400 STRUCTURAL STATES OF CHROMATIN, Donald E. Olins, University of Tennessee Graduate School of Biomedical Sciences and the Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830.

Evidence accumulating in numerous laboratories supports the conception that the functional activities of chromatin might involve conformational changes in the fundamental subunit, the ν (Nu) Body, and alterations in the higher-order packaging of ν Bodies. One of the goals of this laboratory is to define the conformational states of ν Bodies in response to solvent and macromolecular perturbants. Monomer Nu Bodies (ν_1) isolated by zonal ultracentrifugation, and extensively characterized by hydrodynamic and spectroscopic techniques, have been examined in solutions of varying urea concentration (0-10 M urea, 0.2 mM EDTA, pH 7). Although urea does not dissociate histones from DNA, profound cooperative effects are observed on the α-helical-rich histone "core" of the ν Body. Urea effects on the DNA of ν Bodies, and overall size and/or shape are also dramatic, although not markedly cooperative. Coupling of structural alterations of the ν Body "core" to its DNA-rich "shell" could form a basis for regulation of ν Body conformation.

Inactive chromatin exhibits characteristic fibers 250-300 Å in diameter. We have suggested that a close-packing of ν Bodies into helical arrangements could generate such threads. Ultrastructural studies of ruptured nuclei reveal ν Bodies close-packed in the thicker chromatin fibers. It is reasonable to postulate that the nature and stability of this higher-order packaging of ν Bodies is, in part, a consequence of the very lysine-rich histones (H1 or H5) and must be modulated prior to (or simultaneous with) transcription.

401 HISTONE INTERACTIONS, Irvin Isenberg, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331

Histones H3 and H4 have been strongly conserved during evolution, but H2a and H2b have not. Pea H2a consists of two species, one of which is 8% larger and the other 19% larger than calf thymus H2a. Pea H2b is 20% larger than calf thymus H2b. Pea H2a and H2b have amino acid compositions markedly different from their calf thymus counterparts. Despite these differences, the binding pattern of the pea histones is the same as that of the calf histones.

Histone H1 has subfractions which are structurally distinct. These subfractions bind selectively to the non-histone chromosomal proteins HMG-1 and HMG-2.

402 DNA SEQUENCE ORGANIZATION IN EUKARYOTE GENOMES, E. H. Davidson, B. R. Hough-Evans, W. H. Klein, W. R. Crain, S. G. Ernst, G. A. Galau, A. S. Lee, T. L. Thomas, R. C. Angerer, F. Costantini, M. M. Davis, B. J. Wold, M. E. Chamberlin, and R. J. Britten, Division of Biology, California Institute of Technology, Pasadena, CA 91125

Eukaryote DNA sequence organization has several unique features. In most organisms single copy sequences are interspersed with short repetitive sequences only a few hundred nucleotides long. In a few unusually small protostome genomes including that of Drosophila the repetitive sequences are thousands of nucleotides in length. Recent studies have focused on sequence organization in the vicinity of transcribed regions of the genome. In the sea urchin genome short interspersed repetitive sequences are adjacent to structural genes transcribed during embryogenesis. At each stage a special list of structural gene sequences is transcribed. New studies demonstrate that a special subset of repetitive sequences is adjacent to the genes transcribed at one particular stage of development. Analyses of cloned fragments of sea urchin DNA have confirmed that interspersed repetitive sequences transcribed in hnRNA and are set in a "scrambled" array, with nearest neighbor sequences in general non-homologous.

403 HISTONE ANTIBODIES AND CHROMATIN STRUCTURE, Michael Bustin, N.I.H., Bldg. 6, Rm. B1-32, Bethesda, MD 20014

Antibodies elicited by purified histone fractions specifically recognize each of the five main histone classes and can distinguish between various H1 subfractions present in a single tissue. Because these antibodies bind specifically to the nucleus of a cell, to isolated nuclei, purified chromatin, isolated metaphase and polytene chromosomes, and to purified nucleosomes they can serve as probes for histone molecules in their "native" chromatin-bound, state.

Only part of the histone antigenic determinants are exposed in chromatin. Sonication of chromatin increases the number of exposed antigenic determinants. The exposed determinants in chromatin-bound histones are spatially separated so that the binding of one type of antibody does not cause steric hindrance to the binding of a second type of antibody. All the various H1 subfractions present in a tissue are arranged in chromatin in a similar manner so that the determinants which are shared among the H1 subfractions are exposed and available to interact with antibodies.

Indirect immuno fluorescence reveals that each of the histone fractions is located along the entire length of each chromosome present in a tissue. When polytene chromosomes are stained with antihistone sera the resolution of individual bands observed by fluorescence microscopy is of the same order as that obtained by orcein staining or by phase contrast microscopy. The amount of exposed antigenic sites in chromosome bound histones is proportional to the ammount of DNA present in a band. Each band contains each type of histone. Puffing of specific bands result in changes which can be detected using antihistone sera.

The detailed organization and composition of histones in chromatin subunits can be directly visualized by immunolectron microscopy. Upon specific binding of antibodies to chromatin subunits a significant increase in the diameter of the nucleosome is observed. Each nucleosome contains, histone H_2B. Nucleosomes which interact with antihistone antibodies can be visualized in Drosophyla embryos prepared in a manner which allows detection of transcribed regions.

Antihistone antibodies purified by affinity chromatography interact with isolated nucleosomes to yield a particle of an increased mass which is separable from unreacted 11 S nucleosomes by centrifugation on sucrose gradients. Using this technique it was conclusively shown that each nucleosome contains equal ammounts of each of the histones H_2A, H_2B, H_3 and H_4.

404 OLD AND NEW SYNDROMES, John L. Hamerton, Division of Genetics, Department Pediatrics, University of Manitoba, Winnipeg, Manitoba, Canada.

The development of a variety of chromosome banding techniques has resulted in a great improvement in the resolution with which human chromosomes or chromosome segments can be identified. Application of these techniques to clinical cytogenetics has resulted in an improvement in genotype/phenotype correlation for a number of syndromes in which the chromosome abnormality had originally been identified by conventional methods. Thus, it is now clear from studies of patients with Down Syndrome in which the chromosome abnormality involved a rearrangement of chromosome No. 21, that trisomy of band 21q22 only is required for the full expression of the Down Syndrome phenotype. In addition, banding techniques have revealed an array of new cytogenetic syndromes involving the duplication/deficiency of specific chromosome segments which result from the segregation of a balanced chromosome rearrangement in one or other parent. Phenotypes resulting from tertiary trisomy or monosomy in affected offspring are variable, and the chromosome imbalance may involve tertiary trisomy or monosomy for any one of the human chromosomes. Since 1973, new chromosome abnormalities involving duplication of deficiency of segments of each of the human chromosomes have been described and this data will be briefly reviewed.

405 NONRANDOM CHANGES IN MALIGNANT CELLS, Janet D. Rowley, The University of Chicago, Chicago, IL 60637

Recent evidence, obtained with banding techniques, has demonstrated that nonrandom karyotypic patterns are present in many (perhaps all) human neoplasms examined to date. For example, in CML, 85% of patients have the Philadelphia (Ph^1 or 22q-) chromosome which, in 93% of these patients, is the result of a 9;22 translocation. In blast crisis, the most common patterns are the addition of a second Ph^1, and extra No. 8, a rearrangement of No. 17, or an additional No. 19. In AML, 50% of patients have abnormalities, which most often involve an extra No. 8, a missing No. 7, or a translocation between Nos. 8 and 21, with or without the loss of a sex chromosome. In other myeloproliferative disorders, such as polycythemia vera and myelofibrosis, nonrandom changes also occur, particularly plus 8, or minus 7, but also trisomy for part or all of No. 1, plus 9, or deletions of No. 20. Lymphoid disorders show abnormalities of the long arm of No. 14, including a translocation between Nos. 8 and 14 recently identified in Burkitt's lymphoma. In meningiomas, one No. 22 is lacking. Since the genetic content of these chromosomes is largely unknown, the relationship of any particular chromosomal change to altered cell function and malignant transformation is unclear.

406 OCCURRENCE AND SIGNIFICANCE OF CHROMOSOME VARIANTS

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The majority of people have 5-10 variations in size or staining qualities of certain chromosomes with combined conventional staining, Q, C and R banding. This high frequency contrasts with the 0.5-1% of people having definite chromosome abnormalities. These "normal" variants or polymorphisms most frequently involve chromosomes 1, 9, 16, Y and the acrocentric chromosomes and are of interest both in terms of their biological significance and their potential usefulness in clinical cytogenetics. These regions are now known to contain varying amounts of the several classes of redundant DNA. A number of studies of consecutive newborn infants and older randomly selected children, as well as several series of children referred for evaluation of mental retardation and anomalies, have shown statistically significant positive correlations between certain variants (especially large satellites or the secondary constriction region of chromosome 9) and mental retardation or congenital anomalies. In one population study, a significant correlation between a long 9qh region and low I.Q. was found in Black seven year old children, but not in Whites. However, in no case have the majority or even a quarter of children with a particular variant been found to be clinically abnormal. Thus, in interpreting diagnostic studies and antenatal studies these variations should still be interpreted as "normal" variants.

Further research using more sophisticated technics, such as annealing various classes of redundant DNA or RNA to these regions or combinations of several banding technics might reveal subclasses of a particular variant in which the majority of children have an associated clinical abnormality. These clinical correlations might also be explained if the variations act as one genetic factor in abnormalities that are multifactorial in origin. Better family studies would help to distinguish between these possibilities.

Other studies have shown that there are significant racial and ethnic differences in the frequencies of most variants and that there may be an increased frequency of certain variants in abortuses. The latter studies are not well controlled, however. There is also some evidence for non-random segregation of 9qh variants although most variants segregate in a Mendelian fashion and can be used as markers in linkage studies. Use of these variants to distinguish maternal cells from female fetal cells is a particularly important clinical application. Paternity determination is another. Finally, several studies suggest that the frequency of certain variants is increased in chromosomally abnormal children. In summary, these studies, to date, have revealed much that is useful to clinical medicine but the major question of the possible clinical significance remains a subject for further study by better technics.

407 CHROMOSOME BANDING AND CHROMOSOMAL PROTEINS

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Chromomeres observed in pachytene cells represent a basic subdivision of the chromosomes in higher organisms. There is an excellent correlation between pachytene chromomeres and the C- and G-bands of mitotic chromosomes (1). G-band techniques bring out the latent chromomere pattern which is obscured by the high degree of condensation of metaphase chromosomes. The major factors appear to be (a) some rearrangement of the chromatin fibers (2), and (b) the inhibition by non-histone proteins (NHP) of the binding of Giemsa dyes to the DNA of R-band regions (3). Studies of isolated heterochromatin of *Drosophila virilis* and mouse suggest C-band, satellite rich, heterochromatin is predominately a histone-DNA complex with little NHP (4). The AT-richness of Q-bands and the GC-richness of R-bands are the major factors in Q- and R-banding (5,6). EM studies show that chromatin is attached to the nuclear matrix at multiple sites throughout the nucleus, not just at the nuclear membrane (7,8). DNA binding studies indicate the nuclear matrix has preference for the T-strand of AT-rich DNA. This may play a role in organizing the chromosome into chromomeres.

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408 LOCALISATION OF HUMAN SATELLITE DNAs IN MAN AND THE HOMINOID APES

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The location of four human satellite DNAs in human chromosomes has been determined by in situ hybridisation (1). The method used for analysing the results has permitted a quantitative distribution of these DNA sequences to be calculated.

DNA sequences which hybridise with RNAs complementary to human satellite DNAs (satellite cRNAs) are present in the hominoid apes, and the location of these sequences in the chimpanzee (Pan troglodytes) gorilla (Gorilla gorilla) and orangutan (Pongo pygmaeus) has been investigated by the same method.

The distribution of these human DNA sequences in the hominoid apes confirms that their sequences are quite distinct, and that their common hybridisation at many sites in man does not arise from sequence contamination or from cross reaction. The relationship between the distribution of these sequences and chromosome homology determined by banding patterns (2) suggests that such homology does not necessarily include repeated DNA sequences. In several cases homologous chromosome sites do contain the same satellite DNA, but other sites show differences which cannot always be explained by chromosome rearrangement. The distribution of human satellite DNAs in relation to the phylogeny of the Hominoidea allows us to speculate about the mode of evolution of these satellites.

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409 IMMUNOLOGICAL APPROACHES TO CHROMOSOME BANDING

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Metaphase chromosome banding can be produced using immunofluorescence or immunoperoxidase methods with antibodies to some chromosomal components. Antibodies directed against nucleosides or oligonucleotides have been most informative. These antibodies are highly specific and react with single-stranded but not native DNA. Their reaction with chromosomal DNA is determined by both the denaturing method and the antibody specificity. Some combinations of denaturation plus antibody produce fairly standard (Q-,G- or R-) banding patterns; others give more restricted binding, providing specific tags for a small number of chromosome bands. Antibodies to 5-bromodeoxyuridine can be used to demonstrate incorporation of this agent into chromosomes and may be useful in high resolution studies of DNA replication and sister chromatid exchange. While immunofluorescence is useful in all these studies, immunoperoxidase is a more sensitive technique and can also be used with the electron microscope.

Photooxidation of guanine residues in methanol:acetic acid (3:1) fixed, airdried metaphase chromosomes generates single-stranded regions in DNA that is rich in GC base pairs. Since antibody binding after photooxidation produces a reverse (R-) banding pattern, this DNA must be concentated in the bands that do not fluoresce brightly after quinicrine staining. Ultraviolet irradiation (UV) produces pyrimidine and especially thymine dimers in DNA. After UV, anti-adenosine but not anti-cytidine produces generally a Q-banding pattern, confirming the presence of AT rich DNA in these bands. In addition, intense binding occurs on chromosomes 1,9,16 and the Y at sites known to contain satellite DNAs. After UV, anti-cytidine binds intensely to a single band, on chromosome 9. In some individuals it also binds to the centromeric heterochromatin of chromosomes 15 and 22. After either UV or photooxidation, anti-5-methyl-cytidine binds to these sites on chromosomes 1,9,15 and 16 and the distal part of the Y. A specific tag for one of the acrocentric chromosomes has made it possible to demonstrate the frequent origin of abnormal acrocentrics from chromosome 15 and their probable mechanism of origin.

410 THE SYNAPTONEMAL COMPLEX AND MEIOSIS, Montrose J. Moses, Department of Anatomy, Duke University Medical Center, Durham, North Carolina 27710.

The synaptonemal complex (SC) forms the proteinaceous axis of pachytene bivalents and appears to be prerequisite for crossing-over. Unpaired homologues have single axes that become the lateral elements of the SC at synapsis when joined to a central element by transverse filaments. Attachment plaques anchor SC ends to the nuclear envelope. Studies of SC's in serial sections (1) and whole mount spreads (2) indicate that the chromosome axis reflects chromosome behavior. A simple spreading method (3) yields whole complements of selectively stained SC's in which kinetochores are represented by differentiations of the lateral elements. Bivalents are identifiable by length and kinetochore position, and thus pachytene karyotypes have been obtained (2,4). Meiotic prophase has been studied quantitatively in mammalian spermatocytes (4). Autosomal SC's change in length during pachytene, but their relative lengths and arm ratios remain constant, excluding major biological variation and preparative distortions, and implying that autosomal length is under uniform control. A 1:1 relationship between relative lengths of SC's and mitotic autosomes indicates that the control operates in meiotic and somatic cells alike. Axial elements develop from terminal attachments on the nuclear envelope at or just prior to synapsis. Synapsis is asynchronous; it usually begins at the nuclear envelope and proceeds toward the kinetochore; the longest chromosomes tend to be the last to complete pairing. The axial element disassembles in different species before, during, or following desynapsis. Although axial elements evidently do not participate directly in chiasma formation, transient recombination nodules (5) are observed in occasional sets of SC's. X and Y chromosomes operate on an independent schedule. They may be paired over an SC region that is longest at early pachytene; precocious desynapsis often occurs during pachytene, though the SC of the paired region may persist until diplotene in some species. Unpaired X and Y axes undergo elaborations to become the framework of the "sex vesicle". Studies of acrocentric-metacentric trivalent formation in lemur hybrids, and translocations in the mouse (6) show that SC analysis in spreads is uniquely applicable to cytogenetic analysis. (Research supported by grants from the NSF (GB-40562), and NIH (GM-23047; also 5-S01-RR-05404 and CA-14236 to Duke University).

411 FACTORS AFFECTING STRUCTURE OF CHROMATIN, Roger Chalkley and Ross Hardison, Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242

Chromatin structure can be manipulated in a variety of ways in order to probe the nature of the underlying structure itself. The compact conformation of interphase chromatin can be extended by removing divalent cations and lowering the ionic strength. When this is done under gentle conditions the regular subunit structure of chromatin is disrupted, we will argue that the disruption of chromatin structure by shearing is simply due to extension of chromatin rather than to the physical violence in itself. Thus provided the ionic strength is maintained in the range 0.01-0.1 and in the presence of 0.001 M $MgCl_2$ native chromatin structure is resistant to most violent shear forces. We will discuss the effect of urea upon chromatin structure, the effect of fixing histone to DNA and the organization of H1 within the nucleosome organization. Finally, all these lines of argument will be contained in a generalized model for chromatin structure.

412 ORGANIZATION AND FUNCTION OF THE RIBOSOMAL GENES IN PHYSARUM POLYCEPHALUM. Vincent G.Allfrey*, Edward M.Johnson*, Irene Y.-C. Sun*, Virginia C.Littau* Harry R.Matthews** and E.Morton Bradbury**, *Rockefeller University, New York NY 10021 and **Portsmouth Polytechnic Institute, Portsmouth PO1 2DZ, Hants, U.K.

The reiterated ribosomal cistrons of Physarum polycephalum can be isolated as protein-associated components of highly-purified isolated nucleoli (1) and in the free state as a dense DNA satellite separable from the main-band DNA by isopycnic density gradient centrifugation. The structure of the free ribosomal DNA, as revealed by restriction endonuclease digestion, is palindrome-like — in which two ribosomal genes of opposite polarity are separated by a long non-transcribed spacer sequence (2,3). The transcription units at either end of this spacer have now been visualized directly by electron microscopy.

The organization of the ribosomal genes in nucleoli involves their interaction with sets of structural and regulatory proteins. Nuclease and protease digestions have been employed to compare the subunit organization of ribosomal genes and main-band DNA in chromatin (4) and to evaluate the role of histones in nucleolar chromatin as compared to bulk chromatin structure.

Nucleolar non-histone proteins have been extracted from purified nucleoli and fractionated by chromatography on Physarum DNA covalently attached to Sephadex G-25. DNA-binding fractions have been compared with regard to their affinities for rDNA and main-band DNA sequences. Evidence for selective protein interaction with rDNA will be presented. Tests for effects of rDNA-associated proteins on transcription of the ribosomal genes require a cell-free RNA-synthesizing system. Conditions have been developed for labelling ribosomal RNA in isolated nuclei and identifying the newly-synthesized transcripts by hybridization to intact rDNA and to its restriction fragments.

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413 THE SIGNIFICANCE OF STRUCTURAL VARIATIONS OF LYSINE-RICH HISTONES, R. David Cole, Myrtle M. Hsiang, George M. Lawson, Rodney O'Neal and Shirley L. Welch, Department of Biochemistry, University of California, Berkeley, CA 94720

The complete amino acid sequence of one of the H1 histones of rabbit thymus and the partial sequences of two others from rabbit thymus, one from calf thymus, and one from chicken erythrocyte can be compared. All these histones possess an amino terminal region of about 40 residues that is high in lysine and proline and extremely low in hydrophobic residues. This region is followed by a region of about 70 residues that is high in hydrophobic character and devoid of proline. Finally there is a region of about 115 residues high in lysine and proline. The amino terminal 40 residues display substantial variation even among various H1 histones of a single tissue, but the hydrophobic region is highly conserved, and the carboxyl terminal hundred residues varies only modestly.

There seem to be only two clusters of cationic residues in H1 histone larger than pairs. These clusters lie in the region from about residue 18 to residue 40, just where most of the variation occurs between the different H1 histones of a single tissue. Moreover the distance between these clusters appears to vary among the various H1 histones so that the histones can be arranged in a series where the cationic clusters are separated by 4, 5, 6---, 10 residues. We speculate that the spacing between clusters determines the degree of conformational constraint in chromatin from relatively stringent constraint to relaxed. Since different tissues seem to require different relative amounts of the different kinds of H1 histone, it can be postulated that the quantitative recipe of H1 histone is a reflection of a particular cell's requirement for certain amounts of stringent constraints, intermediate constraints and relaxed ones.

The various types of H1 histone can be shown to differ in their interactions with DNA by observing the circular dichroic spectra of H1-DNA complexes, by measuring binding affinities in filter assays, and by eluting the H1 histones differentially from DNA-cellulose columns. Different H1 species can also be selectively extracted from chromatin.

414 CHROMOSOME MAPPING: IN SITU HYBRIDIZATION, MARY LOU PARDUE, DEPARTMENT OF BIOLOGY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE, MASSACHUSETTS 02139

Techniques of annealing two molecules of single-stranded nucleic acid and characterizing the resulting hybrid molecule make it possible to compare nucleic acid sequences even though the actual order of nucleotides in the sequences may not be known. When combined with cytological procedures that permit in situ detection of the cellular or chromosomal localization of the hybridized nucleic acid, these techniques can be used in several ways to study the organization and function of the genetic material in higher organisms. (1) in situ hybridization to condensed chromosomes can be used to map the sites of DNA sequences that have not been mapped by genetic breeding experiments. (2) Just as experiments with known nucleic acid sequences in the hybridizing solution can be used to identify chromosomal sites, hybridization to known chromosomal regions can be used in characterizing the nucleic acid sequences in the hybridizing solution. (3) Hybridization to cytological preparations gives information on the functional organization of particular sequences in the diffuse chromatin of interphase nuclei. This permits comparison of the organization in cells of different types. (4) Because cytological techniques permit analysis at the level of single cells, in situ hybridization can be used to obtain preliminary evidence on gene amplification or underreplication in single cell types in tissues composed of several cell types or cell stages. (5) The distribution of specific RNA species within a cell or in mixed cell populations can be studied by hybridization with DNA transcribed in vitro from that RNA by RNA-directed DNA polymerases.

415 "MAPPING" WITH ANTIBODIES TO NONHISTONE CHROMOSOMAL PROTEINS, Sarah C.R. Elgin and Lee M. Silver, The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

The *in situ* distribution of nonhistone chromosomal proteins (NHC proteins) in *Drosophila* polytene chromosomes is being studied using an indirect immunofluorescent "staining" technique (1). Formaldehyde fixation is used to prevent extraction or rearrangement of the proteins during preparation and staining of the chromosomes. Results obtained using antisera against total NHC proteins demonstrate that these proteins, purified from isolated chromatin, are indeed preferentially associated with the chromosomes *in vivo*. Recent studies using antisera against three molecular weight subfractions of the NHC proteins indicate that while some of the NHC proteins may be widely distributed in chromatin, others have a limited and specific distribution which may be indicative of their function. A highly selective staining pattern is obtained using antiserum against subfraction ρ; puffs (loci very active in RNA synthesis) and many nonpuffed chromomeres which are known to puff at other times during the third larval instar or prepupal stages are brightly fluorescent. In *Drosophila* new RNA synthesis can be induced at specific chromomeres, such as 87A and 87C1,by heat shock treatment; these loci, previously stained at low levels, are stained brightly using the ρ serum after heat shock. The sum of the available evidence suggests that a particular chromatin structure or configuration, indicated by staining using the ρ serum, is a necessary but not sufficient condition for gene activity as indicated by puffing. This correlation is being studied in greater detail.

The immunofluorescent "mapping" technique should be useful in studying the NHC proteins in two ways. First, the distribution patterns obtained using serum against individual NHC proteins isolated by two-dimensional gel electrophoresis may suggest the functions of these proteins, as above. Second, the distribution patterns of proteins isolated by other properties (such as enzyme activity) may be obtained to test hypotheses concerning the role of such proteins in chromosome structure and function. Both approaches are being used in current work.

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416 MECHANISMS OF X CHROMOSOME INACTIVATION

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The molecular mechanism of X chromosome inactivation remains a mystery. However, some experimental and theoretical progress has been made, and this will be critically reviewed. Three models to explain this important chromosomal differentiation phenomenon will be discussed: (A) episomal activators or inactivators (1); (B) nonhistone proteins (2, 3); and (C) DNA methylation (4).

As a direct biochemical approach to this problem, we have begun studies on X chromosome isolation. To facilitate X chromosome purification, we have searched for a mammalian species where the X chromosome is normal sized (5% of genome) and yet smaller than the autosome. The American opossum (Didelphys virginiana) has such a favorable karyotype. We have established and characterized a permanent cell line from this species and chromosome isolation studies are in progress now.

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417 TWO MAJOR REGULATORY GENES OF THE MAMMALIAN SEX DETERMINING MECHANISM

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The mammalian male development is due to successive interventions by two major regulatory genes of the inherently feminine embryonic plan. The first residing on the Y-chromosome acts upon the embryonic indifferent gonad and induces it to organize a testis instead of an ovary. Barring defective mutations affecting subsidiary regulatory systems and their structural gene components, the Y-organized testis then synthesizes testosterone which is responsible for all the extragonadal masculine development. The second residing on the X-chromosome controls a single species of the nuclear-cytosol androgen-receptor protein, and it is this rather ubiquitously expressed regulatory protein which activates a specific set of structural genes in each target cell type in response to testosterone and its active intracellular metabolite (1).

Together with Wachtel, Koo and Boyse of the Sloan-Kettering Institute, I have proposed that an evolutionary conserved plasma membrane protein that has been known as H-Y antigen is the product of the Y-linked testis-determining gene (2). Indeed, in exceptional mammalian individuals whose gonadal sex does not agree with their chromosomal sex, the expression of H-Y antigen strictly coincided with the presence of testicular tissue and neither with the Y-chromosome presence nor with the masculine phenotype manifestation; i.e., XX males of man and mice expressed H-Y antigen, whereas fertile XY females of the wood lemming did not.

On the Y, the testis-determining H-Y antigen gene appears to exist in multiple copies, and when varying fractions of the copies detach from the Y and become inserted into an autosome, they become either a dominant or recessive autosomal testis-determining gene that produces XX males. An X-linked gene appears to exercise the restraining control on the Y-linked multiple copies of the H-Y antigen. In the wood lemming, an over-restraining mutation of this X-linked gene produces fertile XY females by suppressing the expression of Y-linked testis-determining H-Y antigen genes (3,4).

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418 Y-CHROMOSOME-DNA, Louis M. Kunkel, Kirby D. Smith, Samuel H. Boyer, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Reiterated DNA has been isolated from the human Y chromosome by two approaches (1,2). The first, isolation by exclusion, utilizes extensive reassociation of trace amounts of ^{3}H-labelled 47,XYY DNA with excess 46,XX DNA. Radiolabelled DNA not reassociated to 46,XX DNA after two challenges is completely specific for the human Y chromosome (1). Through the use of Y chromosome mutants, these it-Y sequences have been localized to the long arm of the Y chromosome (3). When our usual reassociation criteria (0.12M phosphate buffer (PB),60°) are reduced to 0.14M PB,50°, ∿ 50% of ^{3}H-it-Y DNA reassociates, i.e., cross reacts, with whole genome DNA from a woman. These cross reacting sequences are not found on the X chromosome since at reduced criteria they do not reassociate with DNA isolated from a mouse-human hybrid cell line containing the X as the only human chromosome (AHA-11A, a gift from Dr. Frank Ruddle). Under usual criteria (0.12M PB,60°) the cross reacting sequences do not reassociate with DNA from great apes, under reduced criteria (0.14M PB,50°) they do. Studies utilizing driver DNAs of different sizes, indicate that both cross reacting and non-cross reacting ^{3}H-Y chromosome sequences are clustered in lengths of 600 to 1000 bases in human male DNA.

The second approach utilizes restriction endonucleases. Digesting DNA from human males with Hae III yields two Y-chromosome-specific fragments representing between 30 and 50% of the estimated human Y chromosome DNA (2). The larger of the Hae III restriction fragments (∿ 3400 nucleotide base pairs in length) has been isolated using restriction enzymes Hind III, Bam HI and RII to 70% purity(2). Our reassociation data indicate that (a) ∿ 50% of the ^{3}H-Y chromosome sequences isolated by exclusion are contained in the large Hae III fragment and (b) this fragment contains no single-copy DNA. The small Hae III fragment (∿ 2500 nucleotides in length) does not reassociate either the ^{3}H-Y specific sequences discussed above or ^{3}H-labelled large Hae III fragment DNA. Further uses of these probes will help elucidate the organization of the Y chromosome.

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419 SISTER CHROMATID EXCHANGES: DETECTION AND APPLICATIONS
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Sister chromatid exchanges (S.C.E.'s) represent the interchange of DNA between replication products at apparently homologous loci. These exchanges, which are generally detected in cytological preparations of metaphase chromosomes, presumably involve DNA breakage and reunion, although the molecular basis of sister chromatid exchange formation remains unknown. Detection of S.C.E.'s, which initially utilized autoradiography, has been greatly facilitated by BrdU-dye techniques in which sister chromatid differentiation is reflected by characteristic fluorescence or Giemsa staining patterns.

S.C.E.'s have proved to be highly sensitive indices of the interaction of clastogens with chromosomes, and have provided an additional means of differentiating between hereditary diseases known or suspected to involve a defect in DNA repair. A wide range of chemicals, many known to be mutagenic and/or carcinogenic, markedly increase the S.C.E. frequencies in cultured cells at concentrations sufficient to cause at most minimal chromosome breakage. The biochemical mechanism of S.C.E. formation, as well as the biological importance of S.C.E.'s is currently under investigation. Methods for in vivo analysis of S.C.E. formation in intact animals have recently been developed. These permit the detection of tissue-specific, host mediated responses to potential clastogens, and have led to preliminary studies of sister chromatid differentiation in meiotic cells. Abnormalities in the BrdU-dye dependent "baseline" S.C.E. frequency or in the change in S.C.E. frequency following exposure of cells to clastogens have been reported in Bloom's syndrome, Fanconi's anemia and in xeroderma pigmentosum, while no abnormalities related to S.C.E. formation have been described in ataxia telangiectasia. Elucidation of the mechanism of S.C.E. formation may lead to improved understanding of the molecular basis of these diseases as well as their clinical consequences.

Previous information about the nature and presumptive basis of methods for S.C.E. detection as well as the application of S.C.E. measurements to study clastogen effects and chromosome fragility diseases will be briefly reviewed, and new information about the induction and potential significance of S.C.E. formation will be presented.

420 SISTER CHROMATID EXCHANGE: PLANT AND ANIMAL CHROMOSOMES — A REVIEW

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If cells are grown for two rounds of DNA replication in the presence of a thymidine analogue such as bromodeoxyuridine or iododeoxyuridine, the chromosomes contain sister chromatids that are chemically different from one another. These sister chromatids can be stained differentially, which allows the detection of sister chromatid exchanges (SCEs) without autoradiography. The resolution of the system is such that the SCEs can be easily seen with great clarity and precision. Experiments on SCEs with the new methods have been useful both for fundamental studies on chromosomal organization and for applied studies designed to screen for the effects of mutagenic carcinogens in mammalian cells.

Several conclusions can be reached from the studies on SCE's: They are not caused by the exchange of single polynucleotide strands, which would result in differentially stained chromatids after one round of replication; isolabelling of chromosomes in autoradiograms, which had been construed as evidence for multistranded chromosomes, has now been found to be an artifact of the autoradiography and the imprecision with which cell cycles can be timed; SCEs occur in the interband region of quinicrine stained chromosomes and are less frequent in heterochromatin than in euchromatin or the euchromatin-heterochromatin junctions; SCEs are independent of caffeine-sensitive post-replication repair mechanisms; the lesions responsible for SCEs are different from those that lead to the induction of ordinary chromosome exchange aberrations; ultraviolet radiation or alkylating agents induce long-lived lesions that lead to SCEs when the cells proceed through S; thymine dimers do not lead to SCE; SCEs can be found in cell cycles subsequent to the one treated with inducing agents.

The induction of SCEs in cultured cells has proved to be the most sensitive mammalian system available for the detection of the cytogenetic effects of mutagenic carcinogens. A ten-fold increase in SCEs can be detected at chemical concentrations 100-fold lower than those required to detect increases in ordinary chromosome aberrations. If whole animals are injected with the chemicals and SCEs detected in cultured lymphocytes, then this system can be used to detect the effects of compounds that require metabolic activation, as well as those that do not. Cells in tissue culture can also be used to detect both types of chemical agents if an activating system containing liver microsomes is added to the cultures along with the chemicals. Studies with a variety of agents that cause different chemical lesions and with cells containing different DNA repair defects are giving insights regarding the lesions responsible for the induction of sister chromatid exchange.

421 DNA REPAIR AND MOLECULAR MECHANISMS OF SISTER CHROMATID EXCHANGE

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Sister chromatid exchanges (SCEs) are induced by low levels of most DNA damaging agents and it is tempting to make simple comparisons between DNA repair systems and SCEs in order to explain the mechanisms of SCE formation. SCEs involve exchange of long stretches of chromatids containing double stranded DNA but the known DNA repair systems in mammalian cells do not contain double strand exchange as integral steps. Also, a consideration of SCE formation in human diseases in which DNA repair defects have been characterized indicates that the involvement of these repair systems in SCE formation is complex. Xeroderma pigmentosum cell lines which are excision defective (A,B,C,D,E) produce higher SCE frequencies than do normal cells when their DNA is damaged, thus implicating unexcised DNA lesions in SCE formation (1,2,3). The XP variant however, which exhibits defects in post-replication repair during the first few hours after DNA damage occurs, produces the same frequency of ultraviolet light induced SCEs as do normal cells (2), even though such cells show a higher than normal UV induced mutation frequency (4). Blooms syndrome, by contrast, shows high levels of spontaneous SCEs and has no detectable defect in any repair system; the only known molecular defect is a slight reduction in DNA chain growth rate (5). At the very least it is clear that many SCEs are produced when DNA replication occurs on damaged DNA, and the increased number of persistent damaged sites in excision defective cells results in high SCE frequencies. But post-replication repair as currently understood is not involved in SCE formation because it is: (i) confined to the first few hours after damage, (ii) is only known to involve small single strand exchanges (less than 1000 nucleotides) if any, (iii) is altered in the XP variant without effect on SCE formation. Therefore the mechanisms involved during semiconservative DNA replication which produce SCEs must involve new facets of known repair systems or a hitherto unknown double strand exchange mechanism. Work supported by U.S. Energy Research and Development Administration.

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422 STRUCTURE IN RELATION TO FUNCTION OF EUKARYOTE CHROMATIN, James Bonner, Division of Biology, California Institute of Technology, Pasadena, CA 91125

It is only through a deep and thorough understanding of human genetics and human cytogenetics that we can hope that humankind can escape the natural expectation for any and all species, namely, that will become extinct. Through such understanding, wisely used, we can little by little produce better people more fitted to our environment and, hopefully, reduce the proportion of humans not optimally so fitted. Genetic engineering (bad word) can do little except help us to understand in depth and detail the operation of the human genome so that we can not only understand our genetic endowment but also manipulate the genome as a control of gene expression to useful ends.

423 SATELLITE DNA AND CYTOGENETIC EVOLUTION, F.T. Hatch, A.J. Bodner, J.A. Mazrimas, and D.H. Moore,II, Lawrence Livermore Laboratory, Livermore, CA 94550

Simple, highly reiterated DNA sequences, often observed in density gradients as satellite DNAs, exist in condensed heterochromatin. This material is predominantly centromeric, occasionally telomeric or intercalated, and in a few species occupies entire chromosome arms. It is a highly variable component of the genome of most higher eukaryotes, but its functions have remained speculative.

The genus of kangaroo rats (Dipodomys) exhibits a remarkable interspecies variation in content of three satellite DNAs and in species karyotypes (1-3). Variations in diploid DNA content (2C) are related principally to satellite DNA content and secondarily to chromosome number (2N). Satellite DNA content is correlated positively with predominance of biarmed over uniarmed chromosomes (high fundamental number FN) and with number of subspecies, and is correlated inversely with two anatomical indices (leg bone lengths) of specialization for the jumping gait. Karyotypic variation is achieved via chromosomal rearrangements, e.g., Robertsonian fusion, C band heteromorphism, and pericentric inversion. Environmental adaptation is achieved, in part, by reassortment of gene linkage groups as a result of the chromosomal rearrangements. The foregoing relationships led us to propose that highly reiterated DNA sequences play a supragenic, global role in environmental adaptation and the evolution of new species.

In the kangaroo rat genus over evolutionary time progressive reiteration of certain simple DNA sequences (3-10 base pairs long) has apparently been paralleled by partial elimination of an intermediate-density class of DNA. Concomitantly, conversion of uniarmed to biarmed chromosomes has occurred.

Kangaroo rats are the most successful mammals of North American semi-arid and arid regions. These severe environments place a premium on genetic flexibility and adaptive capacity. The variability of the genome, mediated in part by satellite DNAs, is described above. The animals live in small populations of limited mobility, a feature favorable to the fixation of chromosomal rearrangements and the development of new species. They also exhibit major adaptations in anatomy (for jumping) and physiology (for water conservation). Thus molecular, behavioral, and environmental systems have been integrated for coping.

(Work performed under the auspices of the U.S.ERDA, Contract No. W-7405-ENG-48.)

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424 NONHUMAN PRIMATE EVOLUTION; Pearson, P.L., Instituut voor Anthropogenetica, Leiden Holland

The extensive use of various chromosome banding techniques has permitted the accurate identification of primate chromosomes and for comparisons to be made with the human karyotype (1). Many of the chromosomes present in the great apes appear to have human counterparts and except for the postulated telomeric fusion of two pongid chromosomes to give rise to the human chromosome 2 (2), chromosome evolution appears to have been very conservative. In the closely related Symphalangidae (Gibbons and Siamangs) there are no autosomal similarities, but members of the Cercopithecoidea (Old world monkeys) represented by the Macaques and Baboons, although more distantly related to man than the Symphalangidae, appear once again to have some of the human chromosome banding characteristics. We have made use of somatic cell hybridization (3) to investigate whether primate chromosomes, defined as being homologous to particular human chromosomes on the basis of banding pattern, also contain the same gene loci. Similarities and exceptions to the human pattern have been observed and these will be discussed in interpreting the relationship between banding pattern and gene content, chromosome evolution and definitions of genetical homology.

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425 MOLECULAR AND CYTOGENETIC EVOLUTION, Allan C. Wilson and Lorraine M. Cherry, University of California, Berkeley, CA 94720 and California State University, San Diego, CA 92182.

We review evidence for the existence of a molecular evolutionary clock. With the clock, one can estimate approximately the time elapsed since a pair of living species had a common ancestor. Two applications are considered. The first concerns estimation of the time when the human lineage diverged from that leading to apes. The second concerns estimation of rates of chromosomal evolution. An improved method for estimating rates of chromosome evolution is described. It is a phylogenetic method, which uses molecular information regarding phylogenetic relationships and divergence times among the species of known karyotype. There are correlations between rate of chromosomal evolution, rate of speciation and rate of morphological evolution for the major groups of vertebrates as well as for the major groups of seed plants. Rates of macromolecular sequence evolution are rather steady, however, and do not correlate with the above rates. To explain these results, one may propose that chromosomal evolution facilitates adaptive evolution. This facilitation could result from a direct mechanism, in which chromosomal mutations act as regulatory mutations; alternately, the mechanism could be indirect. Chromosomal mutations might, for example, act as sterility barriers, thereby facilitating speciation.

441 CHANGES IN CHROMATIN STRUCTURE DETECTED WITH THE SV40 MINICHROMOSOME, Jack D. Griffith and Gunna Christiansen, Stanford University Medical School Stanford California 94305

A chromatin, containing simian virus 40 (SV40) DNA and histone was used to develop a fixation procedure which best preserved its native sedimentation properties yet rendered it resistant to harsh physical and chemical treatments. Under this fixation the chromatin in a 0 02M phosphate, 0.01 M NaCl buffer (pH 7.5) was treated with 1% formaldehyde for 15 min on ice followed by 0 6% glutaraldehyde for 15 min more on ice. Using this fixation we have detected an increase in the length of the DNA segment joining the basic chromosomal subunits following shearing SV40 chromatin depleted of histone H1 exhibited identical sedimentation coefficients in 6M urea, 0.01 M Tris pH 8.0 with or without prior fixation showing that high concentrations of urea does not change the beaded conformation. SV40 chromatin examined by electron microscopy following fixation was a chain of 21 beads joined by short thin bridges Without fixation it changed into a relaxed loop of a fiber 2-3 nm in diameter and 1.1 μm long

442 ELECTRON MICROSCOPIC STUDIES ON NUCLEOSOME STRUCTURE, John C. Wooley and John P. Langmore, Biophysics Dept., University of Chicago, Chicago, IL 60637.

Our scanning transmission electron microscopy studies on unstained unfixed chromatin have suggested nucleosomes are about 110 Å by 55 Å discs with two tightly packed DNA loops (radius roughly 40 Å), each about 70-80 base pairs long, wrapped around a histone core (PNAS 72, 2691, 1975; J. Biophysics, in press). We have now examined the effects of solvent and fixation and find the fine structure to be essentially the same after formaldehyde fixation at 1mM $NaPO_4$, 0.15M KCl or 0.25mM $MgCl_2$ as in unfixed chromatin prepared from 1mM $NaPO_4$. We can now reconcile our observed widths for unstained nucleosomes with widths observed for stained nucleosomes based on observations on the nature of stain-chromatin interactions and the spatial distribution of nucleosomes in a layer of negative stain. Our studies provide further indications for a chemical difference between the outside and the center of the nucleosome (e.g., a central cleft and/or the carboxyl-rich C-terminal histone segments). The regular DNA-like 60 base pair long thin internucleosomal unit fiber we visualize, now supported by nuclease digestion data, leads us to predict the existence of multiply repeated (contiguously over 200 base pairs and/or spaced out-of-phase with the 200 base pair subunit repeat) copies of DNA sequences representing important control elements, thus permitting sequence-specific regulatory proteins to bind without serious inhibition by or disruption of nucleosome structure.

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443 IMMUNOELECTRONMICROSCOPIC IDENTIFICATION OF HISTONE PROTEINS IN TRANSCRIPTIONALLY ACTIVE CHROMATIN. Steven L. McKnight*, Michael Bustin∞ and Oscar L. Miller, Jr.* *University of Virginia, Charlottesville, Va., 22901. ∞N.I.H., Bethesda, Md., 20014.

Ultrastructural studies of transcription in Drosophila embryos have shown that chromatin within transcriptionally-active, non-ribosomal genes is morphologically similar to inactive nucleosomal chromatin (McKnight and Miller, J.C.B. 67:276a, 1975; and, Laird and Chooi, Chromosoma, in press). Such observations suggest that histone proteins remain associated with template-active DNA sequences. Here we report initial results obtained using immunological methods in conjunction with chromatin spreading techniques.

Antibodies elicited to calf thymus histones H2b and H3, purified by affinity chromatography (Simpson and Bustin, Biochemistry, 15:4305, 1976), were tested for cross reactivity with purified D. melanogaster histones (Bustin, Reeder and McKnight, manuscript in preparation). Immunological data confirm the cross reactivity of such anti-histones, and show no immunological difference between thymus H2b and Drosophila H2b, and only ∿4% amino acid difference between thymus H3 and Drosophila H3. To probe chromatin regions we dispersed single D. melanogaster embryos in pH 9.0 H_2O and incubated with ∿0.2mg/ml of anti-H2b or anti-H3 in 20mM NaCl. After 10min, goat anti-rabbit IgG, coupled to ferritin, was added to ∿0.5mg/ml, and the sample was spun through 0.5M sucrose (in 10% formalin) onto a carbon coated E.M. grid. Such preparations show morphologically altered nucleosomes, and up to 30% are ferritin-tagged. Templates of transcribed, non-ribosomal genes show a distribution of ferritin tags equal to that found on inactive chromatin of the same preparation. These preliminary results indicate that non-ribosomal DNA is complexed with histone proteins during transcription. Supported by NSF grant BMS73-01134-A01.

444 VARIANT DNA CONTENT IS AN INHERITED HUMAN CHROMOSOMAL HETEROMORPHISM. B.H. Mayall, A.V. Carrano and D.H. Moore II. Biomedical Research Division, Lawrence Livermore Laboratory, Livermore, Calif. 94550. Scanning cytophotometry quanitates the DNA stain content of pre-identified human metaphase chromosomes. Typically, ten cells are measured for each individual. The measurements are tested for differences between the members of each homologous pair of chromosomes and are compared with a standard data set drawn from ten phenotypically normal Caucasian adults. The present study involves two families, each with one son. Analysis of the chromosomal DNA measurements reveals that the two sons have respectively 6 and 5 chromosome pairs where the two homologous chromosomes differ from one another at the 90 percent level of significance. Comparison with measurements of the parental chromosomes allows assignment of the homologous chromosomes. In 19 of the 22 assignments, it appears that chromosomal DNA content is inherited as a dominant characteristic. The remaining three assignments show significant differences between the parental and offspring chromosomes; this may reflect unequal crossing over at meiosis. (This work was conducted under the auspices of U.S. ERDA contract No. W-7405-ENG-48 and was supported in part by USPHS Grant GM 20291).

445 CHROMATIN REPLICATION IN VITRO, Ronald L. Seale, Division of Molecular and Cellular Biology, National Jewish Hospital and Research Center, 3800 E. Colfax Ave., Denver, CO 80206.

An in vitro replication system, using HeLa cell nuclei, was characterized for the study of chromatin replication. Isolated nuclei incorporated ^{3}H-TTP for at least 30 min into a DNA product, and this DNA was synthesized from active replication forks. The DNA replicated in vitro accumulated as immature DNA precursor fragments; the ligation functions are extracted into the cytosol during cell fractionation.

The association of DNA replicated in vitro with nucleosomes was investigated by micrococcal nuclease digestion. The in vitro DNA was degraded to 75-80% acid soluble material, compared to 50% in controls, indicating that half the new DNA is associated with histones. The nuclease-resistant DNA was shown to be associated with nucleosomes since early digestion products were multiples of the 180 bp unit particle and limit digest products were the complete set of oligonucleotides of 30-130 bp, as in controls. Analysis of the sizes of oligonucleotide digestion intermediates showed that nucleosomes are partitioned to one arm of the replication fork.

Since nuclei in vitro accumulated DNA precursor fragments, it was possible to isolate nucleosome-associated Okazaki fragments and demonstrate the association of these precursors with the nucleosome. By denaturation of monomer, dimer, trimer and tetramer-sized double-stranded units of in vitro DNA, the Okazaki fragment termini were shown to map outside the nucleosome.

446 PARTIAL PURIFICATION AND CHARACTERIZATION OF THE CENTROMERE REGION OF MOUSE CHROMOSOMES, G. Krystal, J.B. Rattner and B.A. Hamkalo, Depts. of Mol. Biol. & Biochem. & Dev. & Cell Biol., University of California, Irvine, Ca. 92717

Metaphase chromosomes prepared from mouse L929 cells by non-ionic detergent lysis after colcemid arrest or shake-off exhibit distinct heterochromatic centromere regions with associated kinetochores when viewed by electron microscopy after preparation by the Miller procedure. Separation of chromosomes from debris by differential centrifugation, followed by DNase I treatment results in preferential digestion of chromosome arms; the material remaining, consisting of centeromeric heterochromatin and associated kinetochores as assayed by electron microscopy, is utilized for biochemical analysis. Enrichment in centromere material after digestion is quantitated by the relative increase in 100,000xg pellets of the 1.691 satellite DNA known to be located at the centromere by in situ hybridization. Density gradient centrifugation of centromeric DNA suggests the existence of 2 or 3 satellites in addition to the 1.691 band. The protein compositions of whole chromosomes and of centromeric fragments were compared by 2-dimensional gel electrophoresis after in vitro labelling with Na^{125}I. Labelling preparations before and after dissociation with SDS provides data on the total protein composition of kinetochore-centromere fragments and their 3-dimensional conformation. Preparations in which surface proteins are labelled show enrichment for at least 3 polypeptides in the centromere region, and those labelled after dissociation exhibit approximately 5 to 10 polypeptides in the range of 20,000 to 60,000 daltons which appear to be unique to the kinetochore-centromere complex. Current studies are directed toward kinetochore purification and assignment of kinetochore-specific proteins. (Research supported by NSF GB 41939;Am. Can. Soc.; NIH GM23241-01 to BAH and Natl. Can. Inst. of Canada to GK).

447 BIVALENT ASSOCIATIONS OF C-HETEROCHROMATIN AT HUMAN MEIOTIC PROPHASE, Daniel J. Driscoll and Catherine G. Palmer, Dept. of Med. Genetics, Ind. U. Sch. of Med.

Nonhomologous associations of chromosomal regions have been seen at meiosis in a variety of species. We have studied nonhomologous associations of heterochromatin in human spermatogenesis at pachynema. Meiotic tissue was obtained by testicular biopsy performed for subfertility and from orchiectomy for prostatic cancer. The preparations were studied with the aceto orcein, Giemsa, G-11, Q or C-band methods.

In many pachynema there are nonhomologous associations between the C-heterochromatin of several bivalents in which the heterochromatic regions coalesce and/or have thin connections. C-banding was the most useful procedure for demonstrating these heterologous associations. The thin filaments connecting the heterochromatic regions of the nonhomologs were observed between regions of similar or different size. Associations between bivalents bearing 2 magenta areas, a magenta and a non-magenta area, and 2 non-magenta areas were seen with the G-11 technique. These connections may reach long distances across the nucleus. Occasionally a bivalent may be associated with the sex vesicle. Multiple associations may be seen with several bivalents connected at the C-heterochromatin.

The repetitious DNA of C-heterochromatin may be significant in homologous pairing and nonhomologous associations in meiotic prophase. Bivalent associations of first meiotic prophase in species with paracentromeric heterochromatin may play a role in chromosomal orientation in meiosis.

448 Differential fluorescent labelling of chromosomes and DNA with base pair specific DNA binding antibiotics, J. H. van de Sande and C.C. Lin, Division Med. Biochemistry The University of Calgary, Calgary, Alberta, Canada. Orange-red fluorescent bands (Q bands like) are obtained by treating human chromosomes with daunomycin or adriamycin (Science, 190, 61, 1975). Solution interaction of these antibiotics with DNA of known base composition indicates that differential fluorescent quenching by DNA regions with specific base sequence arrangement can account for the observed bands on chromosomes. The carbohydrate moiety of daunomycin, daunosamine, stabilizes the interaction of this antibiotic with DNA or chromosomes, but it is not essential for the appearance of fluorescent bands. Recently, the G-C specific DNA binding antibiotic olivomycin was found to produce characteristic reverse fluorescent banding patterns (R-bands) on human, bovine and mouse metaphase chromosomes. In solution, the repeating polymer poly d(G-C).d(G-C) enhances the olivomycin fluorescence, while the antibiotic fluorescence is not affected by poly d(A-T).d(A-T). Natural DNA's show an enhancement of fluorescence related to their G-C content, which is an expression of the degree of binding of olivomycin to the DNA's. This mechanism is different from the mode of binding of quinacrine or daunomycin to DNA, where the binding is not sequence specific, but the variable quantum efficiency is a function of the base composition at the binding site. These results strongly support the suggestion that nucleotide sequence arrangement along the chromosome is a primary determinant for the appearance of fluorescent bands. (Supported by the Medical Research Council of Canada).

449 THE ROLE OF VARIANT HETEROCHROMATIN IN INTERINDIVIDUAL DNA CONTENT VARIATION MEASURED BY FLOW-MICROFLUOROMETRY, Holger Hoehn and James Callis, Div. Genetic Path., University of Washington Medical School, Seattle, Washington 98195

Under standardized conditions of staining time, sample concentration and sample flow rate differences in sex-chromosome constitution between individuals can be readily detected by flow-microfluorometry (FMF). We obtained a correlation coefficient of 0.95 when we compared DNA content differences between a series of euploid and aneuploid individuals with differences expected on the basis of single chromosome scanning microphotometry. These studies gave no evidence for systematic effects, on the FMF measurements, of different chromatin moieties (euchromatin vs. facultative heterochromatin). However, when we compared a series of cytogenetically normal males and females by FMF, within group variation of the respective DNA contents in the order of 1% was observed. In addition, it was found that individuals occupying extreme positions of the mean DNA content distributions within a given group maintained their respective positions on subsequent runs. The analysis of the distribution of C-band heterochromatin in conventional karyotypes from these individuals revealed no major differences with respect to the number and size of C-band variants. At present, we are analyzing the DNA content distributions of male or female individuals with enlarged or unusually small areas of C-band heterochromatin of chromosomes 1, 9, 13-15, 16, 21-22 and Y. If no consistent associations between C-band pattern and position within the DNA content distribution of a given sex can be made, other reasons (e.g. fluctuations in nuclear shape, stain binding coefficients, dye permeability, fluorescence quantum yields, etc.) for the interindividual DNA content variation have to be explored.

450 REGIONAL MAPPING OF THE MAJOR HISTOCOMPATIBILITY COMPLEX ON HUMAN CHROMOSOME 6,
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Interspecific somatic cell hybrids containing defined parts of human chromosomes 1 and 6 were used for regional mapping of gene loci previously assigned to chromosome 6: the HLA region (MHC), phosphoglucomutase-3 (PGM_3) and malic enzyme-1 (ME-1), and to chromosome 1: PGM_1 and 6-phosphogluconate dehydrogenase (6-PGD).

Human fibroblasts containing a balanced reciprocal translocation between the short arms of chromosomes 1 and 6 [t(1;6)(p3200;p2100)] were fused with an established Chinese hamster cell line. Hybrid clones segregating human chromosomes were studied for the presence of the translocation chromosomes 1^T and 6^T and the normal homologues 1 and 6. Informative clones that had retained 1^T or 6^T, and three clones with the normal 6 and 6^T as controls,were analyzed for expression of HLA-A antigens by a microabsorption test,and for expression of human PGM_1, PGM_3, ME-1 and 6-PGD by electrophoretic methods. The results indicate intrachromosomal mapping of the major histocompatiblity complex (MHC) in region 6p2100→6pter and of the genes for PGM_3 and ME-1 in region 6p2100→6qter. Genetic map distances as established by family studies, allow rather precise mapping of MHC and PGM_3 near the translocation breakpoint in band 6p2100.

On chromosome 1, our results indicate that the gene for PGM_1 maps in band 1p31 proximal to the translocation breakpoint, while 6-PGD is located distal in region 1p3200→1pter.

Quantitative correlation between the density of HLA antigens on the hybrid cell surface and the number of copies of the respective HLA gene-bearing chromosome suggests a gene dose effect for cell surface molecules, as it exists for intracellular gene products.

451 CHROMOSOMAL RNA-DNA HYBRIDS, George T. Rudkin, The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

An antiserum specific for RNA-DNA hybrids (Stollar, B. D., Science 169: 109, 1970) has been used to probe for such hybrid molecules in acetic acid fixed polytene chromosomes by means of an indirect immunofluorescence technique. The specificity of the immunocytochemical reaction was demonstrated by showing 1) that Drosophila melanogaster chromosomes subjected to denaturing conditions would not bind the antiserum, 2) that annealing 5S RNA to such denatured chromosomes caused antibody binding only at the locus of 5S RNA (56F on the right arm of chromosome 2), and 3) that poly(rA)·poly(dT) blocks the reaction of this antiserum with in situ hybridized 5S RNA and with naturally occurring hybrids. Hybrids were detected in chromosomes of salivary glands of the giant phenotype during the last four days of larval development and the first eight hours of prepupal development (25°C). The most conspicuous positive reactions were in puffs, such as at three prominent sites on the right arm of chromosome 3, although other sites on the third and on other chromosomes also gave a positive reaction at some time (or times) in development. Many puffed regions displayed no hybrids while some regions in which no puff was visible gave positive reactions, in some places apparently in bands, in others seemingly in interband regions. A unique distribution of hybrid sites appears to be characteristic of a developmental stage.

452 SILVER STAINING AS A POSSIBLE INDICATOR OF ACTIVE NUCLEOLUS ORGANIZER REGIONS IN NORMAL AND NEOPLASTIC HUMAN CELLS. Hubbell, H. R., Lau, Y. -F. and Hsu, T. C.,
Section of Cell Biology, The University of Texas System Cancer Center M. D. Anderson Hospital & Tumor Institute, and The Graduate School of Biomedical Sciences, Houston, Texas 77030.

Recent investigations have employed ammoniacal or aqueous silver nitrate to specifically stain the nucleolus organizer regions (NORs) in the chromosomes of several species. Studies of man-mouse hybrids, in which only mouse 28S rRNA is found in the cytoplasm, show that only the mouse NORs are stained with silver (Miller, et al., 1976). We have studied the NORs of 14 aneuploid human tumor lines to further elucidate the relationship between silver staining and active NORs. If the multisomy of chromosomes and chromosomal segments, commonly seen in tumors, is more or less random, the increase in the NORs should be roughly proportional to the stemline number. In all of the tumor cell lines, the modal number of Ag-NORs was approximately the same as that found in normal human lymphocytes, although the stemline number varied. Conventional Giemsa staining of control metaphases, followed by silver staining, indicated that all D and G group chromosomes with stalks also stained with silver. In D and G chromosomes without visible stalks, silver staining may be present. Cell cycle analysis, using Hoechst 33258 to stain RNA, shows that silver-staining material is present during the active transcription of the rRNA genes throughout interphase, but is dispersed, as is the nucleolus, just before and after mitosis. Our results indicate that silver nitrate may be able to identify metaphase NORs which were active in rRNA transcription.(Supported in part by PHS Training Grant No. CA-05047 from NCI and Research Grant VC 21 from ACS.)

453 EVIDENCE FOR THE GENETIC ACTIVITY OF BOTH X-CHROMOSOMES IN FEMALE TERATOCARCINOMA STEM CELLS, Gail R. Martin, Shaul Yatziv, Michael Bishop, Shirley Clift, Charles J. Epstein and David W. Martin, Jr., The University of California, San Francisco, California 94143.

Undifferentiated stem cells have been isolated from a spontaneous ovarian teratocarcinoma in the LT strain of mouse. The levels of both X-linked and autosomal enzymes have been measured in these cells and compared with the levels in male teratocarcinoma stem cells isolated from spontaneous testicular or embryo-derived teratocarcinomas. The level of enzyme activity in the female cells has been found to be twice that in the male cells for the X-linked enzymes G6PD, HGPRTase and alpha-galactosidase. In contrast, the levels are the same in the female and male cells for all autosomal enzymes tested (ICDH, 6PGD, beta-galactosidase, beta-glucuronidase, etc.). Control experiments using livers from adult mice indicate that these results are not due to intrinsic strain differences between the cells. These results have therefore been interpreted as indicating that in the LT cells, both X-chromosomes are genetically active. Since the cells can be made to differentiate in a manner which parallels normal mouse embryogenesis in vitro, experiments are currently in progress to determine whether or not the cells can undergo X-chromosome inactivation in vitro, and if so, at what stage in the differentiative sequence this process occurs.

454 SOMATIC CELL DAMAGE AND ADJUVANT CANCER TREATMENT; A GROWING DILEMMA. John E. Byfield, University of California, San Diego, Calif. 92103

The growing enthusiasm for "adjuvant" cancer therapy,i.e. aggressive multi-modal treatment early in the disease course with increased patient cure as its goal, is laudable but may introduce a new spectrum of human "genetic" diseases. All useful anti-cancer agents lead to lethal cell damage but most (all?) non-cycle specific drugs also induce sub-lethal damage of a non-oncogenic nature. Particularly troublesome are the intercalating agents (Act D, adriamycin etc.) and the alkylating agents, all of which stimulate extensive DNA repair mechanisms in both normal and malignant cells. These repair mechanisms include both endonucleolytic and/or excision steps or a mix of the two. In somatic cells such mechanisms may be incomplete leading to varying degrees of genetic damage. A functional result of this pattern appears to be chronic organ failure. Cardiotoxicity from the anthracyclines, marrow failure from x-rays or alkylating agents, lung damage from bleomycin, and diabetes from streptozotocin are examples of such "diseases". As the number of useful drugs increases and the population at risk expands, new "diseases" are likely to emerge. A central problem in reducing this hazard is the quantitative variation in repair activity found when species are compared which may hamper the development of animal models. The molecular mode of action of several useful drugs will be demonstrated and their somatic "disease" outlined as illustrations.

455 AN ALTERNATE MODEL FOR THE PREDOMINANCE OF NON-SISTER CHROMATID EXCHANGE DURING MEIOSIS, Rolf Martin, Dept. of Chemistry, Brooklyn College, Brooklyn, NY 11210

Previous investigations have indicated that the frequency of sister chromatid exchange during meiosis "is insignificant, or well below" that of homologous (non-sister) exchange (see Egel, R., 1973). Egel has suggested that this may be due to locally asymmetric DNA synthesis. I would like to describe an alternate model: the predominance of non-sister exchange may be a consequence of DNA repair mechanisms which mediate the transfer of sequence information between homologous chromosomes during synapsis. Such a repair system may be helpful or even necessary to control the accumulation of those kinds of genetic lesions that cannot be corrected by recombinational repair involving sister chromatids. As noted previously (Martin, R., 1976), this mechanism may help to explain certain aspects of aging and rejuvenation in microorganisms and germinal cells. Evidence concerning the relationships between DNA damage, pachytene DNA synthesis, gene conversion and recombination is taken into account. The organization and timing of meiotic processes are also discussed in this context.

456 EVIDENCE FOR SISTER CHROMATID EXCHANGE INVOLVING HALF HELICES OF DNA FOLLOWING TREATMENT OF CHROMOSOMES WITH MITOMYCIN C, Herman E. Wyandt and Ann Nicholas, University of Oregon Health Sciences Center, Portland, Oregon 97201.

Sister chromatid exchanges (SCE) are evident in chromosomes that have gone through two replication cycles in BrdU and are then stained with Hoechst 3325 (Latt, 1973). Normally such exchange is an all or none phenomena with complementary light and dark patterns invloving the two chromatids. We have found that occasionally, after two replications in BrdU in the presence of mitomycin C, sister chromatids do not show the typical complementary pattern. Instead, both chromatids are lightly or darkly stained over identical regions. This asymmetrical pattern can theoretically occur if exchange has involved only half of the DNA per chromatid during the preceding replication. These patterns appear to be rare and may represent a unique effect of mitomycin C.

456A GIEMSA COMPONENTS IN G-BANDING, Herman E. Wyandt and Ruth S. Anderson, University of Oregon Health Sciences Center, Portland, Ore. 97201

Dye components of Giemsa, including azures A, B and C, methylene blue, toluidine blue and thionin were studied for their ability to give chromosome banding following treatments with heat, alkalinity, trypsin and various routinely used G-banding procedures. G-banding was achieved by all of the dyes, without any special treatment of chromosomes, in the above descending order of quality. The concentrations of dye (0.003-.005%) and buffer (.05M phosphate) were critical. Various routinely used G-banding procedures did not alter the relative quality of banding by the different dyes. Absorption spectra of band and interband regions of human chromosome no.9 were measured *in situ* following various treatments and staining procedures. Three separate phenomena were observed: (1) a shift in spectra between band and interband regions; (2) a difference in staining intensity; (3) a combination of (1) and (2). Azure B, for example, showed a shift from 575 nm to 550 nm (for band and interband regions respectively) after no treatment or after treatment with trypsin. Treatment of chromosomes by ASG (heat-saline) technique or with 70°C temperature, followed by staining with azure B, showed no absorption shift, but did show intensity differences (i.e., at 580 nm). Results with toluidine blue after similar treatments showed intensity differences between band and interband regions, but no shifts in absorption. These results verify subtle differences in mechanisms involving polymerization (side-stacking) of dye and/or extraction of chromosomal components depending on the dye or treatment used.

457 THE INDUCTION OF SISTER CHROMATID EXCHANGES (SCEs) IN PHA-STIMULATED HUMAN LYMPHOCYTES BY MITOMYCIN C (MMC), Raymond Tice and Yutaka Ishii, Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973

At least one type of SCE has been suggested to strongly correlate with a post-replication type of repair event in mammalian cells. To assess the utility of SCE measurements for detecting acquired or inherited abnormal DNA repair capability among humans, MMC-induced SCEs, aberrations, and cellular inhibition were measured in PHA-stimulated human lymphocytes under a variety of experimental conditions. We investigated a wide range of MMC concentrations, dose durations and timing effects, possible sensitization by bromodeoxyuridine, and dependency on cell number and replicative history. Results indicate that this system when compared with other cytogenetic techniques for assessing DNA repair capability offers greater flexibility and increased resolution while providing information concerning both threshold and saturation responses.

458 ACCENTUATED ACROCENTRIC INTERCHROMOSOMAL CONNECTIONS IN BRDU TREATED HUMAN LYMPHOCYTES Karen Kurvink and Jaroslav Cervenka, University of Minnesota, Minneapolis, Minnesota, 55416.

Lymphocytes treated in vitro with 10^{-5}M of BrdU (bromodeoxyuracil) for sister chromatid differential staining demonstrate accentuated acrocentric interchromosomal connections compared to regular chromosome preparations. These interconnections stain clearly with Giemsa stain. If the slides are destained and restained for N (NOR) bands, the interconnections and satellites stain the same dark color.

Additional evidence for accentuation of the interconnections comes from observations of pulverized chromosomes. In the BrdU treated cultures occasional pulverized chromosomes are present. They are usually acrocentric chromosomes. If the chromosome is a member of an interconnected group of acrocentrics, the entire cluster will be pulverized.

It is probable that the observed interchromosomal connections represent the normal condition of acrocentric chromosomes before exposure to routine harvesting and slide preparation. The variability of the acrocentric satellites (as well as their presence or absence) could reflect length differences in the interconnected segment as well as varied breakage sites. Rupturing of the interconnecting segment would produce dangling tails which probably recoil--perhaps forming the satellites.

459 INVERTED REPETITIVE SEQUENCES IN THE RAT GENOME, James W. Posakony, William R. Pearson, Jung-Rung Wu and James Bonner, Div. of Biol., CA Inst. of Technol., Pasadena, CA 91125

Approximately 6% of the rat genome consists of sequences whose inverted complements are present on the same DNA strand. These sequences form duplexes by a relatively rapid first-order reaction. Such "foldback" duplexes are isolated by renaturing long (>10 kb) DNA fragments to very low Cot (<.0005), digesting single-stranded tails and loops with S-1 nuclease, and collecting the resistant DNA on hydroxyapatite. After shearing, the complexity and repetition frequency of this fraction were investigated by self-renaturation and by annealing to a large excess of whole rat DNA, respectively. The results indicate that at least 60% of foldback duplex DNA in the rat consists of moderately repeated sequences. Furthermore, as determined by Biogel A-50 chromatography, the size distribution of S-1 resistant duplexes after renaturation to Cot 0.005 is quite similar to that of the S-1 resistant DNA at Cot 5. In particular, there is in both cases an apparently discrete class of sequences of 250-450 nucleotides long. Finally, preliminary results from experiments in which repetitive DNA is used to drive labeled foldback duplex DNA are consistent with a considerable degree of sequence homology between these two fractions. These observations appear to suggest that the majority of foldback sequences in the rat genome are very similar to the ordinary repetitive sequences, the major distinguishing characteristic being their inverted pattern of repetition. Indeed, some of the mechanisms postulated for the generation of ordinary repeats in higher genomes could also yield inverted repetitive sequences. A more detailed examination of the relationships between ordinary and inverted repeats is in progress.

460 CHROMOSOME AND PROTEIN EVOLUTION IN THE EQUIDAE, Oliver A. Ryder, Naomi C. Epel, Arlene T. Kumamoto and Kurt Benirschke, Research Dept., San Diego Zoo, San Diego, CA 92112

The horse family, Equidae, consists of seven species including the domestic horse, Equus caballus, the Przewalski's wild horse, E. przewalskii, the donkey, E. asinus, the so-called wild asses, E. hemionus, and three species of zebra, E. burchelli, E. grevyi, and E. zebra. The diploid chromosome numbers vary from 2n=66 in E. przewalskii to 2n=32 in E. zebra hartmannae. G-banded, C-banded, and Ag-AS stained karyotypes of the various Equidae species will be compared. Plasma protein electrophoresis in polyacrylamide gels has been performed in order to establish genetic distance estimates among present-day equine species. The phylogenetic data derived from the gel studies may be utilized to assess the rate of karyotypic changes within this taxon.

This work was supported by the Zoological Society of San Diego, the Bank of America-Giannini Foundation, and the National Institutes of Health.

461 RELATIVE ITERATION FREQUENCY OF RIBOSOMAL GENES AS DETERMINED BY IN SITU MOLECULAR HYBRIDIZATION. Loh-chung Yu, Paul Szabo, and Wolf Prensky. Sloan-Kettering Institute, New York, New York 10021.

Iodinated 5S and 18 + 28S RNA's were annealed to human and domestic cat chromosomes, and to filter bound DNA from these species. The extent of hybrid formation was controlled by varying the concentration of the probe in the reaction mixture. In the human genome it was known that 5S rRNA genes are located on chromosomes 13, 14, 15, 21 and 22. In the cat we found the 5S rRNA genes on one pair of the D group chromosomes (probably D3 or D4), and the 18 + 28S genes on the E1 chromosome pair.

For quantitative measurement of the extent of in situ hybridization, grain counts were taken from the relevant human and cat chromosomes. The data showed that the cat has about 2X the number of 5S genes and 3X the number of 18 + 28S genes per genome as compared to the human. These observations were repeated and reproduced by RNA:DNA hybridization to DNA bound to nitrocellulose filters.

This study demonstrates the feasibility of studying the relative concentration of mammalian DNA sequences by in situ hybridization. Studies have been initiated to measure the concentration of 5S DNA in man. Preliminary evidence suggests that the number of 5S DNA copies varies among individuals.

462 NUCLEASE-RESISTANT CHROMATIN DNA: A GC-RICH, HIGH MOLECULAR WEIGHT COMPONENT SHOWING EVOLUTIONARY CONSERVATION, Kenneth A. Marx, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

A small fraction of mouse liver chromatin DNA (< 1%) remains resistant to digestion by micrococcal nuclease under conditions where the remaining chromatin DNA is digested predominantly to monomer units (about 200 base pairs or 1.3×10^5d). The nuclease-resistant chromatin DNA is of high molecular weight (> 1×10^7d) and appears to be largely a single GC-rich component based upon its CsCl buoyant density (1.715 g/cm^3) and T_m (78°C in .018 M Na^+) relative to total chromatin DNA. A labeled cRNA copied from this DNA template was used as a tracer in DNA driven hybridization reactions. Hybridization of a consistent sizable fraction of the tracer (40%) proceeded bi-molecularly and was complete before a driver DNA C_0t of 40. When the tracer was driven with Drosophila DNA (C_0t of 50) a similar proportion hybridized, indicating that the nuclease-resistant mouse chromatin DNA fraction is highly conserved. To examine the trivial possibility that this DNA represented ribosomal genes, hybridization was carried out in the presence of mouse ribosomal RNA at a 1000:1 mass excess over the rDNA sequences. This did not compete out the formation of tracer cRNA:DNA hybrids, demonstrating that the origin of this fraction is other than condensed rDNA chromatin. One possible biological function of such a conserved high molecular weight chromatin DNA fraction might be to organize the centromere. To test this hypothesis the nature of a similar DNA fraction in humans is currently being investigated for comparison and for use in cytogenetic studies. (Supported by a Muscular Dystrophy Association of America, Inc. fellowship).

463 GENETIC BASIS OF THYMIDINE RESISTANCE IN CULTURED HAMSTER CELLS, John Morrow and Douglas Stocco, Dept. of Biochem., Texas Tech University School of Medicine, Lubbock, Texas, 79409.

High levels of thymidine will inhibit the growth of V79 Chinese hamster cells, and the level of resistance of the wildtype cells increased gradually over a one year period. This was demonstrated by experiments in which the resistance level was determined on several different occasions followed by retesting of the original line. The spontaneous mutation rate of thymidine resistance was determined both by the Luria-Delbruck fluctuation test, and the clonal variance test of Szybalski. Resistant mutants were stable, and occurred at rates ranging 1.5×10^{-2} to 5.3×10^{-4} per cell per generation. The mutation rate was higher at lower thymidine concentrations, and resistant mutants were stable for long periods in the absence of the selecting agent. Discrete classes of mutants were not observed, but rather a broad spectrum of variants was detected. Mutagenesis tests with UV showed a slight increase, while ethyl methane sulfonate and ethidium bromide were ineffective as mutagens.

Extensive testing for mycoplasm was negative. The biochemical basis of thymidine resistance was not due to variation in thymidine kinase levels, but rather was due to an alteration in the rate of uptake of thymidine as shown by experiments employing this radiolabelled precursor. There was an inverse correlation between the rate of uptake and the level of thymidine resistance. Thymidine resistance in Hamster cells has properties not observed in other systems, and further experiments, including cell hybridization, will be required to resolve its genetic nature.

464 ISOCHROMOSOME FORMATION: A GENERAL MODEL, D.L. Van Dyke, M. Logan, L. Weiss, G.S. Pai, Henry Ford Hospital, Detroit, MI 48202

Karyotyping was performed on a young boy with mental retardation, hyperactivity, and strabismus. Three cell lines were found. 30 cells exhibited a normal karyotype and 54 had an extra E-group-sized acrocentric with satellites on both the long and short arms. Twenty other cells each had, in addition to the first marker (M1), a tiny bisatellited chromosome (M2). Both ends of M1 and M2 participated in acrocentric associations. C-banding showed that both markers were dicentric; however M1 had only one primary constriction. Thus one centromere was apparently inactive, analogous to that seen in certain tandem X-X translocations and X isochromosomes. G-, C-, and Q-banding data were consistent with the dicentrics having originated from chromosome 15 material, resulting in a partial tetrasomy 15 syndrome.

We suggest that M1 was an isochromosome derived from a breakage and homologous chromatid fusion in the proximal long arm of chromosome 15 during meiosis. This produced a symmetrical isodicentric chromosome, plus one or two acentric fragments. M2 then resulted from a dicentric bridge-break-synthesis-reunion phenomenon during mitosis.

This general model for the evolution of extra dicentric isochromosomes requires only a single exchange event during a parental meiosis. Modification of the model to exchange between sister chromatids can explain the origin of dicentric X or Y isochromosomes in subjects with no normal cell line.

465 UV-IRRADIATION STUDIES IN MOUSE MYELOMA CELLS, Tatsuo Matsushita and Aaron D. Simms, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL 60439

Mouse tumor lines are inefficient in removing pyrimidine dimers and have been reported to be excision repair deficient. Mouse myelomas are end cell tumors which have not been well defined in their excision repair capacity. We are studying UV mutagenesis in the mouse myeloma subclone 66.2 of the MPC 11 cell line (Kuehl, W. M. and Scharff, M. D., J. Mol. Biol. [1974] 89, 409) using ouabain-resistant variants. Both ouabain variants and wild-type 66.2 cells are being examined for survival after UV irradiation, excision of pyrimidine dimers, and gross chromosome changes (karyotype analyses). Survival after UV irradiation is determined by three methods: soft agar cloning, viable cell counting by trypan blue staining, and electronic cell number counting. Both cell number and size are measured with a Coulter Counter multichannel analyzer system using a sensing aperture 50 μm in diameter (Kubitschek, H. E. and Claymen, R. V., J. Bacteriol. [1976] 127, 109). The persistence of pyrimidine dimers in UV-irradiated myeloma cells is determined by gentle extraction of DNA, treatment of the DNA with crude M. luteus UV-endonuclease extract, and alkaline sucrose gradient centrifugation.

(Work supported by the U. S. Energy Research and Development Administration.)

466 ROTATIONAL MOBILITY OF NON-HISTONE PROTEINS IN CHROMATIN, B.L. Malchy, G.B. Strambini and W.C. Galley, Dept. of Biochemistry, Queen's University and Dept. of Chemistry, McGill University.

Non-histone proteins are a highly heterogeneous group of nuclear components. While some of these proteins are enzymes involved in gene expression, others may play a more structural role as control elements maintaining the state of differentiation of the cell. In the present study, the rotational mobility of these proteins as a general class has been monitored from measurements of the anisotropy of the phosphorescence of chromatin in highly viscous solvents. Using the tryptophane emission, which serves as a specific marker for non-histone proteins, the lifetimes and anisotropies of the phosphorescence were measured from -100°C to room temperature in 70% (w/w) glycerol - water. The steady state anisotropy values were corrected for variations in the lifetimes and a transition in the lifetime-normalized anisotropy was observed between -70 and -20°C. The midpoint of the transition which corresponds to a rotational correlation time of 3 sec. occurs at about -52°C. A viscosity of 380 poise at this temperature would suggest that if the average reorienting unit were a rigid sphere in this solvent it would possess a hydrodynamic volume corresponding to a radius of greater than 200A°. These data indicate that the bulk of the non-histone proteins cannot be undergoing motions as essentially free proteins but are hindered in their motions as a consequence of macromolecular interactions. The restrictions to rotational freedom implied in the large "apparent hydrodynamic volume" would suggest that a significant fraction of non-histone proteins are involved in a degree of organization which is higher than the nu bodies observed by electron microscopy.

467 CYTOFLUOROMETRIC AND CYTOCHEMICAL COMPARISONS OF NORMAL AND ABNORMAL HUMAN CELL CHROMATIN, James E. Gill, Carol Hanna-Madden, Richard J. Marisa, and Leon L. Wheeless, Jr., Department of Pathology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

Slit-scan cytofluorometry provides fluorescence contours of cells as they pass a slit aperture or a narrow band of exciting light. These contours are analyzed for cell diameter, nucleus diameter, nuclear fluorescence, and other low resolution morphological information (Wheeless, L. L. Jr., Patten, S. F. Jr., and Cambier, M. A., Acta Cytol. (1975) 19, 460). Acridine Orange (AO) or Propidium Iodide (PI) staining of exfoliated cells from the human female genital tract results in nuclear and cytoplasmic fluorescence. When cells are classified on the basis of morphology, then slit-scanned for total nuclear fluorescence intensity, abnormal cells are found to give values from two to ten times the mean value for normal diploid cells. Abnormal cells often have increased nuclear Feulgen absorption (Bohm, N.. and Sandritter, W., Current Topics in Pathology (1975) 60, 152) but this apparent increase in DNA content is not sufficient to account for the observed increase in nuclear fluorescence after AO or PI staining. Fluorescence emission spectra from the nuclei of both normal and abnormal cells are consistent with the hypothesis that DNA is the substrate binding AO and PI. It appears that the chromatin of abnormal cells is altered in such a way as to allow more AO or PI binding per unit of DNA.

468 ANALYSIS OF DNA PURIFIED BY DNA BINDING NHC-PROTEINS. S. Tasi, L. L. Jagodzinski and J. S. Sevall, Chemistry Dept. Texas Tech Univ., Lubbock, TX 79409.

Purification of a class of rat liver nonhistone chromosomal polypeptides which have a preferential affinity for rat DNA has been accomplished by ion exchange, molecular seiving and affinity chromatography. Initial characterization indicates a 200,000 MW complex consisting of a 50,000 MW polypeptide which preferentially binds rat DNA. A crude protein fraction can fractionate the rat genome by removal of the protein bound DNA sequences that are retained on nitrocellulose filters. At protein to DNA mass ratios of 3:1, 20% EcoRI restricted rat DNA is isolated as protein bound DNA. In vitro labeling with DNA polymerase I and shearing restricted fragments to 350bp size allowed DNA reassociation kinetics to be followed. Second order reaction curves were fit to individual components of each reassociation curve. The bound DNA was represented by three components: 19.1% reassociating very fast, 33.1% reassociating with a $Cot\frac{1}{2}$ of 0.0585, and a slow component of 40.3% with a $Cot\frac{1}{2}$ of 588.2. The total rat DNA sequences are comprised of four components: a fast (9%), two moderate components (17.6% of $Cot\frac{1}{2}$ 0.93 and 12.2% of $Cot\frac{1}{2}$ 145), and a slow component (58.1%; $Cot\frac{1}{2}$ 2460). The unbound DNA shows similar reassociation components as total rat DNA. DNA excess reassociation of total rat DNA and trace amounts of labeled bound DNA indicate we have enriched for sequences that are rapidly reassociated and moderate repetitive DNA sequences in the fast component of intermediate repetitious sequences. These results suggest that a class of rat liver NHC-proteins can interact with a subset of DNA sites which can be isolated for further analysis of protein-DNA interactions.

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469 PRODUCTION OF HUMAN-MOUSE HYBRIDS FOR REGIONAL MAPPING OF HUMAN CHROMOSOME 5, Donna L. George and Uta Francke, Dept. Pediatrics, Univ. Calif. San Diego, La Jolla, CA 92093

Human leukocytes containing a balanced translocation, consisting of the insertion of a region from the chromosome 5 long arm into the long arm of a chromosome 3 [46,XY, t(3;5)(q27;q13q15)], were fused to mouse 3T3 (TK^-) cells and hybrid clones isolated in HAT selective medium. The chromosome constitution of hybrid cells was determined by trypsin-Giemsa banding. Two independent clones containing the 5q- chromosome and one containing the 3q+ chromosome were informative for regional mapping of genes assigned to chromosome 5: hexosaminidase B (HEX B), diphtheria toxin sensitivity (DTS), and interferon production (IF-2). Hybrid cells containing a normal chromsome 5 or the 5q- chromosome were sensitive to killing by diphtheria toxin; cells containing the 3q+ chromosome, but not a normal 5 or an intact 5q- chromosome were resistant to diphtheria toxin. Chromosome and biochemical studies now in progress on informative clones, before and after diphtheria toxin treatment, should allow regional localization of genes involved in expression of Hex B and interferon production.

470 SEGREGATION AND ASSORTMENT OF C BAND VARIANTS OF CHROMOSOME 1, 9, 16. C. G. Palmer, P.L. Yu, L.Y. Wang, A.D. Merritt, P.M. Conneally, R.E. Magenis, M.L. Rivas, Dept. of Med. Genetics, Ind. U. Sch. Med. and Div. Genetics, U. of Ore.

C-band variants of chromosome 1, 9, and 16 were analysed for homolog segregation in 816 individuals from 77 families. When evaluating 1qh, no segregation distortion was present when parents were classed as N (5/4 or 5/6) but when the parent was heterozygous 5/Inv; 5/-(2,3);5/+(7,8), there was a significant deficiency of the normal homologs among offspring in all classes. Several studies have reported an excess of large variants in chromosome 9 segregation; however, the limited number of 9qh+ variants in the present study show no significant segregation distortion.

Pairwise assortment of 1, 9, 16 variants and the Y chromosome was evaluated in 269 progeny from 100 matings. In six pairwise analyses combinations of 1qh normal (5) with minus (3) variants of 9qh were recovered together less frequently in progeny than expected and the normal (5) variants of both chromosomes were recovered together more frequently in offspring than expected when a parent had two heteromorphic pairs. Combinations of chromosome variants 1 and 16 or 9 and 16 did not differ from expected for these pairs in progeny of a parent with heteromorphisms in both chromosomes. The assortment of Y with plus (7 or 8) variants of 1qh was significant.

These data suggest that there may be preferential segregation of plus and minus variants of chromosome 1. Further, assortment of chromosomes with these C-heteromorphisms may not be independent.

471 REDUCED EXCISION OF O^6ALKYLATED GUANINE IN XERODERMA PIGMENTOSUM CELLS AFTER TREATMENT WITH ALKYLATING CARCINOGENS, Regine Goth-Goldstein, Laboratory of Radiobiology, University of California, San Francisco, CA 94143

The alkylation of DNA and the elimination rate of alkylated guanines was compared for normal human fibroblasts and fibroblasts derived from a Xeroderma Pigmentosum (XP) patient. The cells were treated for 45 min with either ^{3}H-methylnitrosourea (MNU) or [1 - ^{14}C]ethylnitrosourea (ENU) and their DNA isolated 0, 24 and 48 hrs after treatment. After mild acid hydrolysis of the DNA the purine bases were separated on a Sephadex G10 column and the amount of guanine alkylated at the N7(7-Alk Gua) or at the O6 position (O^6-Alk Gua) measured. The initial amount of alkylated guanine and the loss of 7-Alk Gua after 48 hrs were very similar in normal and XP cells both after MNU and ENU treatment. But there was a considerable difference between these cell lines in the loss of O^6-Alk Gua. The normal cell line reduced its O^6-Alk Gua content to 28% and 9% for MNU and ENU respectively. In contrast there was still 63% and 70% of the original amount of O^6-Alk Gua in XP cells after this time. This means that XP cells have a reduced capacity to eliminate O^6-Alk Gua from their DNA. Since O^6-Alk Gua is a promutagenic base this deficiency may be a significant aspect of the high cancer susceptibility of XP patients. It also might be connected with the increased sister chromatid exchange in XP-cells after treatment with alkylating agents. Work was performed during the tenure of a fellowship by the Deutsche Forschungsgemeinschaft and under the auspices of the U.S. Energy Research and Development Administration.

472 CHARACTERIZATION OF THE PACKAGING PROTEINS OF CORE 40S HnRNP PARTICLES, Ann L. Beyer, Barbara W. Walker, Mark E. Christensen and Wallace M. LeStourgeon, Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235

The hnRNA of HeLa cells is associated in a repeating ribonucleoprotein particle structure with a simple complement of low molecular weight basic proteins which apparently function structurally in the packaging and stabilization of hnRNA in a manner analogous to the histones of nu bodies. The proteins involved in protein-RNA and protein-protein interactions to form the core 40S monomer particle have been identified and characterized. Through complete sucrose gradient analyses and controlled salt dissociation of particles, 6 polypeptides are identified as the protein constituents of core particles, in association with rapidly-labeled non-ribosomal nuclear RNA. These particles appear in the EM as 210 Å spheres. The 6 proteins migrate in polyacrylamide gels as 3 doublets (groups A,B and C) and are present in a simple stoichiometry. The C proteins (42K and 44K daltons) interact directly with RNA to form a smaller high salt-resistant complex. The A proteins (32K and 34K D) are major nuclear proteins and constitute 60% particle protein mass. These 2 proteins are basic with isoelectric points near 9.2 and 8.4. respectively, they interact electrostatically with nucleic acid (including HeLa DNA) and are characterized by similar unusual amino acid composition, including 25% glycine and the unusual basic residue, N^G,N^G-dimethylarginine. 40S hnRNP particles of Chinese hamster lung fibroblasts have a strikingly similar protein composition and stoichiometry, consistent with a conserved particle structure in eukaryotes. Crosslinking studies with dimethylsuberimidate and formalin, and molecular weight sieving of dissociated particle proteins demonstrate specific protein-protein interactions in the core RNP complex. (Supported by NSF BMS 7503105, NIH 5T01-GM01395)

473 C-band Chromosomal Polymorphisms: Racial Differences and Similarities. Ray M. Antley and Jonggu Park, Indiana University Medical Center, Indianapolis, IN 46202.

Studies indicate a high degree of C-band polymorphisms within individuals. Data emerging from population studies suggest racial differences. To evaluate the influence of race on frequency of C-band polymorphisms, a sample of Koreans, Chinese, Japanese and Caucasians were compared. The variable C-band regions were scored as H-variants (secondary constrictions), C-variants (centromere), S-variants (satellite), and P-variants (position variation of the secondary constriction or centromere). A total of 678 C-band variants (averaging 3.4 ± 0.1 per subject) were scored in 200 subjects. On the average, Orientals were found to have slightly higher frequencies of C-band variants than Caucasians (but the difference was not statistically significant). Most C-band variants showed similar frequencies among Orientals. Combining the results of this study with those of previous studies of Caucasians gave the following results: 1 qh-, Yq+, 19C+, and 9P (position variant) were significantly more frequent in Orientals than in Caucasians, and S-variants of No. 22 were significantly more frequent in Caucasians. From a population cytogenetic point of view, these differences appear all the more interesting when compared to an overall pattern of similarity in frequency for most polymorphisms.

474 DNA FRAGMENT SIZES OF MOUSE TLT HEPATOMA CHROMATIN AFTER AUTODIGESTION OR TREATMENT WITH VARIOUS NUCLEASES, Jacob D. Duerksen and K. Wayne Connor, Department of Biology, The University of Calgary, Calgary, Alberta, Canada T2N 1N4.

Autodigestion of chromatin apparently releases euchromatin-enriched segments (Paul & Duerksen, Biochem. Biophys. Res. Commun., 68, 97(1976); Arch. Biochem. Biophys., 174, 491 (1976)). This autodigestion proceeds slowly producing 21% acid-soluble material after 17 hr. whereas the action of micrococcal nuclease produces 27% in 35 min at 46 U/mg chromatin and DNAase I produces 24% in 25 min at 1.2 µg/mg chromatin. DNA fragments isolated from timed aliquots of these treated chromatin preparations were separated under denaturing conditions by slab gel electrophoresis and fragment sizes were determined with a [5'-^{32}P]p(dT)$_{10}$ polymer mixture standard (10 base pair series from T_{10} to approximately T_{200}; gift from Dr. J.H. van de Sande). DNAase I treatment rapidly produced a fragment pattern 10 bp apart spanning the range from 10 bp (smallest fragment was actually 13 bp) to approximately 200 bp with a prominent band at 80 bp and weak bands at 60 and 100 bp. Short micrococcal nuclease digests gave primarily a prominent doublet band at 140-160 bp. Continued digestion produced in addition smaller fragments approximately 10 bp apart similar to the DNAase I pattern. (Patterns resulting from DNAase II digestion are comparable.) Autodigestion for 1-3 hr, however, produced primarily multiple bands larger than the nucleosome-sized fragment. Continued autodigestion up to 17 hr produces in addition smaller-sized fragment bands 10 bp apart. This pattern caused by 1-3 hr autodigestion is consistent with the previously reported EM pictures showing the multi-nucleosome (370±70Å diameter/nucleosome) size of euchromatin-enriched fractions. Examination of the bp size of the DNA fragments isolated from such euchromatin-enriched fractions is now in progress.

475 IMMUNOLOGICAL MAPPING OF REPLICATING SEGMENTS OF CHROMOSOMES, Howard G. Gratzner, Papanicolaou Cancer Research Institute, Miami, Florida 33123.

Antibodies specific for 5-bromodeoxyuridine (BrdU) or iododeoxyuridine (IdU) have been employed to visualize regions of chromosomal replication. Highly specific antibodies were produced in rabbits by a "carrier-switch" procedure, in which iodouridine (IU) coupled to ovalbumin (IU-oval) was injected, followed 2 weeks later by bovine serum albumin (BSA), followed then by IU-BSA. This procedure results in large amounts of specific antibody. Antibody, subsequent to affinity chromatography on AH-Sepharose 4B-broumouridine, was used to treat spreads of metaphase chromosomes from cells pulsed in S phase with BrdU.

Bands were produced on the chromosomes by the immunoperoxidase procedure, which employs goat anti-rabbit IgG labeled with horseradish peroxidase and 3',3"-diaminobenzidine and H_2O_2. The identification of the bands with incorporated BrdU was verified with ^{3}H-BrdU and autoradiography.

The primary feature of this immunological procedure, aside from its sensitivity, is that it is applicable to electron microscopy. We have also devised a method for preparing chromosomes for scanning electron microscopy, by which combined light and SEM studies of immunocytochemically stained chromosomes can be performed on the same structures. This method entails centrifuging chromosomes onto glass slides containing a numbered grid which can be used to relocate a specific chromosome in the SEM.

476 FINE STRUCTURE OF THE X AND Y CHROMOSOMES, Alberto J. Solari, Departments of Anatomy and Zoology, Duke University, Durham, N.C. 27710

Using the micro-spreading technique of Counce and Meyer (Chromosoma, 44, 231, 1973) as employed by Moses et al. (Science 187,363,1975) in mammals, synapsis and repulsion of the X and Y chromosomes of several mammals has been shown to follow a regular pattern. The axes of the X and the Y appear during zygotene as separate structures. They first approach by one end and then begin to form a pairing region with a synaptonemal complex at late zygotene. The extent of the pairing region is at a maximum in very early pachytene. The differential regions of the axes do not present special modifications up to mid-pachytene. These modifications differ in various species. In the armenian and chinese hamster they consist of thickenings, bulgings and shortenings during mid-pachytene, followed by the appearance of a thinner and branching structure in late stages. The pairing region progressively shortens in some species as the mouse, while it remains intact up to diplotene in the armenian hamster. In both groups during diplotene the pairing region is reduced to a single region of association between the X and Y axes. This single region is confined to the ends in most mammals but it is near the centromeres in the armenian hamster. Although this regular behavior occurs in most species, at least in one mammal the axial cores associate to each other only by the ends and do not show special modifications.

477 EFFECTS OF HEPARIN ON NUCLEOSOME STRUCTURE, Bryant Villeponteaux and Isaac Harary, Laboratory of Nuclear Medicine and Radiation Biology, University of California, 900 Veteran Ave., Los Angeles, Calif. 90024

Nucleosome structure and its possible modification by acidic proteins were studied using heparin and staphylococcal nuclease as probes. The natural polyanion heparin was found to enhance markedly the nuclease sensitivity of DNA within chromatin. Electrophoresis of the products of nuclease digestion reveal that the larger size limit digest DNA fragments are preferentially digested in heparin treated chromatin. Pretreatment of chromatin with trypsin also leads to an increased sensitivity of chromatin to staphylococcal nuclease. The electrophoretic banding pattern of the nuclease resistant fragments from heparin treated chromatin and from trypsin digested chromatin were compared and were found to closely resemble each other. Trypsin is known to cleave only 20 to 30 amino acid residues from the N terminal segments of the nucleosome core histones H2a, H2b, H3, and H4. Thus the similarity of the nuclease resistant DNA products from heparin treated and trypsin digested chromatin indicates that heparin interacts with the same N terminal segments of the core histones as does trypsin. These results suggest that nuclear acidic proteins may interact with the N terminal segments of the core histones to alter nucleosome structure in preparation for transcription. (This work was supported by contract E (04-1) GEN-12 between ERDA and the Univ. of Calif.)

478 CYTOGENETIC ANALYSES OF HUMAN OOCYTES, Frederick W. Luthardt, University of California, Los Angeles, Cal. 90024.

Oocytes were obtained by follicular aspiration from in situ or excised ovaries from 35 women between the ages of 22 and 45 years. Of 50 oocytes, 7 (14%) were classified as atretic, 30 (60%) as nonovulatory, and 13 (26%) as preovulatory. Oocytes were cultured in microdroplets of Ham's F10 medium supplemented with 10% fetal calf serum for 24-28 hr at 37°C in 5% CO_2 in air environment. Cytogenetic analysis indicated that 27/39 (69.3%) of cultured oocytes were undergoing maturation at the time of culture termination. Chiasma frequencies evaluated during diakinesis and early first meiotic metaphase were estimated between 35 and 51. At present, this data is not sufficient to correlate chiasmata frequency with maternal age. Specific staining procedures were also utilized to facilitate centromere localization, identification of specific bivalents, and evaluation of meiotic chromosome association patterns. Cytogenetic data obtained from this investigation may help to elucidate possible consequences of maternal age upon the meiotic process.

479 DNA-AFFINITY CHROMATOGRAPHY OF IN VITRO PHOSPHORYLATED H1 AND H5 HISTONES, Thomas M. Fasy[1], Akira Inoue[2], Edward M. Johnson and Vincent G. Allfrey, The Rockefeller University, New York, NY 10021. Present addresses: [1]The Roche Institute of Molecular Biology, Nutley, NJ 07110, [2]Osaka City University Medical School, AbenoKu,Osaka,Japan.

Phosphorylation of the extranucleosomal histones, H1 and H5, has important and diverse consequences on the structure of the DNA-histone complex which may influence replication, transcription and chromosome condensation. We have used affinity chromatography to compare the DNA-binding properties of phosphorylated and non-phosphorylated histones. Pure H1 histones from calf thymus (C.T. H1) and two highly basic H1 homologues from condensed inactive nuclei, duck erythrocyte H5 (Duck H5) and Strongylocentrotus purpuratus sperm H1 (S. purp. H1) were applied to DNA-Sephadex G-25 columns and eluted with salt gradients. The histone sample contained a small portion (<1%) which had been phosphorylated in vitro by γ-^{32}P-ATP using a cAMP-dependent kinase from calf thymus. Most of the C.T. H1 eluted from the DNA-column between 0.30-0.55 M NaCl with peaks around 0.40 M. The more basic Duck H5 eluted between 0.56-0.78 M NaCl with peaks at 0.60 and 0.65 M. These salt concentrations are quite consistent with those required to extract these proteins from chromatin or nuclei. The extremely basic S. purp. H1 eluted from the DNA column between 0.74-1.03 M NaCl with a peak at 0.84 M. Again this molarity range corresponds very well with our incremental salt extractions of H1 from S. purp. sperm nuclei. In every case, the phosphorylated species eluted from the DNA column at lower salt concentrations than the main histone peak. Experiments are presented which indicate that phosphorylation of H1 causes it to elute from DNA columns at lower salt concentrations.

480 ROLE OF HISTONE PROTEINS IN ULTRAVIOLET LIGHT-INDUCED PROTEIN-DNA ADDUCTS IN CHROMATIN. S. C. Rall and G. F. Strniste, Cellular and Molecular Biology Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87545

We have isolated and purified the nucleosome classes, monomer through tetramer, from nuclease-treated Chinese hamster cell nuclei. In agreement with the predicted model for chromatin substructure, our results indicate that, on the average, there exist two molecules each of histones H2A, H2B, H3, and H4 per chromatin subunit in each nucleosome class. The relative amount of histone H1 increases from monomer through tetramer. Using nucleosomes as a basic model system representing the protein:DNA interactions existing in chromatin, we have been investigating the role of the various histone proteins in the process of ultraviolet (uv) light (254 nm) induction of protein-DNA adducts. Analysis of uv-irradiated nucleosomes on SDS-polyacrylamide gels shows a depletion of all five histones from their normal migrating positions in the gel. Histones H2A and H2B are removed more rapidly than are histones H3 and H4. In addition to protein-DNA adducts, at least 50% of the affected histones are induced to form other photoproducts. One of the primary protein-protein photoproducts observed appears to be the H2A-H2B dimer. In the crosslinking process, irradiated nucleosomes differ from irradiated, sheared, soluble chromatin in that there is more histone involvement but less total protein linked, the latter result most likely being a consequence of the involvement of nonhistone protein in the crosslinking phenomenon observed in sheared, soluble chromatin. (This work was performed under the auspices of the U. S. Energy Research and Development Administration.)

481 SEROLOGIC DETERMINATION OF ALTERED BASES IN DNA AND CHROMATIN, McConlogue, L., H.L. Lewis, A. Norman and J.F. Ward. Laboratory of Nuclear Medicine and Radiation Biology, 900 Veteran Avenue, University of California, Los Angeles, Los Angeles, Calif. 90024.

We are developing serologic assays for DNA base damage induced by UV, ionizing radiation, and chemical mutagens and carcinogens. The goal of this research is to quantitate and to localize lesions and repair of lesions in isolated DNA, chromatin fractions, and in chromosomes and nuclei in situ. The mammalian repair system appears to be heterogeneous with respect to rates and location in the nucleus. Wilkins and Hart (Nature 247,35,1974) provided evidence that heterogeneity is due to decreased repair of DNA masked by chromosomal proteins. We are using a radioimmunoassay (RIA) to study the distribution of DNA repair of UV induced lesions in fractionated chromatin. The RIA, a modification of the procedure of Seaman et al. (J.Biol.Chem. 247, 5709,1972), can detect lesions in 1 µg DNA extracted from cells irradiated with 3 J/M^2 (254 nm), corresponding to ~ 10" dimers. It has been used to study the rates of UV induced lesion production in cellular DNA. Measurements of the rates of lesion production and removal in "histone bound" and "histone free" chromatin and in "active" and "inactive" chromatin will be presented.

Antibodies have also been prepared to 5 hydroxymethyl uridine (5HMU), an ionizing radiation product of thymidine. Using a phage neutralization assay picogram levels are detected. The G value for the production of 5 HMU in irradiated solutions of thymidine is 0.04. 5HMU has been measured in irradiated DNA and data will be presented on the yields within chromatin fractions. (Supported by USPHS Grant #CA 13437 and by USERDA)

482 A CHROMOSOMAL AND DNA REPAIR LOOK AT INCONTINENTIA PIGMENTI. J.M. Rary and P.C. Huang, Department of Gynecology and Obstetrics, School of Medicine and Department of Biochemical and Biophysical Sciences, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland 21205.

Incontinentia pigmenti, a dominant X linked disorder that is lethal in affected males, was originally reported as a chromosomal breakage syndrome by DeGrouchy et al. (1976 Ann. Genet. 15, 61) and has been recently confirmed by Kelly et al. (1976 J. Heredity 67, 171). Surviving affected females are characterized by a three to four fold increase in the chromosomal aberration frequencies in leukocytes and fibroblasts. Most of the aberrations scored are of the chromatid type, although the frequency of the chromosomal type aberration is also elevated. The results were obtained from measurements with leukocytes from three affected females and skin fibroblasts from one affected female and one affected male fetus. Since the aberration frequency is further increased by gamma irradiation in the G_2 phase of the cell cycle, enzyme activities presumably related to DNA strand breakage-reunion as well as removal of modified bases were examined. The results on single and double stranded DNase, N-glycosidase and apurinic site specific endonuclease in lysates of these and normal cells are to be reported. (Supported by National Foundation/March of Dimes).

483 CRITICAL DNA DAMAGE AND HAMSTER CELL REPRODUCTION, H.J. Burki, Biology and Medicine Division, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720

Synchronous V79 Chinese hamster cells accumulated I-125 induced DNA damage in the G2 stage of the life cycle at 4 degrees centigrade. The position of the I-125 within the nuclear DNA was changed by incorporating I-125 Iododeoxyuridine at various times during the previous DNA replication period. The efficiency of cell inactivation induced by the decay of this isotope depended on the position of the isotope within the nuclear DNA. It was found that the efficiency of inactivation was less when decays occurred in the DNA usually associated with euchromatin, i.e., early replicating DNA. The decays were found to be most toxic when they occurred in late replication DNA, or in heterochromatic and centromeric regions. These results suggest that damage in late replicating DNA may critically affect cell reproduction.

484 SISTER CHROMATID EXCHANGES AND CHROMOSOME ABERRATIONS IN COCKAYNE'S SYNDROME, A POSSIBLE DNA REPAIR DEFICIENCY DISEASE, Harlow K. Fischman and Catherine Joy, Department of Medical Genetics, New York State Psychiatric Institute, New York, N. Y. 10032. Cockayne's Syndrome is a genetic disease with autosomal recessive inheritance. It is characterized by microcephaly, dwarfism, pigmentary retinal degeneration, precocious senility, and sensitivity to sunlight. Cockayne's syndrome cells have an increased sensitivity to U V irradiation, and depression of unscheduled DNA synthesis. Chromosome damage is increased in conditions known to include DNA repair deficiency. We investigated the possibility of such a defect in a 16 year old female with Cockayne's syndrome. A chromosome analysis using G- and Q- banding, revealed a normal 46,XX karyotype. A 72 hour leucocyte culture was examined for spontaneous chromosome damage. There was a high level of both total chromosome aberrations (26/100 cells) and chromosome and chromatid breaks (11/100 cells). A 72 hour leucocyte culture was subjected to a modification of the BUdR/Benzimidizole technique, and revealed an elevated number of sister chromatid exchanges (SCE's) (26.3/cell) when compared to the control (12.5/cell). The number of SCE's was directly proportional to chromosomal length in longer chromosomes but was significantly reduced in the F and G groups. The high level of both chromosome damage and SCE's, supports the conclusion that Cockayne's syndrome involves a defect in the DNA repair system.

485 STUDIES OF MITOMYCIN INDUCED SISTER CHROMATID EXCHANGE AS A FUNCTION OF CELLULAR AGING IN VIVO AND IN VITRO, Edward L. Schneider, David Kram, Raymond R. Tice, Peter Gianas and Brian Gilman, Laboratory of Cellular and Comparative Physiology, Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

There is considerable evidence that sister chromatid exchanges (SCE) may represent a form of postreplication DNA repair. It was, therefore, decided to examine if the mechanisms involved in SCE are altered by aging both in vitro in cultured human cells and in vivo in mouse and rat bone marrow cells. For the in vitro studies, cultured human skin fibroblasts derived from old (65+ yrs) and young (20-30 yrs) volunteer members of the Baltimore Longitudinal Study were incubated with 10 μg/ml bromodeoxyuridine (BrdU) together with varying concentrations of Mitomycin C (MMC) and chromosome preparations were made 48 hours after BrdU addition. For the in vivo studies, young (12 mo) and old (24 mo) rats and mice were infused intravenously with 50 mg/kg wt/hr BrdU. MMC was administered one hour after commencement of the infusions and chromosome preparations were made at 26 hours. Preliminary results indicate that both in vivo and in vitro MMC induced SCEs occur with relatively equal frequencies in both old and young cells at low MMC concentrations. However, at high MMC concentrations (7.5 ng/ml in vitro, 5 mg/kg in vivo), there is a significant decrease in MMC induced SCE in the fibroblasts derived from old human subjects when compared to young subject cell cultures (36.7 ± 2.2 vs 44.2 ± 1.3) as well as from old rat bone marrow cells when compared to young rat bone marrow cells (46.3 ± 2.4 vs 76.7 ± 2.7). These findings suggest that with increasing age, mammalian cells both in vitro and in vivo may lose their ability to form SCE in response to high levels of MMC.

486 SEQUENCE COMPLEXITY OF TOTAL POLY(A) CONTAINING RNA FROM MOUSE SIMPLE EMBRYOID BODIES AND TERATOCARCINOMAS AS DETERMINED BY C-DNA HYBRIDIZATION, Stephen E. Harris and Sandra Gipson, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

The mouse teratocarcinoma system appears to be an ideal system for studying the molecular mechanisms of cellular differentiation in a mammalian system. The OTT 6050 teratocarcinoma was used in the following studies. Total poly(A)-containing RNA was isolated from simple embryoid bodies, less than 100 μ in diameter, and from tumors derived from these embryoid bodies, which were 1 cm to 2 cm in diameter, using oligo dT-cellulose chromatography. The RNA samples were further purified by another oligo dT-cellulose column. These preparations contained 2.3-3% poly(A) and the poly(A) containing molecules have a number average nucleotide length of 1600-1900 nucleotides as determined by denaturing formamide sucrose gradients. The size of the poly(A) region is approximately 70 nucleotides in both preparations. A complementary DNA labeled with tritiated dCTP of both poly(A) containing RNA preparation was synthesized using reverse transcriptase. The mean size of the cDNA was about 400 NT and the probe used for the complexity study was fractionated on alkaline sucrose gradients and contained molecules 250 to 1200 NT with a mean size of 750 NT. The sequence complexity of the poly(A) containing RNA from the embryoid bodies and the tumors was then determined by hybridization with their respective complementary DNAs. By computer analysis the hybridization curves were resolved into high abundancy-low complexity and low abundancy-high complexity poly(A) containing RNA classes.

487 THE SIGNIFICANCE OF STRUCTURAL VARIANTS OF COMPLEX-FORMING HISTONES. Alfred Zweidler, Samuel G. Franklin and Patricia Goldman, The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

We have characterized a number of primary structure variants of the histones 2a, 2b and 3, which have been preserved in parallel throughout the evolution of at least the mammals. The variants differ by one to three conservative amino acid substitutions in their hydrophobic regions. We have found that the histones can be transferred quantitatively from chromatin to Amberlite CG-50 (in 10 mM Tris-Cl pH 7.0), as H2a-H2b dimers, H3-H4 dimers and H1 monomers, and that the histone complexes which contain the more hydrophobic variants are more resistant to dissociation by urea. Careful quantitation of the histone components resolved by gel electrophoresis in presence of nonionic detergents showed that although the relative amounts of the variants vary significantly, the four complex-forming histone classes occur in equimolar amounts in all examined tissues. We conclude that all H2a, H2b and H3, H4 histones occur as heterodimers which in association with DNA assemble into oligomeric nucleohistone complexes containing equimolar amounts of the two dimer types. We propose that the whole genome of eukaryotes is organized as nucleohistone complexes, but that different regions have different conformations according to their functional state. While transient changes in conformation could be induced by charge modification of the histones, stable conformation differences could be due to the presence of different variants. The tissue-specific variation in the relative amounts of the histone variants could therefore reflect the differential gene activity associated with cell differentiation. (Supported by grants from the NIH and the Commonwealth of Pennsylvania.)

488 KARYOTYPE-PHENOTYPE CORRELATION IN THREE CASES OF STRUCTURALLY ABNORMAL X CHROMOSOME. Dagmar K. Kalousek, The Montreal Children's Hospital, Montreal, Canada.

1. 46,X,del(X)(p11) A case of sex-chromatin positive in other aspects classical Turner syndrome in 14 years old girl.

2. 46,X,del(X)(p21) A case of sex-chromatin positive 12 years old female with short stature, multiple nevi, short IV and V metacarpi, normal levels of gonadotropins and irregular menstruation.

3. 47,XY,i(Xq) A case of sex-chromatin positive phenotypically normal male investigated for sterility.

Karyotype-phenotype correlation and the tabulated review of the related literature will be presented.

489 A SLOWLY SEDIMENTING COMPONENT FROM NUCLEASE DIGESTED CHROMATIN
Caroline S.M. Tahourdin and Hannah J. Gould, King's College, London WC2

Chicken reticulocyte chromatin was prepared in a "gentle" way, minimizing the concentrations of salt and divalent cations and minimizing shearing, in order to prevent the migration of proteins from their original attachment sites on DNA. This chromatin was digested under mild conditions with deoxyribonuclease from Staphylococcus aureus and fractionated by zonal centrifugation in a sucrose gradient. In addition to the series of nucleosome oligomers observed by other workers, it has been possible to resolve a more slowly sedimenting nucleoprotein fraction which has interesting properties. This fraction contains equal quantities of nucleic acid and protein. The nucleic acid component is 50% DNA and 50% RNA and the DNA length has been estimated to be about 30 nucleotides. The DNA represents about 2 - 4% of the original DNA and is single-stranded. The protein content of the slowly sedimenting fraction differs dramatically from that of nucleosomes for it is made up almost exclusively of non-histone proteins rather than histones. Most of the nuceic acid and protein appears to be non-covalently associated. Non-histone proteins have been implicated in globin gene activation in reticulocytes. Thus the DNA sequence isolated may be of special significance in transcription.

490 THE PHOTOADDITION OF TRIMETHYLPSORALEN TO DROSOPHILA MELANOGASTER NUCLEI; A PROBE FOR CHROMATIN SUBSTRUCTURE, John E. Hyde, Gary P. Wiesehahn, and John E. Hearst, Department of Chemistry, Univ. of Calif. Berkeley, California 94720

Derivatives of the furocoumarin, psoralen, which can covalently cross-link the DNA double helix, have been used as a probe for chromatin structure. The DNA in Drosophila melanogaster nuclei and intact cells is protected from light-induced psoralen binding to the extent of about 90% relative to purified DNA. Psoralen treated nuclei have been digested with nucleases and the location of the bound drug examined by gel electrophoresis. Micrococcal nuclease was found to attack photoreacted nuclei in such a way that psoralen-containing regions of the DNA were preferentially excised. Psoralen derivatives thus appear to bind at the regularly disposed sites in chromatin that are particularly sensitive to nuclease digestion.