizing high vacuum transmission electron microscopy of whole mount and freeze-fracture preparations has led to two novel concepts: the continuity of the cisterna karyothecae and the existence of nuclear-rings,the sole structure that we observed between the chromatin network and the nuclear membranes using these techniques.Densitometric tracings and countings by unit of area,interpherence contrast and integrated photometry confirmed to us that the optical densities and dry weight determinations in so-called "nuclear-pores" are similar to or higher--not less- than in non "pore "regions of the nuclear membranes. Nuclear-rings are attached to the inner nuclear membrane at "pore"places,apparently anchored at eight points (origin of earlier"octogonality"and"eight points of symetry"findings)and conected with six chromatin fibers to the chromatin network.Microfilament anchorings,probably of actin nature,were detected on the outer nuclear membrane at the ring areas forming pseudorings.Isolated nuclear-rings without chromatin fibers or microfilament anchorings were also observed(see electromicrograph at 300.000 X magnification).The ocurrence of chromatin fibers or microfilament connections may correspond to various functional states of the cell nucleus.

0006 LAMIN A IS SYNTHESIZED AS A PRECURSOR IN BHK CELLS, Viviane Bibor-Hardy, André Dagenais and René Simard, Institut du Cancer de Montréal, 1560 est, rue Sherbrooke, Montréal, Québec, Canada, H2L 4M1.

Lamins are structural proteins found in the fibrous lamina underlining the nuclear envelope. In vitro translation of mRNAs followed by immunoprecipitation with a serum raised against BHK nuclear matrix proteins showed that lamin A (72 Kd) is synthesized as a high molecular weight precursor (74 Kd). These results prompted us to investigate if in the cell lamin A is present in a precursor form and if other nuclear matrix proteins behave similarly. We have thus analyzed the kinetics of nuclear matrix proteins during pulse and chase experiments by in vivo labelling and immunoprecipitation of lamin proteins. Only lamin A is synthesized in vivo as a precursor. This molecule has an identical MW as the precursor previously described and possess a half-life of 60 min. In 2-D gel, the isovariants of the 74 Kd are more alkaline than their counterparts in lamin A. The cellular processing and functions of nuclear matrix lamins are a lot more complexe than once thought. Immunoprecipitations of BHK cell lysate also point out that a 63 Kd protein immunologically related to lamins coexists with the 62 Kd lamin C. The presence of many isovariants for the 63 Kd and the 62 Kd on 2-D gels and their similarity with the partial digest of lamin C argue for a same origin. However, they do not share a precursor-product relationship since both are always present even after long period of chase, the 63 Kd being the most abundant cellular form. The fact that the 63 Kd protein is not present in translated products of BHK mRNAs suggest that it originates from a post-trancriptional modification of the 62 Kd protein.

0007 ENZYMATIC MODIFICATION OF NUCLEAR LAMINA PROTEINS IN NOVIKOFF HEPATOMA, Judith A. Briggs, William F. Glass II, Michael J. Meredith, Robert C. Briggs, and Lubomir S. Hnilica, Depts. of Biochemistry and Pathology, School of Medicine, Vanderbilt University, Nashville, TN 37232

A Ca^{++} dependent upward shift (by approx. 2Kd) in the molecular weight of lamin C and to a lesser extent, lamin A, can be induced in whole cell homogenates or isolated total chromatin of Novikoff hepatoma as well as other rodent ascites-type tumor cells but not in similar preparations from normal rat liver. This phenomenon has been visualized and quantitated using the immuno-blot technique with a polyclonal antiserum which recognizes only lamins A and C. The specific in vitro requirements indicate that this is an enzymatically mediated reaction activated by Ca^{++} at a pH optimum of 8.0-8.5 and is inhibited by sulfhydryl binding agents as well as by Cu-bleomycin. It is also inhibited competitively by putrescine. Our results suggest that the enzyme involved in this reaction is a form of transglutaminase. Since no association of ^{14}C-putrescine with the lamins (after Ca^{++} activation) could be detected, we believe that the lamins may serve as the amine donors in the reaction while the γ-glutamyl acyl group is being contributed by a low molecular weight moiety. Supported by NCI Grant CA 27338.

0008 THE NUCLEAR MATRIX IN TWO HORMONE RESPONSIVE TISSUES, Klaus Brasch, Keith Peters and Ann Jensen, Faculty of Biological Sciences, University of Tulsa, Tulsa, OK 74104

The hormonal control of vitellogenesis in insects and oviparous vertebrates are prime models of gene regulation. In chicken liver the process is under estrogenic control and males can be induced, while in locust fat body it is juvenile hormone (JH) dependent and males cannot be induced. We have examined large-scale nuclear events associated with the response in both species with emphasis on structural changes and the nuclear matrix. In fat body, JH affects all aspects of nuclear development including, enlargement, DNA synthesis and polyploidization, elaboration of inclusion bodies (NB) and proliferation of the nuclear matrix. While all nuclear responses are far more pronounced in females, none can be linked exclusively with the sex limited induction of Vg synthesis. Hepatocyte nuclei enlarged 50% after induction with sharp rises in total protein and RNA content. Major restructuring includes partial disaggregation of chromatin, proliferation of interchromatin components and of prominent NB. Quantitative fluctuations in nucleoplasmic and matrix proteins occured, notably the lamins, Hn-RNP polypeptides, a~175 kD component. Full differentiation was evident 24h post hormone and persisted for several days before gradually regressing. All major inductive effects had passed by 4 wks, but several key nuclear modifications persisted, including enlargement, elevated NHP content and modified matrix proteins. These may constitute part of the irriversible "priming" effect common to steroid target cells, whereby a second, more pronounced reaction can be triggered after regression of the primary response.

0009 TEMPORAL AND SPATIAL RELATIONSHIPS OF NUCLEAR MATRIX ANTIGEN REORGANIZATIONS AND SPINDLE FORMATION' N. Chaly and D.L. Brown, Department of Biology, University of Ottawa, Ottawa, Ont. K1N 6N5

We have examined the relationship between spindle microtubule assembly and nuclear matrix antigen redistribution during mitosis in mouse 3T3 fibroblasts, using antibodies against individual nuclear matrix antigens, as well as anti-tubulin, anti-centromere and the DNA-specific fluorochrome Hoechst 33258. Nuclear matrix antibodies P1 and PI1 were used to monitor mitotic stages and the 3-dimensional organization of nuclei and mitotic figures. P1 stains the periphery of nuclei and mitotic figures. PI1 stains both peripheral and internal components of interphase nuclei; at prophase the internal component disperses and two involutions are revealed at the nuclear periphery. Double immunofluorescence labelling with anti-tubulin and PI1 shows that the spindle poles at prophase are located at the nuclear involutions defined by PI1. With nuclear envelope breakdown the condensing chromosomes become ordered into a characteristic prometaphase configuration which encloses the spindle. At this stage centromeres are ordered at the surface of the spindle and kinetochore microtubules are first evident. The chromosomes are then reordered into a classic metaphase plate. The data suggest that chromosome ordering at the metaphase plate is a two-step process consisting of 1) the translation of interphase chromatin into an ordered prometaphase chromosome configuration with minimal intervention of the mitotic spindle, and 2) the formation of the metaphase plate after the appearance of kinetochore microtubules. (Supported by MRCC and NSERC).

0010 THE NUCLEAR MATRIX POSSESSES BINDING SITES FOR SPECIFIC DNA SEQUENCES. P.N. Cockerill and W.T. Garrard, Department of Biochemistry, The University of Texas Health Science Center at Dallas, Dallas, TX. 75235.

We have developed an *in vitro* DNA-binding assay to investigate specific interactions between the nuclear matrix and segments of cloned genes. Nuclear matrices, prepared by DNase I digestion and 2M salt extraction of isolated nuclei, are mixed with ^{32}P-end labeled restriction fragments in the presence of unlabeled, competitor *Escherichia coli* DNA. After centrifugation, bound and unbound DNA fragments are electrophoretically resolved and visualized by autoradiography. Using this assay, matrices prepared from cultured murine plasmacytoma cells exhibit specific binding of a 254 bp AluI fragment of the mouse κ-immunoglobulin gene. This fragment resides immediately upstream of the κ-enhancer, between the J and C regions. DNA fragments containing this region are also specifically retained by nuclear matrices prepared from cultured murine mastocytoma and fibroblast cells. Similar analysis of the SV40 genome reveals specific binding of Taq I-linearized SV40 DNA to murine nuclear matrices and the DNA sequences responsible for this binding are localized primarily on a 306 bp BstNI-HinfI fragment within a T-antigen exon. Interestingly, limited sequence homology exists between the 254 bp Alu I fragment of the κ-gene and the 306 bp BstNI-HinfI fragment of SV40 DNA. A limited survey of other genes reveals specific binding of the *Drosophila* histone gene repeat unit and the human β-globin gene but not the human α1-globin gene. (Supported by NIH and The Robert A. Welch Foundation).

0011 v-*myc* AND c-*myc*-ENCODED PROTEINS ARE ASSOCIATED WITH THE NUCLEAR MATRIX, R.N. Eisenman, C.Y. Tachibana, H.D. Abrams, and S.R. Hann, Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

We have applied a series of extraction procedures to avian nuclei which have allowed us to define three types of association of v-*myc* and c-*myc* encoded proteins with nuclei: 1) a major fraction (60-90%) which is retained in DNA- and RNA-depleted nuclei following low and high salt extraction, 2) a small fraction (1%) released during nuclease digestion of DNA in intact nuclei in the presence of low salt buffer, and 3) a fraction of myc protein (<10%) extractable with salt or detergents and found to have affinity for both single- and double-stranded DNA. Immunofluoresence analysis using anti-*myc* peptide sera on cells extracted sequentially with nucleases and salts confirmed the idea that *myc* proteins are associated with a complex residual nuclear structure (matrix/lamin fraction) which also contains avian nuclear lamin protein. Dispersal of *myc* proteins into the cytoplasm was found to occur during mitosis. Both c-*myc* and v-*myc* proteins are associated with the matrix/lamin, suggesting that the function of *myc* may relate to nuclear structural organization.

0012 FORMATION OF NUCLEUS-LIKE STRUCTURES AROUND BACTERIOPHAGE DNA MICROINJECTED INTO XENOPUS EGGS, Douglass J. Forbes[#], Marc W. Kirschner[+], and John W. Newport[#], # = Univ. of Calif. at San Diego, La Jolla, CA, 92093, + = U.C.S.F., S.F., CA 94143

The major structural elements of the eucaryotic nucleus appear to form independently of specific DNA sequence information. We find that injection of bacteriophage lambda DNA into unfertilized Xenopus eggs causes the assembly around the DNA of structures resembling typical eucaryotic cell nuclei. These spherical structures begin to form 60-90 minutes after injection. They contain lambda DNA and are bounded by a phase-dense envelope. Immunofluorescent staining of the lambda-DNA-containing structures with anti-lamin antibody reveals the presence of the lamin nuclear proteins at the periphery of the structure, a pattern identical to that of embryonic nuclei. Electron microscopy reveals that the injected DNA is surrounded by a double bilayer nuclear membrane containing nuclear pore complexes. The "nuclei" containing lambda DNA respond to modulators of the Xenopus cell cycle in a manner that mimics the response of embryonic nuclei to these modulators during mitosis. These results suggest that: 1) many of the structural features of the nucleus are not dependent of the presence in the DNA of specific eukaryotic sequences, 2) the response of the nucleus to cell cycle signals is also independant of specific DNA sequences, at least in some cases, and 3) the Xenopus egg contains a stored pool of all the structural components (with the exception of DNA) necessary to form hundreds to thousands of nuclei.

0013 PRESENCE OF LAMINS DURING SPERMATOGENESIS IN MICE, Bernhard French, Kathleen Bechtol and Gerd G. Maul, The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, PA 19104

Lamins A, B and C constitute the bulk of the nuclear envelope in the vertebrate somatic cell nucleus. Recently, another set of three proteins has been described that is localized on the nuclear envelope during interphase, but that envelopes the forming chromosomes during prophase before the nuclear envelope disintegrates and the lamins disperse. It was postulated that these proteins are essential for chromatin attachment in interphase and for the orderly release of the chromosomes during mitosis. This concept was challenged by reports (Stick and Schwarz, Cell Differentiation 11:235, 1982; Cell 33:949, 1983) that the lamins are absent during spermatogenesis and in pachytene during oogenesis. We have detected lamin B during mouse spermatogenesis using human autoimmune antibodies, although not in elongating spermatids. Epitopes of lamins A and C are recognized by a human autoantibody present only in the Sertoli cells. However, a monoclonal antibody prepared against rat liver antigens and detecting the lamin A C epitope shows strong reactivity with most spermatogenic cells in cryosections. The peripheral proteins are seen in all cells during spermatogenesis but not in the mature sperm head. Thus, the lamins and the peripheral proteins, thought to interact with the nuclear envelope and chromatin, are present until the nuclear envelope is replaced with a specialized membrane covering the sperm head. After fertilization, the sperm acquires both lamin A C and B as well as the peripheral proteins in the early pronuclear stage.

0014 THREE-DIMENSIONAL CHROMOSOME PACKAGING AND TRANSCRIPTIONAL ORGANIZATION IN SALIVARY GLANDS NUCLEI OF DROSOPHILA, Yosef Gruenbaum, David Mathog, Mark Hochstrasser, Harald Saumweber, David Agard & John Sedat, Dept. Biochem., UCSF, San Francisco, CA 94143 (YG,DM,MH,DA,JS) Max-Planck-Institut fur Entwicklungsbiol., D7400 Tubingen, FRG (HS)

We have focused on the polytene nucleus of Drosophila melanogaster salivary glands in our initial investigations of the three-dimensional organization of interphase chromosomes. This data base now includes over 20 nuclei from several different animals. The primary observations can be summarized as follows: 1) Each of the five major chromosome arms lies within its own restricted spatial domain, 2) Chromosomes usually extend from one pole of the nucleus to the other through a series of irregular right-handed coils, 3) The chromocenter is always and the telomeres are usually at the nuclear envelope, 4) A number of specific chromosomal loci are consistently located at the nuclear surface, 5) These loci correlate very strongly with a subset of intercalary heterochromatin sites, 6) The arms are non-randomly arranged with respect to one another. We have also been assaying the spatial organization of gene activity both by pulse-labeling glands with tritiated uridine and by immunofluorescent staining of specific RNP structures by use of monoclonal antibodies. Both approaches have revealed close associations of several active loci which appear as bridges of grains or antibody stain between puffs. On the other hand, transcriptional activity is not concentrated as first approximated in either the interior or periphery of the nucleus; the distribution of grains with respect to nuclear radius coincides almost exactly with that of the chromosomes.

0015 STUDIES ON THE MECHANISM OF NUCLEAR PROTEIN LOCALIZATION IN YEAST, Michael N. Hall, Dept. of Biochemistry and Biophysics, UCSF, San Francisco, CA 94143

In order to identify determinants governing nuclear protein localization, we have constructed a set of gene fusions coding for hybrid proteins containing varying amounts of the yeast nuclear protein α2 (a DNA-binding, regulatory protein) at the amino terminus and a constant, active portion of β-galactosidase (LacZ) at the carboxy terminus. (Targeting of E. coli β-galactosidase to the nucleus in yeast. Cell 36, 1057-1065, 1984) The properties of these hybrid proteins are summarized in the following table.

HYBRID	α2 AA's	LOCALIZATION	BINDS DNA	LETHAL TO HOST WHEN OVERPRODUCED
3α2-LacZ	3	cyto.	-	-
13α2-LacZ	13	nuc.	n.d.	+
25α2-LacZ	25	nuc.	-	+
67α2-LacZ	67	nuc.	-	-
210α2-LacZ	210	nuc.	+	-

The above results argue that nuclear protein localization is not by passive diffusion through nuclear pores with subsequent retention by binding to a non-diffusible intranuclear substrate. Proteins may be selectively translocated across the nuclear envelope. I am selecting mutants to identify presumed components of a selective translocation apparatus in the nuclear envelope.

0016 DETERGENT EXTRACTS OF NUCLEI AS A SOURCE FOR NUCLEAR DRUG-METABOLIZING ENZYMES
David E. Moody[1,2], Gary A. Clawson[1], Bruce D. Hammock[2], and Edward A. Smuckler[1]
University of California, [1]School of Medicine, San Francisco, CA 94143, and [2]Davis, CA 95616

The similarity of the nuclear envelope to the endoplasmic reticulum necessitates consideration of nuclear enzymes as sites of activation and detoxification of carcinogens, drugs, and other xenobiotics. A method for the rapid preparation of a nonturbid suspension of nuclear enzymes is now described along with some pertinent applications of this procedure. Purified nuclei were incubated for 15 min. with buffered 0.6% sodium cholate at 4°C and then centrifuged to provide the detergent extract. From livers of un-, phenobarbital(PB)-, and 3-methylcholanthrene(3-MC)-treated rats protein and cytochromes P-450 and b_5 were extracted with equal efficiency over a broad range of starting nuclear protein. The activities of NADPH cytochrome P-450 reductase, NADH cytochrome b_5 reductase, epoxide hydrolase, and NAD(P)H menadione reductases in extracts were proportional to protein added to the assays. DT diaphorase activity was also found in the nuclear extracts. The response of these enzymes to treatments with PB and 3-MC was qualitatively similar to that found in microsomes with 3-MC having a singular inductive effect on cytochrome P-448, while PB increased P-450, b_5, NADPH cytochrome c reductase, and epoxide hydrolase. The quantity of nuclear and microsomal polypeptides of 45-60 kilodaltons was also increased by both treatments. Detergent extracts of nuclei offer a rapid procedure for preparing nuclear enzymes with 5-10 times the recovery and similar specific activity to nuclear membrane preparations.

0017 NUCLEAR ASSEMBLY IN VITRO, Donald D. Newmeyer, John M. Lucocq, Denice Smith and Eddy M. De Robertis, Biocenter, University of Basel, Switzerland

Studies of Forbes, Kirschner and Newport (Cell 34, 13-23, 1983) have demonstrated that nucleus-like structures are formed around bacteriophage DNA injected into Xenopus eggs. Lohka and Masui (Science 220, 719-721, 1983) have shown that demembranated sperm nuclei are remodeled into structures resembling pronuclei in extracts of Rana pipiens eggs. Stimulated by these observations, we have begun to study the assembly in vitro of artificial nuclei made from exogenous DNA added to Xenopus egg extracts. Electron microscopic evidence suggests that these structures are formed through accretion of membrane vesicles around a core of DNA (presumably already assembled into chromatin) and subsequent flattening and fusion of the vesicles into a bilaminar envelope. Pore complex assembly need not accompany this process, since it is possible to form artificial nuclei which contain few pores, if any. Some of the nuclei assembled in vitro are able to accumulate radiolabeled nucleoplasmin added to the extract. With this system we intend to explore some of the mechanisms of nuclear assembly and protein migration into nuclei.

0018 THERE ARE AT LEAST TWO DISTINCT PATTERNS OF LOCALIZATION FOR NUCLEAR ENVELOPE ANTIGENS THROUGH THE MITOTIC CYCLE, Michael R. Paddy, Harald Saumweber, and John W. Sedat, Dept. Biochem., UCSF, San Francisco, CA 94143 (MRP and JWS) and Max-Planck-Institut fur Entwicklungsbiol., APB, D7400 Tubingen 1, FRG

Using indirect immunofluorescence, we have carefully characterized the intracellular localization of two of our nuclear envelope specific monoclonal antibodies through the mitotic cycle of Drosophila early embryos. One monoclonal is directed against antigens with molecular weights of 74 and 76 kD, while the other is directed against an antigen with molecular weight 176 kD. Both monoclonals yield bright patches of staining on the nuclear envelope during interphase, but have distinctly different staining patterns through the rest of the mitotic cycle. In prophase, the staining observed from the 74/76 kD antigen invaginates and breaks into many small particles which remain excluded from the nuclear region throughout mitosis . The staining from the 176 kD antigen, on the other hand, loses its particulate, ring-like staining pattern in prophase and then appears to fill the entire nuclear region throughout mitosis. Both antigens again produce a particulate ring of staining in early interphase. The different staining patterns from the two antigens are particularly clear when the three dimensional staining patterns are computationally reconstructed from optical sections of embryos labeled simultaneously with both monoclonals. We are currently determining the ultrastructural localization of these antigens in the nuclear envelope using EM and 50A gold bead conjugated secondary antibody.

0019 PROSTATEIN GENE ASSOCIATION WITH NUCLEAR MATRIX, Valerie E. Quarmby, David H. Viskochil, Elizabeth M. Wilson and Frank S. French, Laboratories for Reproductive Biology, University of North Carolina, Chapel Hill, North Carolina 27514

Certain transcriptionally active genes have been reported to be closely associated with the nuclear matrix, and it has been proposed that the matrix may be involved in the regulation of gene transcription.

In the rat ventral prostate, androgen stimulation results in the synthesis and secretion of prostatein. Transcription of the mRNA's coding for the C1, C2 and C3 subunits of prostatein is under androgenic regulation.

We have examined the association of the C3 gene with nuclear matrix of ventral prostate cells following androgenic stimulation and withdrawal. Dot blot analysis of nuclear and matrix associated DNA from ventral prostates of castrated animals did not show any preferential association of the C3 gene with the matrix. However, analysis of corresponding samples from intact or intact androgen stimulated animals showed that the C3 gene was enriched two to three fold on the matrix. In the liver, where the C3 gene is not transcribed, it does not appear to be preferentially associated with the nuclear matrix. Experiments are in progress to determine whether there may be multiple attachment sites for these C3 genes on the matrix.

0020 NUCLEAR TARGETING AND TRANSCRIPTIONAL ACTIVITY OF ADENOVIRUS E1a, Joel Richter[1], Blair Ferguson[2], Martin Rosenberg[2], and Nicholas Jones[3], Worcester Foundation, Shrewsbury MA[1], Smith, Kline, and French, Philadelphia, PA[2], and Purdue Univ., W. Lafayette, IN[3]

The adenovirus E1a gene product is a positively acting transcription factor required for the transcription of certain other adenovirus genes (e.g., E3). We recently have undertaken studies to examine the mode of action of E1a (MCB 3:2131, 1983; Science 224:1343, 1984). To obtain sufficient amounts of the protein for biochemical analysis, E1a was overproduced in E. coli. This protein was shown to be active as assessed by its ability to enhance transcription of cloned E3 in microinjected Xenopus laevis oocytes. In this report, determinants of E1a which specify nuclear localization and transcriptional activity are examined. When E1a is injected into the cytoplasm of oocytes, it undergoes a post-translational modification such that its apparent molecular weight increases by about 2000 daltons. Only the high molecular weight form of the protein is found in the nucleus. In an attempt to localize the site of the modification, several DNA deletion mutants were constructed and the resulting truncated peptides were overproduced, isolated and injected into oocytes. These peptides, which lack carboxy terminal, amino terminal, and internal residues, all are modified, enter nuclei, and enhance E3 transcription in injected oocytes. These experiments, together with further deletion studies, suggest that the modification is required for nuclear localization and perhaps also transcriptional activity.

0021 CHANGES IN THE NUCLEAR LAMINA DURING SPERMATOGENESIS IN XENOPUS LAEVIS, Michael S. Risley, Cornell University Medical College, New York, NY 10021

The presence of a nuclear lamina in developing male germ cells has been controversial. Data is presented here to demonstrate that a distinct nuclear lamina is a constituent of spermatocyte nuclei and nuclear matrices that are isolated under conditions which minimize proteolysis. Cells from different stages of spermatogenesis were collagenase dissociated and isolated following unit gravity sedimentation and density gradient centrifugation. Nuclei were isolated at -20°C and demembranated with Triton X-100. Nuclear matrices were isolated by DNAse I digestion and extraction with 2M NaCl or polyanions. Matrices from spermatogonia and spermatocytes, but not sperm had distinct nuclear laminas which, in some spermatocytes, were directly associated with the termini of synaptonemal complexes. Nuclei and nuclear matrices contained two proteins which comigrated electrophoretically with Lamins I and II of erythrocytes, but were present in low abundance relative to erythrocyte lamins. Erythrocyte Lamin I and presumptive spermatocyte Lamin I yielded similar V8 protease peptide maps. Immunofluorescence microscopy of isolated nuclei (unfixed) reacted with LS-1 serum demonstrated strong reactivity with spermatogonia, spermatocytes and spermatids, but not metaphase chromosomes or sperm. The results strongly suggest that a delicate nuclear lamina is present in spermatogonia and spermatocytes but disassembles during maturation of spermatids into sperm.

0022 MODIFICATION OF NUCLEAR MATRIX PROTEINS BY ADP-RIBOSYLATION. ASSOCIATION OF POLY(ADP-RIBOSE)SYNTHETASE WITH THE NUCLEAR MATRIX. G. Sauermann and J. Wesierska-Gadek. Institute of Tumorbiology-Cancer Research, University of Vienna, A-1090 Vienna, Austria.

A number of ADP-ribosylated proteins in the range of 40 to 300 kd exist in nuclear matrices isolated from HeLa cells. The modified proteins were found in two experimental approaches. They were identified after isolation of the ADP-ribosylated proteins of the nuclear matrix in aminophenyl-boronate columns. Their formation was also detected after incubation of permeabilized cells with ^{32}P-NAD.

A portion of the nuclear poly(ADP-ribose)synthetase (about 1-2 per cent) was tightly associated with the isolated nuclear matrix. Nuclear matrix isolated from unlabeled cells catalyzed the formation of (ADP-ribose) protein conjugates in a time-dependent aminobenzamide-inhibited reaction. Other data suggest that the enzyme is directly associated with the nuclear matrix and not indirectly bound by fixation to the residual DNA attached to the matrix.

0023 SURFACE MORPHOLOGY OF LIPID AND DETERGENT FREE RAT LIVER NUCLEI. Melvin Schindler, Department of Biochemistry, Michigan State University, East Lansing, MI 48824
The nuclear surface of isolated rat liver or Xenopus laevis oocyte nuclei when viewed by scanning electron microscopy (SEM) appears as a complex landscape of ribosomes, membrane fragments, bits of chromatin and pore complexes. Small ribosomal-like particles termed annular subunits define the nuclear pore complex periphery. SEM attempts at viewing the submembranous architecture of the nuclear surface comprised of nuclear lamina and the pore complexes have generally relied on the use of non-ionic detergents, specifically Triton X-100, to remove lipid components. This results in a view of nuclear cytoskeletal surfaces with bound detergent. The advent of non-ionic detergents of high critical micelle concentration that can be easily removed from proteins by a short period of dialysis, e.g., β-octylglucoside and octylpolyoxyethylene have provided a means for examining the nuclear surface devoid of lipid and detergent. This offers the possibility of an enhanced view of fine structure that may be masked by detergent binding. Experiments to be reported using a dialyzable, non-ionic detergent, octylpolyoxyethylene (POE) in conjunction with scanning electron microscopy present a view of rat liver nuclear surfaces quite different than previously observed by Triton X-100 treatment. A highly pebbled surface is exposed consisting of an interlocking array of circular particles with diameters of 80-100 nm with a surface density approximately 2-3 times that of similar structures observed in Triton X-100 treated nuclei. Many of these particles have holes in their center. Their appearance suggests pore complexes without the octagonal bonnet of annular subunits. (This work was supported by NIH Grant GM 30158).

0024 CYCLOSPORIN A CHANGES THE NUCLEAR MORPHOLOGY OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES AND CLONED HUMAN OK-T4 LYMPHOCYTES, Jonathan W. Simons, Steven Noga, Donald S. Coffey and Allan Hess, Oncology Center, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.
Cyclosporin A is a natural eleven amino acid cyclic peptide with powerful immunosuppresive properties when administered to humans and experimental animals. It is used widely to prevent allograft rejection in bone marrow and organ transplantation. Cyclosporin A, at immunosuppressive dosages, causes dramatic in vivo alterations in the nuclear volume and morphology of a select group of human peripheral blood lymphocytes. Using cloned human OK-T4 lymphocytes in vitro, similar changes occur in 35-40% of the nuclei. Nuclear volume is dramatically increased 6 to 8 fold. Nuclear morphology is changed from ovoid shape to distinct splayed lobulations. These nuclear changes occur within 90 minutes of cyclosporin A treatment at 37°C and are rapidly reversed by cessation of treatment without loss of viability. After lymphocyte incubation with a dansylated derivative of cyclosporin A, the drug is bound to isolated lymphocyte nuclei and the nuclear matrix. The mechanism of this drug induced alteration in nuclear morphology will be discussed.

0025 NUCLEAR ENVELOPE LOCALIZATION OF AN ADENOVIRUS TUMOR ANTIGEN MAINTAINS THE INTEGRITY OF CELLULAR DNA, Eileen White and Bruce W. Stillman, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.
The adenovirus early region 1B 19,000-molecular weight tumor antigen is required for oncogenic transformation of cells by adenovirus. We have demonstrated that this tumor antigen is located in the nuclear envelope of infected and transformed cells and that within the nuclear envelope, a fraction of the protein is associated with the nuclear lamina. During cell division in transformed cells, the nuclear envelope containing the tumor antigen is dissociated at metaphase and then reforms around the separated daughter chromosomes at telophase. Adenovirus mutants carrying lesions in the gene encoding this tumor antigen are defective for transformation and cause degradation of host cell chromosomal DNA. In these mutants, the intracellular localization of the 19,000 dalton protein is altered. These results demonstrate that components of the nuclear envelope function in the organization of chromatin in infected and transformed cells and that a virus encoded protein plays a critical role in this process.

0026 DEVELOPMENTAL REGULATION OF XENOPUS RIBOSOMAL PROTEIN GENES, Michael Wormington, Ellen Baum, Janet Donahue & Helen Romanczuk, Rosenstiel Center, Brandeis University, Waltham, MA 02254

The massive synthesis of ribosomes during amphibian oogenesis represents a developmental process amenable to study at the molecular level. Our research is focused on the coordinate regulation of the genes encoding the cytoplasmic ribosomal proteins (rps). We have isolated full length cDNAs complementary to 10 rp-mRNAs encoding ribosomal proteins for both 60S and 40S subunits. These clones have been used as probes to measure quantitative and qualitative patterns of rp-mRNA synthesis at different developmental stages, as well as to isolate and determine the structural organization of genomic clones encoding these proteins. In order to elucidate the regulation of rp-gene expression and ribosome assembly during oogenesis, we are utilizing a novel approach in which oocytes are injected with "antisense" RNAs, complementary to rp-mRNAs, synthesized in vitro with sp6 RNA polymerase. These transcripts can form specific and stable hybrids with endogenous oocyte rp-mRNAs, precluding their expression. By injecting antisense transcripts for individual or groups of rp-mRNAs we anticipate determining whether coordinate expression is achieved at post-transcription, translation, or at the level of subunit assembly. We are using the sp6 transcription system to synthesize pure rp-mRNAs in vitro which, upon translation, yield primary translation products to functionally determine, upon microinjection into oocytes, the sequences responsible for targeting ribosomal proteins to the nucleus and subsequently within the nucleolus to the appropriate subunit.

0027 NUCLEAR STRUCTURAL CHANGES PRECEEDING CELL DIFFERENTIATION, Andrew Yen, University of Iowa, Iowa City, IA 52242

Differentiation of HL-60 promyelocytic leukemia cells along the myeloid lineage due to retinoic acid (RA) involves a precommitment memory state. Precommitment results after an RA exposure of duration corresponding to one cell division cycle. Cells in pre-commitment require only an abbreviated subsequent exposure to RA to differentiate. This pre-commitment, memory of RA exposure is labile. The precommitment memory state is associated with a change in nuclear structure detected by reduced narrow angle light scatter intensity measured by flow cytometry. This nuclear structural change is not cell cycle phase specific, does not involve loss of nuclear protein, and apparently reflects a change in nuclear membrane conformation. This alteration persists only for as long as pre-commitment memory exists. It is associated with changes in specific calcium binding, anionic proteins observed in the cytosol.

Nuclear Structure

0028 Structural Considerations for Gene Expression. Bert W. O'Malley, Baylor College of Medicine, One Baylor Plaza, Houston, Texas, 77030, U.S.A.

It is clear that the cellular forces involved in steroid hormones induction of gene expression are complex. Our studies and the studies of others on steroid hormone regulation of egg-white protein synthesis in the chick oviduct suggest that the following major structural determinants are required for induction of gene expression: (1) steroid receptor is the obligatory and active intermediate to transmit the informational signal inherent in the hormone to the regulatable gene; work on the molecular structure and synthesis of steroid receptors will be presented; (2) the primary or linear sequence of the gene itself contains the "promoter" and "hormone control element" sequences which together allow preferential binding of receptor and determine its maximal rate of expression; these regions have been defined by deletion and linker-scan mutations; (3) inducible genes are located within "giant" chromosomal domains (distinguished on the basis of DNase 1-sensitivity) which are a product (or cause) of molecular differentiation and which are responsible for maintaining the "capacity" of genes to respond to inductive influences; these domains have been defined and potential regulatory DNA sequences and proteins have been identified; and (4) the expressible genes within these domains are selectively attached to the nuclear matrix, thereby preventing their packaging into higher order chromatin and enhancing their interaction with the transcriptive apparatus. The matrix appears to provide the structural skeleton for transcription in these cells.

0029 HORMONAL REGULATION OF INDUCIBLE AND CONSTITUTIVELY EXPRESSED GENES,
Jamshed R. Tata, Alan P. Wolffe, Wee Chit Ng and Andrew J. Perlman,
National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

Many processes of transcription, RNA maturation and DNA synthesis involved in hormonal regulation of gene expression are thought likely to be associated with structural elements of the nucleus, including nuclear matrix and envelope. For example, it has recently been reported that the nuclear receptor for steroid hormones, the processing of steroid-induced transcripts and the intranuclear distribution of hormone-activated genes may all somehow be linked to the nuclear matrix. The disruption of several nuclear activities, particularly transcription, is known to be associated with the nuclear uptake of heat shock proteins and their intranuclear distribution. In this presentation, the following aspects of transcription of specific hormone-activated and -deinduced genes and overall DNA synthesis during estrogen induced vitellogenesis in Xenopus hepatocyte culture, studied in the our laboratory, will be reviewed.

1. The differential activation of individual gene members of the vitellogenin multigene family during development and in adult hepatocytes.
2. How estradiol added to primary cultures of hormonally naive male Xenopus hepatocytes causes the upregulation of its own receptor and now the latter is tightly coupled to the transcription of vitellogenin genes de novo.
3. The transient paralysis of hormonal response of these cells by heat and culture shocks which produce a loss of hormone receptors.
4. The rapid de-induction of the constitutively expressed albumin genes and the destabilization of their mRNA during the early phase of activation of vitellogenin genes in cultured hepatocytes by estrogen.
5. The reciprocal relationship between DNA synthesis and inducibility of vitellogenin genes in short-term cultures.

These findings will be discussed in the context of the structural organization of nuclear activities.

Transcription and Processing

0030 RNA PROCESSING IN A PROKARYOTIC CELL, David Apirion, Geza Dallmann, Michael Gurevitz, Andras Miczak, Bela Pragai, Makam N. Subbarao, Jozef Szeberenyi, Tihamer Tomcsanyi, and Ned Watson, Department of Microbiology and Immunology, Washington University Medical School, St. Louis, MO 63110.

A short overview of RNA processing in Escherichia coli will be presented. The known endoribonucleases involved in these processes, RNase III, RNase E and RNase P will be discussed. While RNase III and RNase E seem to be pure proteins, RNase P is an RNP particle where the RNA moiety can, under certain conditions, carry out in vitro the reaction performed by the whole enzyme (1). In the other extreme, at least in some of the reactions, the RNA possess the capacity to cleave itself (2), however, in the cell there is a protein factor(s) that accelerate the reaction (3). While the evidence is overwhelming that RNase P recognizes primarily secondary and teritary structure in its substrates, it seems, on the other hand, that in the reactions carried out by RNase III and RNase E an element of sequence recognition is involved. In order to deal with these issues, minigenes producing substrates for RNase E were prepared. These minigenes contain a truncated rRNA transcription unit, they include the promoters terminators and the complete 5S rRNA gene. When RNase E was inactivated, in an rne mutant, such a plasmid gave rise to 7S RNA (4) which contains 5S rRNA and the termination stem. This 7S RNA is a specific substrate for both enzymes RNase E and RNase III. Their cleavage sites are only eight nucleotides apart (5). These minigenes are very suitable for manipulation via site specific mutagenesis, and they should help to determine what is necessary and sufficient in an RNA molecule to become a substrate for the RNA processing enzymes RNase III and RNase E. While the enzymes RNase III and RNase P have a fair number of natural substrates, thus far, RNase E had a single substrate, 9S rRNA, the precursor to 5S rRNA. Recently we found that RNA I, the RNA that is inhibiting DNA replication of the colE1 plasmid, is a substrate for RNase E. RNase E introduces a cleavage at the 5' end of this molecule. This cleavage inactivate the biological activity of the molecule, and in the absence of RNase E RNA I is stabilized in plasmid-containing strains. Remarkably, there are nine identical nculeotides in the two substrates around the cleavage sites. In order to obtain large quantities of these two RNA processing enzymes, their genes have been cloned. RNase III is overproduced in a plasmid containing strain. The RNase III gene is encoding a protein of about 25,000 daltons. It has been localized to a 1.3 kb of DNA that contains its own promoter.

1. Gurrier-Takeda, C. et al. (1983). Cell, 35, 849-857.
2. Watson, N. et al. (1984). J. Mol. Biol. 172, 301-323.
3. Gurevitz et al. (1982). E. J. Biochem. 124, 553-559.
4. Szeberenyi, J. and Apirion, D. (1983). J. Mol. Biol. 168, 525-561.
5. Szeberenyi, J. et al. (1984). Biochemistry 23, 2952-2957.

0031 MECHANISM OF SELF-SPLICING OF THE RIBOSOMAL RNA PRECURSOR OF *Tetrahymena*, Thomas R. Cech, Brenda L. Bass, Michael D. Been, Tan Inoue, Francis X. Sullivan and Arthur J. Zaug, Department of Chemistry, University of Colorado, Boulder, CO 80309

The ribosomal RNA precursor of the ciliated protozoan, *Tetrahymena thermophila*, undergoes accurate RNA splicing *in vitro* in the absence of any protein. Splicing is initiated by covalent addition of a guanosine nucleotide to the 5'-end of the intervening sequence (IVS) in a transesterification reaction. Ligation of the exons is accomplished by a second transesterification reaction. These cleavage-ligation reactions are mediated by the folded structure of the RNA molecule.

We have identified three specific things that the IVS does to accomplish its own splicing. (1) It provides a binding site for the guanosine nucleotide in proximity to the 5' splice site. Evidence for guanosine binding has come from comparison of kinetic parameters (K_M and V_{max}) with different guanosine analogs.[1] Recently competitive inhibition experiments at 42°C have provided further evidence for this idea; 2',3'-cyclicGMP, dGTP and ddGTP at high molar excess inhibit the reaction of guanosine with the IVS. (2) The IVS provides a binding site for the last three nucleotides of the 5' exon or, in the case of the IVS cyclization reaction, the last three nucleotides of the fragment that is released upon cyclization. Miniexons as short as UpUpU or CpU, when added to the pre-rRNA in the absence of guanosine, act as 5' exons and become ligated to the 3' exon. Recombinant DNA manipulations have been used to alter the sequence of the IVS near the cyclization site. Analysis of the cyclization reaction with these mutants provides additional evidence for the importance of tripyrimidine sequences preceding sites of cleavage-ligation. (3) The IVS makes the phosphates at the reaction sites particularly susceptible to hydrolysis and, presumably, to nucleophilic attack in general. The lability of the phosphodiester bond at a splice site was first seen with the circular IVS RNA,[2] and has more recently been observed at the 3' splice site of the pre-rRNA. We propose that the combination of specific binding interactions and activation of phosphates at the reaction sites largely explains how the IVS RNA structure accelerates the rate of such specific transesterification reactions.

[1]Bass, B. L. and Cech, T. R., *Nature* 308, 820-826 (1984).
[2]Zaug, A. J., Kent, J. R. and Cech, T. R., *Science* 224, 574-578 (1984).

0032 IN VITRO SPLICING OF mRNA PRECURSORS, Goldenberg, Carlos J., DiMaria, Peter R. and Kaltwasser, Gustavo Washington University School of Medicine Department of Pathology, 660 S. Euclid Avenue, St. Louis, Missouri 63110

The pre-mRNA splicing activity present in nuclear extracts prepared from MOPC-315 mouse myeloma cells was characterized and partially purified. Precursor RNA substrates for splicing reaction were synthesized in vitro from a plasmid DNA in which the early region 2 gene of adenovirus 2 was fused to an efficient bacteriophage promoter (Salmonella phage 6). The pre-mRNA splicing activity was partially purified 108-fold by three chromotographic steps. The in vitro splicing reaction catalized by the partially purified fractions was efficient and accurate at the nucleotide level. The reaction occur without any detectable lag and nucleotides (ATP or GTP) were absolutely required. Monoclonal anti-Sm antibodies totally inhibit the splicing activity of the purified fractions indicating that U1 snRNPs copurify with the activity and are absolutely required for the splicing reaction. Consistent with this result, we have directly detected U1 snRNAs and proteins carrying Sm determinants in the purified fractions with splicing activity.

0033 ULTRASTRUCTURAL ANALYSIS OF RNA SYNTHESIS AND PROCESSING, Ann L. Beyer and Yvonne N. Osheim, Dept. of Microbiology, Univ. of Virginia, Charlottesville, VA 22908

Using the "Miller" chromatin spreading technique to visualize active genes, we have shown that ribonucleoprotein assembly on nascent transcripts is site-specific and that the resultant RNP complex apparently mediates an early RNA processing event. Our current goal is to visualize specific active genes in order to correlate RNP structure with known processing pathways on nascent transcripts of defined sequence. To date we have analyzed the tripartite leader region of the Adenovirus major late transcript and two of the chorion genes of Drosophila. In addition, we are developing a system to visualize specific genes on plasmid vectors after microinjection into Drosophila embryos.

The Ad leader region has three short exons (40-90 bp) and two longer introns (1-2.4 kb), while the 1.6 kb s38 chorion gene has a short intron (227 bp) located near the 5' terminus (B. Wakimoto, L. Kalfayan, J. Levine, A. Spradling, pers. comm.). Large RNP particles essentially cover the short exons in the Ad transcript, while in the chorion transcripts, 1-2 large particles cover the short intron region. Taken together, these data suggest that RNP particles may be associated with splice junction sequences, while introns and exons occur in a smoother RNP fibrillar form. Careful analysis of the s38 transcripts reveals that two particles (25-30 nm) form at or near the donor and acceptor splice junctions soon after the sequences are transcribed and, in some cases, apparently coalesce to form one particle (40 nm) on the more mature nascent transcripts. Since all of the particles seen are larger than expected for snRNP complexes, they are either different than snRNPs or are snRNPs with additional (hnRNP?) proteins.

The Drosophila embryo microinjection system will allow visualization of specific genes on multi-copy extrachromosomal plasmids. Since we intend to use the hsp 70 (heat shock) promoter to turn on fused genes, we are currently determining the feasibility of spreading heat-shocked embryos and are investigating the state of RNP structure during heat shock. Surprisingly, we have observed no obvious differences between heat shocked (37°,30') and controls in non-nucleolar transcriptional levels or in RNP assembly on nascent hnRNA transcripts. Due to the high level of transcriptional activity, we have been unable to identify specific heat shock loci. We do observe a difference in nucleolar genes after heat shock. Most, but not all, display a lower polymerase density than control rpRNA genes as well as more extended nascent RNP fibrils. This different RNP structure may correlate with the well-documented breakdown in rpRNA processing after heat shock. It is also interesting that the reported decrease in rpRNA synthesis during heat shock is not averaged out over all the gene copies, but is rather a gene-specific phenomenon. (Supported by NSF PCM-83-09131 and subawards from ACS IN-149A and NIH 5 S07 RR05431-21.)

0034 SnRNAs, NUCLEAR PROTEINS AND GENE REGULATION. H. Busch, R. Reddy, J. Saba, D. Henning, and R. K. Busch. Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77030.

The small nuclear ribonucleoproteins found in "interchromatin dense granules" have been linked to splicing of pre-messenger RNA transcripts, transport of hnRNP particles and to autoimmune diseases. The "excision" hypothesis of Lerner and Steitz is that U1-snRNP "guides" bind to the "GU-AG" consensus sequence by its UCCA and adjacent 5' intron sequences, i.e. the ACUU 5' portion of U1 RNA. The enzymatic cleavage mechanisms are not defined but more convincing data for this possible role of the U1-snRNP have been presented by Sharp and Steitz. Other U-snRNPs may be involved in transport or other processing events.

The sequencing and characterization of the U-snRNAs have been completed first by RNA sequencing and later with recombinant DNA sequencing. After the six U-snRNA species were identified, no new "capped" small RNAs have been identified recently. Similar types of U-snRNAs and other snRNAs have been found in Dictyostelium and dinoflagellates. The Sm antigen of the U-snRNPs is also evolutionarily conserved.

The most remarkable common feature of the small RNAs is the 5' m_3G cap which differs from that of mRNA by its 2, 2' dimethyl groups. The small nuclear RNAs specifically localize to the nucleoplasm (U1,U2,U4,U5,U6 RNA) or the nucleolus (U3 RNA).

Many other snRNAs are of interest, including those recognized by La antibodies (RNA Pol III transcripts) and Ro antibodies. The 7S RNA originally sequenced by us and then by DNA sequencing by Ullu and Melli, is in an snRNP signal recognition particle found by Walter and Blobel.

Many genes for small nuclear RNAs are being cloned and sequenced. Among these are genes for U1, U2, U3, 4.5S, and 7S RNA species. A surprisingly large number of genes have been found to be "pseudogenes", some of which are remarkably similar to the "true genes". The "true" genes range from 1-10 in the haploid genome and lack "TATA" boxes. The controls and rates of their transcription by RNA Pol II are not defined. In the case of 4.5S RNA (Saba, Reddy and Busch), one of the genes is colinear with all but six of the nucleotides in the 4.5S RNA. In U3 pseudogenes, colinarity has been found for several pseudogenes with minor insertions, deletions and base substitutions. The regulation of these genes is an area of considerable interest which will become approachable as more genes are cloned and sequenced.

RNA Modification

0035 POST-TRANSCRIPTIONAL MODIFICATION OF RNA: RNP ASSEMBLY OF PRE-MESSENGER RNA AND SMALL NUCLEAR RNA's, Thoru Pederson, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Two obstacles in the study of pre-mKNP (hnRNP) have been the inability to isolate particles containing a single pre-mRNA and the lack of an assay for hnRNP function. We have investigated the RNP packaging of a pure pre-mRNA in vitro. Polyadenylated human β-globin pre-mRNA was transcribed using Salmonella phage SP6 RNA polymerase and its promoter. The pre-mRNA was capped and added to a HeLa cell nuclear extract under conditions permissive for mRNA splicing (Krainer et al., Cell 36, 993-1005, 1984). This resulted in the formation of a ribonucleoprotein complex. The RNP has a density of 1.35 g/cm^3 in Cs_2SO_4, the same as native hnRNP, and reacts with a monoclonal antibody against hnRNP core proteins. The extent of RNP assembly appears to reach a maximum at approximately 30 minutes of incubation, and decreases thereafter. Intron-lacking β-globin RNA transcripts show a different pattern of RNP assembly.

The small nuclear RNA's and their associated proteins (snRNP's) have been implicated in mRNA processing reactions. We have mapped the localizations of snRNP's by indirect immunofluorescence with polyclonal and monoclonal antibodies in polytene chromosomes of Chironomus tentans. Both U1 and U2 snRNP's are present at all sites of transcription, and are promptly released when gene transcription ceases, indicating that nascent pre-mRNA is the major chromosomal binding site of snRNP's. The co-localization of U1 and U2 snRNP's at loci producing a single mRNA (e.g. BR2) is of particular interest. The retention of snRNP antigens when U1 and U2 RNA's are associated with pre-mRNA confirms our recent biochemical demonstration of this point, using psoralen-mediated U1 snRNP:pre-mRNA cross-linking (Calvet and Pederson, Cell 26, 363-370, 1981; Setyono and Pederson, J. Mol. Biol. 174, 285-295, 1984).

U1 and U2 snRNP complexes can be assembled in vitro and this has been used to define conserved protein binding sites on U1 RNA (Wieben, Madore and Pederson, Proc. Nat. Acad. Sci. 80, 1217-1220, 1983). We have now studied snRNP assembly in vivo. Precursors of U1, U2 and U4 small nuclear RNA's have been identified in HeLa cells by hybrid-selection of pulse-labeled RNA. These precursor molecules contain trimethylguanosine caps and are 1 to approximately 12 nucleotides longer than the mature species. The precursors are found in snRNP complexes soon after transcription, but additional experiments indicate that nascent U1 RNA is not complexed with snRNP proteins. Thus, unlike hnRNP which assembles on nascent pre-mRNA, snRNP assembly appears to be post-transcriptional. Finally, an in vitro system has been developed in which pre-U2 RNA:snRNP complexes are accurately processed to mature U2 snRNP. The reaction involves 3' processing, is ATP-dependent and requires Mg^{++}, and in these respects resembles the processing of histone mRNA's.

RNA Transcription, Processing, Modification and Transport

0036 UI RNP SECONDARY STRUCTURE DETERMINED BY LOCALIZATION OF ANTIGENIC SITES. Paul F. Agris, Andra Boak, Shirley Kovacs, and Theodore Munns, University of Missouri, Columbia, MO. 65211 and Washington University, St. Louis, MO. 63110.

UI RNP has been shown to be involved in mRNA processing by its removal from in vitro processing systems with addition of the autoimmune antibodies RNP or Sm. We had previously shown that the two antigenic determinants for these antibodies are conferred by different peptides and are both part of the UI RNP complex. We had also shown the structural relationship between nucleotides 40-90, of the 165 nucleotide UI RNA, and the protein antigenic determinant for RNP antibody. Others demonstrated the localization of the Sm determinant at the 3' terminal nucleotide sequence. We have now defined by two independent methods the structural positions of these antigenic determinants relative to each other and two other structural positions. A newly recognized autoimmune antibody, Me defined the third locale; and antibody directed against m^7G defines the fourth position that of the 5'-cap of UI RNA within the complex. This approach has demonstrated the closeness of the Me and Sm peptides at the 3'-terminus of the RNA with each other and the 5'-cap, and the distance of the RNP determinant. A two dimensional picture of UI RNA and UI RNP peptides results.

0037 ALTERED mRNA AND SnRNA RESULTING FROM FLUOROURACIL INCORPORATION. R.D. Armstrong and E.C. Cadman, Cancer Research Inst., Univ. of Calif., San Francisco, CA 94143
Studies were initiated to examine the effect of abnormal base substitution, e.g. 5-fluorouracil (FUra), on mRNA and SnRNA. Studies of mRNA were completed using cDNA-mRNA hybridization methods to specifically examine dihydrofolate reductase (DHFR) mRNA. C3-L5178Y cells were exposed to FUra, the nuclear and cytoplasmic RNA isolated, and DHFR-mRNA levels determined following hybridization with 32P-DHFR-cDNA. FUra produced a dose-dependent increase in nuclear DHFR-mRNA levels. Experiments using 3H-Cyt pulsing to allow measurement of only mRNA synthesized during FUra exposure, showed a marked increase in nuclear DHFR-mRNA with a corresponding decrease in cytoplasmic DHFR-mRNA. These results suggest that FUra produces either an inhibition of mRNA processing or an inhibition of nuclear-cytoplasmic transport. We are currently assessing nucleoside-triphosphatase mediated transport of FUra altered mRNA. SnRNA are known to be involved in RNA processing, and if altered by abnormal base incorporation, may be unable to function in that role. Our studies have identified 3 potential alterations of SnRNA resulting from FUra. The migration pattern of the U4 and U6 SnRNA in nondenaturing electrophoresis is altered with FUra incorporation, suggesting 2^{o} structure differences. Also observed was an accumulation of the U1 SnRNA synthesized during exposure to FUra. Studies are underway to attempt to determine if these changes contribute to the inhibition of mRNA or rRNA processing that appears to result in cells treated with cytotoxic concentrations of FUra.

0038 POST-TRANSCRIPTIONAL REGULATION OF CASEIN GENE EXPRESSION, Terry L. Brown, John R. Rodgers, Jeffrey M. Rosen, Baylor College of Medicine, Houston, TX 77030
The goal of this research is to determine the co- and post-transcriptional mechanisms by which the hormones prolactin and hydrocortisone regulate the expression of α-, β- and γ-casein in developing mammary gland. Transcription rates and mRNA stability, along with potential pre-mRNA processing pathways, have been examined. We have quantified the induction and deinduction of casein mRNAs in mammary explant cultures, following prolactin stimulation and withdrawal. Nuclear run-off transcription experiments and pulse-chase data have suggested that transcription rates and cytoplasmic mRNA stabilities are only minimally involved in the prolactin induced expression of these genes. Rather, the stability of nuclear casein transcripts appears to be markedly influenced by prolactin stimulation. To further pursue the molecular mechanism of this action, the role of nuclear processing has been investigated. Distinct patterns of nuclear precursor RNAs, after size separation and detection with different genomic and cDNA probes, have consistently been observed. Comparison of the putative nuclear pre-RNA patterns revealed by the various probes, suggests that there are major differences in the steady-state levels of the pre-RNAs, and in the complexity of the processing pathways. Finally, using computer analysis, evolutionary comparison of α-casein sequences from several species have suggested that there are possible common secondary structural features in these mRNAs, especially in the 3'-noncoding regions. To study this possible functional role of these sequences, gene transfer experiments using several mammary cell lines have been initiated. (Supported by NIH fellowship GM-09392 and grant CA-16303)

0039 IDENTIFICATION AND cDNA CLONING OF PROTEINS INVOLVED IN SN-RNA PROCESSING, Jasemine Choy Chambers, Michael G. Kurilla and Jack D. Keene, Duke University Medical Center, Durham, NC 27710

We are investigating the processing, assembly and transport of small RNAs by blocking these processes with viral infection and by cloning of the snRNA-binding proteins. The earliest event detected following infection with vesicular stomatitis virus is a disruption of cellular snRNA (Pol II and Pol III transcripts) processing and assembly. Subsequent to this effect, cellular RNA and protein synthesis are shut off. We have used human lupus antibodies to identify proteins bound to the precursor and mature forms of these snRNAs. In addition, we have obtained cDNA clones of several of these snRNA-binding proteins using lupus antibodies as probes. One of these proteins, La, associates with a variety of Pol III and viral transcripts and may play a role in the transcription or processing of small RNAs. We have now mapped an antigenic site of the La protein at the terminal 15% of the carboxyl end of the molecule and are presently mapping the RNA-binding domains by subcloning fragments of the La-cDNA into expression vectors.

0040 IN VITRO PROCESSING OF MOUSE PRECURSOR RIBOSOMAL RNA, Nessly Craig and Barbara Sollner-Webb, Johns Hopkins School of Medicine, Baltimore,MD 21205.

Accurate transcriptional initiation of cloned rRNA genes in vitro using a S-100 extract was first accomplished several years ago by Miller and Sollner-Webb (Cell 27:165). This work also identified a previously undetected primary rRNA processing site at position +650 for mouse rRNA and showed that this processing could occur in vitro. This processing occurs very efficiently in vivo, and thus the majority of cellular '45S' rRNA has its 5'terminus at position +650. We are using this coupled transcription/processing S-100 system to study the mechanism of this rRNA processing in terms of its biochemistry and the sequences specifying the cleavage site. Experiments conducted so far have demonstrated that: (1) concurrent transcription is not necessary for processing, (2) the required optimal concentration of available Mg^{++} is lower for processing than it is for transcription, (3) processing requires a ribonucleoside triphosphate, (4) processing occurs steadily over a 45' period without an obvious lag in the coupled system, and (5) the rate and extent of processing appear to be independent of the amount of transcribed rRNA, suggesting a limiting amount of a component(s) needed for processing. Construction and testing of 3' deletions of cloned rRNA genes indicate that sequences extending 150-200 nucleotides 3' of the processing site are required for efficient cleavage; the sequences of this region (but not flanking regions) are highly conserved between mouse and human rDNAs.(Supported by a grant from March of Dimes)

0041 CHARACTERISATION OF RNA 3'-TERMINAL PHOSPHATE CYCLASE FROM HeLa CELLS, W.Filipowicz[+*],K.Strugala[+],M.Konarska[+] and A.J.Shatkin[Δ],*Friedrich Miescher Inst.,4002 Basel,[+]Inst. of Biochem. & Biophys.,Polish Academy of Sciences,Warsaw and [Δ]Roche Inst. of Molecular Biology,Nutley,N.J.07110.

RNA 3'-terminal phosphate cyclase, an enzyme that catalyses conversion of the 3' phosphate to the 2',3'-cyclic phosphodiester at the end of RNA, has been identified previously in extracts of HeLa cells (Filipowicz et al.NAR 11:1405, 1983). Because no processes other than RNA ligation are presently known to require terminal cyclic phosphate, it is likely that cyclase functions to generate and/or maintain cyclic forms at the 3' ends of ligation substrates.

The cyclase, partially purified from HeLa cell extract, requires ATP and Mg^{+2} for activity.The mechanism of 3'phosphate cyclisation was studied with oligonucleotides containing terminal 2'-deoxy or 2'-O-methylribose.Incubation of these substrates with cyclase and ATP results in formation of adenylylated intermediates with terminal dN(3')pp(5')A and N^{m}(3')pp(5')A, respectively. It is proposed that transfer of the adenylyl group from ATP to the 3' phosphate of RNA is an intermediate step in cyclisation. It is normally followed by attack of the adjacent 2'-OH, resulting in elimination of AMP and formation of cyclophosphate. Cyclase preparations can be labeled with [α-^{32}P]ATP suggesting that an earlier step is formation of an AMP·protein intermediate.

0042 REGULATION OF HUMAN HISTONE GENE EXPRESSION IN VITRO AND IN VIVO, Nathaniel Heintz, Olga Capasso and Sarah Hanly. Rockefeller University New York, NY 10021

The major objective of the current studies in my laboratory is to understand the mechanisms governing the transcription and stabilization of histone mRNA during the mammalian cell cycle. Previous studies of transcription of a human histone H4 gene in extracts from synchronized HeLa cells (Heints and Roeder, 1984) established that the limiting activity for transcription of this gene is present at elevated levels in S phase nuclear extracts, is specific for the H4 gene, and can be specifically sequestered by the H4 template DNA in a competition assay. To assess whether this trans-acting H4 specific transcription factor is involved in cell cycle regulation in vivo, we have used a nested series of promoter deletion mutants to determine whether the sequences required for cell cycle regulation in vitro are similar to those necessary for regulated H4 expression in vivo. The results of these studies clearly indicate that upstream promoter elements in the H4 gene are necessary for intereactions of the H4 specific transcription activity in vitro, and that these same sequences are also utilized in vivo.

Heintz, N. and Roeder, R.G. 1984, Proc. Nat. Acad. Sci. 81, 2713-2717.

0043 THE ACUTE-PHASE RESPONSE OF PLASMA PROTEIN SYNTHESIS IN THE RAT. Geoffrey J. Howlett, Tu Guo-Fen, Angela Aldred, Phillip Dickson, Tim Cole, Helen Birch, Mariko Nagashima and Gerhard Schreiber. Biochemistry Department, University of Melbourne, Parkville, Victoria 3052, Australia.

A cDNA library for rat liver polyadenylated RNA has been constructed using the expression vector λgt11.amp3. Clones expressing antigenic determinants for rat serum albumin, transferrin, transthyretin (prealbumin), major acute phase α_1-protein and α_1-acid glycoprotein have been isolated and their identity established either by restriction mapping or by nucleic acid and protein sequencing. The sequence data indicates that rat transferrin possesses a presegment rich in hydrophobic amino acids but lacks a prosegment. Radiolabeled derivatives of the above cDNA clones have been used to quantitate the tissue levels of mRNA and to confirm that the liver is the major organ for the synthesis of these proteins. Analysis of mRNA levels following the induction of inflammation by the subcutaneous injection of turpentine indicates that albumin mRNA decreases over a period of 48 h to approximately 25% of the normal level whilst the mRNA for α_1-acid glycoprotein increases 25-fold in 36 h. For transferrin there is little change in the mRNA level for 24 h followed by a three-fold increase after 48 h. The maximal changes in the mRNA levels during inflammation are similar in magnitude but occur slightly earlier than the changes in the incorporation rate of radiolabeled amino acids previously reported (Schreiber et al. (1982) J. Biol. Chem. 257, 10271-10277).

0044 ORIGIN OF THE BRANCHES IN POLY(ADP-RIBOSE). Miyoko Ikejima and D. Michael Gill. Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Mass. 02111.

Poly(ADP-ribose), the nuclear polymer, is synthesized at DNA breaks and may be involved in DNA repair and DNA recombination. Its turnover in situ is rapid; newly synthesized poly(ADP-ribose) has a heterogeneous size distribution and is branched. Purified poly(ADP-ribose) polymerase seems to ADP-ribosylate several nuclear proteins including itself and both to insert occasional branches. Most workers have assumed that new residues are added at the enzyme-distal ends of growing chains. Although three different catalytic sites on the polymerase have been expected for different reactions, namely ADP-ribosylation of proteins, elongation and branching, those sites have not been identified.

Recently we found that branches can be formed by non-enzymic ADP-ribosylation of poly(ADP-ribose) at the middle of chains. This non-enzymic reaction was slow, but the product was identical to that found in vivo. We suggest that natural branch points may be created non-enzymically. This result fits with an alternative mechanism of poly(ADP-ribose) synthesis, a ping-pong mechanism, adding new ADP-ribose at the proximal ends of growing chains.

RNA is also spontaneously ADP-ribosylated, but not DNA.

0045 TRANSPORT OF ß-GLOBIN mRNA FROM NUCLEI OF MURINE FRIEND ERYTHROLEUKEMIA CELLS. REVERSIBLE REDOX-DEPENDENT INHIBITION OF mRNA TRANSPORT. I. Kindås-Mügge, G. Sauermann, Inst for Tumorbiology, Univ. Vienna, Austria.

An in vitro assay system for analysis of ß-globin mRNA transport is described. Nuclei isolated from murine Friend erythroleukemia cells induced to synthesize globin mRNA, are incubated in micro assays under conditions excluding RNA degradation. The RNA released from the nuclei was hybridized with a plasmid containing a fragment from the murine ß-globin gene. The size of the hybridizing RNA was the same as that of the cytoplasmatic ß-globin m-RNA.

The rate of ß-globin mRNA release from isolated nuclei was determined by the "dot hybridization method" of White and Bancroft. The inducibility of the transport of ß-globin mRNA by ATP and other agents corresponded to that of the release of rapidly labeled RNA in other nuclear systems previously reported by various authors.

Low concentrations of an oxidizing sulfhydryl reagent inhibited the release of ß-globin mRNA from nuclei of erythroleukemia cells as well as that of rapidly labeled RNA from rat liver nuclei in nuclear column experiments. The inhibitions could be reversed by postincubation of the nuclei with a reducing agent. The possible role of dithiol-disulfide interchanges in transport processes is discussed.

0046 TRANSCRIPTS OF ALU FAMILY DO NOT BIND LA ANTIGEN IN VITRO, Ryszard Kole, Lulu Fresco*, Philip L. Cohen, Robert A. Eisenberg and Phyllis Golden Andrews, University of North Carolina, Chapel Hill, NC 27514; *Duke University, Durham, NC 27710

We have screened sera from patients with systemic lupus erythematosus against RNAs transcribed in vitro in HeLa whole cell extracts. We find that sera from 6 out of 70 patients precipitate RNA polymerase III transcripts derived from the Alu family sequence located upstream from the human γ^G-globin gene. These transcripts are not precipitated by anti-La, anti-Sm, anti-RNP or anti-Ro antibodies. Protein is required for antigenicity. This protein seems to be Alu specific since adenovirus VA RNAs are not precipitated by the same sera. In contrast to La antigen which binds at the 3' end of the RNA polymerase III transcipts, Alu-antigen binds within 200 nucleotides from the 5' end.

0047 SPLICING OF mRNA PRECURSORS IN VITRO: STRUCTURE OF INTERMEDIATE AND PRODUCT RNAs, Maria M.Konarska, Paula J.Grabowski, Richard A.Padgett and Phillip A.Sharp, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Precursor RNA of Adenovirus 2 major late transcription unit is efficiently spliced when added exogenously to a HeLa whole cell or nuclear extract. The first characterized step in the reaction is a cleavage of the precursor RNA at the 5' splice site producing a bipartite intermediate consisting of a 5' exon terminating in a 3' hydroxyl group and a lariat structure containing the remaining part of the RNA. At a later stage of the reaction the two exons are joined via a 3'-5' phosphodiester bond utilizing a phosphate group from the 3'splice site. The excised intervening sequence terminates in a 3' hydroxyl group. The branch in the lariat RNAs results from a joining of the 5' terminal guanosine of the intervening sequence to the 2' hydroxyl group of an adenosine residue positioned 24 nucleotides upstream of the 3' splice site. The phosphate group in the 2'-5' phosphodiester bond of the branch structure originates from the 5' splice site. Interestingly, there is a distinct complementarity between the sequences preceding the adenosine at the branch site and the 5' terminus of the intervening sequence. A model of mRNA splicing incorporating these findings will be presented.

0048 TRANSCRIPTION OF U1 SMALL NUCLEAR RNA IN ISOLATED NUCLEI, G. R. Kunkel and T. Pederson, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

We have previously described precursors of U1 small nuclear RNA (Madore et al.,J. Cell Biol. 98, 188, 1984), which are 1-12 nucleotides longer than mature U1 RNA (165 nucleotides). These precursor molecules appear promptly in the cytoplasmic fraction of pulse-labeled HeLa cells and are complexed with small nuclear RNP proteins as revealed by the use of snRNP-specific antibodies. To examine the more transcription-proximal stages of U1 small nuclear RNA biosynthesis, we have now investigated the in vitro transcription of U1 RNA in isolated HeLa cell nuclei. ^{32}P-labeled RNA was hybridized to nitrocellulose-immobilized, single-stranded M13 DNA subclones corresponding to several regions within and flanking a human U1 RNA gene. No hybridization was detected to DNA immediately adjacent to the 5' end of mature U1 RNA (-6 to -105 nucleotides), indicating that transcription starts at or very near the U1 RNA cap site. In contrast, we find that some transcription continues well beyond the 3' end of mature U1 RNA. Significant hybridization was observed to DNA fragments as far as approximately 400-500 nucleotides beyond the 3' end of mature U1 RNA. The in vitro transcription is markedly sensitive to α-amanitin at 1 μg per ml, consistent with previous evidence that U1 RNA is transcribed by RNA polymerase II. Nascent U1 transcripts in these nuclei are not assembled with the major set of small nuclear RNP proteins as the ^{32}P-labeled U1 RNA is not immunoprecipitated with Sm or RNP autoantibodies. This raises the possibility that snRNP assembly is post-transcriptional.

0049 ALTERNATIVE RNA PROCESSING OF RAT CALCITONIN GENE TRANSCRIPTS: A TRANSFECTIONAL ANALYSIS IN LYMPHOID CELLS. Stuart Leff, Susan Amara, Charles Nelson, Ronald Evans and Michael G. Rosenfeld, Univ. of Calif., San Diego and The Salk Institute, La Jolla, CA 92093. The rat calcitonin gene is comprised of six exons. In thyroid C-cells, splicing of the first four exons and usage of an upstream polyadenylation (A_n) site produces mature calcitonin mRNA Alternative processing in several neural and endocrine cells yields predominantly mature calcitonin gene related peptide (CGRP) mRNA. This mRNA contains the first three exons spliced to the fifth and sixth exons, and thus it uses a downstream A_n site. We have introduced pSV_2gpt derived plasmids containing this gene and several deletion mutants into B-lymphomas expressing either high or low ratios of membrane-bound vs secreted IgM (μ_m and μ_s respectively). Since mRNAs encoding μ_m utilize a downstream splice acceptor and A_n site, we tested whether processing of calcitonin/CGRP transcripts might be coordinate with IgM processing in these cell lines. Our results indicate that all lymphocyte lines transfected with calcitonin DNA accumulate predominantly calcitonin mRNA and partially processed products. Deletion of the fourth exon splice acceptor prevents the accumulation of mature calcitonin mRNA while mature and immature species polyadenylated after the CGRP exon accumulate. Cells transfected with a calcitonin A_n site deletion mutant yield only a trace of mature CGRP mRNA but accumulate immature species polyadenylated after the CGRP exon. These data suggest that putative factors regulating RNA processing during B-cell development may not coordinately regulate calcitonin/CGRP RNA processing. Deletion mutation experiments suggest that alternative splice choices as well as A_n site choice may both be relevant in regulating RNA processing for this gene.

0050 SITE-SPECIFIC CLEAVAGE AND POLYADENYLATION OF EUKARYOTIC mRNA IN A CELL-FREE SYSTEM, Claire L. Moore and Philip A. Sharp, Massachusetts Institute of Technology, Cambridge, Mass. 02139

We have developed a cell-free system using HeLa nuclear extract which accurately cleaves exogenously-added RNA at the L3 (hexon) site of adenovirus RNA, and adds a tract of approximately 200 adenine residues. The reaction requires ATP and exhibits a 15 minute lag before appearance of product. The reaction proceeds in the presence of 1 mM EDTA, and under these conditions, an RNA species corresponding in size to sequences downstream of the p(A) site can be detected, suggesting that an endonuclease rather than an exonuclease is responsible for the site-specific cleavage. Under normal conditions, polyadenylation is tightly coupled to cleavage. There is no accumulation of cleaved but not polyadenylated product unless the α-β analog of ATP is added to the reaction. The structure of the ends of these two cleavage products is now under investigation and should yield insight into the molecular mechanisms of this process. The *in vitro* system will be used as an assay in the purification of the enzymatic activities involved and in characterization of the specific RNA sequences which serve as signals for cleavage-polyadenylation *in vitro*.

0051 REVISED MODEL FOR THE NUCLEOSIDE TRIPHOSPHATASE-MEDIATED POLY(A)(+) mRNA EFFLUX FROM NUCLEUS TO CYTOPLASM, Werner E.G. Müller, H.C. Schröder, M. Bachmann and A. Bernd, Universität, 6500 Mainz, West Germany. The activities of nuclear envelope-associated protein phosphokinase and protein phosphatase were determined in nuclear ghosts from liver and oviduct of quails. The protein kinase was found to be inhibited by poly(A) by 75%. The phosphoprotein phosphatase from liver was stimulated by 190% by poly(A), whereas only a slight enhancing effect by this polymer was determined with the oviduct enzyme (to 125%). Comparative determinations of the nuclear ghost-associated enzyme activities revealed the following values (in nmol P_i/min per 10^8 ghosts); oviduct: phosphokinase, 0.015; phosphatase, 0.004 and nucleoside triphosphatase, 39.4. These data indicate that phosphorylation/dephosphorylation proceeds independently of the nucleoside triphosphatase cycle. This assumption is supported by analytical results revealing that no marked dephosphorylation occurs after poly(A) binding to the nuclear envelope. Moreover, stoichiometrical data showed a nearly 1:1 molar ratio between ATP-binding and phosphorylation of nuclear envelope protein. From these finding a new model for the nucleoside triphosphatase-mediated poly(A)(+)mRNA efflux from nuclei is deducted, proposing phosphokinase and phosphatase only to modulate the affinity of the "carrier structure" for poly(A)(+)mRNA, but not to constitute the nucleoside triphosphatase. (supported by DFG)

0052 PURIFICATION OF THE NUCLEAR POLY(A) BINDING ACTIVITY FROM RAT LIVER, Alan B. Sachs and Roger D. Kornberg, Stanford University, Stanford, CA 94305.

The exchange of proteins on mRNA is a key feature of mRNA maturation. In particular, the nuclear poly(A) binding protein(s) are exchanged for the 75,000 dalton cytoplasmic poly(A) binding protein. As a first step in analyzing this exchange, we have begun to characterize the major nuclear poly(A) binding activity from rat liver. A 150-fold purification of this activity, which yielded a doublet of 53,000 and 55,000 daltons on SDS-polyacrylamide gel electrophoresis, was achieved by applying a hnRNP nuclear extract to oligo (dT)-cellulose and affi-gel blue chromatography. The purification was monitored using a nitrocellulose filter binding assay based on the activity's specificity for binding poly(A). The doublet could be excised from an unstained gel, renatured, and shown to contain greater than 90% of the original activity. The activity showed at least a 100-fold higher affinity for poly riboadenylic acid over poly deoxyadenylic acid, poly (2'-O-methyl) adenylic acid, and poly ($1,N^6$-ethenoadenylic acid). These two proteins, which may or may not be encoded by a single gene, are distinct from the cytoplasmic poly(A) binding protein in their chromatographic characteristics, molecular mass, and cellular compartmentalization.

0053 DEVELOPMENT OF AN SV40-BASED SHUTTLE VECTOR WHICH EFFICIENTLY EXPRESSES THE SV40 LATE REGION IN TRANSIENT EXPRESSION ASSAYS, Nancy J. Soo and Luis P. Villarreal, University of Colorado Health Sciences Center, Denver, CO 80262

When compared to SV40 DNA, an SV40-based shuttle vector similar in design to commonly used vector expresses the SV40 late region poorly in transient expression assays. To circumvent this difficulty, we constructed a new vector, pSVPiC, which contains the 885 base-pair plasmid, PiAN7, and duplicate SV40 origin/promoter elements. Transient expression of the SV40 late region of pSVPiC is as efficient as that of SV40 DNA and more efficient than that of commonly used vectors. Nuclease S1 analysis shows that the splice acceptor sites and the site of 3' terminal cleavage are identical in late RNAs made from pSVPiC and SV40 templates; therefore, pSVPiC is useful in the genetic analysis of the processing of the SV40 late RNAs. The gene for rabbit β-globin is also expressed at high levels when placed in the late region of pSVPiC, indicating that this vector is generally useful for expressing foreign genes.

0054 BASE PAIRING BETWEEN NUCLEAR CAD RNA AND U1 SMALL NUCLEAR RNA IN VIVO
Joseph Sperling+, Fanny Szafer*, Perah Spann* and Ruth Sperling*, +The Weizmann Institute of Science, Rehovot, *The Hebrew University of Jerusalem, Jerusalem, ISRAEL

Syrian hamster cells were treated with soluble derivatives of psoralen and irradiated with long-wave UV light in order to induce crosslinks in double-stranded regions of their nucleic acids. Hybridization of Northern blots of nuclear RNA with a cDNA probe homologous to CAD RNA (CAD mRNA encodes the enzymatic activities of Carbamyl-p-synthetase Aspartate trans-carbamylase and Dihydro-orotase on a single polypeptide chain) revealed the formation of crosslinked species of that RNA. Crosslinked CAD RNA products were formed in a dose dependent fashion. Discrete crosslinked bands of CAD RNA are of lower mobilities than mature CAD mRNA and may represent entraped processing intermediates. The presence of U1 small nuclear RNA (U1 snRNA) in crosslinked total RNA has been demonstrated by probing the blots with a cloned U1 DNA probe, and its presence in crosslinked CAD RNA has been demonstrated by hybridization to purified crosslinked CAD RNA. These results indicate that U1 snRNA is base paired to CAD RNA, as well as to heteronuclear RNA, in vivo. They are also compatible with out observation that U1 snRNP is associated with CAD RNP in vivo and support the notion that U1 snRNA has a role in splicing of nuclear RNA.

0055

U1 snRNP IS ASSOCIATED WITH CAD NUCLEAR RNP

Ruth Sperling+, Perah Spann+, Dan Offen* and Joseph Sperling*, +The Hebrew University of Jerusalem, *Weizmann Institute of Science, Rehovot, Israel

Nuclear RNA transcripts are complexed with proteins to form ribonucleoprotein complexes (RNP) on which nuclear RNA processing occurs. We have focused our investigation on mutant Syrian hamster cell line in which the gene for a multifunctional enzyme abbreviated CAD (for Carbamyl-p-synthetase, Aspartate transcarbamylase, Dihydro-orotase) is amplified 200 fold, this cell line overproduces CAD RNA. We have previously shown that CAD nuclear RNP can be released from nuclei as particles that sediment at 200S in a sucrose gradient. In this study we have shown that U1 small nuclear RNP (U1 snRNP) is tightly associated with the 200S CAD nuclear RNP. This was demonstrated by: 1) the presence of U1 RNA in the 200S region. 2) immunoprecipitation of 200S nuclear CAD RNP along with U1 snRNP by anti-U1 RNP antibodies from patients with systemic lupus erythematosus (SLE). These results are in accord with the proposed role of U1 RNA in splicing and suggest that the 200S nuclear RNPs are structurally and functionally close to the native particles on which RNA processing occurs.

0056

FORMATION OF TRYPANOSOME CALMODULIN mRNAs REQUIRES FUSION OF NON-CONTIGUOUS SEQUENCES, Christian Tschudi, Alexander S. Young and Frank F. Richards, Yale University, School of Medicine, New Haven, CT 06510.

The calmodulin genes of Trypanosoma brucei gambiense are arranged in three tandem repeats. We have determined the DNA sequence of the calmodulin locus which covers 2.5 kb of genomic DNA. One repeat unit of 843 bp contains 450 bp of coding and 393 bp of intergenic sequences. By comparing the primary structure of the three repeats, we note only three base changes which are located in the intergenic region. Northern analysis revealed a transcript of 840 nucleotides. To analyse the 5' end(s) of the calmodulin mRNA(s), we determined the nucleotide sequence of primer extended calmodulin mRNA. Our data showed a 35 nt. leader sequence at the 5' end of the calmodulin mRNA. Originally, this leader sequence was identified as an essential component for the expression of variant specific surface glycoproteins. This leader is not encoded by DNA contiguous to the calmodulin locus. In addition, we note two different recognition sites for the fusion of this leader to the mRNA. Our experiments indicate that trypanosome mRNAs are not produced by conventional splicing mechanisms.

0057

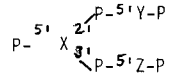
ARCHITECTURE OF PRE-MESSENGER RIBONUCLEOPROTEIN (RNP) MONOPARTICLES, John C. Wooley, Su-yun Chung and Wallace LeStourgeon, NSF, NIH and Vanderbilt, Nashville, TN 37235

Mild ribonuclease digestion or autolysis of mammalian nuclei releases 40S pre-messenger ribonucleoprotein complexes or monoparticles which contain nascent RNA and 6 major (nucleus-restricted) polypeptide species termed A1 (mol. wt. 32K), A2 (34K), B1 (36K), B2 (37K), C1 (42K) and C2 (44K), respectively (Beyer et al, Cell 11, 127ff, 1977). We have used biochemical methods and electron microscopy to begin a structural characterization of monoparticles (from HeLa cells) and also other RNP complexes derived from monoparticles. Gel filtration data and mass analysis by scanning transmission electron micoscopy (in collaboration with Joseph Wall, BNL) indicate that the mass of RNP monoparticles is about 2 million. Thus there is a high copy number for the 6 major structural polypeptides, and of the order of 50 polypeptides per RNP monoparticle from the protein stoichiometry (below). This suggests an internal symmetry to the monparticle, that is, that there is an oligomeric protein structure, repeated several times, which serves to fold the RNA. Densitometric analysis of SDS gels suggest that A1, A2 and C1 are equimolar and are 3-fold more abundant than B1, B2 and C2. In addition, A1,A2 and C1 can be readily crosslinked by two different reagents (one a "zero-length crosslinker) up to homotrimers; (A1-A2)n complexes are also formed. Other studies support the existence of $(A2)_3B1$ tetramers free in solution and as building blocks in RNP assembly; similarly, C1-C2 contacts with each other and with RNA are indicated. RNP architecture appears to be built from complex oligomeric protein interactions that may have important implications for RNA processing and transport.

RNA Modification

0058 Branched RNA in the Nucleus of HeLa Cells. Mary Edmonds, J.C. Wallace and J.D. Reilly. Dept. of Biological Sciences. University of Pittsburgh.

The branched structures found in polyadenylated nuclear RNA molecules of HeLa cells (Wallace, J.C. and Edmonds, M. (1983) PNAS 80, 950) shown below are apparently present in splicing intermediates.



Such intermediates are generated by the esterification of the cleaved 5' phosphorylated end of an intron to the 2'hydroxyl group of an internal nucleotide within the same intron, resulting in the lariat structures recently recovered from splicing extracts (Ruskin, B., et al., (1984) Cell 38, 317; Padgett, R.A., et al., (1934) Science 225, 89. Examination of the location of branches within polyadenylated nuclear RNA of HeLa cells has shown they are not exclusively associated with 3' or 5' regions, but could be either centrally located or randomly distributed in RNA molecules. Analysis of the number of branches in different size classes of polyadenylated nuclear RNA shows that in all size classes about one molecule in eleven is branched. This observed stoichiometric distribution implies that a small molecule is as likely to bear a branch as a large one. These results will be discussed in relation to the lariat structures isolated from in vitro splicing reactions. Branches have also been detected in adenovirus specific polyadenylated RNA recovered from the nucleus of HeLa cells after adenovirus infection. The properties of these viral branches will be compared with the branches found in host cell nuclear RNA.

0059 INTERSTRAND DUPLEXES IN NUCLEAR RNA, A. Oscar Pogo, Kenton S. Miller, Shelley A. Grubman, Valerie Zbrzezna, Julie Rosenbaum, L.F. Kimball Research Institute of the New York Blood Center, New York, New York 10021.

Native RNA-RNA complexes were studied in nuclei isolated from mouse erythroleukemia cells (MEL), human leukemia cells (K562) and Drosophila Kc embryonic cells. These complexes were obtained by a procedure that yields protein-free RNA without the use of phenol, guanidinium isothiocynate or cesium chloride. The procedure consists of obtaining DNA-depleted nuclei under conditions in which endogenous RNAases are fully inhibited and of lysing the nuclear structure with lithium dodecylsulfate. By using this procedure, we have discovered intermolecular duplexes among large nuclear RNAs and between small nuclear RNA (snRNA) and heterogeneous nuclear RNA (hnRNA). We observed that the bulk of hnRNA in MEL and K562 formed large RNA networks having a sedimentation coefficient larger than 45S. Thermal denaturation reduced the sedimentation coefficient to between 18S and 28S. In Drosophila Kc cells, however, the bulk of hnRNA sediments between 18S and 28S. Only a small fraction (∿10%) sediments greater than 45S and is sensitive to thermal denaturation. Proof of the existence of interstrand duplexes prior to deproteinization was obtained by the introduction of interstrand 4'-aminomethyl-4,5',8'-trimethylpsoralen (AMT) cross-links. On the basis of oligo(dT) chromatography, sucrose gradient centrifugation and reannealing experiments, we were able to define two distinct classes of nonpolyadenylated hnRNA. The first or class I poly(A)$^-$ RNA represents approximately 10% of the total hnRNA and it is tightly bound to polyadenylated RNA. It is present in mouse, human, and Drosophila cells. The other, or class II poly(A)$^-$ RNA, represents the bulk of hnRNA, does not interact with polyadenylated RNA, but interacts with snRNA in the three cell types. In MEL cells, the hybrids included all the U-rich snRNA 4.5S, 4.5SI and a novel species designated W. In mouse and human cells, most of the class II poly(A)$^-$ RNA forms multiple molecular complexes but in Drosophila cells, only a small fraction has this feature. Therefore, the small number of RNA-RNA complexes vis-a-vis the size of RNA networks in Drosophila nuclei is correlated with the small number of short-period interspersed repetitive sequences in the Drosophila genome. By using a HIND III fragment of the mouse ß-globin gene which was hybridized to fractions of the sucrose gradient, we observed that mature globin mRNA yielded a dispersed profile across the gradient. However, its precursor only sedimented in the 15S region of the gradient. These studies have uncovered a novel aspect of intermolecular interactions of nuclear RNA, i.e. these RNAs not only form complexes with proteins but with other RNA molecules. The further characterization of these two classes of poly(A)$^-$ RNA is in progress using recombinant techniques.

RNA Transport Systems: Basic Properties

0060 RNA PROCESSING RNA TRANSPORT AND NUCLEAR STRUCTURE, Paul S. Agutter, Dept. Biological Sciences, Napier College, Edinburgh EH10 5DT, U.K.

An overview of nucleocytoplasmic mRNA transport is presented including an attempt to relate existing knowledge of RNA modification and nuclear structure to this process(1,2). It is argued that functional mRNAs and precursors are not diffusible in vivo but exist only in a solid-phase state. Given this view, the transport process minimally comprises three stages: release of RNA from its intranuclear solid-phase support, translocation through the nuclear-envelope pore-complexes and cytoskeletal binding. To understand release we require (i) detailed mechanistic understanding of RNA splicing and its control, (ii) the relationship between nuclear RNP structure and its attachment to the matrix. Translocation is more fully characterised: its energy requirements, the role of the poly (A) tail and the mechanism of action of the cytoplasmic factor described by Webb's group (3) are discussed. Cytoskeletal binding, less well understood, may be less crucial for an understanding of transport. Methods for studying mRNA transport are reviewed briefly with emphasis on in vitro models (1).

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0061 RNA TRANSPORT STUDIES UTILIZING THE ACUTE PHASE RESPONSE. G. A. Clawson and E. A. Smuckler, Department of Pathology, University of California, San Francisco, CA.
The acute phase response of rat liver to subcutaneous administration of turpentine involves dramatically increased production of a number of "acute phase" proteins, with a 100X increase in abundance of message for α_1-acid glycoprotein (αAGP). This seemed an admirable model to study the regulation of RNA transport. In spite of the increase in message for acute phase reactants, message for albumin and other "maintenance" proteins decreases, and we measured decreases in total RNA transport in vitro, and in nuclear envelope and nuclear matrix nucleosidetriphosphatase activity. This caused us to reinvestigate transport systems and to establish conditions appropriate for study of RNA transport. Our assay includes a detergent-rinse for removal of nucleus-absorbed RNA sequences, inclusion of 50 μM polyvinylpyrrolidone (PVP), and substitution of purified RNase inhibitor for cell sap. Inclusion of PVP resulted in 80-90% decreases in transport. Northern blots of electrophoretically-separated transported RNA were hybridized to nick-translated αAGP sequences cut from plasmid pAGP633. Hybridization occurred only to appropriately-sized, poly(A)+ RNA transported from nuclei from turpentine-treated rats. If PVP was omitted, or if cytosol replaced RNase inhibitor, most hybridization occurred to transported poly(A)- RNA of smaller, more heterogeneous size. Mixing experiments qualitatively documented contamination of purified nuclear preparations by nucleus-absorbed sequences, which amount to 20-75% of transported RNA, depending upon nuclear isolation and incubation conditions.

0062 ALTERED PROCESSING AND TRANSPORT OF NUCLEAR RNA DURING CHEMICAL CARCINOGENESIS. Viktor Holoubek, Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550.

After the application of hepatocarcinogens some RNA sequences which in normal adult liver cells are restricted to the liver cell nuclei are released into the cytoplasm. In our laboratory we investigated a 3'-methyl-4-dimethylaminoazobenzene induced release of sequences of small nuclear RNA (fr 3-RNA) which are transcribed from middle repetitive DNA (1). In liver of control animals the same sequences are present only in the nuclei and can be isolated as metabolically highly active 29 nucleotides long RNA associated with nonhistone chromosomal proteins. This RNA originates by splitting from large transcripts of hnRNA. This RNA is not tissue specific and is conserved in evolution since the same RNA can be isolated from the chromatin of chicken reticylocytes. From the screening of rat genomic library with fr 3-RNA isolated from rat liver the minimal number of copies of sequences of fr 3-RNA in rat genome must be at least 7,800. In saturation-hybridization experiments fr 3-RNA hybridized with 1.63% of repetitive DNA. Hybridization of fr 3-RNA with blots of total genomic DNA established that some sequences homologous of this RNA are dispersed through the genomic DNA and others present on one Eco R1 restriction fragment, which is approximately 6,000 base pairs long. Genomic DNA of four randomly selected clones which hybridized with fr 3-RNA contained complex assemblage of sequences homologous to fr 3-RNA, each variable in size, and surrounded by different restriction sites (2). By Southern analysis of restriction fragments of rat albumin and α-fetoprotein genomic clones, DNA sequences complementary to this RNA were located approximately 1800 bp downstream from the termination exon of the albumin gene and 10 kbp downstream from the last exon of the α-fetoprotein gene (3). In this 3'end flanking regions of both genes DNA sequences complementary to the fr 3-RNA are preceded by a 542 bases long conserved sequence and surrounded by various shorter repeats. After the application of the carcinogen the sequences of fr 3-RNA are released from the nucleus as part of larger RNA molecules. The release of transcripts containing sequences complementary to fr 3-RNA is not random, and sequences of fr 3-RNA originating from divers transcripts and combined with different sequences are released, depending on the duration of the application of the carcinogen.

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RNA Transport Systems: Regulation

0063 REGULATION OF RNA TRANSPORT BY CYTOPLASMIC FACTORS, Dorothy E. Schumm and Thomas E. Webb, Department of Physiological Chemistry, The Ohio State University, Columbus, Ohio 43210

Two RNA transport factors, which induce the release of messenger RNA from isolated nuclei, have been identified in the cytoplasm of mammalian cells. A 35Kd phosphoprotein is involved in messenger RNA transport in normal adult cells (1). A 60 Kd oncofetal form of this factor is restricted to fetal and neoplastic cells and tissues (2). The transport of messenger RNA in response to these factors is equally efficient from intact and membrane-denuded nuclei (3). Despite the presence of ribosome-like structures within the nuclear pore complex, the factor-mediated transport of messenger RNA does not appear to involve a translation-like mechanism since release is not affected by aurintricarboxylic acid, puromycin or cycloheximide, which are potent inhibitors of eucarytic or procaryotic translation. Even insulin stimulation of messenger RNA transport which requires intact nuclear membranes, is not affected by inhibitors of protein synthesis. Furthermore, an active reticuloycte lysate could not substitute for the RNA transport factors. During a serial incubation experiment in which nuclei are first incubated in the absence of transport factors, followed by incubation in their presence, all of the predicted RNA was transported during the second incubation, indicating that the factors do not merely protect the transported RNA, but rather induce its transport. Competitive hybridization studies show that the 60 Kd factor from embryonic and tumor tissue elicits the transport of similar repetitive sequences from normal liver nuclei. These sequences are different from those transported by the 35 Kd factor. Neither the 35 Kd nor the 60 Kd factors possess ATPase activity, thus other proteins must utilize the ATP in the transport process. However, like the putative RNA-dependent ATPases, these proteins may associate with the RNA. These data together with data from earlier studies suggest that the 35 Kd factor is a single phosphoprotein with a single specificity, which is for messenger RNA transport.

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0070 ISOLATED NUCLEI AS A MODEL TO STUDY RNA TRANSPORT, R. J. Patterson, J. D. Fetherston, R. M. Denome, E. A. Werner, Department of Microbiology and Public Health, Michigan State University, East Lansing, MI 48824-1101

We have studied the transport of kappa light chain mRNA from isolated myeloma nuclei (1). By hybridizing transported and retained RNA to exon- and intron-specific probes, we have established that more than 70% of kappa light chain mRNA exons are transported, while greater than 80% of kappa intron-specific sequences are retained within the nucleus.

Because the myeloma system lacks an easily detected nuclear restricted RNA, we have used adenovirus-infected HeLa cells as an alternative model for RNA transport studies. Flint and collaborators (2,3) have shown that, late in infection, HeLa cells transport only adenovirus-specific sequences from the nucleus, even though transcription and processing of cellular sequences appear normal. Recombinant probes for human actin mRNA, rRNA, and adenovirus RNA were employed to assess the distribution of these sequences following transport in vivo and from isolated nuclei.

By 20 hrs. after infection, there was a dramatic inhibition of actin-specific mRNA transport in both the in vivo and in vitro systems. Ribosomal RNA transport in both systems was reduced by about 50%. Adenovirus-specific sequences were transported at high levels in both systems. These data indicate that the in vitro transport model mimics the in vivo system faithfully.

Studies are in progress to assess the intranuclear distribution of the various RNAs in infected and uninfected cells. DNA-depleted nuclei were fractionated into matrix-associated and salt-extractable RNA pools and examined with actin, ribosomal, pHe 7 (4), and adenovirus probes. While matrix-associated RNA is a mixture of precursor and mature species, only mature species can be detected in the salt-extractable pool. This RNA is being analyzed by northern and dot blot hybridization techniques.

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Nucleocytoplasmic Regulation: I

0064 TRANSPORT AND ASSEMBLY OF NUCLEAR PROTEINS, R.A. Laskey and C. Dingwall, CRC Molecular Embryology Group, Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, England.

Nucleoplasmin is a pentameric, acidic protein from eggs of Xenopus laevis which assembles nucleosome cores in vitro by binding histones and transferring them to DNA.[1] Nucleoplasmin migrates rapidly into the nucleus after introduction into oocyte cytoplasm. A carboxyterminal domain of nucleoplasmin is responsible for migration.[2] One such domain is sufficient to carry a pentamer of the amino terminal core into the nucleus by allowing entry across the nuclear envelope rather than retention after diffusion into the nucleus. The properties and structure of this domain will be considered and compared with other proteins which enter the nucleus including the carboxyterminal domain of histone H1. This domain also contains information for accumulation in the nucleus.[3]

The role in nucleosome assembly of nucleoplasmin and other acidic proteins which bind histones will be discussed, together with the possibility that nucleoplasmin could be involved in the transport of other proteins into the nucleus.

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Nucleocytoplasmic Regulation: II

0065 NUCLEAR ENVELOPE NTPase AND RNA EFFLUX, Paul S. Agutter, Dept. Biological Sciences, Napier College, Edinburgh EH10 5DT, U.K.

The evidence for the role of this enzyme in mRNA translocation across mammalian liver nuclear envelopes is now very strong (1). However (a) it includes kinetic studies about which there are disagreements in the literature; (b) it is not clear which of the phosphorylated polypeptides of the nuclear envelope are relevant to the enzyme or to translocation. An investigation of these issues is described and the main conclusions are as follows. (a) The general kinetic behaviour depends on the envelope isolation procedure and on the assay conditions, particularly on the Mg/ATP ratio. (b) The Mg/ATP ratio also affects the phosphorylation of certain polypeptides, notably ones of 110 KDa and 68 KDa, also described by Müller's group (2). The 68 KDa seems, on antibody-inhibition evidence, to be an intermediate in the NTPase reaction. The 110 KDa component shows poly (A)-sensitive phosphorylation and may regulate the NTPase. Finally, the effect on the NTPase of the cytoplasmic regulatory factor described by Webb's group (3) is discussed.

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0066 NUCLEAR REGULATION OF A THYROID HORMONE RESPONSIVE GENE AT A POST-TRANSCRIPTIONAL LEVEL, Howard C. Towle, Prema Narayan, Teh-Yi Tao and Julie Engle, Department of Biochemistry, University of Minnesota, Minneapolis, MN 55455.

Administration of triiodothyronine (T_3) to a hypothyroid rat results in a rapid and dramatic increase in the level of hepatic mRNA coding for spot 14 - a polypeptide with a Mr of 17000 and a pI of ~4.9. Thus, relative levels of spot 14 cytoplasmic mRNA begin to increase with a lag time of less than 20 min after hormone treatment and by 4 hr reach a maximal level which is 8-12 fold higher than in the hypothyroid animal (1). We have recently found that the relative level of nuclear precursor to spot 14 mRNA increased with a lag time of less than 10 min. The increase in nuclear precursor preceding that for cytoplasmic mRNA suggested that this gene is regulated at least in part at the nuclear level (1). To further investigate the actual site of T_3 regulation, we have compared the effects of T_3 on relative levels of spot 14 cytoplasmic mRNA, nuclear precursor and gene transcription. Nuclear precursor RNA levels were measured by the RNA "dot blot" procedure using a probe to a unique region of the single intervening sequence of the spot 14 gene. In all situations that we have examined, the relative levels of nuclear precursor change proportionally with alterations in cytoplasmic mRNA. Thus, the regulatory site must precede accumulation of the nuclear precursor. Measurements of spot 14 gene transcription by the "nuclear run-on" procedure yielded unexpected results. The hepatic spot 14 gene was transcribed actively even in the hypothyroid animal. Following injection of T_3, a transient increase of approximately 2-fold was found within 30 min. However, the relative rate of gene transcription then leveled off and began to fall by 1 hr. In chronically-treated animals, the relative transcription rate was no greater than 1.6 times that of hypothyroid animals and, thus, could not account for the 8- to 12-fold changes seen in nuclear and cytoplasmic RNA levels. The authenticity of the spot 14 gene transcripts measured in the transcription assay was indicated by the sensitivity of their appearance to a low concentration of α-amanitin and their ability to hybridize to only the template strand of the spot 14 gene. In addition, simultaneous measurements of α_{2u}-globulin, another T_3 responsive mRNA in rat liver, indicated that changes in transcription rate could account for a large portion of the changes observed in the mRNA levels of this gene product. Thus, these data suggest that the primary site of regulation of spot 14 mRNA is at the level of stability of the nuclear precursor. In other words, the rate of production of primary transcripts is roughly the same under all conditions, but in the presence of T_3, a greater proportion of primary transcripts are processed and transported to the cytoplasm than in the absence of T_3. Control at this level could represent another major pathway by which eucaryotic cells regulate their genetic expression.

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0071 THE MOLECULAR ARCHITECTURE OF THE DROSOPHILA NUCLEUS, Paul A. Fisher, Miguel Berrios, David E. Smith, Adele J. Filson, Neil Osheroff[1], and Kathleen Doyle, Department of Pharmacological Sciences, S.U.N.Y. at Stony Brook, Stony Brook, NY 11794, and [1]Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232.

A subcellular fraction prepared from isolated Drosophila embryo nuclei by nuclease digestion followed by extraction with 2 % Triton X-100 and 1 M NaCl has been shown to contain morphologically identifiable remnants of nuclear pore complexes, peripheral lamina, and internal matrix. It has therefore been termed the nuclear matrix-pore complex-lamina (NMPCL) fraction. SDS-PAGE analyses have shown this fraction to be heterogeneous. Major polypeptides ranging in mass from 15-kD to nearly 200-kD have been identified by one-dimensional procedures. Two of these polypeptides, with apparent molecular weights of 74-k and 76-k, have been shown to be immunochemically related to each other as well as to the three vertebrate nuclear lamina polypeptides, lamins A, B, and C. Indirect immunofluorescence analyses of larval cryosections have shown these two Drosophila polypeptides to be restricted to the nuclear periphery in situ. Developmental analyses performed in conjunction with in vitro translation experiments have shown the 76-kD polypeptide to be a specific precursor of the 74-kD form. A third polypeptide, identified as a glycoprotein with an apparent molecular weight of 188-k, has also been shown to be a specific component of the Drosophila nuclear periphery. In this respect, as well as several others, this Drosophila polypeptide appears to be homologous to a 190-kD glycoprotein (gp 190) found to be a specific component of the rat liver nuclear pore complex (1). A fourth polypeptide, identical in SDS-PAGE mobility to the putative pore complex glycoprotein, has been shown to be the active-site containing subunit of the major ATPase activity associated with the NMPCL fraction. Immunochemical analyses have shown this polypeptide to be a form of myosin heavy chain. In situ localization studies are currently in progress. Finally, a fifth major polypeptide component of the Drosophila NMPCL fraction with an apparent molecular weight of 166-k has been identified as the enzyme, DNA topoisomerase II. Indirect immunofluorescence analyses have shown this polypeptide to be a specific component of the nuclear interior (i. e. internal nuclear matrix). Thus, in summary, we believe we have identified major polypeptide markers for each of the three architectural "subdomains" of the Drosophila NMPCL fraction.

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Gene Expression and Altered Regulation in Disease

0067 TISSUE SPECIFIC DEVELOPMENTAL AND HORMONAL REGULATION OF SUBSETS OF THE ALPHA 2U GLOBULIN GENE FAMILY, P. Feigelson, R. M. Gubits, M. McLaughlin, A. Kulkarni, and P. Hollander-Unger, Institute of Cancer Research, Columbia University, New York, N. Y. 10032

Our laboratory has been exploring tissue specific developmental and endocrine control of gene expression. The alpha 2u globulins are isoproteins encoded by a family of highly homologous rat genes, which have been shown to be expressed in the liver, the submaxillary salivary gland and the lachrymal gland. In each tissue they are subject to complex developmental and endocrine control. Isofocusing gels of heterologous translational products, S1 nuclease analysis and cDNA sequencing demonstrate that different subsets of the alpha 2u globulin gene family are expressed in these tissues. Dot blot and Northern analysis indicate tissue specific developmental and endocrine control of alpha 2u globulin RNA levels. Nuclear runoff studies, using nuclei derived from animals in varying developmental and endocrine states, confirm transcriptional control. In addition to the cDNAs, one of the alpha 2u globulin genes and its flanking regions has been sequenced. The gene contains within intron 3 an alternating GT pair repeated 27 times. It remains to be determined whether this region of potential Z DNA configuration affects the transcription of this highly transcribed gene. This cloned alpha 2u globulin gene has been transfected into L cells, where it is transcribed, generating RNA capable of directing heterologous synthesis of alpha 2u globulin. Sequence analysis of alpha 2u globulin cDNAs indicates the presence of sequences of varying lengths at a specific locus within the 3' noncoding region. Comparison with the gene sequence indicates that these segments are derived from the 3' end of the last intron. Since the translational termination signal is in the preceding exon, this genetic heterogeneity is not reflected in the protein structure, and hence presumably is not subject to selective pressure.

0068 ALTERED REGULATION OF TRANSPORT IN NEOPLASIA. E.A. Smuckler and G.A. Clawson, Department of Pathology, University of California, San Francisco, CA.

The expression of a neoplastic phenotype is currently without explanation. Malignant cells frequently show surface phenomena and constituent enzymes that are not usually expressed in their mature normal counterpart. The identification of nuclear restricted RNA sequences, an active and selective transport mechanism, and the apparent lack of stringency in restriction of nuclear RNA suggest both a heuristic explanation of the altered phenotype and that the transport process is disrupted in neoplasia. ATP-independent transfer of RNA to a surrogate cytoplasm was found in malignant cells and liver cells exposed to carcinogens. Enhanced NTPase activity and increased transport are associated with carcinogen exposure. The changes precede appearance of malignant cells, suggesting that this change may be related to an event associated with development of a permissive gene expression and carcinogenesis.

0069 THE MOLECULAR BASIS OF HUMAN GENETIC DISEASE - DELETION, MISPROCESSING, TERMINATION, Robert Williamson, Department of Biochemistry, St. Mary's Hospital Medical School, University of London, W2 1PG, England.

There are over three thousand different inherited diseases of man, the majority of which are due to defects in a single gene. Using recombinant DNA techniques, over 200 human genes have now been isolated, most of which cause genetic disease when mutated. These genes can be used as disease-specific probes, and can identify the level of dysfunction which occurs in a person.

There are three broad classes of dysfunction: a structural gene deletion; a point mutation which causes an amino acid substitution; and a point mutation which causes a failure of transcription, processing or translation. This last class of defect, which includes misprocessing of nuclear RNA to mRNA, is surprisingly common, and accounts for many of the defined mutations causing disease, including Cypriot beta-thalassaemia.

A gene probe that is disease-specific can be used as a diagnostic tool, either with patients or antenatally. For deletions, a restriction fragment will be absent or reduced in size. Approximately one-third of single base changes alter restriction sites, and these can be identified easily using Southern blots. Any other single base change can be distinguished by differential melting of an oligonucleotide probe. Additionally, each probe can be used to study the inheritance of variants in a family, either directly or by linkage. With the creation of a total human gene map, it will become possible to determine the interaction of several genes with each other, and with the environment, in the causation of complex conditions such as coronary heart disease and hypertension.