CELL REPRODUCTION: Honoring Daniel Mazia
E. R. Dirksen and D. Prescott
March 19 – March 24, 1978

Plenary Sessions and Workshops

Poster Sessions

Macromolecular Control of Cell Proliferation

713 THE CELL PERIPHERY AND PROLIFERATION CONTROL, Max M.Burger, Kurt Ballmer and Ralph J. Mannino, Department of Biochemistry, Biocenter of the University of Basel, Basel, CH 4056, Switzerland.

Proliferation control in culture at high cell density remains still a problem without a solid explanation on molecular grounds. In debate are inhibitory effects by the cell periphery (1) of crowded cells on the uptake of growth factors from the medium, of nutrients or of ions as Ca^{++}. On the other hand not sufficiently considered are neighboring effects of crowded cell populations on the cytoskeleton or mechanical effects in general.

An increasing list of surface alterations found originally in transformed cells has now been observed in untransformed cells as well, but only during mitosis and early G_1 (2). If this alteration reflects or is somehow causally related to the maintenance of the cell cycle, then some surface modifications of untransformed cells may alter the cell cycle but only if applied during the sensitive period, namely M and early G_1. Succinyl Con A (suc Con A) inhibits 3T3 cell growth only if present during M or early G_1 (2).

In search for a signalling mechanism between the plasma membrane in mitosis and the nucleus the question was raised whether some novel proteins are incorporated into the newly assembled nuclear membrane after mitosis. Isotopically labelled nuclear membrane proteins before mitosis reappeared, however, in the new nuclear membrane after mitosis without significant changes (3).
As expected suc Con A inhibited also the growth of transformed cells to higher densities. This inhibition was reversible with α-methyl mannose, density dependent and the cells were, against a widespread dogma, primarily arrested in G_1. Possible mechanisms of action of suc Con A will be considered.

Suc Con A reversed also another phenotype of the transformed cell: anchorage independence in soft agar could be suppressed, and this effect was inhibited with just about the concentration of α-methyl mannose which corresponds to its dissociation constant with suc Con A.

1. Stoker, M.G.P., Nature, 246, 200-203 (1973)
2. Mannino, R.J. and Burger, M.M., Nature, 256, 19-22 (1975)
3. Sieber-Blum,M. and Burger, M.M., Biochem. Biophys. Res. Communs., 74, 1-8 (1977)

714 THE ROLE OF RNA PROCESSING IN GENE EXPRESSION, Robert P. Perry, The Institute for for Cancer Research, Philadelphia, PA 19111.

In living cells the expression of heritable information involves the selective transcription of genes into a variety of RNA molecules, which in turn participate in the synthesis of proteins. In most instances the initial products of gene transcription are not identical to the functionally competent, mature forms of RNA. Rather, the transcripts are larger molecules which must be converted to the mature forms by molecular alterations known as "processing". In the case of messenger RNAs (mRNAs) which contain the information specifying the amino acid sequences of proteins, processing may include cleavage of the transcripts and selective degradation of the non-conserved portions, formation of 5' terminal cap structures and 3' terminal poly A segments, and the methylation of particular internal adenine residues. Collectively these reactions could be used as a means of regulating the functional output of various genes and of verifying the authenticity of gene transcripts. Moreover, the recent discovery that several eukaryotic genes are organized in such a way that two or more non contiguous DNA segments are used to specify a single covalently continuous mRNA molecule suggests that processing may be the principal way in which higher organisms cope with extensive scrambling of DNA sequences. In this presentation I will cover some of the current ideas about processing pathways for messenger RNA and will discuss the possible biological significance of the processing reactions.

715 ON THE FIDELITY OF DNA REPLICATION. Lawrence A. Loeb, The Institute for Cancer Research, Philadelphia, Pa. 19111

The high accuracy of DNA replication is achieved by a multistep process. Differences in free energy between correct and incorrect base-pairs account for a fidelity of 10^{-2}. DNA polymerases can provide additional selectivity at the catalytic site increasing the fidelity to 10^{-3} - 10^{-5}. Procaryotic DNA polymerases contain an associated exonuclease which could excise mis-incorporated bases during polymerization or during post-replicative repair. Evidence exists for cellular endonucleases that can excise non-complementary nucleotides after the replication process is completed.

Different polymerases copy homopolymer templates with varying fidelity.

Enzyme	Error Rate
Reverse Transcriptase	1/500 - 1/5000
Human Placenta DNA polymerase-α	1/8000
Human Placenta DNA polymerase-β	1/20,000 - 1/40,000
T_4 DNA polymerase	1/10,000
E. coli DNA polymerase I	1/30,000 - 1/80,000

If the reaction mixture is modified by alterations in nucleotide pools, depurination or alkylation of templates the addition of activating or non-activating metal cations or other mutagens, fidelity is decreased. Thus, measurements of the fidelity of DNA synthesis may provide a means of testing for certain classes of mutagens and/or carcinogens.

An assay has been developed for measuring mutagenesis in vitro with a natural DNA template. The template, ØX 174 DNA containing an amber mutation with a restriction DNA fragment as a primer is copied by a DNA polymerase in vitro. Errors are determined by infecting E. coli spheroplasts with the copied DNA and measuring the reversion of progeny phage to wild type. Using this assay, E. coli DNA polymerase I makes less than 1 in 9000 mistakes, while the error rate of a DNA polymerase from an RNA tumor virus was estimated as 1/706 ± 300. These initial results indicate that measurements of fidelity with homopolymers correlate with those obtained with ØX DNA. This assay provides a new probe for determining the role of replicative proteins in the fidelity of DNA synthesis.

Control of Nuclear Expression

716 GENETIC CONTROL OF RNA METABOLISM, Barry I. Kiefer, Department of Biology, Wesleyan University, Middletown, CT 06457.

rDNA-deficient mutants of Drosophilia melanogaster have been utilized to discover and elucidate a variety of compensatory strategies associated with different genetic and developmental circumstances. We have demonstrated that the rate of accumulation of rRNA is not directly dependent on the amount of rDNA present as measured by saturation hybridization, and that the phenotype (bristle length, development time) is directly related to rate of rRNA accumulation rather than rDNA content. A specific Y chromosome (YbbSuVar-5) stimulates an increased rate of 28S + 18S, 5S, 4S, and Poly-A RNA synthesis in rDNA-deficient genotypes, but has no effect when paired with a wild-type X chromosome. This demonstrates the presence of a regulatory region on the Y, and suggests that what is being monitored is the rDNA content of the X chromosome. Spermatogenesis in rDNA-deficient males is defective. In many spermatids Nebenkern and axoneme differentiation is abnormal and many spermatids degenerate. However, functional sperm are produced but they are 20-30% shorter than wild-type. The defects in spermiogenesis can be related to the fact that although rDNA-deficient adults have approximately the same total amount of RNA as wild-type, spermatids in rDNA-deficient males contain significantly fewer ribosomes. The presence of the YbbSuVar-5 chromosome restores wild-type bristle length and development time in rDNA-deficient males but has no effect on spermatogenesis. These results suggest the existence of cell specific regulatory mechanisms for a given set of genes (rDNA). (Supported by NIH grant HD19130).

717 SEA URCHIN HATCHING PROTEASE: TEMPORALLY CONTROLLED TRANSLATION OF MATERNAL MESSAGE, Dennis Barrett, Dept. of Biological Sciences, University of Denver, Denver, CO 80208.

Minutes after fertilization, the sea urchin egg begins to synthesize protein at a sharply increased rate, by newly engaging mRNAs pre-transcribed in the oocyte. The kinds of proteins thus made on the maternal messages are diverse, but are not novel; their synthesis generally resumes the patterns of oogenesis. Sixteen hours later at blastula stage, a hatching protease enables the embryo to digest its way out of the confining fertilization envelope. The demonstration that this enzyme is a novel product, employing maternal message whose activation is delayed until blastula, requires two parts.

A. Transcription of mRNA for hatching protease is completed during oogenesis. Two indirect lines of evidence support this contention: anucleate half-eggs, activated by parthenogenesis, are devoid of nuclear transcription, but hatch nonetheless; reciprocal hybrids between Strongylocentrotus purpuratus and S. franciscanus develop normally, and in each case the hatching protease, characterized by sensitivity to Mn^{2+}-inhibition, reflects only maternal information.

B. Translation of hatching protease does not begin until blastula stage. Timing of synthesis has been directly determined in pulse-chase experiments. Embryos are exposed to a 4-h pulse of ^{14}C-valine, and then a chase of ^{12}C-valine; after the hatching protease is secreted it is purified to homogeneity and counted. Embryos pulsed at 0 to 12 h after fertilization secrete unlabelled enzyme; embryos pulsed at 12 to 16 h secrete labelled enzyme.

Thus the egg stores a large class of inactive messages, to be activated en bloc shortly after fertilization; but these results suggest that it stores another class of messages, to be individually activated at specific stages, at least as late as blastula, when the progress of embryogenesis requires the appearance of their products.

718 PREREPLICATIVE PHASE IN DIFFERENTIATED CELLS STIMULATED TO DEVIDE IN REGENERATION SYSTEM, M. Gontcharoff, Biologie Cellulaire, Université de Reims, 51062 Reims Cedex.

During embryonic development cells differentiate and stop to devide. Adult cells are arrested in G_1 or G_0 (1).

In two regeneration system plants *Vicia faba* and newts *Triturus cristatus*, adult cells stimulated to proliferate before regeneration, it can be shown that cell division prior to morphogenesis is a prerequisite for regeneration.

Stimulated to devidé important events occur before mitosis, during the prereplicative phase of the cell cycle, Protein synthesis, Poly(A)RNA synthesis, early DNA synthesis (2)(3).

Structural properties of chromatin are modified, nuclear chromatin is spread in the nuclear sap, scanning cytophotometry of stimulated and quiescent cells show striking chromatin dispersion (4).

Chromatin extracted from nuclei of stimulated and quiescent cells were separated by affinity chromatography using *E.coli* RNA polymerase immobilized on agarose gel (5).

The percentage of euchromatin increased in cells stimulated to proliferate.

Inhibition of RNA protein or early DNA synthesis in prereplicative phase prèvents regeneration.

REFERENCES.

1. BASERGA R. - Multiplication and Division in Mammalian Cells. Vol. 6, in "The Biochemistry of Disease. M. DEKKER inc. New-York (1976)
2. DESSELLE J.C. - Cytophotométrie des acides nucléiques dans les cellules musculaires de membres en régénération, de membres irradiés aux rayons X, de membres irradiés réactivés par implant de cartilage chez *Triturus cristatus*. Acta Embryologiae Experimentalis, 3, 207-235 (1974)
3. JEANNY J.C. et M. GONTCHAROFF. - Synthèse de l'ADN au cours des phases précoces de la régénération des membres postérieurs de *Desmognathus fuscus*. C.R. Acad.Sc.Paris 278, 1513-1516 (1974)
4. JEANNY J.C. et M. GONTCHAROFF. - Etude en microscopie électronique et par cytophotométrie "scanning" de la structure et de la distribution de la chromatine dans les noyaux de cellules cartilagineuses de *Triturus cristatus* au cours de la sénescence. (sous presse)
5. JALOUZOT R., M. GONTCHAROFF et D. BRIANE. - Fractionation of Plant chromatin with immobilized RNA-polymerase. Feb Letters 59, 2, 221-224 (1975)

719 CONTROL OF SPERM CHROMATIN STRUCTURE BY EGG CYTOPLASM IN THE SEA URCHIN, Dominic L. Poccia,* Geoffrey Krystal,* David Nishioka,** and Jane Salik,* Department of Biology SUNY, Stony Brook, NY 11794,* and Hopkins Marine Station, Pacific Grove, CA 93950**

Sea urchin eggs activated by ammonia begin succesive cycles of DNA synthesis and chromosome condensation but do not divide (1). These activated eggs may be polyspermically fertilized at any time during the cell cycle, thus synchronously introducing large numbers of identical nuclei into the egg cytoplasm. When fertilization is shortly after activation and moderate (male pronuclei/egg < 20), the male chromosomes will replicate within the first cell cycle and condense synchronously with the female chromosomes; at higher degrees of polyspermy the cell cycle is progressively lengthened but both male and female chromosomes still condense at the same time. When fertilization is during G_2, the sperm chromatin first decondenses, then without having replicated condenses synchronously with the maternal chromosomes. This premature chromosome condensation does not require transcription from the female or male pronuclei or mitochondria, nor mRNA carried by the sperm cell, and is independent of protein synthesis after late S or early G_2. Conditions promoting premature chromosome condensation can be achieved in enucleated cytoplasms prepared by density gradient centrifugation or manual bisection, then activated by ammonia. Although initiated at the same time in both nucleated and enucleated egg halves, the conditions for premature chromosome condensation are much more labile in the enucleates. Thus the conditions for promoting chromosome condensation appear to be cytoplasmically initiated but stabilized by some element of the maternal pronucleus. The potential utility of the polyspermic sea urchin egg as a system for the biochemical study of changes in chromatin during major structural and functional transitions is discussed. Preliminary results indicate a loss of sperm specific H1 histone during male pronuclear decondensation.

1. Mazia, D., Proc.Natl.Acad.Sci.USA 71: 690 (1974).

Molecular Control of Cell Proliferation and Growth

720 SELECTION AND EVOLUTION OF YEAST CELLS IN A CHEMOSTAT, Thomas W. James, Department of Biology, University of California, Los Angeles, CA 90024

Selection based on the energetic cost of gene maintenance has important implications in cell evolution (1). Yeast is one eucaryotic system in which selection can be measured readily with a chemostat. Two methods were used in which respiratory deficient cytoplasmic mutants, petites, were examined relative to parental wild type cells in anaerobic chemostats in which glucose was used as the energy-limiting substrate. The first method involves the comparison of growth rate vs substrate concentration plots. Under these conditions linear Lineweaver-Burk plots are obtained and Michaelis-Menton kinetics apply. Consequently, a specific strain or mutant can be characterized by a growth rate maximum (V_{max}) and a glucose affinity constant (K_m). These characteristics were obtained by setting the chemostat to a series of growth rates (dilution rates) and measuring the glucose concentration at each with a glucose electrode. When these characteristics are compared for wild type and petite cells by superimposing their Michaelis-Menton plots, it can be seen that at lower substrate concentrations the petite mutant has the selective advantage. A plot of the numerical difference between any two Michaelis-Menton curves against the substrate concentration allows a pairwise comparison of strains and gives regions of substrate concentration where growth rate differences go through a maximum. This difference function indicates that for a specific pair selection is in favor of the mutant in regions of low concentration or growth rate, while at higher concentrations the wild type ought to have the selective advantage. This suggests that there is a substrate concentration at which selection is neutral for the two strains. Since the function obtained by this method can be determined by another method, a direct test was undertaken. It consists of measuring the change in ratio of wild type to mutant cells which occur with time in a mixed culture chemostat (2,3,4). The slope of the logarithm of this ratio against time yields a value which is the difference in growth rates of the two strains and is identical to the differences obtained by the previous method. When this procedure is applied to mixed wild type and petite cells maintained at a growth rate of 0.092/hr, i.e., a generation time of 7.5 hr., selection is in favor of the mutant. The differences in the growth rate at this generation time and substrate concentration are 0.045/hr for the first and 0.047/hr for the second indicating that the two methods are compatible. Comparable data at other substrate concentrations are being obtained. This work is in good agreement with what has been found in procaryote cells (3,4). It also indicates that eucaryotic mitochondria are subject to the same energetic selection pressures as are nuclear systems.

1) James, T.W. (1969). In The Cell Cycle (G. Padilla & I.Cameron, eds.) Vol.1, Acad.Press, N.Y., p.3.
2) Moser, H. (1958). Carnegie Institution of Washington Publication #614.
3) Zamenhof, S. & H. Eichhorn (1967). Nature, 216:456.
4) Dykhuizen, D.E. (1971). Ph.D. Dissertation, University of Chicago.

721 BIOCHEMICAL GENETICS OF COLCEMID SENSITIVITY, Seymour Lederberg and Dan Sackett, Div. Biology & Medicine, Brown Univ., Providence, RI.

Nuclear division, cell wall deposition and cell division of the fission yeast, Schizosaccharomyces pombe, are inhibited by concentrations of colcemid in the millimolar range (1). Mutants resistant to this level of colcemid have been isolated (2). These mutants do not modify the drug and are still as permeable as the parent strain to colcemid (3). Our presentation deals with the interaction of colcemid with cellular elements and with the perturbation of membrane functions and growth control by colcemid and by mutation to colcemid resistance.

1) Stetten, G. and Lederberg, S. (1973) J. Cell Biol. 56, 259-262.
2) Lederberg, S. and Stetten, G. (1970) Science 168, 485-487.
3) Lederberg, S., Gourse, R. and Sackett, D. (1977) J. Bacteriol. 129, 198-201.

722 RIBOSOMAL PROTEIN PHOSPHORYLATION AND CONTROL OF CELL GROWTH, Edwin H. McConkey, Stanley M. Lastick, & Peter J. Nielsen, Dept. of Molecular, Cellular & Developmental Biology, University of Colorado, Boulder, Colorado 80309.

The small ribosomal subunit protein S6 is phosphorylated in many eukaryotic cells in response to a wide variety of stimuli. In high density, slowly growing suspension cultures of HeLa cells, S6 is essentially unphosphorylated. Transfer of cells to fresh medium plus serum induces phosphorylation of S6, culminating in two hours, when virtually every molecule of S6 has acquired approximately 5 atoms of phosphorus. Stimulation of phosphorylation is even more rapid if insulin is substituted for serum. At physiological levels of insulin, amino acids are required for the phosphorylation; amino acids alone cause variable phosphorylation.

Cyclic AMP and cyclic GMP and their dibutyryl and 8-bromo derivative do not stimulate phosphorylation of protein S6 either in the presence of fresh medium lacking serum or in Earle's salts. Intracellular levels of cAMP and cGMP do not rise when cells from a high density culture are resuspended in fresh medium plus serum or insulin - i.e., conditions that lead to maximal phosphorylation of S6. Two-dimensional gel analyses of total cell proteins synthesized in ½ hour by control and by serum-stimulated or insulin-stimulated HeLa cells show no major differences. The functional implications of these and related observations will be discussed.

723 PROTEIN SYNTHESIS AND RADIATION-INDUCED MITOTIC DELAY, Ronald C. Rustad, Division of Radiation Biology, Dept. of Radiology, Case Western Reserve University, Cleveland, Ohio 44106

The radiation-induced inhibition of cell division was discovered over 100 years ago. The obvious cytological effects of a G_2 and/or prophase delay are know to include inhibition of chromosome condensation and of elaboration of the mitotic apparatus. We* have been testing our hypothesis that radiation interferes with the synthesis of "mitotic proteins" and/or creates the need for the synthesis of "recovery proteins".

Initially, it was shown that the protein synthesis inhibitor puromycin extended the period of radiation sensitivity and vice versa in the mitosing sea urchin egg. Both prefertilization and post fertilization radiation recovery were later shown to be inhibited by the drug. These results were generalized in other laboratories working with mammalian cells in culture and with higher plants. In a sychronously dividing plasmodial slime mold, we find that the sensitivity first to the protein synthesis inhibitor cycloheximide and then radiation are lost when the division of irradiated nuclei can still be blocked by the drug.

The reduction of radiation-induced mitotic delay be caffeine in other mitotic systems has been confirmed. Structurally similar chemicals are found to have the same effect, but some drugs which elevate cyclical AMP content actually increase radiation-induced mitotic delay. Thus, the possible involvement not only of protein synthesis per se but also processing of proteins in the mitosis in irradiated cells will require further investigation.

*Collaborations with and individual investigations by E.N. Brewer, B.M. Burchill, P.M. Failla, and N.L. Oleinick are acknowledged. Supported by the Department of Energy.

Mitosis and the Mitotic Apparatus I

724 ADVANCES IN VISUALIZATION OF MITOSIS IN VIVO, Gordon W. Ellis, Program in Biophysical Cytology, Department of Biology, University of Pennsylvania G-7, Philadelphia, PA 19104.

A new form of light microscopy has been devised. It is single-sideband edge-enhancement (SSEE) microscopy (G.W. Ellis, 1977, J. Cell Biol., 75, 247a). Microscopes of this form extend our ability to monitor mitosis in individual living cells. Using illumination that is not absorbed by the object (this is important for cell survival), the image is intensity modulated as a function of optical path difference in the specimen or as a function of rate of change of optical path through the specimen or as combinations of both. Thus, the image can resemble a phase contrast image with a much reduced halo and improved resolution, a differential interference contrast (DIC) image, or intermediate combinations of both.

The optical path differences to which the microscope responds are both those due to isotropic differences in refractive index and those due to birefringence. In the latter case the effective direction of the electric vector of the light probing the specimen is selected after the light has passed the objective and may be changed without moving the specimen. In common both with phase contrast and DIC systems, contrast is enhanced for higher spatial frequency components of the specimen image, thus yielding improved definition at edges. Unlike most phase contrast systems and in common with all DIC systems image contrast in SSEE is a function of specimen orientation. However, unlike DIC images, the directional dependence of image contrast in SSEE images is in addition spatial-frequency dependent with the highest spatial frequencies being the last to be lost. Consequently, the directionality of image contrast is less obtrusive with SSEE than with DIC. Nevertheless a rotatable stage should be used with either system.

In its preferred form, the single-sideband edge-enhancement microscope does not require special objectives or condenser turrets, does not require expensive crystalline components and does not require that the objective and condenser operate between crossed polars. As a result, such microscopes should be economical and extremely flexible in application. Culture chambers, perfusion chambers and temperature and/or pressure control chambers, which can be difficult to use with polarizing or DIC microscopes because of chamber birefringence, are readily accessible to SSEE microscopy. Therefore, observation of mitotic events under many different experimental conditions can be facilitated by use of single-sideband edge-enhancement microscopy.

Supported by NSF Grant #BMS 7500473 and NIGMS Grant #23475.

725 CALCIUM-REGULATING SYSTEMS OF THE MITOTIC APPARATUS, Christian Petzelt, Institut f. Zellforschung, Deutsches Krebsforschungzentrum Heidelberg, D 6900 Heidelberg 1, West Germany.

The mitotic calcium-activated adenosine triphosphatase (E.C. 3.6.1.3.) was first described in sea urchin eggs to occur (1,2). Subsequently, the enzyme was found in nearly all eucaryotic cells studied, and its involvement in the establishment and maintenance of the mitotic apparatus was tentatively identified (3). We have now purified the enzyme to almost total homogeneity using the following procedure: A cell homogenate is washed in several buffers of different ionic strengths, each time the supernatant of the homogenate after a high speed centrifugation is discarded. A protein fraction of the pellet containing the enzyme is brought into the solution by adding Triton X-100 in a ratio of protein to detergent =1. This protein mixture is further purified by ion exchange chromatography on DEAE-cellulose using a continuous gradient of 0-0.2 M NaCl and 0.1%-0.5% Triton X-100. The enzyme consists of several identical subunits each being a dimer with a molecular weight of 55K and 56K, respectively. The molecular weight of the native enzyme is approximately 250K (4). These results and the recently discovered preferential localization of a calcium-dependent regulator protein in the mitotic apparatus (5) and its known capacity to activate calcium-dependent enzymes may give us a better understanding of the regulation of calcium-ions in the mitotic apparatus.

1. Mazia, D., Petzelt, Ch., Williams, R. O., Meza, I., A Ca-activated ATPase in the mitotic apparatus of the sea urchin egg. Exptl. Cell Res. 70, 325-332 (1972).
2. Petzelt, Ch., Ca^{2+}-activated ATPase during the cell cycle of the sea urchin Strongylocentrotus purpuratus. Exptl. Cell Res. 70, 333-339 (1972).
3. Petzelt, Ch., Auel, D., Synthesis and activation of the mitotic Ca^{2+}-ATPase during the cell cycle of mouse mastocytoma cells. Proc. Nat. Acad. Sci. (U.S.) 74, 1610-1613 (1977).
4. Auel, D., Ph.D. thesis, University of Heidelberg, 1978.
5. Welsh, G. J., Dedman, J. R., Brinkley, B. R., Means, A. R., Calcium-dependent regulator protein associated with the mitotic apparatus of mammalian cells. J. Cell Biol. 75, 262a (1977).

726 COMPARISON OF MITOTIC AND OTHER TUBULINS, T. Bibring and J. Baxandall, Dept. of Molecular Biology, Vanderbilt Univ. Nashville, TN 37235

Three functionally distinct tubulins from the sea urchin have been compared by electrophoresis in polyacrylamide gels containing SDS and urea (1) and by gel electrofocusing of cyanogen bromide peptides (2). Tubulins from mitotic apparatus of the embryo, from the A microtubule of cilia of the hatched blastula, and from doublet microtubules of mature sperm were compared after purification of the proteins by selective extraction with organic mercurial and DEAE-cellulose chromatography of the reduced and carboxymethylated proteins. Small but definite differences are observed in the electrophoretic mobilities of the tubulin subunits in SDS-urea gels and in the peptides displayed by gel electrofocusing. Thes differences presumably reflect differences in the covalent structure of the tubulins which play a role in the determination of the structural and functional differences between microtubule systems.

(1) T. Bibring and J. Baxandall, J. Cell Biol. 69, 301 (1976)
(2) T. Bibring and J. Baxandall, Analyt. Biochem., in press.

Regulation of Mitotic Events

727 IN VIVO ANALYSIS OF MITOTIC SPINDLE DYNAMICS, Shinya Inoué and Daniel P. Kiehart, Department of Biology, University of Pennsylvania G-7, Philadelphia, PA 19104.

In vivo analysis of changes in birefringence and morphology of mitotic spindle fibers (occuring naturally in mitosis and induced artificially by low temperature, high hydrostatic pressure, colchicine, D_2O etc.) led to a dynamic equilibrium model of mitotic spindle fiber organization. The birefringence measures the concentration of oriented, assembled microtubules which equilibrate labily with a pool of unassembled tubulin dimers or oligomers. These relationships have since been verified in vitro. Microtubules are generally assembled at organizing centers such as centrioles, kinetochores, cell plate etc. In anaphase, chromosomal fiber microtubules are disassembled at or near the centrosome. This microtubule disassembly rate-limits the poleward velocity of chromosomes (1).

Using microinjection we have shown that steep gradients of Ca^{++} can exist in dividing sea urchin cells and that Ca^{++} in the 5-10 micromolar range depolymerizes mitotic microtubules (2). We therefore suggest that local, endogenous changes in the Ca^{++} concentration could orchestrate assembly and disassembly of microtubules in the mitotic spindle and therefore could control the dynamic equilibrium between monomer and polymer of tubulin.

Since we have demonstrated that microtubule disassembly is necessary for chromosome to pole movement, we are now testing whether controlled disassembly of microtubules is alone sufficient to move chromosomes or whether other force producers are involved. Thus we have physiologically evaluated the contribution of actomyosin for force production: Microinjection of antibody against egg myosin inhibits 8 successive cleavages in starfish eggs but does not inhibit either chromosome to pole movement or pole to pole elongation (3). Therefore, despite cytochemical studies on fixed cells, it is unlikely that chromosomes are moved poleward by an actomyosin system.

Supported by NSF Grant #BMS 7500473, NIGMS Grant #23475 and NIH 5 T01 HD00030.

1. Inoué, S. 1976. In Cell Motility edited by R. Goldman, T. Pollard and J. Rosenbaum. Cold Spring Harbor Laboratory, N.Y. pp. 1317-1328.

2. Kiehart, D.P. and S. Inoué. 1976. J. Cell Biol., 75: 230a.

3. Kiehart, D.P., S. Inoué and I. Mabuchi. 1977. J. Cell Biol., 75: 258a.

728 REGULATION OF SPINDLE MICROTUBULE ASSEMBLY-DISASSEMBLY: LOCALIZATION AND POSSIBLE FUNCTIONAL ROLE OF CALCIUM DEPENDENT REGULATOR PROTEIN. B.R. Brinkley, J.M. Marcum, M.J. Welsh, J.R. Dedman and A.R. Means. Department of Cell Biology, Baylor College of Medicine, Houston, TX. 77030.

Intracellular calcium has been proposed as a regulator of microtubule (MT) assembly-disassembly in the mitotic spindle as well as in the cytoplasmic microtubule complex. Recent work in our laboratories has focused upon the distribution and function of a low molecular weight, thermostable calcium dependent regulator (CDR) protein present in all non-muscle cells (1). Using indirect immunofluorescence we have identified CDR as a dynamic component of the mitotic apparatus in a variety of eucaryotic cells in culture (2). During prophase diffuse immunofluorescence was apparent throughout the cytoplasm, but as the cells progressed from prometaphase to metaphase, intense staining was localized in each half-spindle. During anaphase staining was confined to the chromosome-to-pole region but was excluded from the interzonal region of the spindle. However, at late anaphase when poleward movement of chromosomes had ceased, CDR appeared abruptly in the interzone region where it presisted in the midbody until cell division was completed. The localization of CDR in the half-spindle coincided with anaphase chromosome movement and concurrent disassembly of kinetochore-associated microtubules. The presence of CDR in the mitotic spindle coupled with experimental evidence that calcium ions depolymerize spindles (3) suggests that this protein may function in calcium mediated microtubule depolymerization. In order to test this hypothesis, we have investigated the effect of purified rat testes CDR and an homologous Ca^{++} binding protein, skeletal muscle tropronin-C (TnC), on the assembly-disassembly of microtubule protein (MTP) in vitro. MTP was purified from rat brain by three assembly-disassembly cycles, and MT polymerization was monitored by the change in turbidity at 320nm (ΔOD_{320}). At low free Ca^{++} concentrations ($\sim 10^{-7}$M), CDR or TnC had no effect on MT polymerization. However, when the free Ca^{++} concentration was increased to micromolar levels samples containing CDR or TnC at stoichiometric concentrations relative to MTP displayed essentially total inhibition of polymerization. Samples of MTP alone at the same free Ca^{++} concentration (micromolar), showed a reduction of approximately 20-25% in ΔOD_{320}. In addition to inhibiting MT assembly, CDR and TnC were also shown to reverse the MT polymerization reaction in a Ca^{++} dependent manner. These results together with the immunofluorescent localization of CDR in the mitotic spindle suggest that this protein is involved in the Ca^{++} dependent regulation of MT assembly-disassembly in vivo. (Supported by NIH Research Grant CA-23022 and HD-07503).

1. Dedman, J., J.D. Potter, R.L. Jackson, J.D. Johnson and A.R. Means 1977. J.Biol.Chem. (In Press)
2. M.J. Welsh, J.R. Dedman, B.R. Brinkley and A.R. Means 1977. J.Cell Biol. 75:262a.
3. Salmon, E.D. and Robert Jenkins 1977. J. Cell Biol. 75:295a.

729 INDUCTION OF CHROMOSOME MOVEMENT IN THE ISOLATED MITOTIC APPARATUS AS A FUNCTION OF MICROTUBULES, Hikoichi Sakai, Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Bunkyo-ku, Hongo, Tokyo 113.

Isolation media using microtubule assembly buffers have been proved to be capable of isolating cold-labile and Ca-sensitive MAs (1-4), which provide a model system for the investigation of molecular events associated with chromosome motion. MAs isolated using 1 M glycerol containing GTP, EGTA, Mg ions, GSH, ascorbic acid, cAMP and MES at pH 6.2 retain birefringence approximately equal to that of *in vivo* MAs. The birefringence disappears when MA is treated with cold, Ca ions or SH-blocking reagent (3). The growth of the spindle and asters *in vitro* can be controlled by exogenous porcine or flagellar tubulin concentration. At a tubulin concentration a little less than *in vivo* tubulin pool size, the length of the spindle is maintained to be almost constant during two hour's incubation. Addition of Mg-ATP to the isolated anaphase MA induces motion of daughter chromosomes toward the poles at a speed of 0.1 μm/min at 27.5°C (5). When MA is isolated in the glycerol medium at pH 6.7, the initial velocity of the half bivalents increases to 0.5 μm/min. The motion is totally inhibited by μM level of colchicine. PCMPS, a mercaptide forming reagent, immediately blocks the motion at a concentration of 5 μM by causing tubulin to be incapable of polymerizing into microtubules as well as by depolymerizing pre-existing microtubules (6). Anti-Fragment A (tryptic fragment of dynein) serum also causes daughter chromosomes to cease the motion. However, γ-globulin fraction of anti-starfish myosin serum has no effect on the motion in isolated starfish MAs at a final concentration of 1 mg/ml. These observations suggested that the *in vitro* motion is not induced by myosin-actin system but by dynein-microtubule interaction.

1. Rebhun,L.I.,Rosenbaum,J.,Lefebvre,P. & Smith,G. Nature,249:113-115(1974).
2. Inoué,S.,Borisy,G.G. & Kiehart,D.P. J.Cell Biol.,62:175-184(1974).
3. Sakai,H.,Hiramoto,Y. & Kuriyama,R. Devel.Growth & Differ.,17:265-274(1975).
4. Salmon,E.D. & Jenkins,R. J.Cell Biol.,75:295a(1977).
5. Sakai,H.,Mabuchi,I.,Shimoda,S.,Kuriyama,R.,Ogawa,K. & Mohri,H. Devel.Growth & Differ.,18:211-219(1976).

730 REGULATION OF EGG METABOLISM DURING FERTILIZATION AND MITOSIS, David Epel, Hopkins Marine Station, Department of Biological Sciences, Stanford University, Pacific Grove, California 93950

The "activation" of non-dividing cells to enter mitosis generally involves an interaction of the cell surface with some extracellular messenger. This interaction results in a generalized change in the cell effecting all cell compartments, such as transport (plasma membrane) cytoplasm (protein and RNA synthesis), nucleus (DNA synthesis), and various cell organelles (such as secretory granules of mitochrondria). The major problem in understanding cell activation is elucidating how these pervasive effects are initiated following the interaction of the cell surface with the extracellular messenger.

This paper shall analyze the events emanating from contact of the sperm with the plasma membrane of the egg. The sequence of events shall be reviewed and the hypothesis proferred that the entire program of post-fertilization events that culminate in mitosis results from an increase in intracellular calcium and that the major consequence of the calcium increase is the activation of a Na^+-H^+ exchange system which increases intracellular pH. The mechanisms by which this pH increase might activate the cell to begin synthesis and mitosis will be discussed, as well as recent work on other factors emanating from the calcium increase that might be involved in regulating cell division.

Control of Fertilization Phenomena

731 MECHANISM OF SPERM ADHESION TO SEA URCHIN EGGS, Victor D. Vacquier, Department of Zoology, University of California, Davis, CA 95616.

Sea urchin sperm acrosome granules contain a single insoluble protein, named bindin, that mediates species-specific recognition and adhesion of sperm to the egg surface. Bindin can be isolated in essentially pure form and milligram quantities by sieving Triton-X-100 extracted acrosome granules through a glass fiber column. Bindin has an apparent MW of 30,500 daltons, is 100% protein by dry weight and does not stain for carbohydrate. Bindin from S. purpuratus agglutinates trypsinized glutaraldehyde-fixed rabbit red cells in microtitre-V plate assays. Red cell agglutination is inhibited by micromolar amounts of galactose leading us to propose that bindin is a galactose recognizing lectin. Two lines of evidence support the idea that bindin is the sperm-bourne component of the sperm-egg bond. First, a monospecific horseradish peroxidase-conjugated rabbit anti-bindin localizes bindin on the surfaces of the sperm acrosome process and on the egg microvillus to which the sperm is attached. Localization is especially prominent on the inner surface of the acrosome membrane; the membrane which fuses with the oölemma. Second, particulate bindin is a species-specific agglutinin of unfertilized sea urchin eggs. Bindin from S. purpuratus and S. franciscanus have identical mobilities on SDS gels and both have tyrosine as the N-terminus. The pI of Sp bindin is 6.62 and of Sf 6.59. Amino acid analysis reveals almost identical composition between the two species for 16 amino acids (average variation 1.3 residues per molecule). Only two amino acids, Asx and Pro show large species differences. Tryptic peptide maps show similar patterns, with 24 spots of identical correspondence, 10 spots unique to Sf and 13 to Sp. Sequence analysis shows the N-terminal end of Sp bindin to be hydrophobic with no net - or + charge in the first 46 amino acids. We thus suppose the N-terminal end is buried in the hydrophobic domain of the sperm membrane. A large glycoprotein can be isolated from the vitelline layer of Sp eggs which has specific affinity for bindin. It may be the bindin receptor. The receptor is released from ^{125}I-labeled eggs by parthenogenetic activation with A23187 in the presence of trypsin inhibitor. About 10% of the surface-released ^{125}I has affinity for bindin as measured by a simple glass fiber filter assay. Binding of ^{125}I-receptor to bindin exhibits time dependent saturation kinetics. Competition with cold receptor for ^{125}I-receptor-bindin complexes shows species-specificity. Also, whole egg protein is not an effective competitor. The active bindin receptor is only found in the V_0 of a Biogel A5m column indicating a MW $\geq$ 5 x 10^6 daltons. Isoelectric focusing of the V_0 peak in a pH 3-5 gradient resolves two peaks of pI 4.0 (receptor) and 2.5 (egg jelly). 64% of the pI 4.0 peak binds to bindin with bindin in excess. The receptor is 34% neutral hexose which are galactose and mannose in a molar ratio of 5 to 1. We hope to characterize bindin and its receptor from several species and to reconstruct their interaction under in vitro conditions. Supported by NIH Grant No. HD-08645.

732 SURFACE GROUPS AND FERTILIZATION OF SEA URCHIN EGGS ., Roger Lallier, Station Zoologique, 06230 Villefranche-sur-Mer, France

The role of chemical groups at the periphery of eggs was studied in connection with the fertilization in the sea urchin Paracentrotus lividus. Unfertilized eggs with or without their jelly coat were incubated in solutions of specific reagents for various chemical groups and fertilization took place in these solutions. For blocking of sulfhydryl groups we used 6'6-dithiodinicotinic acid, 5,5'-dithio.bis (2-nitrobenzoic) acid and the p.chloromercuribensoic, p.chloromercuriphenylsulfonic and o.iodosobenzoic acids. The splitting of disulphide bonds was studied with the following thiols: mercaptoethylgluconamide, β-mercaptopropionylglycine and thiomalic acid. Eggs are impermeable or only slightly permeable to these agents. The non permeable sulfhydryl blocking agents did not interfere with fertilization. Fertilization occured in the presence of agents splitting the disulphide bonds, but the fertilization membrane was not formed or is progressively dissolved This phenomenon is attributed to the splitting of disulphide bonds formed in the first phase of fertilization membrane elaboration. Blocking of reactive amino groups accessible at the surface of eggs by action of pyridoxal, succinic anhydride or dimethylmaleic anhydride does not inhibit fertilization. Anionic groups were blocked with polycationic agents such as Polybrene, protamine, polymyxin and polylysine. The polycationic agents agglutined spermatozoa. Nevertheless it is possible to obtain fertilization in the presence of Polybrene or polymyxin. Polylysine and protamine were more toxic for spermatozoa, but fertilization is possible in their presence. The effects of blocking the cationic groups were examined by using a polysulfonic dye, Evans blue, and various small molecules such as SITS and ANS, used as label for cell membrane. Relatively high concentrations of anionic agents such as SITS and ANS inhibited fertilization. Evans blue is a very effective inhibitor at very low concentration (20 µg/ml). The inhibition is reversible by washing. An effect upon the cell surface appeared implicated. Monosulfonic acids, such as benzenesulfonic and toluenesulfonic did not inhibit fertilization and did not suppress the inhibitory effect of Evans blue. In contrast with the 100 % inhibition of fertilization, the acrosomal reaction of sperm is inhibited by about 50 % with the Evans blue. It is suggested that the inhibitory effect of Evans blue upon fertilization may be due to the formation of a complex with a material, probably proteins, located at the unfertilized egg surface. The egg membrane thus stabilized would be unable to interact with spermatozoa (1,2) .

(1) R.Lallier, Acta embryol.et morphol.exp. 3, 255-265, 1974 . (2) R.Lallier, Arch.Biol. 87, 137-155, 1976 .

733 ACROSOMAL ENZYMES AND THE CONTROL OF FERTILIZATION, Maurice H. Bernstein, Department of Anatomy, Wayne State University, Detroit, MI 48201.

Consideration of the acrosome as a modified lysosome has been a most useful paradigm in the analysis of sperm participation in fertilization. Most of the data has been developed from studies of mammalian fertilization.

The acrosome reaction initiates the release of acrosomal enzymes, some of which can be roughly correlated with functional considerations; hyaluronidase with dispersal of cumulus cells, esterase with corona dispersal, and proteinase (acrosin) with penetration of the zona. Expectations that this ordered sequence of reactions would be reflected in a specific acrosomal structure have not been realized. Acrosin and hyaluronidase have been found dispersed through the acrosomal matrix. Only the esterases appear to have discrete localizations.

Both by its persistence through the acrosome reaction and the absence of demonstrable enzymes, the equatorial segment of the acrosome occupies a special position and appears to be the most likely candidate for a specific receptor-mediated role in the actual sperm-egg interaction phases of fertilization.

Supported in part by U.S.P.H.S. Grant No. RR 05384.

734 FERTILIZATION OF SEA URCHIN EGGS IN SODIUM-FREE SEA WATER, David Nishioka, Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950; and Nicholas Cross, Department of Biology, University of California, San Diego, La Jolla, CA 92093

Embryonic development is arrested in sea urchin eggs fertilized in normal sea water and transferred to Na^+-free, choline-substituted sea water (Ch-SW), pH 8.0, two minutes after fertilization (1). This arrest may be reversed while maintaining Na^+-free conditions by raising the pH of the egg suspension to 9.0 with KOH for ten minutes and then lowering it to 8.0 with HCl. Protein synthesis, DNA synthesis, and development to a 32-64 cell embryo are fully stimulated suggesting that a transient rise of intracellular pH is an important metabolic signal (2). We report here that sperm metabolism may be stimulated similarly under Na^+-free conditions. When sperm are suspended in Ch-SW, pH 8.0, motility and O_2 consumption are arrested. If, however, the pH of the sperm suspension is raised to 9.0 with KOH, motility and O_2 consumption resume. For both fertilized eggs and sperm, pH is the only effective variable since KCl is present in the Ch-SW and molar additions of KCl equal to those of the KOH and HCl used to adjust the pH are ineffective in stimulating embryonic development or sperm motility. This implies that in both fertilized eggs and sperm there are Na^+-dependent pH stats which cannot function under Na^+-free conditions allowing intracellular pH to be experimentally manipulated and suggesting that increased intracellular pH is important in both egg and sperm metabolism. If sperm are reactivated in Ch-SW, pH 9.0 with unfertilized eggs present, the eggs are polyspermically fertilized. This result shows that cellular processes preceding sperm incorporation such as sperm-egg recognition, sperm-egg binding, the sperm acrosome reaction, and the egg cortical reaction can occur in the absence of external Na^+. Additionally, this result lends further support to the existence of a rapid electrical block to polyspermy since these eggs cannot undergo the very early Na^+-influx and membrane depolarization which normally prevent supernumerary sperm from binding to the egg (3). If these eggs are administered the same ten minute pulse of pH 9.0 which stimulates normal development in eggs fertilized in normal sea water and then transferred to Ch-SW, no cleavage is observed, suggesting that without the early Na^+-influx, a transient rise of intracellular pH is ineffective in activating development.

(1) Chambers, E. L. (1976). J. Exp. Zool. 197, 149-154.
(2) Nishioka, D. and Epel, D. (1977). J. Cell Biol. 75, 40a.
(3) Jaffe, L. A. (1976). Nature 261, 68-71.

Supported by a grant from the National Science Foundation to Dr. David Epel. N.C. supported by a postdoctoral fellowship from the Rockefeller Foundation.

735 THE ROLE OF THE EGG SURFACE IN THE ESTABLISHMENT OF THE BLOCKS TO POLYSPERMY. Gerald Schatten, Department of Biological Science, The Florida State University, Tallahassee, Florida 32306.

The egg at fertilization is under a stiff constraint; it is required to incorporate one, and only one, spermatozoon. To prevent the entry of supernumerary sperm the egg possesses means to limit fertilization which overlap in duration and are the blocks to polyspermy. Since sperm which have entered the egg cytoplasm contribute a centriole at mitosis to result in a multipolar spindle, the polyspermy blocks in the sea urchin are at the level of the cell surface. In order to examine the relative importance of each surface layer, eggs were treated with surface modifying agents and tested for polyspermy. The sperm concentrations used were insufficient to cause polyspermy in untreated controls and cytological criteria were employed for assaying the extent of supernumerary penetrations.

Treatments which prevent the normal elevation of the fertilization coat, such as Dithiothreitol, result in polyspermy implying that the fertilization coat itself plays a role in the block. However refertilization of these eggs demonstrates that the extent of polyspermy diminishes with time to approach zero. Refertilization of fertilized eggs whose fertilization coat was stripped in iso-osmotic urea is more dramatic; the fertilizability drops to zero after ten minutes. This argues that a late block to polyspermy exists which is independent of the fertilization coat.

Agents which artificially activate the unfertilized egg and which disrupt the outer surface so that the fertilization coat cannot elevate, result in very high polyspermy. However these agents which cause the secretion of the cortical granules, e.g. brief treatments in iso-osmotic Urea, also establish a late block to polyspermy. This late block is not altered by subsequent retreatments.

Regulation of Developmental Events

736 GENE CHANGES DURING SEA URCHIN DEVELOPMENT, Ralph Hinegardner and Nancy O'Rourke, Division of Natural Sciences, University of California, Santa Cruz, CA 95064.

Sea urchins can be raised through their life cycle in the laboratory. This allows for convenient examination of all stages in the life cycle and for the maintenance and control of genetic crosses (1). Using two unrelated inbred lines of Lytechinus pictus we have been able to follow changes in three different types of enzyme activity during development and to demonstrate something about the inheritance of enzyme activity. Enzyme differences were measured using disc electrophoresis on polyacrylamide gels. Isozyme bands were stained using the procedures of Shaw and Prasad (2).

We examined malic dehydrogenase, MDH, glucose phosphate isomerase, GPI, and non-specific esterases, EST, in the egg, blastula, pluteus, feeding larva, early and late rudiment stages and young adult. All enzymes show at least some differences in their gel patterns at different stages of development and between the two inbred strains. MDH showed the most variation during development and between strains. GPI remained more constant. The most dramatic changes occurred between the egg and feeding larva. Esterase activity was maternally inherited in both of the reciprocal crosses we used. There is about as much isozyme difference between major developmental stages as there is between the two strains we used.

1. Hinegardner, R.T., 1969, Biol. Bull., 137:465-475.
2. Shaw, C.R. and Prasad, R., 1970, Biochem. Genet., 4:297-320.

737 TUMOR CELL PROLIFERATION, S. Gelfant, Depts. of Dermatology & Cell and Molecular Biology, Medical College of Georgia, Augusta, GA 30902

Tumors are viewed as discrete proliferative ecosystems where various categories of cycling and noncycling cells, including prediversified subpopulations, serve as a complex adaptive system to fulfill particular proliferative needs and to increase tumor survival.

There are four major categories of tumor cells: cycling cells (moving through the cell cycle); noncycling G_1-blocked cells (arrested in late G_1); noncycling G_2-blocked cells (arrested in late G_2); and noncycling G_0-blocked cells (arrested in early G_1). These represent the potential proliferating pool in tumors *in vivo*.

Transformation is viewed as a compensatory proliferative reaction to compensate for impaired release of normal noncycling cells--which become refractory to normal physiological controls because of chronological age or other reasons. The behavior of transformed cycling cells which under certain conditions lose their ability to return to the noncycling state would account for unabated tumor growth.

It is suggested that the cancerous properties of tumor dormancy, metastasis, and repopulation reside in a minority system of tumor cells compatible with our descriptions of noncycling G_1- and G_2-blocked cells and their subpopulations; and that the properties of tumor growth reside (for the most part) in the major system of cycling $\rightleftarrows$ noncycling G_0-blocked cell transitions.

738 DIRECT EVIDENCE FOR IONIC MESSENGERS IN THE TWO PHASES OF METABOLIC DEREPRESSION AT FERTILIZATION IN THE SEA URCHIN EGG, Richard A. Steinhardt, Department of Zoology Univerity of California, Berkeley, CA 94720.

It has been proposed that the release of intracellular ionic calcium may be the univeral factor promoting activation of egg metabolism at fertilization (1,2). If this hypothesis is valid then fertilization and all parthenogenetic agents which evoke the entire program of activation would necessarily involve an intracellular rise in ionic calcium. Fertilization or parthenogenetic activation of *Lytechinus pictus* eggs was monitored after injection with the Ca-sensitive photoprotein aequorin. We estimated the peak calcium transient at 2.5-4.5 μM free calcium 45-60 sec after activation and lasting 2-3 min, assuming equal distribution throughout the cytoplasm (3). The calcium release at fertilization was shown to be from intracellular store. The threshold for the discharge of the cortical vesicles was between 9 and 18 μM calcium, suggesting that the transient calcium release is confined to the inner subsurface of the egg. Parthenogenetic treatments, ionophore A23187, non-electrolyte 1M urea, and hypertonic medium 1.6 X concentrated sea water, all acted to release calcium from intracellular stores. Sperm, ionophore and non-electrolyte release from the same calcium store which can be recharged after 40 min. Hypertonic medium releases from a different store.

It has been proposed that the second ionic messenger is an increase in intracellular pH, which is responsible for the late steps in activation such as protein and DNA syntheses (4, 5, 6). For this hypothesis to be valid, treatment with weak bases which only evoke late steps in activation should not release intracellular calcium but should raise intracellular pH. Measurements with aequorin injected eggs showed some calcium entry from external solutions with NH_4Cl exposures but that it was not essential to weak base activation. Measurements with micro-electrodes of intracellular pH in intact eggs did show a rise in pH with NH_4Cl and other weak bases as well as in normal fertilization. The increase in pH lasted over 30 min. The relation of this increase to the increase in protein synthesis will be discussed (see also Shen and Steinhardt, this meeting). Supported by NSF PCM 74-09430A01 and 77-04260.

1. Steinhardt, R. A. and Epel, D. Proc. Nat. Acad. Sci., 71: 1915-1919, 1974.
2. Steinhardt, R. A., Epel, D., Carroll, E. J., Jr. and Yanagimachi, R. Nature, 252: 41, 1974.
3. Steinhardt, R. A., Zucker, R. and Schatten, G. Devel. Biol., 58: 185-196, 1977.
4. Steinhardt, R. A. and Mazia, D. Nature, 241:400-401, 1973.
5. Epel, D., Steinhardt, R., Humphreys, T. and Mazia, D. Devel. Biol., 40: 245-255, 1974.
6. Johnson, J., Epel, D. and Paul, M. Nature, 262: 661-664, 1976.

Mitosis and the Mitotic Apparatus II

739 COMPARATIVE ANALYSIS OF STABILITY CHARACTERISTICS OF HEXYLENE GLYCOL AND GLYCEROL/DMSO ISOLATED MITOTIC APPARATUS, Arthur M. Zimmerman, Department of Zoology, University of Toronto, Toronto, Ontario M5S 1A1 and Arthur Forer, Biology Department, York University, Downsview, Ontario.

Recent studies from our laboratory have shown that mitotic apparatus isolated with glycerol/dimethylsulfoxide are able to support chromosome movement after they are injected into enucleate frog eggs. In order to ascertain properties of the glycerol/DMSO mitotic apparatus that are essential for sustaining cleavage we have investigated the stability and chemical composition of the mitotic apparatus under various conditions of isolation. The stability of the glycerol/DMSO mitotic apparatus was compared to mitotic apparatus isolated with hexylene glycol medium and with glycerol/Mg^{++} - Triton X isolation medium. Birefringence of hexylene glycol mitotic apparatus are dependent upon the hydrogen ion concentration of the isolation medium; the higher the pH the lower the birefringence. The stability of the hexylene glycol mitotic apparatus is also dependent upon the pH of isolation medium; the lower the pH the slower the rate of birefringence decay. Glycerol/DMSO isolated mitotic apparatus have more stable birefringence than hexylene glycol mitotic apparatus. The birefringence decay of the glycerol/DMSO isolates were about 1000 times slower than the isolates prepared with hexylene glycol. We are currently studying the proteins found in the mitotic apparatus under various isolation and storage conditions using polyacrylamide gel electrophoresis. In addition we are further investigating the movements of sea urchin zygote chromosomes after mitotic isolates are injected into frog eggs.

Work supported by National Research Council of Canada.

740 CONTROL MECHANISMS IN THE ORGANIZATION OF THE MITOTIC APPARATUS, Patricia J. Harris, Department of Biology, University of Oregon, Eugene, OR 97403.

Since microtubules were the first structures shown to be a consistent part of the mitotic traction system, the search for mitotic control mechanisms has naturally focused on the conditions necessary for microtubule polymerization. In vitro studies have shown that tubulin concentration, divalent cations, presence of nucleating centers, and cytoplasmic pH, for example, may act as possible control factors. More recently, evidence for the presence of actin and other muscle proteins in many types of cells, as well as a mechanism for controlling calcium ions, has added further options for control, not only of microtubule polymerization, but of contractile proteins as well.

It might be expected that structural changes during the course of the cell cycle would reflect the nature of the control mechanisms in the living cell. On the basis of this expectation, a reexamination of the much-studied sea urchin first cleavage division was made with both light and electron microscopy. Among the observations to be considered here are the following:

1. The role of "heavy bodies" in tubulin storage and release.
2. Pigment migration to the cortex as a monitor of "activated" cytoplasm.
3. Significance of the migration of mitochondria into the aster centers.
4. Membrane-bounded vesicles associated with the mitotic apparatus and their role in calcium ion regulation.
5. The spacial and temporal control of cytoplasmic calcium ion concentrations. Is there a calcium wave?

741 UNEQUAL CLEAVAGE: CAUSE AND SIGNIFICANCE, Katsuma Dan Department of Biology,, Tokyo Metropolitan Univ. Tokyo

In 1956, Dan & Nakajima[1] succeeded in isolating MAs of the 8-cell stage within the intact fertilization membrane by using the classical method of Mazia & Dan[2]. By obtaining a complete set of 8 MAs, it was revealed that the animal 4 cells which were to become mesomeres had 2 equally sized asters while in the vegetal 4 cells which were to give rise to macromere and micromere, one aster was of the usual radiate shape whereas the other one has astral rays spreading in one plane.

The cause of flatness of the micromere aster was analyzed both by fixation and by in vivo observations. The following is the finding. (a) Immediately after the 3rd cleavage, the nuclei of both animal and vegetal cells are centrally situated. (b) Next, the nucleus of the vegetal cell rotates making one of the 2 centrosomes face toward the vegetal pole. (c) The nucleus moves as a whole toward the vegetal pole. (d) The nucleus apparently attaches itself by the foregoing centrosome to the inside surface of the vegetal pole. (e) The nuclear membrane breaks down at the site. (f) The result is a radiate aster at the center of the cell and a flat aster is sandwitched between the cell surface and a spindle pole.

Tanaka[3] found that sea water containing 20-30 μg/ml Na-lauryl-sulphate could suppress the micromere formation, making the cleavage revert to an equal type. In this case, the nucleus of the vegetal cell remains at the center, giving rise to a spindle with 2 equally sized asters. Interestingly enough, in this cleavage, the spindle orients itself horizontally in the same direction as mesomers so that 16-cell larva gets two tiers of 8 similar cells. The 5th division is vertical again like the mesomers.

1) 1956 Embryologia 3 : 187-200. 2) 1952 Proc. Nat. Acad. Sci. USA 38 : 826-838. 3) 1976 Develop. Growth & Different. 18 : 113-122

742 CENTRIOLE DUPLICATION IN SAND DOLLAR EGGS, Hans A. Went, Department of Zoology, Washington State University, Pullman, Washington 99164, U. S. A.

Developing sand dollar eggs were treated with actinomycin D, 5-bromodeoxyuridine (BUdR), chloramphenicol or mercaptoethanol for carefully controlled periods of time prior to second cleavage in order to ascertain their effects on centriole duplication. Zero, one or two periods of centriole duplication were included in the treatment periods. After treatment the cells were assayed for mature centrioles by the indirect method involving benzimidazole (1). All four of the reagents tested interfered with centriole duplication. However, the time patterns of interference fell into two distinctly different categories. Chloramphenicol and mercaptoethanol clearly interfered with centriole duplication when only one period of centriole duplication was included in the period of exposure to these reagents. However, the period of exposure to actinomycin D or BUdR had to include two successive periods of centriole duplication before the effects manifested themselves as a reduction in the number of mature centrioles. Excess thymidine neutralized the presence of BUdR. The data support the "generative" mechanism for centriole duplication suggested by Mazia (2).

The effects of these same four reagents upon cytaster formation was also investigated. Cytaster formation was induced by the "double-method" of Loeb. The data show that the time course of cytaster development and the number of cytasters was unaffected by actinomycin D and BUdR. On the other hand chloramphenicol and mercaptoethanol completely suppressed the formation of cytasters, provided that the exposure to these reagents was initiated early enough. If the exposure began after a certain time and before any cytasters had become visible, cytaster formation appeared to be unaffected. This suggests that the unfertilized egg may contain many procentrioles which will develop into mature centrioles under certain conditions. This interpretation appears to be consistent with the data of Weisenberg and Rosenfeld (3).

Amino acid incorporation from a mixture of C^{14} labeled amino acids into the 10% TCA insoluble fraction of eggs activated by the "double-method" of Loeb was also examined. The data do not reveal a meaningful difference in the level and pattern of incorporation between the cells exposed to mercaptoethanol and the control cells. This suggests that the absence of cytasters in the cells treated with mercaptoethanol is probably not caused by the supression of synthesis of a major structural component of the cytasters.

(1) Went, H A, J cell biol 30 (1966) 555; (2) Mazia, D, The cell (ed J Brachet & A E Mirsky) vol. 3, p. 134. Academic Press, New York (1961); (3) Weisenberg, R C & Rosenfeld, A C, J cell biol 64 (1975) 146

743 MEMBRANES IN THE MITOTIC APPARATUS OF MAMMALIAN CELLS, Neidhard Paweletz, Institut für Zellforschung, Deutsches Krebsforschungszentrum, D-6900 Heidelberg, F.R.G.

In the last few years it has been shown, that the mitotic apparatus of plant (1) and animal cells (2) often contains large amounts of vesicles. Though their function is not yet clear it is important to know more about their structure and distribution in the course of mitosis. We have studied the distribution of membraneous systems in the mitotic apparatus of HeLa cells in various stages of mitosis. Different regions of the mitotic apparatus have to be distinguished: a) The pericentriolar region, b) the perichromosomal region c) the spindle region between poles and kinetochores, d) the midbody in postmetaphase stages and e) the periphery of the myxoplasm. While only a few vesicles are present near to the condensed chromosomes, and which probably belong to the smooth endoplasmic reticulum, vesicles and cisternae of the endoplasmic reticulum can be found in larger amounts in the polar regions. Between the microtubuli of the spindle itself a few vesicles can be found during all mitotic stages. In the midbody their number increases with progression of mitosis. The myxoplasm is always surrounded by large cisternae of the endoplasmic reticulum. Their number can be drastically increased by treatment with colchicin. Part of these cisternae belongs to the disintegrated nuclear envelope and is then probably incorporated into the new nuclear envelope of daughter-nuclei. Up to now a direct contact between these membraneous systems and microtubuli could not be found.

1. Hepler, P.K.: Membranes in the Spindle Apparatus: Their Possible Role in the Control of Microtubule Assembly In: Mechanism and Control of Cell Division (eds. Rost and Gifford) in press.

2. Harris, P.: The Role of Membranes in the Organization of the Mitotic Apparatus. Exp.Cell Res. 94, 409-425, 1975.

Microtubule Assembly – In Vitro and In Vivo

744 TUBULIN ASSEMBLY IN CULTURED CELL EXTRACTS, J. Bryan, B. Nagle, Biology Dept., Univ. of Penn., Philadel., Penn., 19104. Measurements on the tubulin content of several cultured cell lines (NA, N-18, C_6, CHO and SV-3T3 cells) indicate that tubulin is approximately 3-5% of the protein in all the 100,000 x g cytosol preparations. The tumor cells of neural origin have a markedly lower tubulin content than brain and in this respect closely resemble a general cultured cell phenotype. All of the tubulin concentrations are ten to twenty times higher than the C_c of neurotubulin assembly, but spontaneous assembly under equivalent conditions is marginal. The addition of cell cytosol to purified neurotublin results in some inhibition of assembly. This inhibition is attenuated and spontaneous assembly will occur in a cytosol supplemented with glycerol. A comparison of the assembly products obtained from NA, C_6, and CHO cells shows that the copurification of tubulin and a 49K dalton protein are enhanced by increased glycerol concentrations. These preparations include the tau complex, but show a distinct lack of the high molecular weight MAPS found in neurotubulin. The 49K protein will copurify with tubulin at nearly constant stoichiometry through several assembly cycles. This protein and the tau complex are removed by phosphocellulose (PC) chromatography. This treatment results in a loss of polymerizability which is restored by reconstitution. It is not clear if the 49K protein alone will promote assembly. The brain-cultured cell MAP differences are paralleled by an absence of rings in the cell tubulin. The addition of brain MAPS to NA tubulin produces rings and promotes assembly of PC treated NA tubulin. The addition of NA-MAPS to PC-neurotubulin will promote assembly. These MAP differences and the absence of rings argue that an assembly model based on neurotubulin alone will not be completely general. (Supported by NSF grants BMS-75-20302 and RCM-77-17930.)

745 MICROTUBULE POLYMERIZATION: OPPOSITE END ASSEMBLY AND DISASSEMBLY OF MICROTUBULES AT STEADY STATE IN VITRO, Leslie Wilson and Robert L. Margolis, Department of Biological Sciences, University of California, Santa Barbara, CA 93106

Antimitotic drugs such as vinblastine and colchicine inhibit microtubule polymerization through a potent substoichiometric poisoning mechanism. In the case of colchicine, we have found that the substoichiometric poisoning is brought about through addition of colchicine-tubulin complex (es) to microtubule ends during assembly (1). Substoichiometric blockage of assembly can only occur if microtubules are resistant to disassembly at the drug addition site, since otherwise disassembly would first release the drug blockage and free the microtubule for further assembly. One possible mechanism for the success of substoichiometric drug poisoning is that while microtubules predominantly assemble at one end, they disassemble at the other end. Another possibility is that a common microtubule assembly-disassembly site is " capped " by the drug-tubulin complex, so that neither assembly nor disassembly can occur at that site. To distinguish between these two possibilities, we have measured the flux of tubulin into and out of microtubules at steady state in vitro using ^{3}H-GTP as a pulse-chase marker for tubulin incorporation and loss rates. Our results indicate that the microtubule assembly-disassembly "equilibrium" is a steady state summation of two different reactions which occur at opposite ends of the microtubule, and that assembly and disassembly occur predominantly and perhaps exclusively at the opposite ends under steady state conditions in vitro (2).

The functional implications of opposite end assembly and disassembly of microtubules within cells seem very great. Due to their intrinsic unidirectional flow characteristics, microtubules may conceivably function as passive carriers of materials dependent upon them for transport. If they assembled near the nucleus and disassembled near the cell membrane, cytoplasmic microtubules could passively transport secretory products toward the cell surface. Similarly, neuronal microtubules, by assembling at the junctional region between the neuronal cell body and axon and disassembling at the axonal tip, might passively transport linked materials distally in the process of slow axonal transport.

Based upon our conclusions, we have developed a model for the mitotic mechanism. In this model, opposite end assembly and disassembly of microtubules as well as sliding forces between antiparallel microtubules operate concertedly in the orderly segregation of chromatids.

1. R.L. Margolis and L. Wilson (1977) Proc. Natl. Acad. Sci., USA, 74, 3466-3470.
2. R.L. Margolis and L. Wilson (1978) Cell, 13, 1-8.

746 THE CENTRIOLE AS A MICROTUBULE ORGANIZER: CILIOGENESIS IN MULTICILIATED CELLS AS A MODEL SYSTEM, Ellen Roter Dirksen, Department of Anatomy, University of California, Los Angeles, California 90024.

Considerable in vitro evidence has accumulated recently that in mammalian cells in culture, centrioles are responsible for microtubule organization. The analysis of ciliogenesis in mammalian ciliated cells provides a particularly good system for the study of microtubule assembly in vivo, since both centriolar and ciliary microtubule formation can be analyzed. In the developing oviduct, cilium formation during ciliated cell differentiation is preceded by the formation of centrioles. The centriolar microtubules had been assembled earlier from precursor structures having little resemblance to the mature organelle and consisting of particulate tubulin. This particulate tubulin is derived from the parent centrioles, which remained after the last mitosis of the cell (1,2). After the centriole microtubules are assembled from this particulate tubulin, the now matured centrioles move to the surface of the cell, where, as basal bodies, they generate the ciliary microtubules. While this is occurring, another wave of centriole replication is taking place within the cell (3). With age, there is an increase in the number of cilia per cell and in the number of cells. During the most rapid period of ciliated cell differentiation, ^{3}H-leucine incorporation experiments show that the tubulin assembled into centriole and ciliary microtubules is synthesized de novo (4). Using pulse-chase conditions, we further investigated the incorporation of ^{3}H-leucine into tubulin in isolated oviducts. Label appears in solubule, particulate and axonemal fractions minutes after incubation (5). It is postulated that a complex transfer of synthesized tubulin from a soluble fraction through centriole precursors to assembled ciliary axonemes occurs during ciliogenesis. This and other evidence leads to the conclusion that centrioles influence microtubule formation and structural patterning by a mechanism still to be understood.

1) Dirksen, E.R. (1971) J. Cell Biol. 51, 286-302.
2) Staprans, I. and Dirksen, E.R. (1974) J. Cell Biol. 62, 164-174.
3) Dirksen, E.R. (1974) J. Cell Biol. 62, 899-904.
4) Dirksen, E.R. and Staprans, I. (1975) Dev. Biol. 46, 1-13.
5) Dirksen, E.R. and Staprans, I. (1977) Cell Biol. Int. Reports 1, 275-281.

747 REGULATION OF FLAGELLAR TUBULIN SYNTHESIS IN NAEGLERIA, Chandler Fulton, Elaine Y. Lai, and Peter A. Simpson, Dept. of Biology, Brandeis University, Waltham, MA 02154

The α- and β-subunits of both outer doublet and central pair microtubules are synthesized de novo during the hour-long differentiation of Naegleria gruberi NEG from amebae to flagellates The synthesis of outer doublet tubulin begins about 20 min after the initiation of differentiation and reaches its maximal rate at about 60 min, near the time of flagellum assembly. Flagellar tubulin is one of the major proteins synthesized during differentiation. Amebae have tubulin, as well as other 55,000 dalton proteins that comigrate with tubulin in several systems of polyacrylamide gel electrophoresis. Ameba tubulin differs from flagellar tubulin in at least two respects: it is not utilized in the assembly of flagellar microtubules and it is not recognized by an antiserum that is specific to flagellar tubulin. Translation of poly(A)-containing RNA from differentiating cells in a cell-free wheat-germ system yields flagellar tubulin that has been characterized by several criteria, including immunoprecipitation by the specific antiserum. Flagellar tubulin accounts for 10% of the in vitro products. No detectable flagellar tubulin is synthesized when the cell-free system is directed by RNA either from amebae prior to initiation of differentiation or from cells whose RNA synthesis is inhibited by actinomycin D while they are incubated under differentiation conditions. The time course of appearance of translatable flagellar tubulin mRNA during differentiation closely parallels the time course of in vivo synthesis of outer doublet tubulin. These results are consistent with two working hypotheses: that flagellar tubulin differs in primary structure from ameba tubulin (the "multi-tubulin hypothesis"[1]), and that synthesis of flagellar tubulin is regulated at the level of transcription. (Supported by NSF.)

[1]Fulton, C., and P.A. Simpson (1976) Selective synthesis and utilization of flagellar tubulin. The multi-tubulin hypothesis. In Cell Motility (R. Goldman, T. Pollard, and J. Rosenbaum, eds.), Cold Spring Harbor Laboratory, pp. 987-1005.

Structural and Molecular Basis of Cell Movement

748 FACTORS CONTROLLING THE ELONGATION OF CILIA AND FLAGELLA: A MODEL INVOLVONG ANTAGONISTIC GROWTH-ZONES, Frank M. Child, Department of Biology, Trinity College, Hartford, CT 06106

After the shafts of newly forming cilia and flagella begin to elongate, or when they elongate during regeneration from the stump left after amputation, the rate of elongation constantly decreases without reaching zero. At the limits observable, the very slow rate of elongation is difficult to measure and may be masked by secondary events affecting the length. The kinetics of elongation are affected by temperature, by a number of drugs, and by a variety of factors which will be discussed. A general scheme needs to distinguish between effects on the synthesis of precursors, the size of the available pool of precursors, and the assembly of the shaft itself. Measurements on synchronized Chlamydomonas show that the flagella continue to elongate during interphase as the cells approach division at the end of a 24-hour dark-light cycle. A few cells delay division by two or more hours (after the light goes off); these have flagella up to 20% longer than average pre-division cells. During interphase, the cell-body-pool of precursor protein (PCP)(measured as length regenerated in cycloheximide (CH)) doubles, but has little effect on regenerative capacity. The total store of PCP changes during interphase, but most PCP accumulates during the divisions. Euglena does not store PCP; nevertheless, the kinetics of elongation do not vary between 24° and 34° C. Temperatures outside this range slow the elongation, mimicking the effects of CH. At 39° regeneration does not occur; neither does PCP accumulate although total protein synthesis is only partially inhibited.

These and other facts to be presented argue that given 1) an adequate rate of synthesis and 2) an abundance of precursors, the rate of assembly is regulated by events occuring in the shaft itself. A number of models of self-regulating assembly have been applied to explain the kinetics of elongation. Some of these can be rejected, at least for the present, for a number of reasons. A new model will be presented which accounts for the deceleratory rate of assembly. The model requires two overlapping axial elements to be assembled in opposite directions: --one element by a distal growth-zone, the other by a proximal growth-zone. The addition of units to the growth-zones is restricted to the regions near overlap ends. The overlapped elements are connected sideways by links possessing the stochastic property of being either open or closed. Elongation of the shaft is permitted when enough links are open (so that the two axial elements may slide with respect to each other), but is not permitted when too many links are closed. Elongation of the shaft occurs, therefore, with an expectation which is a function of 1) the number of links and 2) the probability enough links are open. This model gives a good fit to the observed rates of elongation. It is still, however, a matter of conjecture which structural elements of the shaft correspond to the elements of the model. The two axial elements may be either membrane-axoneme, or peripheral-central fibers, or other structures not yet considered.

749 CALCIUM CONTROL OF CILIARY MOTION, Peter Satir, Marika Walter and William Reed, Department of Anatomy, Albert Einstein College of Medicine, Bronx, N.Y. 10461 USA and Department of Physiology-Anatomy, University of California, Berkeley, Cal. 94720 USA

Lateral (L) cilia of freshwater mussels (e.g. Elliptio), can be arrested momentarily in ca. 10^{-2}M Ca^{2+} while other gill cilia,-e.g. laterofrontal (LF) and frontal (F) cilia-continue to beat. This ciliary arrest is mimicked by perfusion of the gill with the ionophore A23187 in the presence of 10^{-2}M Ca^{2+} but not 10^{-2}M Mg^{2+}, Na^{+} or K^{+}. We suggest that arrest is caused by a temporary increase in Ca^{2+} within the ciliary axoneme. This may model effects of Ca^{2+} on other microtubule systems. When Ca^{2+} returns to a low level (ca. 10^{-7}M), ciliary beat resumes. To test this further, we have isolated ciliated epithelial cells from the gill. Cell type can be identified by ciliary morphology. About half of the cells are motile. In 10^{-5}M A23187 and 10^{-2}M Ca^{2+}, motility falls dramatically for all cell types, but is unaffected by ionophore or Ca^{2+} alone. Cells can be treated with Triton X-100, and reactivated to normal beat by addition of 4mM ATP and 8mM Mg^{2+} in EGTA, HEPES and KCl solution. $Ca^{2+} > 10^{-6}$M added as Ca^{2+}-EGTA buffer inhibits reactivation. Inhibition is differential in that 75% of the reactivatable L cells are inhibited by ca. 8×10^{-7}M Ca^{2+}, which has only minimal effect on LF or F cells. Motility ceases entirely for all cell types at 10^{-2}M Ca^{2+}.

Cilia can be obtained from the isolated cells, and then treated with triton followed by trypsin. Under dark field, addition of ATP to the trypsin-treated axonemes causes (1) disintegration, presumably as linkages within the axoneme relax, and (2) sliding, when dynein arms are activated. In the presence of 10^{-3}M Ca^{2+} (as Ca-EDTA) or 10^{-2}M Ca^{2+}, the sliding of microtubules in such axonemes proceeds as in controls without added Ca^{2+}. Neither the percent of cilia which slide nor the form of sliding is altered. Although Ca^{2+} controls ciliary motion in this system, it does not act directly on dynein-tubulin sliding.

Supported by USPHS grant HL22560

750 MOLECULAR FACTORS IN THE AUTO-ASSEMBLY OF CONTRACTILE ULTRASTRUCTURE. Teru Hayashi and Wallace S. Ip, Department of Biology, Illinois Institute of Technology, Chicago, IL 60616

The interaction and complex formation of the contractile proteins actin and myosin will result in the formation of a bipolar, hexagonally-associated "contractile unit" which is the structural unit of the myofibril. The self-assembly of this organized macromolecular aggregate occurs only when precise initial conditions of molecular state are met (1). The results of this study indicate that the molecular reactions underlying the self-assembly process are dependent on a facilitated, unidirectional polymerization of actin (2, 3) and a polarity-specific actin-myosin interaction. The factors of facilitation and polarity-specificity were studied by in vitro measurements of actin-myosin complex formation concomitant with actin polymerization (4), utilizing myosin and its complex-forming derivatives as heavy meromyosin (HMM), one-headed HMM, and the S_1 subfragment. Facilitation of actin polymerization is measured both by viscometry and by the preferred formation of "herringbone" complexes monitored by electron microscopy. Myosin, HMM, and one-headed HMM effectively facilitate polymerization, whereas S_1 does not. Detailed analysis of the several types of complexes formed and the pattern of their formation indicate that the facilitation of actin polymerization is polarity-specific, and that this specificity is dependent on the presence of the S_2 subfragment. The findings of these in vitro studies, if applied to the in vivo formation of muscle ultrastructure in development, indicate that the participating proteins carry the requisite information in their gene-coded structure and reactivities, and that special cytoplasmic machinery for ultrastructure formation is unnecessary.

1) Hayashi, T., R. B. Silver, W. S. Ip, M. L. Cayer, and D. S. Smith, J. Mol. Biol. (1977) 111, 159 - 171

2) Hayashi, T., and W. S. Ip, J. Mechanochem. Cell Motility (1976) 3, 163 -169

3)Kondo, H., and Ishiwata, S. J. Biochem. (1976) 79, 159 - 171

4) Ip, W. S., and T. Hayashi, Fed. Proc. (1977) 36, 625

751 MOTILITY IN DICTYOSTELIUM DISCOIDEUM: BIOCHEMICAL AND GENETIC STUDIES. Margaret Clarke, Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, NY 10461.

We are studying the molecular basis of motility in the cellular slime mold Dictyostelium discoideum. Actin and myosin have been purified from Dictyostelium amoebae and examined in vitro. In order to analyze the roles of the actin and myosin and to identify other proteins that affect their organization and interaction within the cell, we are seeking mutants with conditional defects in movement-requiring processes such as phagocytosis. Bacteria that have bromodeoxyuridine substituted for thymidine in their DNA, are fed to Dictyostelium amoebae under restrictive temperature conditions. The amoebae are then exposed to 310 nm light, which kills cells that have incorporated the bromodeoxyuridine. Approximately 1% of the cells survive; those that prove to be temperature-sensitive for growth are further tested for motility defects. The mutants obtained will be characterized biochemically in hopes of correlating motility defects with alterations in specific proteins.

752 CYTOPLASMIC ACTIN FROM SEA URCHIN EGG EXTRACTS, Robert E. Kane, Pacific Biomedical Research Center, University of Hawaii, Honolulu, HI 96822.

The unfertilized sea urchin egg contains a relatively large amount of G-actin, which remains unpolymerized in extracts at 0°C. After dialysis to remove low molecular weight components, polymerization can be induced by warming to 35-40°C in the presence of 1 mM ATP. If the calcium concentration is kept low through EGTA chelation, the F-actin formed immediately combines with two other proteins in the extract, of 58,000 and 220,000 MW, to form a filamentous gel visible in the light microscope. When negatively stained and examined in the electron microscope, the filaments are seen to be arrays of F-actin in register which display an unusual banding pattern with an approximately 110 Å repeat. Although the polymerization of actin in the extract is temperature reversible if gelation is blocked, combination of the F-actin with the other proteins yields a gel which is stable at 0°C. The gel material is soluble in 0.5 M KCl, which releases the F-actin from combination with the other proteins. If the actin concentration in such a dissolved gel solution is sufficiently high, the F-actin filaments will spontaneously aggregate to microscopic bundles in the presence of ATP. These bundles can be removed by centrifugation and this provides a simple method of preparing purified actin from this material. The proteins of 58,000 and 220,000 MW remain in the supernatant solution and will recombine with actin at salt concentrations in the range of 0.1 M to form gel identical to the original material. In experiments in collaboration with J. Bryan, the 58,000 and 220,000 MW proteins have been separated through the use of agarose and DEAE-cellulose columns and their roles in gelation determined. Combination of F-actin with the 58,000 MW protein gives rise to microscopic needles which display the characteristic cross banding pattern and addition of 220,000 MW protein causes these needles to aggregate to form gel. Optical diffraction studies carried out by D. DeRosier, et al., have demonstrated that this banding pattern is generated by the association of the 58,000 MW protein with the F-actin filaments, with the ratio of 58,000 protein to actin monomers approximately 1:4. The combination of these proteins with actin to form structural units and their sensitivity to calcium in the micromolar range suggests that they may play a cytoskeletal role in the cell, with calcium acting to control the shift of the actin from interaction with these structure-forming proteins to interaction with myosin to induce contractility. (Supported by NIH grant GM 14363.)

The Differentiated Cell

753 DIFFERENTIATED FROG GASTRIC PARIETAL CELLS IN CULTURE, Gertrude H. Blumenthal and Dinkar K. Kasbekar, Department of Physiology and Biophysics, Georgetown University School of Medicine, Washington, D.C. 20007.

The multicellular nature of the gastric epithelium presents certain difficulties when one attempts to elucidate the basic mechanisms involved in acid and pepsinogen secretion. For this reason, we have undertaken the isolation and culture of enriched populations of frog gastric tubular cells, the homologue of the mammalian oxyntic and chief cells in that the tubular cell secretes both acid and pepsinogen. Since in vitro preparations of the frog gastric epithelium have been studied in considerable detail with regard to the basic mechanisms of acid secretion, there is a significant body of information available for comparative studies at the cellular level. Additionally, the gastric tubular cell offers the unique opportunity for studying variations in stimulus-secretion coupling in a cell where the same physiologic stimulus can elicit two distinct and differing secretory responses. For example, at concentrations of colchicine which predominantly inhibit pepsinogen secretion, stimulation of acid secretion is noted. Similarly, burimamide, an H-2 receptor antagonist, and thiocyanate are more effective in inhibiting acid than pepsinogen secretion. In order to reproduce and extend these observations to the cellular level, we have studied some of the pertinent physiologic properties of the tubular cells isolated from the gastric mucosae of R. catesbeiana and maintained in short term culture. During the first few days after isolation and culture, cell surface damage was manifest by an enhanced leakage of intracellular pepsinogen and lack of response to known gastric secretagogues with respect to pepsinogen release and respiration. After several days in culture, cellular pepsinogen levels increase and the spontaneous leakage of pepsinogen shows a marked decline, indicating repair of the initial membrane or surface damage. Variable response to gastric secretagogues is observed with regard to respiratory and pepsinogen secretory activities. After about 30 days in culture, the pepsinogen content of the cells as well as the respiratory response to secretagogues begins to decline. This is accompanied by morphological changes. The feasibility of preparing an in vitro reconstituted epithelium comprised of a homogeneous tubular cell population is currently under investigation.

754 HORMONAL REGULATION OF LIVER GROWTH, Nancy L.R. Bucher, Joan A. McGowan, and Usha Patel, Huntington Laboratories, Massachusetts General Hospital, Boston, MA 02114.

Adult liver cells are arrested in G_0, but proliferate actively following partial hepatectomy or acute liver injury This growth process is under humoral control, and we are seeking the responsible effectors. Hepatocyte proliferation, as determined by DNA synthesis, is greatly diminished in partially hepatectomized rats that have been previously deprived of pancreatic and enteric hormones and nutrients by excision of the gastrointestinal tract, pancreas, and spleen. In such animals growth is restored to normal by infusion of insulin and glucagon in combination, but not separately. In normal rats following partial hepatectomy, portal venous glucagon levels rise and insulin levels fall. Consistent with this pattern, DNA synthesis in eviscerated, partially hepatectomized rats is promoted by high glucagon-low insulin treatment. The efficacy of insulin and glucagon together, but not separately, is further supported by their striking promotion of survival of mice with massive liver necrosis from lethal infection with murine viral hepatitis.

Introducing another model, Short et al. (1) induced hepatic DNA synthesis in normal rats by feeding an amino acid meal after several days of a protein-free diet. In 3-6-day protein-deprived rats we find normal portal venous glucagon levels, but insulin is only one-third of control values. Amino acid feeding causes an abrupt rise in glucagon, but little change in insulin. If rats are starved instead of protein depleted, amino acid feeding fails to stimulate DNA synthesis, although initial insulin and glucagon levels are the same as in protein-deprived animals and the early glucagon rise is even higher. Hence amino acids themselves are not primary growth stimulators, nor are the early changes in insulin and glucagon induced by these dietary manipulations. This is consistent with our previous inability to initiate DNA synthesis in animals with intact livers by administration of these two hormones, although intact livers can respond to humoral factors, as shown by cross circulation of blood between normal and partially hepatectomized partners. Insulin, glucagon, and amino acids thus emerge as important potentiators, but not primary initiators of liver growth.

On the other hand epidermal growth factor (EGF) weakly stimulates hepatocyte proliferation in intact livers of normal rats. This growth initiating action is greatly enhanced by combination with insulin or glucagon, though all three hormones together are not additive. Hepatocyte proliferation thus depends upon interplay of many factors--hormones, nutrients, and possibly other agents also.

Supported by USPHS Grants #CA 02146-24 and 5 R01 AM19435-02.

1. Short et al. (1973) Biochim. Biophys. Acta 50:430.

755 DIFFERENTIATION OF SEX CHROMOSOMES AND AUTOSOMES, Alberto J. Solari, Department of Histology, School of Medicine, University of Buenos Aires, Buenos Aires 1132, Argentina.

Sex chromosome differentiation from an original homomorphic pair to a distinctive heteromorphic pair is mirrored by the special behavior of sex-chromosome axes during meiotic prophase in the heterogametic sex. In placental mammals most of the species show partial synapsis in the XY pair. In species bearing X chromosomes belonging to the original type, XY synapsis is longer at early pachytene and then desynapsis occurs along all but not at the tip of the pairing segment. In several species of hamsters, pairing is longer and stable during pachytene. Species bearing large C-heterochromatic blocks at the ends of the X chromosome, may show only end-to-end joining of the axes, as in Psammomys obesus. In marsupials, the tiny Y axis does not form a synaptonemal complex with the X axis, but a special dense plate joins the ends of the chromosomes. In birds the Z and W chromosomes form a distinctive heteromorphic pair, in which partial synapsis is shown as an asymmetric synaptonemal complex. In the chicken partial synapsis is not accompanied by heteropycnosis of the ZW pair. In the grasshopper Melanoplus differentialis the single X chromosome in the male shows a characteristic sequence of changes which lead to the folding of the chromosomal axis and the joining of the tips at late pachytene. Rearrangements in autosomes may be detected by the special behavior of their axes during early pachytene. Deletions, reciprocal translocations and tetraploidy have been demonstrated in mammalian spermatocytes.

756 TRENDS IN SEX CHROMOSOME EVOLUTION, J. Wahrman, Department of Genetics, The Hebrew University, Jerusalem, Israel.

Sex chromosome relationships in male mammals and insects were studied by electron microscopy. Chromosome pairing patterns of several species of rodents were elucidated by the spreading of first spermatocytes onto various hypophases (1). These species included *Gerbillus gerbillus* with three sex chromosomes, XY_1Y_2, in the male (2), and *Apodemus mystacinus*, the only mammalian species known with both pre- and postreduction of the XY bivalent (3). Among insects a unique series of multiple sex chromosomes is being examined in beetles of the genus *Blaps*. From 3 to 18 sex chromosomes are present in males of different species. Proper segregation of part of these chromosomes appears to depend on a "segregation body" device rather than on pairing segments (3).

(1) Counce, S.J., and Meyer, G.F., Chromosoma *44*: 231, 1973. (2) Wahrman, J. and Ritte, U., Proc. XIth Inter. Congr. Genet., *1*: 125, 1963. (3) Wahrman, J., Nezer, R., and Freund, O., Chromosomes Today, *4*: 434, 1973. Wahrman, J., and Nezer R., Chromosomes Today, *5*: 464, 1976.

Microtubule Assembly Mechanisms

757 TUBULIN SYNTHESIS IN CELL FREE SYSTEMS, Isaura Meza, Department of Cell Biology, Centro de Investigación del Instituto Politécnico Nacional, México 14, D.F.

The search for tubulin messenger was first started in polysomes isolated from chick brain (1). This polysomal preparation contained a mRNA fraction which in a reticulocyte cell-free system was capable to direct the synthesis of several proteins, among them, the two tubulins, as was corroborated by their electrophoretic mobility. Further characterization of the products synthetized "in vitro" under the direction of brain poly A-containing RNA was made (2,3) by copolymerization of the "in vitro" synthetized products with purified brain tubulin using polymerization cycles. In these experiments the tubulins made "in vitro" in a wheat germ cell-free system, sedimented with the microtubule pellet, but so did actin and other proteins. A better indication of the presence of tubulin was that some of the tubulin made "in vitro" was capable to bind to colchicine-Sepharose columns. Other attempts to purify tubulin messenger from the poly A containing RNA in brain have been done by fractionation of the poly $(A)^+$ RNA in acrylamide- formamide preparative gels (4). The fractions eluted from the gel were tested for their ability to direct the synthesis of tubulin "in vitro". It was found that about 5 fractions close to the 18 S RNA marker contained the mRNA for tubulin. When the purification was attempted utilising formamide-sucrose gradients (5), the mRNA for tubulin was localized in several fractions, again in the 18 S region. These fractions contained, besides the mRNA for the two tubulins, the mRNA for actin. From these experiments it was clear that each tubulin is codified by its individual messenger. The characterization of the tubulin made "in vitro" by the wheat germ cell-free system and the nuclease-treated reticulocyte system was done by 2D electrophoresis using isoelectrofocusing in one direction, and SDS electrophoresis in the second one. The tubulins made by the two "in vitro" systems were identical in Ip and molecular weight to purified tubulins obtained by polymerization cycles. Further purification of the tubulin mRNA which is being attempted at present, will facilitate the study of the tubulin gene.

1. Gilmore, M.A. and S.M. Haywood (1975) Ann. N.Y. Acad. Sci. 253, 349.
2. Gilmore-Herbert, M.A. and S.M. Haywood (1976) B. B. A. 454, 55.
3. Gozes, I., H. Schmitt and U.Z. Littauer (1975) P. N. A. S. USA 72, 701.
4. Chapelinsky, A.B., E. Palmer, J.L. Saborío and I. Meza (1976) J. Cell Biol. 70, 253a.
5. Palmer, E., J.L. Saborío and I. Meza (1977) J. Cell Biol. 75, 335a.

758 MICROTUBULES AND CONJUGATION IN TETRAHYMENA, Jason Wolfe, Department of Biology, Wesleyan University, Middletown, CT 06457.

Colcemid prevents conjugation in Tetrahymena. After mixing starved mating types the cells continue to swim freely and remain single for 1 hour at which time pairs begin to form, reaching 80% paired by 3 hours. During the 1 hour lag period, a cell interaction occurs, one which depends upon a certain degree of positional stability, is sensitive to actinomycin D, and requires an extracellular component. The interaction is complete by 30 minutes, but a maturation period precedes cell pairing. This process involves a distinct morphological alteration at the anterior tip of the cell, exposing a large smooth surface which participates in the specialized junction formed between cells in pairs. In freeze fracture preparations this area is ringed by aggregate rows of intramembrane particles. When mixed cells are incubated in 1 mM Colcemid this morphological transformation does not occur. Presumably the transformation is microtubule dependent, but it is possible that Colcemid inhibits the cellular interation itself, thus blocking the signal for tip transformation. In any event transformation, once it occurs, is stable and is not reversed by Colcemid. Yet, transformed cells do not form pairs in the presence of Colcemid, suggesting a continued role for microtubules in the pairing process. After the cytoplasm of paired cells is joined through plasma membrane fusion, micronuclei are "activated" and increase in length from 1 μ to 50 μ. Inside the elongating micronucleus, just beneath the nuclear envelope, is a basket of parallel microtubules. The intranuclear microtubules are associated with the elongation process, since Colcemid (1 mM) inhibits micronuclear elongation and causes collapse of already elongated micronuclei. Contrary to expectation, collapsed nuclei still retain their microtubules. There is an unusual displacement of tubules in a row together with two layers of nuclear envelope, into a tripartite arch that is continuous with the nucleus. Cytoplasm is entrapped in the space between the arch and the nucleus. This effect of Colcemid is reversible. After meiosis haploid gametic pronuclei are exchanged. At the time of nuclear translocation cytoplasmic microtubules appear attached to the external surface of the nucleus, and are presumably involved in the exchange. Thus microtubules play varied important roles in conjugation of Tetrahymena.

Nucleocytoplasmic Interactions

759 CORTEX-NUCLEUS INTERACTIONS DURING THE CELL CYCLE OF STENTOR. Noël de Terra, Department of Anatomy, Hahnemann Medical College, Philadelphia, PA 19102.

The first visible event of cell division in the ciliate Stentor is the assembly of several thousand basal bodies on the ventral cortex to form an oral primordium which develops into the oral apparatus of the posterior daughter cell. During oral development, the chain macronucleus undergoes a series of changes in morphology and synthesis. The morphological changes (coalescence, elongation, nodulation) double the number of macronuclear nodes in preparation for division; at the time of coalescence, macronuclear DNA synthesis shuts off. These events of nuclear replication can be induced in interphase cells by removing the oral apparatus, which triggers oral development (regeneration); they are therefore presumably initiated during division by whatever endogenous stimulus induces oral development in mature cells. Several lines of evidence have suggested that the timing of oral development is controlled by the size of the oral apparatus in relation to the size of the cell body. In the interphase cell, the presence of the oral apparatus inhibits oral development (1). A new oral apparatus only begins to form when the old one becomes disproportionately small in relation to cell body size and this disproportion develops during interphase because the cell body grows and the oral apparatus does not (2). The most direct evidence supporting this hypothesis comes from experiments involving reciprocal exchange of the oral apparatus between stentors from a large (L) and a small (S) strain. Cell division can be initiated prematurely in large interphase L-strain stentors by replacing their oral apparatus with one from an S-strain cell. Conversely, dividing S-strain stentors resorb the primordium and return to interphase when their oral apparatus is replaced with one from an L-strain cell (3). These results are consistent with the hypothesis that oral development and cell division are normally initiated by the developing disproportion between oral apparatus size and cell body size. Previous work (2) had suggested that the time of oral development is determined by the size of the oral apparatus in relation to cell surface area, rather than cell mass or volume. As the cell surface enlarges during interphase, its properties may change in such a way that basal bodies can be assembled on the ventral cortex in spite of the presence of the oral apparatus. The events of macronuclear replication taking place during cell division therefore seem likely to be triggered by a change in cell surface properties arising as a result of cell surface growth during interphase.

(1) Tartar. V. (1958) J. Exp. Zool. 139, 479-506.
(2) de Terra, N. (1969) Exp. Cell Research 56, 142-153.
(3) de Terra, N. (1977) J. Exp. Zool. 200, 237-242.

760 THERE IS MORE TO MITOSIS THAN JUST MOVING CHROMOSOMES AROUND, Lester Goldstein, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309

At the onset of mitosis numerous processes concerned with the segregation of sister chromatids to daughter cells begin, but other processes unrelated to chromosome movement also commence. The early condensation of chromosomes results in the removal of various (perhaps almost all) non-histone proteins and RNA's and the post-metaphase decondensation of chromosomes is accompanied by the reacquisition of RNA's and non-histone proteins. Some of the latter may differ from those that were associated with the chromosomes just prior to the mitosis that is being observed. Data bearing on various characteristics of these RNA's and proteins will be presented. In addition, I will present the thesis that the "reprogramming" of the genetic expression of a cell may be determined to a large extent by the mitotic loss of certain chromosomal RNA's and proteins and the resultant new associations of RNA's and proteins with post-mitotic chromosomes.

The Cell Cycle in Perspective

761 THE CELL CYCLE IN PERSPECTIVE, D. Mazia, Dept. of Zoology, Univ. of Calif., Berkeley CA 94720.

The lecture will deal with the development of the conceptual frame which has governed the progress of cell cycle studies. The accumulation of information about cell cycles has reached the point where we need to turn from the question "What do we want to know?". The problem of the relation between growth and division will be reexamined in the face of the possibility that no generalizations are to be found; that it is a distinct problem for each kind of cell. The usefulness of the conventional phases of the cycle will be criticized. An alternative frame will be considered, much of it provided by the literature on cell fusion. In effect, the basic cycle will be examined as two states of the cell: one in which chromosomes must decondense and can replicate and one in which chromosomes must condense and a mitotic apparatus must form. Recent work by the lecturer, bearing on the inauguration and termination of the S period, on premature chromosome condensation and spindle formation and on the centriole cycle will be introduced.

Cell Cycle Regulation and Control of Growth

762 THE CYTOSKELETON, Keith R. Porter, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309.

The shapes that cells normally adopt are a reflection of activities and responses of the cytoskeleton. This, for present purposes, comprises microtubules, bundles of 60 Å actin filaments (stress fibers) and a fine three-dimensional lattice of slender (30-50 Å) strands or trabeculae. The latter contains or supports such well-known components of the cell as ER cisternae, microtubules, ribosomes and stress fibers and may be properly regarded as representing the cytoplasmic matrix or ground substance to which it lends gel-like properties.

This paper will focus on the form and behavior of the lattice as it occurs in a variety of cultured cells. Its presence divides the ground substance into two phases, one protein-rich and polymerized, the other water-rich and fluid. The question of artifact has been thoroughly examined with results that will be described. Evidence will be presented to demonstrate, as well, that the system responds with structural changes to variations in the ambient temperature, to the presence of cytochalasin B, and to the movement of cytoplasmic particles such as pigment granules. There are reasons for regarding it as the simplest and most primitive of mechanisms for providing a motive force. These and other features of the lattice will be discussed as time permits.

763 CELL CYCLE CONTROLS AND CELL SYNCHRONY,
Erik Zeuthen, The Biological Institute of the Carlsberg Foundation, 16 Tagensvej, DK-2200 - Copenhagen N, Denmark.

Heat shocks induce division synchrony in amicronucleate strains of Tetrahymena pyriformis (1). We are studying patterns of protein synthesis in strain GL by pulse labelling and gel electrophoresis. The findings will be related to mechanisms in the induction of division synchrony by temperature shocks.

Heat shocks spaced a normal cell generation apart will synchronize divisions and DNA replication periods (2). The synchronized cycle can be dissociated into subcycles of DNA synthesis and cell division by elevated temperature (3), and the subcycles can be conflicted (without use of cell fusion). It has been learned that DNA synthesis, and maturation for it, are agreeable with most, if not all, phases of the cell cycle whereas initiation of synthesis is not. The latter can not occur during G_2 and D (4).

When asynchronously replicating cells pour into a phase with synchronized G_2 and D they collect at the DNA synthesis initiation point. These cells are released jointly for DNA synthesis early in the next cycle, just after D. This mechanism tends to bring dissociated subcycles back in normal phase, and it accounts for the fact that heat shocks spaced a cell generation apart synchronize DNA synthesis with cell division rather than cell division alone.

Once initiated, DNA synthesis runs to completion before next G_2 and D. Together with DNA synthesis controls based on nucleoplasmic ratios, the reported mechanisms account for the sequence D, S, G_2, D seen in synchronized cells, though not for a sequence which includes G_1.

(1) E. Zeuthen & L. Rasmussen: Synchronized cell division in protozoa. In "Research in Protozoology", Vol. 4, 9-145. Ed. T.T. Chen, Pergamon Press.
(2) E. Zeuthen: Synchrony in Tetrahymena by heat shocks spaced a normal cell generation apart. Exp. Cell Res. 68, 49-60 (1971).
(3) E. Zeuthen: Order of cell division and DNA replication experimentally reversed i Tetrahymena. To appear in Exp. Cell Res.
(4) E. Zeuthen: Independent synchronization of DNA synthesis and of cell division in same culture of Tetrahymena cells. Exp. Cell Res. 61, 311-325 (1970).

764 RELATIONS BETWEEN GROWTH AND DIVISION DURING THE CELL CYCLE,
J.M. Mitchison, Dept. of Zoology, Univ. of Edinburgh, West Mains Road, Edinburgh EH9 3JT, Scotland

Cell growth during the cell cycle can be dissociated from the main periodic events of DNA synthesis and cell division since growth will continue when the DNA-Division cycle is blocked. There is, however, a connection between growth and the DNA-Division cycle which operated in normal cell cycles. Growth or cell size appears to be a critical control in initiating either DNA synthesis or cell division in various cell systems. In the normal wild type cells of the fission yeast Schizosaccharomyces pombe, cell division seems to be controlled by the attainment of a critical cell size at the time of mitosis. In the small wee mutants of S. pombe the control seems to operate at the G1/S boundary. There are analogous control systems of these two types in other cells, e.g. Escherichia coli, Saccharomyces cerevisiae, Physarum polycephalum, and mammalian cells. In addition, there are elements of cell growth in S. pombe (CO_2 production, enzyme potential, protein synthesis) which show periodic changes which are independent of the DNA-Division cycle. There must therefore be some kind of growth control which can continue to function when the DNA-Division cycle is blocked.

765 GENETIC ANALYSIS OF THE CELL LIFE CYCLE, David M. Prescott and R. Michael Liskay, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309.

The cell cycle consists of a succession of interconnected events that accomplishes doubling of all of the structural components and functional capacities of the cell, culminating in the division of the cell into two daughters. The succession of events is grossly recognizable by the progression of the cycle through G_1-S-G_2-M. The S and M periods in turn are each made up of a causal chain of events at a finer scale. Presumably, G_1 and G_2 also contain sequences of causally related events, but for these periods of the cell cycle proof is far less conclusive. Discovery of the events that make up the G_1 period is especially important because it contains the stop-start switch that controls the cycle and hence controls the rate of cell reproduction. We will review the temperature sensitive mutations obtained so far that cause the cycle of mammalian cells to stop at specific sites at the restrictive temperature, focusing particularly on the several mutations that cause arrest in G_1. G_1 specific mutations provide a means for identifying genes whose functions are responsible for the stop-start switch and for transit through the G_1 period. However, understanding the genetic basis of G_1 is complicated by the fact that certain cells of multicellular animals can proceed through the cycle without a measurable G_1 period (e.g., early embryonic cells, erythropoietic cells, certain cell lines). Intraspecific fusions between a G_1^- cell and a G_1^+ cell in culture results in G_1^- hybrids, demonstrating dominance of the G_1^- state. Enigmatically, fusion of a G_1^- cell of one species and a G_1^+ cell of a second species results in a G_1^+ hybrid, demonstrating recessiveness of the G_1^- state interspecifically. A G_1^- cell is still capable of expressing at least some G_1 functions. Fusion between a G_1^- cell of Chinese hamster and a mouse cell containing a temperature-sensitive mutation that causes G_1 arrest at the restrictive temperature yields a G_1^+ hybrid that is no longer temperature sensitive. Finally, we shall discuss the derivation of G_1^+ mutants from G_1^- cells. These G_1^+ mutants, all of which are recessive, fall into several complementation groups as defined in cell fusion experiments. This suggests that mutation in any of several different genes can cause a transition from G_1^- to G_1^+. These experiments form the beginning of the analysis of the genetic basis of the G_1 period both *in vitro* and *in vivo*.

This work is supported by grant # VC-193 from the American Cancer Society to D.M. Prescott and by the National Institutes of Health Fellowship #5 F32 CA05203 to R.M. Liskay.

Nucleic Acid Synthesis During Cell Proliferation

766 LIVER mRNA SEQUENCES IN THE hnRNA OF AVIAN ERYTHROID CELLS
Larry Lasky and Allan Tobin, Dept. of Biology, UCLA, LA, Ca, 90024
Comparisons of nuclear and cytoplasmic RNA sequence complexities have shown that a larger proportion of the genome is transcribed into hnRNA than eventually functions as mRNA. This complexity difference has been shown to be about 400 fold in avian erythroid cells (Lasky, L., Nozick, N., and Tobin, A. ms. submitted). Part of the nuclear RNA sequence complexity in avian erythroid cells has been shown to be due to mRNA sequences which are translated in liver tissue. Hybridization of liver mRNA-cDNA transcripts has shown that the majority of liver mRNA sequences are found in the low abundancy class of total and poly (A) hnRNA from erythroid cells. In addition,only about 70% of the liver mRNA sequences found in total hnRNA appear to be polyadenylated. These results suggest that the highly differentiated state of the erythroid cell may be in part due to qualitative selection of mRNAs from a large diversity of hnRNA transcripts, at least some of which may be translated in other cell types.

767 CHROMATIN REPLICATION IN VITRO. Ronald L. Seale. National Jewish Hospital and Research Center, Denver, Colorado 80206 and University of Colorado School of Medicine, Denver, Colorado 80262.
Isolated HeLa cell nuclei synthesize DNA for 60-90 min. Half of this DNA is in nucleosomes spaced with a similar neighbor-neighbor distance as in parental chromatin. By comparison of these structures with models for segregation, it is clear that nucleosomes pass from parent to daughter chromatid by a non-random process.

The structure of newly replicated chromatin is different from mature chromatin in three ways; 1) nucleosomes on newly replicated chromatin have an altered conformation, 2) nucleosome phasing is extraordinarily precise, i.e., spacer DNA heterogeneity is lost, and 3) the nucleosomal repeat size is reduced from 185 bp of mature chromatin to 170 bp in newly replicated chromatin.

During replication the nucleosome undergoes a conformational change. In both micrococcal nuclease limit digest and DNAase I digest patterns, the higher molecular weight oligonucleotides are either diminished in content, or lost. These patterns are strikingly similar to those derived from trypsinized chromatin; a model is proposed for the replicative state of the nucleosome wherein the histone N-terminal arms are neutralized by chemical substitution, thus preventing their interaction with DNA.

A soluble activity resides in cell extracts which catalyzes the conversion of the replicative conformation of the nucleosome to the "normal", non-replicating conformation.

768 CHARACTERIZATION OF TRYPANOSOMA BRUCEI POLYADENYLATED RNA, Richard O. Williams, Kenneth B. Marcu*, John R. Young, Luciana Rovis, Sally C. Williams, Int'l Lab for Res on Animal Diseases, Box 30709, Nairobi, Kenya; *Inst for Cancer Res, Philadelphia, PA.
The hemoparasitic protozoa belonging to the genus Trypanosoma cause extensive disease problems in both livestock and humans in Africa. These parasites are able to maintain a chronic pathologic infection by evading or limiting the immune capabilities of the host. It is believed that one of the mechanisms contributing to this chronic infection is the ability of the parasites to alter their membrane antigens. Populations of T. brucei brucei derived from cloned cells are capable of completely changing the antigenic structure of their membrane surface. The cell membrane of T. brucei contains a single predominant glycoprotein (MW 65,000) on its external surface which represents 95% of the total membrane protein. Polyadenylated RNA has been isolated from T. brucei and translated in a wheat germ cell-free system. The isolated RNA directs the synthesis of 4 predominant proteins. The single predominant membrane antigen responsible for antigenic variation has been identified as one of the 4 proteins by immunoprecipitation of the cell-free products. The poly(A)$^+$RNA has been electrophoresed under denaturing conditions in 98% formamide and shown to contain a predominance of material with an apparent size of 2.1 kb. The RNA in this peak has been eluted fro the formamide gels and shown to direct the synthesis of the membrane antigen and microtubule protein. cDNA has been synthesized from the predominant RNA and data will be presented showing the molecular complexity of the predominant RNA species. The trypanosome membrane represents an interesting system for studying eucaryotic membrane protein synthesis and regulation.

769 TRANSCRIPTION IN HAPLOID CELLS. Norman B. Hecht, Department of Biology, Tufts University, Medford, Massachusetts 02155

Ejaculated bovine spermatozoa incorporate ribonucleoside triphosphate into RNA under sterile assay conditions. The synthesis utilizes an endogenous template, continues for at least four hours, and is linearly dependent upon the concentration of spermatozoa. Maximal incorporation is observed in the presence of $MgCl_2$, β-mercaptoethanol and all four ribonucleoside triphosphates. Digestion studies indicate that the spermatozoa must be disrupted and the RNA extracted before it becomes susceptible to degradation by RNase. The RNA synthesis is strongly inhibited by rifampicin, acriflavine, ethidium bromide, Actinomycin D, and caffeine but is not inhibited by α-amanitin (at concentrations up to 10 ug/ml) or rifamycin SV (at concentrations up to 50 ug/ml). The ability of the α-amanitin and rifamycin SV to enter the permeabilized spermatozoa was not determined. To help localize the site(s) of transcription in the sperm cell, sperm were fractionated into head and tail fractions and then assayed for RNA synthesis. A minimum of 54% of the total synthesis occurs in the tail fraction, the site of the spermatozoal mitochondria. Atractyloside, an inhibitor of ATP transport into mitochondria, reduces the RNA synthesis in the tail fractions to 6% of control values but fails to depress RNA synthesis in the head fraction. These results suggest that the mitochondria synthesize the majority of the RNA transcribed in ejaculated sperm but that a transcriptive activity is also associated with the head fraction of these haploid cells. (Supported by NSF Grant PCM76-02094)

770 ALTERATIONS IN tRNA DURING ERYTHROID DIFFERENTIATION OF THE FRIEND CELL, Lawrence Kleiman and Jo Ann Woodward-Jack, Lady Davis Inst for Med Res, Montreal, Quebec

The Friend cell, a transformed murine cell infected with Friend erythroleukemia virus complex, undergoes erythroid differentiation when exposed to various chemical inducers, and loses the capacity to proliferate. During erythroid differentiation, changes in the tRNA population can be detected using reverse phase chromatography. Lysine tRNA can be resolved in 5 isoacceptor species in mammalian cells, and one of these, lysine $tRNA_4$, has been shown to vary in amount according to the proliferative capacity of the cell. It is absent in non-dividing tissues such as brain or muscle, but represents a major portion of lysine tRNA in rapidly-dividing cells such as are found in tumor or embryonic tissue (Ortwerth, B.J., G.R. Yonuschot and J.V. Carlson, Biochemistry 12 (1973) 3985). Lysine $tRNA_4$ comprises 30% of the total lysine tRNA in rapidly dividing, uninduced Friend cells, but less than 10% of the total lysine tRNA in uninduced cells which have reached a density-dependent stationary growth phase. Friend cells undergoing erythroid differentiation divide more slowly than uninduced cells, and finally cease proliferation, but lysine $tRNA_4$ becomes the major lysine tRNA species (greater than 50%). This does not appear to reflect erythroid properties of the cell, since the lysine tRNA of the mouse reticulocyte contains very little lysine $tRNA_4$. The non-dividing erythroid Friend cell, therefore, represents an exception to the finding that non-dividing cells usually have little or no lysine $tRNA_4$ present.

771 INHIBITION OF DNA CHAIN INITIATION BY 1-β-D-ARABINOFURANOSYLCYTOSINE (ARA-C) IN HUMAN LYMPHOBLASTS, Arnold Fridland, St. Jude Children's Research Hospital, Memphis, TN 38101

A novel biochemical effect of ara-C on DNA replication in exponentially growing human lymphoblasts (CCRF-CEM) has been observed. By incubating the cells with (^{3}H)dThd for 5 min and analyzing the nascent DNA by velocity sedimentation in alkaline sucrose gradients, it was possible to discern the initial effect of ara-C on DNA replication. In drug-untreated cells the most frequent size of single-stranded pulse-labeled DNA was 2.6×10^7 daltons. Since the size of replication units or replicons in mammalian DNA ranges from about 2×10^7 to 10^8 daltons, the labeling profile in CCRF-CEM cells represent replicons in various stages of replication. During the first 30 min of incubation, 5 nM ara-C caused a marked inhibition of the initiation of new replication units of DNA without affecting the elongation of previously initiated units. A later effect of the drug, which was evident about 1 h after treatment, was the reduction of the average rate of DNA chain elongation of individual replication units. It is concluded that the primary effect of ara-C on DNA synthesis is at the level of replicon initiation rather than chain elongation. A model will be presented to account for the differential effect of the drug on DNA chain initiation and elongation in mammalian cells.

772 MUTAGENESIS DURING IN VITRO DNA SYNTHESIS, Thomas A. Kunkel, Lisa A. Weymouth, K.P. Gopinathan and Lawrence A. Loeb, Institute for Cancer Research, Fox Chase, Philadelphia, PA 19111

We have developed a biological assay for measuring the fidelity of DNA synthesis in vitro using a natural DNA template. The template was single-stranded ØX 174 DNA containing an amber mutation (am3 in gene E). A specific DNA fragment, obtained separately from an Hae III digest of ØX 174 RFI DNA, was hybridized to this template and served as a fixed primer terminus 83 nucleotides from the mutation. An in vitro polymerization reaction was performed using different DNA polymerases under various conditions. The copied DNA was used to infect spheroplasts or calcium-treated cells, and errors were quantitated by measuring reversion of progeny phage to wild type. Using E. coli DNA polymerase I, the reversion rate indicated an approximate error rate of 1 in 8000 under normal copying conditions. The error rate was reduced to 1 in 500 using altered nucleotide pools or when Mn^{2+} was substituted for Mg^{2+}. From a comparison of the possible codon substitutions with the effect of altering various nucleotide concentrations in the pool it is concluded that the most likely substitution is a C for a T at position 587 in the amber codon. With the avian myeloblastosis virus DNA polymerase frequent mutations were observed even under normal conditions of copying. The absolute error rate of 1 in 706 $\pm$ 300 obtained using this natural DNA template confirms our earlier findings (using synthetic polynucleotide templates) and indicates that the AMV polymerase is error prone. This assay system is currently being used to determine the accuracy of nucleotide selection by DNA polymerases-α and -β from a variety of eucaryotes.

773 FUNCTION OF POLYAMINES IN CELL PROLIFERATION, David R. Morris, University of Washington, Seattle, Washington 98195

Rapidly proliferating animal cells generally have elevated levels of putrescine, spermidine and spermine. This is true in small lymphocytes which show large increases in cellular polyamines after activation by concanavalin A. To examine the role of these increases, we used two inhibitors of polyamine biosynthesis, methylglyoxal bis(guanylhydrazone) and α-methylornithine. When these inhibitors were used with activated lymphocytes to maintain the unstimulated polyamine levels, the incorporation of ^{3}H-thymidine into DNA and the rate of cell division were inhibited, with no effect on the synthesis of RNA or protein. The effect of polyamine deficiency was on DNA replication per se, since cells entered S phase at the same time in the presence or absence of polyamine synthesis. Parallel results were obtained with nuclei isolated from the polyamine-deficient cells. Several lines of evidence argue that the action of these inhibitors is not a secondary one, unrelated to inhibition of polyamine synthesis. Inhibition of DNA replication was reversed by polyamines, either exogenously added or endogenously synthesized. Also, similar dose response curves were found for inhibition of polyamine accumulation and of DNA replication. As well, examination of a series of bis(guanylhydrazone) derivatives showed a direct correlation between inhibition of polyamine synthesis and of DNA replication. The inhibitors of polyamine synthesis had no effect on in vitro DNA replication when added directly to nuclei. Thus, it seems that elevated intracellular polyamines seen in rapidly growing cells are necessary for optimal DNA synthesis, perhaps through direct interaction with the replication machinery.

774 THE ROLE OF DEFECTIVE INTERFERING PARTICLES IN ATTENUATION OF POLIOVIRUS TYPE 1, Julie B. Milstien, Bureau of Biologics, FDA, Bethesda, Maryland 20014

To assess the role of defective interfering (DI) particles in the attenuation of poliovirus type 1, neurovirulent and attenuated strains were subjected to as many as ten serial undiluted passages in four cell substrates. In all cases, the neurovirulent Mahoney strain generated lower density ^{3}H-uridine-containing particles having lower specific infectivity plus the ability to interfere with replication of standard virus. Thatis, they possessed the characteristics of DI particles. Another neurovirulent type 1 strain, LSa, and Mahoney virus which had been subjected to repeated plaque purification were also capable of generating DI particles after ten serial passages in primary African green monkey kidney cells. Three type 1 non-neurovirulent vaccine strains, including two derivatives of the Sabin original strain LSc-2ab/KP_2, and the CHAT strain, did not produce DI particles when passaged serially under identical conditions. These results suggest that the attenuation of type 1 poliovirus, as measured by the loss of its ability to cause paralysis in the monkey neurovirulence test, may be due to factors other than the generation of DI particles during virus replication.

775 RIBONUCLEOTIDE POLYMERIZATION CATALYZED BY PARTIALLY PURIFIED DNA POLYMERASE α OF THE SEA URCHIN, F.M. Racine and Paul W. Morris, Univ. of Ill. Coll. of Med., Chicago, IL. Fractionation of extracts from Strongylocentrotus eggs or embryos yields two DNA dependent DNA polymerase classes identified as the β-polymerase (3.5s; resistant to N-ethylmaleimide (NEM) or 0.1M $(NH_4)_2SO_4$) and the α-polymerase (6-8s; inhibited by NEM or 0.1M $(NH_4)_2SO_4$). The α-polymerase is heterogeneous by sedimentation and, as well, fractionates into three species on DEAE-Sephadex. The RNA polymerizing activity previously shown to fractionate with DNA polymerase and to be distinguishable from the DNA dependent RNA polymerases. (P.W. Morris and W.J. Rutter, Biochemistry 15:3106-3113, 1976) is now seen to co-purify with one of the α-polymerase species through multiple steps. The purified RNA and DNA polymerizing activities co-sediment (6s) and both are inhibited by NEM. DNA polymerase α polymerizes dNTPs in response to nuclease activated DNA, primed deoxynucleotide polymers, and poly dC templates. In the presence of Mn^{+2}, but not Mg^{+2}, the polymerase incorporates rNTPs in response to nuclease activated DNA and poly dC templates, but not with other primed homopolymers. Incorporation of rNTPs ranges from 2.5 - 20% of the incorporation of dNTP in presence of Mg^{+2} and depends upon the degree of purification. Templated rather than non-templated end addition in the Mn^{+2} induced rNTP polymerization is suggested by template-substrate studies. Mixed rNTP and dNTP reactions result in inhibition of rNTP polymerization. Thus dATP inhibits (K_i=4.5μm) the incorporation of rGTP (Km=11μm) but not dGTP (Km=0.6μm). These observations suggest a possible role for the DNA polymerase α in RNA polymerization under conditions of low, but not high, dNTP concentrations.

776 RIBOSOMAL RNA SYNTHESIS AND A CHANGE IN PROCESSING DURING SEA URCHIN EMBRYOGENESIS, Martin Nemer, Saul Surrey and Irith Ginzburg, The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111
Ribosomal RNA (rRNA) synthesis is readily demonstrable in the sea urchin gastrula, but in earlier stages its detection is hampered partially by an overwhelming proportion of heterogeneous RNA (hRNA) synthesis. This difficulty can be overcome by using methionine as precursor and taking advantage of the approx. 80 specific 2'-O-methylations in 26S+18S rRNA, compared to the two methyls per 5' cap of hRNA, each detected by DEAE chromatography. Lytechinus pictus embryos were pulsed for 1 or 2 hr and > 15S RNA from nucleus or cytoplasm, respectively, was RNased, analyzed on DEAE and the molar ratio of rRNA/capped hRNA (R) noted. In 13-hr early blastulae rRNA was readily detected in nuclei (R=0.08), but barely detected in cytoplasm (R=0.01). The R value increases to approx. 0.3 in both nucleus and cytoplasm of 45-hr late gastrulae. Thus during development, the synthesis of nuclear (precursor) rRNA increases relative to capped hRNA and rRNA emerges into the cytoplasm at an increasing rate compared to capped mRNA. This apparent relative enhancement of processing of nuclear rRNA to cytoplasmic ribosomes occurs abruptly during a period of approx. 4 hr prior to mesenchyme blastula. Measurements of the absolute rates of rRNA and capped hnRNA synthesis indicate a constancy of rRNA transcription and a decrease in capped hnRNA transcription per nucleus. Therefore, the shift in ribosome production may be largely attributable to post-transcriptional processing to cytoplasmic forms.

Studies on Mitosis In Vivo

777 THE DURATION OF METAPHASE IN NORMAL VS. TRANSFORMED CELLS IN CULTURE, Jesse E. Sisken and Susan Bonner, Dept. of Pathology, University of Kentucky, Lexington, KY 40506
According to some reports (e.g. Lewis and Lewis, Am. J. Cancer 16:1153, 1932) metaphase takes more time in tumor than in normal cells. The reverse has also been reported for cultures of normal and neoplastic cervical epithelial cells (Richart et al JNCI 39:571, 1967). Since an increased duration of metaphase might be a useful and informative feature of the transformed state, a further study of metaphase durations seemed in order. In non-transformed WI-38 and human foreskin fibroblasts, metaphase was determined from time lapse films to be 7.6 ± .48 and 11.8 ± .85 min. respectively. The duration of metaphase in SV40 transformed WI-38 cells grown under the same conditions was 35.5 ± 1.67 min. Metaphase times in other hyperdiploid lines of cells such as HeLa are also generally observed to take 20 min. or more on the average. Thus metaphase times of these transformed cells are significantly longer than those of the non-transformed cells we studied. Although the transformed cells are hyperdiploid, there is not a linear relationship between metaphase duration and chromosome number since SV40-WI-38 cells have 60% more chromosomes but a 450% increase in metaphase duration. Since calcium levels affect metaphase duration (Sisken and VedBrat, JCB 75:263a , 1977) and since levels of calcium in transformed cells may be altered (e.g. Balk PNAS 68:271, 1971) the observations suggest that the increased duration of metaphase may reflect fundamental changes associated with the transformed state. Supported in part by the Sanders-Brown Kentucky Research Center on Aging.

778 THE "NUCLEAR MITOTIC APPARATUS" IN SEA URCHIN EGGS, Neidhard Paweletz, Deutsches Krebsforschungszentrum, D-6900 Heidelberg, FRG; and Daniel Mazia, Department of Zoology, University of California, Berkeley, CA 94720

The chromosome cycles induced in sea urchin eggs by treatment with NH_3 include some of the cytoplasmic reorganization associated with the formation of a normal MA but, in the absence of normal mitotic centers, no poles are formed and there is no anaphase separation of replicated and split chromosomes. The formation of this "nuclear mitotic apparatus" has been examined by transmission electron microscopy. (1) No centrioles are found in the aster-like structure. (2) As in normal mitosis, the clear zone is formed by a dense congregation of vesicles. (3) Microtubules appear, initially very few and never as abundantly as in a normal MA. (4) The microtubules radiate from numerous metallophilic foci. (5) Chromosomes begin to condense before the nuclear envelope breaks down. Initially, no kinetochores are seen. Then kinetochores are observed, but without attached MTs. At a late stage, MTs are attached to kinetochores, but not in well-defined bundles. (6) Connections between kinetochores and foci from which MTs radiate are not seen. (7) When eggs containing the "nuclear MA" are fertilized, the number of microtubules increases within 5 minutes. In summary, the "nuclear MA" exhibits a number of structural features of a normal MA, but can not form a bipolar spindle in the absence of mitotic centers.

779 THE EFFECT OF EMETINE ON FIRST CLEAVAGE DIVISION IN THE SEA URCHIN, STRONGILOCENTROTUS PURPURATUS, E. B. Wagenaar and D. Mazia, Dept. of Biol. Sciences, U. of Lethbridge, Lethbridge, Alta, Canada T1K 3M4, and Dept. of Zool., U. of Cal., Berkeley, Ca. 94720

The inhibition of protein synthesis by emetine at specific time periods during the first S-period prevented the completion of definite cytological events in mitosis of the first cleavage division in the sea urchin, Strongilocentrotus purpuratus. Emetine added to the suspension in a final concentration of 10^{-4} Mol. before 30 minutes after fertilization completely prevented nuclear breakdown. The inhibitor, when added between approximately 25 and 29 minutes after fertilization and maintained in the suspension, induced large, arrested nuclei containing strongly condensed chromosomes. The addition of emetine between 30 and 35 minutes gradually allowed more and more cells to break down the nuclei and pass into an irregular metaphase which frequently aborted into highly stretched configuration containing thin, irregular chromosomal strands. After 36 minutes emetine had no apparent cytological effects and cleavage division appeared normal. It is concluded that in all probability during the time periods of approximately 20 to 35 minutes after fertilization, the young sea urchin embryos sequentially produce a number of proteins essential for normal progress of mitosis in the following order: protein(s) essential for chromosomal condensation (before about 25 minutes after fertilization), protein(s) needed for nuclear breakdown (between 25 to 30 minutes), and protein(s) required for regular alignment of chromosomes at metaphase (possibly before 30 minutes to about 35 minutes). (Supported by NRC of Canada grant #A-4926 and USPHS grant #13882.)

780 THE REPRODUCTION OF MITOTIC CENTERS: NEW INFORMATION ON AN OLD EXPERIMENT. Greenfield Sluder and David. A. Begg, Biol Dept, Univ of Pa., Philadelphia, Pa. 19104

In sea urchin eggs the number of functional mitotic centers (spindle poles) doubles in telophase. From the observation that monopolar spindles are formed in mercaptoethanol treated eggs, Mazia et al (J. Biophys. Biochem. Cyt. 7:1, 1960) proposed that center reproduction consists of splitting (oneness to twoness), replication (ability to split at the next cycle), and the physical separation of the daughter centers. We induced monopolar spindles by treating eggs of Lytechinus pictus for one cell cycle with .1M mercaptoethanol as described by Mazia et al 1960. When observed with the polarizing microscope such cells develop in one of two ways: 1) The birefringence of the monopole fades as in telophase and a single nucleus reforms. At the next mitosis, the cell forms a bipolar spindle. That and subsequent divisions are normal. 2) The monopole persists and eventually its aster appears doubled. These two asters separate giving a bipolar spindle of normal appearance without the intervening reformation of a nucleus. Anaphase is normal and is folowed by cleavage and reformation of two nuclei. At the next mitosis the daughter cells form monopolar spindles. Their asters double and the sequence is repeated again and again. Identical results are observed when a spindle is broken into half spindles by micromanipulation. Control experiments show that this is not due to nonspecific damage to the cell. This work demonstrates that center replication and splitting are distinct events that can be put permanently out of phase with each other. This phenomenon is most easly understood in terms of the replication and splitting of centriole pairs. Supported by NSF GB31739, NIH CA10171, NIH GM13882, NIH ST01 GM00849-12 Predoctoral Traineeship to G.S. and American Cancer Society Postdoctoral Fellowships to G.S. and D.A.B.

Control of Fertilization

781 ISOZYMES OF G6PD AT FERTILIZATION AND AMMONIA ACTIVATION, Mary Lee Barber and Nadene Shutt, Biology Dept. California State Univ. Northridge, Ca., 91330.
Glucose-6-P dehydrogenase (G6PD) activity was compared in homogenates and lubrol extracts of unfertilized (U), fertilized (F), and ammonia activated (A) eggs of Lytechinus pictus. Enzyme activities of U, F, or A supernates after 1% lubrol extraction were comparable. 5% acrylamide slab gels were run in tris pH 8.8, followed by assay in 2.5 mM G6P, 50 mM triethanolamine pH 7.4, 10 mM $MgCl_2$, 0.2 µM NADP, 70 µM phenazine methosulfate, 10 mM KCN, 5 mM EDTA, 0.3 mM tetrazolium in a dark box on a shaker for 2 or more hrs. Three total isozymes were seen in the gels and were designated I1,I2,I3 with I3 being furthest toward the anode, from the origin. No Coomassie Blue stained bands could be seen at these locations. U and F both contained I2, more active in F. U also had I3, F also had I1. A had I1,I2,I3. I2 and I3 also used Fructose 6P. I2 utilized to a lesser extent 2-deoxy G6P, 6P-gluconate, and G1P. F-I1 used G1P for a faint band and 2-deoxy G6P to a greater extent than I2 did. No isozymes used NAD.

The isozyme pattern and substrate specificity change at fertilization. The most rapidly migrating isozyme is lost and a new slower one appears. Ammonia activation results in a pattern intermediate between U and F. Ammonia activated eggs are also known to be intermediate in other responses: protein synthesis and other metabolic changes are activated as usually occurs at fertilization, but the eggs don't cleave and there is no cortical reaction. It is interesting that different G6PD isozymes appear so rapidly after fertilization, prior to initiation of cleavage and RNA synthesis, at a time when it has been reported that the pentose shunt becomes active and the NADP concentration rises.

782 FERTILIZATION-INDUCED CHANGES IN MEMBRANE FLUIDITY, Judith Campisi and Carl J. Scandella, Dept. Biochem., SUNY, Stony Brook, N. Y. 11794

The relationship between activation of ova and the fluid state of membranes has been studied in two species of sea urchin. Lytechinus pictus and Strongylocentrotus purpuratus eggs were labeled with a spin-label fatty acid, 5-doxylstearate, by incubation for 10 min. at 15° C. Spin-labeled eggs developed into normal plutei. From the ESR spectra recorded at 12° C, the order parameter, S, was measured graphically. A decrease in S may be interpreted as an increase in membrane fluidity. Following fertilization, S decreases 2.21 ± 0.86% for L. pictus eggs and 3.19 ± 1.45% for S. purpuratus eggs. Eggs partially activated by ammonia sea water (Dev. Bio. 40:245 (1974).) exhibit a similar decrease in S: 2.45 ± 1.69% and 2.21 ± 0.68% for L. pictus and S. purpuratus eggs respectively. The order parameter of unfertilized eggs is not perturbed by the removal of the jelly coat or vitelline layer, nor by treatment with cortical granule proteases. The data indicate that activation is accompanied by a structural alteration which allows membrane lipids to enter a more fluid state. The detection of increased membrane fluidity following ammonia activation suggests that this change is not due to massive membrane reorganization resulting from cortical granule exocytosis and may be a late event accompanying increased ion permeability and transport rates.

Nucleocytoplasmic Interactions

783 USE OF CHLORAMPHENICOL RESISTANT CYTOPLASTS TO TRANSFER MICROTUBULE ORGANIZING CENTERS, Jerry W. Shay and John W. Fuseler, The University of Texas Health Science Center at Dallas, Department of Cell Biology, Dallas, Texas 75235

Centrifugation of mammalian cells growing in monolayer culture in the presence of cytochalasin B results in the production of enucleated cells called cytoplasts. Such cytoplasts, which contain the centrioles and associated microtubules, can be fused to whole cells using polyethylene glycol, resulting in the production of cytoplasmic hybrids termed cybrids. Chloramphenicol resistance can be cytoplasmically transferred and can be used as a selective marker for the source of cytoplasm in cybrid experiments. We now present evidence that microtubule organizing centers (MTOCs) can be cytoplasmically transferred. Centrioles and pericentriolar material are important MTOCs which can be visualized along with cytoplasmic microtubules utilizing techniques of indirect immunofluorescence with antibodies directed against tubulin. In order to help illuminate the presence of MTOCs, the cells or cybrids are first incubated in colcemid (0.06 µg/ml) for two hours, which depolymerizes cytoplasmic microtubules. After 15 minutes of recovery from the colcemid treatment, the cells are fixed and subsequently examined by indirect immunofluorescence. Under these conditions, cells usually contain one juxtanuclear MTOC, except during mitosis when two MTOCs are observed. We have observed cybrids in various stages of the cell cycle that consistently contain two or more MTOCs. In addition, we have produced cybrids that contain as many as 8-10 MTOCs. Preliminary observations indicate that transferred MTOCs may persist in future generations.

784 THE CHROMOSOME CONDENSATION CYCLE IN THE SEA URCHIN EGG, Geoffrey W. Krystal and Dominic L. Poccia, Department of Biology, S. U. N. Y. at Stony Brook, Stony Brook, New York 11794

Mazia (P.N.A.S. 71, 690, 1974) has demonstrated that ammonia-activated sea urchin eggs can induce premature chromosome condensation (PCC) in fertilizing sperm nuclei. We have extended his observations to show that activated nucleated and enucleated egg halves are capable of inducing PCC. The peak of PCC-inducing activity occurs at approximately the same time after activation in both nucleated and enucleated merogones. However, the lifetime of the activity is 5-10 times longer in the nucleated halves than in the enucleated halves. These effects were observed in merogones prepared either by density gradient centrifugation or manual bisection. Transcription of mitochondrial genomes or of the sperm nuclear genome is not required, since the induction of PCC is unaffected by incubation of the eggs in ethidium bromide (10 μg/ml.) or actinomycin D (25 μg/ml). PCC induction becomes independent of protein synthesis in late S or early G2 as determined by blocking translation with 0.1 mM emetine.

In summary, our results indicate that the first chromosome condensation cycle in the sea urchin egg is preprogrammed in the cytoplasm, ready to be set in motion by fertilization or activation. The lifetime of the cytoplasmic chromosome condensing environment, however is prolonged by some nuclear component, possibly the chromatin. For chromosome condensation to occur protein synthesis may only be required prior to late S or early G2.

785 CONSTRUCTION OF MOUSE-HUMAN NUCLEAR-CYTOPLASMIC HYBRID CELLS M.J. Hightower and J. Lucas, Dept. Microbiology, State Univ. of New York at Stony Brook, Stony Brook, N.Y.

Nuclear transplantation has been used to construct viable, growing mouse-human hybrid cells. Using both nuclear and cytoplasmic drug markers, the origin and viability of the hybrids has been established. Cytoplasts from human diploid fibroblasts and karyoplasts from mouse A9 cells were prepared by cytochalasin-induced enucleation; Sendai virus was then used to fuse the two into nuclear-cytoplasmic hybrids. The A9 nucleus lacks hypoxanthine-guanine phosporibosyltransferase(HGPRT), wheras the human cytoplasmic donor contains the enzyme. By the criterion of 8-azaguanine resistence, the hybrids contain the enzyme immediately after fusion, but have no HGPRT after three days of growth. The human cytoplasmic donor contains diphtheria toxin receptors on its plasma membrane; the mouse nucleus does not code for the toxin receptor. The hybrids are sensitive to toxin immediately after transplantation, but not four days later. Thus it has been shown that the hybrid cells contain the markers of the human cytoplasmic donor, but that these markers disappear under the control of the mouse nucleus. Immediately after transplantation, hybrid cells are morphologically identical to the cytoplasmic donor, but 24-48 hours after transplantation, they exactly resemble the shape of the nuclear donor. Using immunofluorescent techniques to visualize actin, myosin and tubulin structures, studies are currently under way to investigate the role of cytoplasm in determining cytoskeletal configurations and cell shape.

786 METHYLATION OF RNA TRANSPORTED IN VITRO, Sarah E. Stuart, Ronald J. Patterson and Fritz M. Rottman, Michigan State University, East Lansing, MI 48824

The observation of a methylated 5'-terminal cap structure in both HnRNA and cytoplasmic mRNA suggests that these post-transcriptional methylation events may function in the intranuclear processing and/or transport of mRNA. Kinetic data indicate that the ratio of cap I to cap II in cytoplasmic mRNA changes suggesting that at least two separate methylation events occur during processing and transport of mRNA in eukaryotic cells. In order to study these methylation events we have developed a system in which it is possible to study the release of RNA from the nucleus in vitro using isolated murine myeloma (MOPC-21) nuclei which have been prelabeled with RNA precursors. Optimum conditions for release of RNA have been established by examining such parameters as the concentration of Mg^{++}, presence of cytoplasmic protein, and effect of temperature on the system. A significant proportion of the released RNA is polyadenylated, and the system appears to accurately reflect transport of RNA to the cytoplasm in vivo. This in vitro transport system has provided the opportunity to dissect events of post-transcriptional processing of RNA by separating nuclear events from subsequent cytoplasmic events. We have examined RNA transported from isolated nuclei in vitro following incubation of the cells with ^{3}H-methyl-methionine. Analysis of polyadenylated RNA released from isolated MOPC-21 nuclei in the presence or absence of added cytoplasmic protein has shown that both cap I and cap II 5'-terminal structures are present.

787 ISOLATION OF CYTOPLASMIC INITIATION FACTOR FOR RNA POLYMERASE I. Mari K. Haddox and Diane Haddock Russell, University of Arizona Health Sciences Center, Tucson, AZ 85724.

The synthesis of ribosomal RNA by RNA polymerase I is a key event in all growth systems. Transcription by RNA polymerase I is regulated by a labile protein which serves to initiate the enzyme on the chromatin template. Isolated nuclei have only a fraction of the total polymerase I engaged in a transcriptional complex. RNA synthesis by isolated nuclei in the presence of α-amanitin (400 µg/ml) proceeds for only a few minutes while those RNA chains which were initiated *in vivo* are completed and then halts as no new polymerase molecules can reinitiate due to the apparent lack of the physiological initiation factor. Total enzyme present can be assessed *in vitro* with exogenous substrates; however, this interaction of the polymerase with the deoxynucleotide template is nonspecific and not similar to the physiologic in that it can be disrupted by the polyanion heparin. Polymerase which is initiated *in vivo* and isolated as such in intact nuclei is resistant to heparin disruption. We have employed aminohexyl-sepharose chromatography to isolate a cytoplasmic factor from the liver of a methylisobutylxanthine-challenged calf which promoted the interaction of RNA polymerase I with endogenous template in isolated nuclei. The apparent action of this factor is to initiate new polymerase molecules since not only is the initial rate of the enzyme increased but also there is an increased formation of RNA product. Both the increase in polymerase activity and the amount of product formed was proportional to the units of factor added. The initiation complex promoted by this factor between the endogenous polymerase and chromatin in isolated nuclei is identical to that formed *in vivo* in that it is resistant to heparin disruption.

788 PROPERTIES OF NATURALLY ACETYLATED HeLa CHROMATIN , Lidia C. Boffa , Giorgio Vidali and Vincent G. Allfrey, The Rockefeller University, New York, NY 10021.

The exposure of HeLa S3 cells to 7 mM sodium butyrate leads to a high degree of acetylation of histones H3 and H4 and the effect of the durg is reversible (Riggs et al. Nature 268 , 462, 1977).We have prepared nucleosomes from butyrate-treated cells and control cells by limited digestion with micrococcal nuclease. Both nucleosomes appear to be similar in some of their physical properties (sedimentation coefficient, DNA-melting curves and circular dichroism spectra) in contrast with some recent reports showing differences between chemically acetylated chromatin and control chromatin (Wallace et al. Proc.Natl.Acad.Sci.U.S. 74, 3244, 1977).The reasons for these discrepancies have not been estabilished but it is known that chemical acetylation modifies sites on histones that are not acetylated in vivo.
Double label experiments where control cells were incubated with ^{3}H thymidine and butyrate-treated cells received ^{14}C thymidine show that chromatin containing highly-acetylated histones is more rapidly degraded by DNAse 1. The view that postsynthetic modification of the histones alter chromatin configuration is supported by electrophoretic analysis of the remaining histones at successive staged during DNAse 1 digestion. The most highly acetylated forms of histone H4 are preferentially released at early times during digestion, as expected if they were localized on regions of the chromatin that had been activated for potential transcription.
(Supported by the The National Foundation March of Dimed (1-440), USPHS (CA 14908) and the Rockefeller Foundation Program in Reproductive Biology).

789 NUCLEAR TRANSPLANTATION WITH SYNCHRONIZED AMPHIBIAN CELLS, Cecilia H. von Beroldingen, Dept of Biol, Univ of Oregon, Eugene, OR 97403

Single cell nuclei from synchronized Rana pipiens cell cultures have been transplanted into enucleated Rana pipiens eggs to determine whether there is a correlation between the phase of the cell cycle from which a donor nucleus is transplanted and its ability to program development of the recipient egg. Synchronous populations of the haploid cell line ICR 2A were obtained by the mitotic selection technique. Nuclei were transplanted from cultures at times corresponding to the G1, mid S, late S, and G2 phases.

The ability of the transplanted nuclei to promote development to the gastrula stage is not significantly different for any of the phases tested. About 10% of the injected eggs formed blastulae, most of which arrested before the gastrula stage was reached. Development to the neurula stage and beyond occurred only transfers involving nuclei from mid S, late S, or unsynchronized logarithmically growing cultures, but only 0.5 to 1% of the injected eggs reached these advanced embryonic stages.

Histological studies of the initial response of the transplanted synchronized nuclei to the egg cytoplasm were carried out. Preliminary results indicate that the G1 nuclei are least responsive to egg cytoplasm as judged by the criteria of nuclear enlargement and chromatin decondensation.

790 REGULATION OF RIBOSOME PRODUCTION DURING AMINO ACID DEPRIVATION, Micnael Centrella, Arnold I. Meisler, University of Rochester Medical Center, Department of Microbiology Rochester, New York 14642

Cell number increase is inhibited in SV40 transformed 3T3 mouse fibroblasts after refeeding with medium severely limited for either leucine, methionine, or phenylalanine individually, as well as for other essential amino acids. In the first several hours after methionine deprivation there is a rapid inhibition in the arrival of newly synthesized ribosomes to the cytoplasm, consistent with a high level of undermethylated 45S precursor rRNA found in the nucleolus. This is not true for leucine or phenylalanine deprivation. While there is an immediate and significant drop in the free methionine pool in methionine deprived cells, and a concomitant expansion of the pool in control cultures, the effects are temporary, and the steady state pool levels are recovered by 4 hours later, when both nucleolar and cytoplasmic rRNA measurements are made. Levels of the methylating substrate, S-Adenosyl-L-methionine, newly labelled rRNA and protein, and transport of uridine are equivalent under either condition. Available evidence therefore indicates that under methionine deprivation the initial signalling event for cytoplasmic ribosome limitation occurs very early, is generated by communication with the external nutritional environment, is sensitive to the perpetuation of external deprivation in the wake of adequate internal stores of methionine recovered by anapleurotic means, and is independent of later events which disallow cell multiplication during deprivation for other essential amino acids. The nature of the effector is presently unknown, but investigation is in progress to elucidate the effects of methionine deprivation on the in vitro activity of nucleolar rRNA methylase in biochemically active nucleoli.

791 THE ROLE OF CYCLIC NUCLEOTIDES IN THE REGULATION OF MITOTIC ACTIVITY IN SSPE VIRUS INFECTED HUMAN BRAIN TISSUE, M. Pat Leuschen, Depts. of Anatomy and Physiology-Biophysics, University of Nebraska College of Medicine, Omaha, NE 68105.

The intracellular level of cyclic GMP was independent of the rate of cell division in cells derived from virally infected brain tissue. The phosphodiesterase inhibitor RO7-2956 (4-dimethoxybenzyl-2-imidazolidinone) increased the intracellular level of cyclic GMP, but did not effect the level of cyclic AMP. There was no correlation between the increase in cyclic GMP levels following addition of RO7-2956 and changes in mitotic activity in the brain cell cultures. Experimental manipulations which increased the cyclic AMP level were accompanied by a decreased mitotic rate indicating a correlation between mitotic activity and the level of cyclic AMP in the same cells. Raising the intracellular level of cyclic AMP by exogenous dibutyryl cyclic AMP or cyclic AMP or the use of other phosphodiesterase inhibitors routinely increased the level of cyclic GMP as well. Conversely increasing the intracellular cyclic GMP level by adding the exogenous cyclic GMP increased the level of both cyclic GMP and AMP.

The culture system was a cell line derived from viral infected human brain tissue originally obtained from a patient with subacute sclerosing panencephalitis (SSPE). The intracellular levels of cyclic AMP and cyclic GMP were monitored by radioimmunoassay following manipulation of the system by addition of exogenous cyclic GMP (0.05 mM), addition of exogenous dibutyryl cyclic AMP (0.5 mM), or cyclic AMP (0.5 mM) and the use of phosphodiesterase inhibitors: theophylline (1.0 mM), papaverine (50 µg/ml), 4-3-butoxy-4-methoxy benzyl-2-imidozalidinone (RO20-1724) and RO7-2956. Cell division was monitored in treated and non-treated cultures at 24 hr intervals by analyzing the cell number and mitotic index.

Regulation of the Cell Cycle

792 CONTROL OF THE NUCLEAR DIVISION CYCLE IN PHYSARUM POLYCEPHALUM, Wilhelm Sachsenmaier, John Tyson and Gregorio Garcia-Herdugo, Inst f Biochem & Exp Krebsforschung, Univ. Innsbruck, A-6020 Innsbruck, Austria.

Mitosis in multinuclear plasmodia of the myxomycete P.polycephalum are naturally synchronous. Mixed plasmodia containing different sets of nuclei can be prepared by fusion of plasmodial pieces representing different stages of the mitotic cycle. Nuclei with a different history become synchronized rapidly after plasmodial fusion. A model is proposed suggesting that the timing mechanism of mitosis involves the stoichiometric interaction of a cytoplasmic initiator with nuclear receptor sites. The initiator appears to accumulate proportional to plasmodial growth whereas the number of nuclear sites doubles stepwise during each mitosis. Nuclear division is triggered at a critical ratio of initiator/nuclear sites which is reflected by a corresponding ratio of mass/DNA. This "titration model" characterizes the control mechanism of mitosis as a relaxation oscillator. The onset of mitosis functions as an essential component of the oscillator as opposed to alternative concepts based on the assumption of continuous limt cycle oscillators. Studies with inhibitors of DNA- and protein synthesis (5-fluoro-2'-deoxyuridine, cyclohexamid, anisomycine), temperature shocks, UV- and X-irradiation further suggest that the mitotic initiator is unstable (half life 1-2 hrs) and cannot be synthesized during the S-period. (Supported by the Fund of Austrian Cancer Research Institutes, NIH-grant No 5 F32 CA 05152-02, and Fundacion Juan March)

793 SYNTHESIS *in vitro* OF CELL CYCLE REGULATED POLYPEPTIDES FROM *Chlamydomonas reinhardi*
Stephen H. Howell, Department of Biology, University of California, San Diego, La Jolla, California 92093

The overall rate of protein synthesis (1) and the individual rates of synthesis of certain "cell-cycle regulated" polypeptides (2) vary at different cell cycle stages in synchronized *C. reinhardi* cells. Variations in the overall rate of protein synthesis result from corresponding changes in both polypeptide initiation and elongation rates (1).

We have translated polyribosomes and poly (A) RNA from different cell cycle stages in an effort to reconstruct these cell cycle patterns *in vitro*. We have found that polyribosomes obtained from cells at different cell cycle stages show variations in the rate of chain elongation *in vitro* in much the same manner as *in vivo* indicating that some of the properties regulating chain elongation efficiency must be vested in polyribosomes. Also, changes in relative rates of synthesis of certain cell-cycle regulated polypeptides are observed *in vitro* when either polyribosomes or poly (A) RNA from different cell cycle stages are translated. The most dramatic changes are seen in the rate of synthesis of cytoplasmic-derived, chloroplast membrane proteins. These observations demonstrate that the synthesis of some cell cycle regulated polypeptides is controlled by the availability for translation of their corresponding messenger RNA.

1. Baumgartel, D.M. and Howell, S.H. (1977) Biochemistry 16, 3182-3189.
2. Howell, S.H., Posakony, J.W. and Hill, K.R. (1977) J. Cell Biol. 72,223-241.

794 SYNCHRONOUS REPLICATION OF A LARGE, CYTOLOGICALLY-DEFINED CHROMOSOME REGION IN A DRUG-RESISTANT HAMSTER CELL LINE,
J. L. Hamlin, Sidney Farber Cancer Institute, Boston, MA 02115, and J. L. Biedler, Sloan-Kettering Institute for Cancer Research Rye, NY 10580.

A Chinese hamster cell line (DC3F/A3) selected for resistance to high levels of methotrexate also displays an unusual, large chromosome in metaphase spreads (J. L. Biedler and B. A. Spengler, Science 191: 185-187, 1976). This marker chromosome is characterized by a region (HSR) on the long arm which fails to band when treated with G-banding or Quinacrine techniques, but which stains intensely when C-banded. The HSR is estimated to contain 3-5% of the cellular DNA, and comprises approximately 50% of the length of the marker chromosome. In both exponential and synchronized cultures of this cell line, the HSR is shown to commence replication synchronously at many sites within its length at the beginning of the S period, and to cease replication synchronously between the third and fourth hr of a 6.5 hr S period. The replicons comprising the HSR thus appear to respond to the same initiation signal for DNA synthesis in early S. In addition to this readily identifiable early-replicating marker, DC3F/A3 has an inactivated, late-replicating X chromosome. Hence, this cell line is extremely useful for studies on time-ordered replication of DNA.

795 TYROSYLTUBULIN LIGASE AND COLCHICINE BINDING ACTIVITY IN SYNCHRONIZED CHINESE HAMSTER CELLS,
Gerald L. Forrest and Robert R. Klevecz, City of Hope National Medical Center, Duarte, CA 91010

Tyrosyltubulin ligase (TTL) was found to be present in CHO and V79 Chinese hamster cells grown in tissue culture. The enzyme is soluble and appears to be the same as TTL originally described in brain tissue (Barra et al., J. Neurochem. 20, 97, 1973). The enzyme requires potassium, magnesium and ATP for maximum activity and uses tubulin and tyrosine as substrates. TTL activity was analyzed through the cell cycle in V79 and CHO cells. In V79 synchronized by mitotic selection TTL displayed two peaks of activity at 4 hours and 7 hours into the cycle, corresponding to early S and mid to late S. In CHO cells synchronized by mitotic selection or by isoleucine deprivation, TTL activity was maximum during mid S with a minor peak or plateau during early S. The major peak of activity during mid S occurs when tubulin appears to be undergoing degradation (Forrest and Klevecz, J. Biol. Chem. 247, 3147, 1972). The level of TTL activity varied 3-4-fold during the cell cycle and occurred at a time out of phase with maximum colchicine binding. Colchicine binding was shown to increase through S phase and reach a maximum late in the cycle during G2 approximately 3 hours after the maximum tyrosyltubulin ligase activity.

796 IN VIVO CONTROL OF MITOTIC ACTIVITY IN NORMAL AND IN CANCER CELLS BY REGULATION OF INTRACELLULAR ELEMENTS, Ivan L. Cameron, Nancy R. Smith, Thomas B. Pool and Rodney Sparks, Department of Anatomy, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284

Elemental x-ray microanalysis was performed on hepatocytes of normal, young adult A/J mice and on host hepatocytes and tumor cells of mice which had been injected in the left flank with transplantable H6 hepatoma cells. Following killing by cervical dislocation, tissue was rapidly removed, mounted on tubular brass holders with minced liver, and frozen in liquid propane. The frozen tissue was sectioned at 4 μm in a cryostat, freeze dried and electron probed in a scanning electron microscope. X-ray spectra were collected and processed with an energy dispersive detector and pulse height analysis system. Data were collected from ten cells (nucleus and cytoplasm) from each of 6 data sets (two animals each for normal hepatocytes, host hepatocytes and hepatoma cells). Six elements of biological significance were detected: Na, Mg, P, S, Cl, K. Normal hepatocytes and host hepatocytes differed very little in their elemental contents; the latter did show a decrease in Mg from normal hepatocytes. However, the Na and Cl concentrations of hepatoma cells were found to be more than double that of normal hepatocytes. These findings are consistent with the hypothesis that a high Na concentration, associated with a low transmembrane potential, is mitogenic. The only element showing an unequal distribution between nucleus and cytoplasm was Cl, which had a nuclear-cytoplasmic ratio of significantly less than unity in normal and host hepatocytes and greater than unity in transformed hepatoma cells. Data from other normal and tumor cell types also support the hypothesis. (Supported by NIH Grant Nos. CA16831 & SO1 RR 05654)

797 INTRACELLULAR pH AND THE CELL CYCLE IN TETRAHYMENA PYRIFORMIS. R.J. Gillies, D.J. Masiel & D.W. Deamer, Dept. of Zoology, Univ. of Calif., Davis, Calif. 95616

We have previously shown (J. Cell Biol. (1977) 75:82a) that T. pyriformis maintains a constant intracellular pH of 7.3 in the external pH range from 5.0 to 7.3. This pH range also corresponds to the growth optimum. We concluded that these organisms have a homeostatic mechanism for control of intracellular pH. The presence of a more alkaline pH inside the cell also suggests that a proton gradient vector may be necessary for growth.

To study changes in pH during the cell cycle, we have synchronized T. pyriformis by two different synchronization methods. In the first series of experiments, the cells were starved for 24 hours and synchronized by resuspension in fresh media. Cell number, thymidine incorporation and intracellular pH were followed after refeeding. Intracellular pH was estimated from the equilibrium distribution of a weak acid, DMO. Two sharp peaks of DMO uptake occur, the first before S phase and the second at the transition between S and G2. These peaks are consistant with alkaline shifts of 0.4 pH units. In the second series, the cells were synchronized by heat shock, one per generation. Again, two alkaline peaks of similar magnitude were seen with the same temporal relationship to S phase as in the first experiments. Although causality is not implied, the data suggest a correlation between the observed pH shifts and DNA replication. Supported by UC CRCC grant # 3-504001-19900

798 MECHANISM OF CALCIUM CONTROL OF CELL DIVISION IN XENOPUS OOCYTES James L. Maller and Edwin G. Krebs, University of Washington, Seattle, Washington 98195

Ripe Xenopus oocytes in 1st meiotic prophase when incubated with progesterone in vitro mature synchronously in 3-5 hr without interphase to 2nd meiotic metaphase where they remain until fertilization or activation. Previous work has shown that a reduction in the level of cAMP-dependent protein kinase is necessary and sufficient to induce maturation. Others have shown that calcium ionophore A23187 in combination with elevated calcium levels is sufficient to induce maturation. The present study has examined the possibility that the reduction in protein kinase activity might be mediated by the calcium dependent regulator protein (CDR) of phosphodiesterase. Microinjection into oocytes of homogeneous calcium dependent regulator from bovine heart to an internal concentration of 4 uM led to maturation in 30-100% of the oocytes, the exact percentage varying with different females. The effect was dependent on pretreatment of the activator with 20 uM calcium and did not occur in the presence of cycloheximide, indicating that the CDR acted prior to the protein synthetic steps needed for maturation. Control experiments utilizing microinjection of BSA with 20 uM calcium did not induce maturation in any oocytes. The results support a model in which the calcium requirement for meiotic cell division is related to the biochemical mechanism of cyclic nucleotide degradation and the regulation of protein phosphorylation reactions.

799 ACCELERATION OF CELLS IN G_0 OR G_1 TO S PHASE BY CYTOCHALASIN B, RIBONUCLEASE OR EPIDERMAL STRIPPING, Simon Rothberg and Grace E. Nanarrow, Medical College of Virginia, V.C.U., Richmond, Virginia 23298

Most proliferative epidermal cells spend most of their cell cycle time in G_0 or G_1. Epidermal stripping, a procedure by which a minimal number of stratum corneum cells are removed, seems to accelerate the normal flow of cells to S phase. Twelve to twenty four hours later thymidine incorporation into epidermal and dermal DNA is stimulated maximally. Several compounds, not previously identified with accelerating normal cells in G_0 or G_1 were found to act in this fashion and their action was measured by thymidine incorporation into epidermal and dermal DNA, $17\frac{1}{2}$ hours after the addition of a 30 minute pulse of the compound, cytochalasin B or ribonuclease. Increased incorporation into epidermal and dermal DNA of 149% and 124% was found for the cytochalasin B 10 ɣ/ml pulse and 202% and 110% for a ribonuclease 13 ɣ/ml pulse. Autoradiographic examination of the basal cells confirmed that the increased synthesis was probably due to the increased number of basal cells incorporating thymidine into DNA. A mechanism is sought to explain how the results obtained from each of these procedures accomplishes the same end, acceleration of G_0 or G_1 cells to S phase. One hypothesis suggests a communication between the cell surface receptors, the microfilament submembrane structure and a relationship between RNA during G_0 or G_1 and microtubular structure. Supported by NIAMDD grant 15285.

800 INVOLVEMENT OF HISTONE PHOSPHORYLATION IN CHROMATIN CONDENSATION, L. R. Gurley, J. A. D'Anna, S. S. Barham, P. G. Hohmann, C. E. Hildebrand, R. A. Tobey, L. L. Deaven, and R. A. Walters, Cellular and Molecular Biology Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, NM 87545

Chromatin has many levels of organization, producing a hierarchy of structures ranging in size from molecular to microscopic dimensions. Our studies in cultured mammalian cells suggest that growth-related histone phosphorylation may be involved in chromatin structural condensations at a variety of levels of chromatin organization. Specifically, our observations suggest the following. (1) Interphase histone H1 phosphorylation increases as cells traverse their cell cycle from G_1 to mitosis, and this H1 phosphorylation is associated with a presumptive interphase chromatin condensation occurring at a submicroscopic or molecular level of organization which can be detected with heparin, a molecular probe of chromatin structure. (2) Histone H2a phosphorylation may be associated with chromatin organization at the level of condensed heterochromatin. In two mouse cell lines (Peromyscus eremicus and Peromyscus crinitus) that have identical euchromatin contents but different heterochromatin contents, it was found that the rate of H1 phosphorylation was identical but that H2a phosphorylation occurred at a greater rate in cells containing greater amounts of condensed heterochromatin. (3) Histone H3 phosphorylation and H1 superphosphorylation are associated only with condensed chromosomes at mitosis. These specific histone phosphorylation states were observed only during prophase, metaphase, and anaphase when fully condensed chromosome structures existed. No such phosphorylated histones were observed during S, G_2, preprophase, telophase, or G_1. (This work was performed under the auspices of the USERDA.)

801 CALCIUM, CYCLIC AMP AND THE CONTROL OF CELL PROLIFERATION, A.L. Boynton, J.F. Whitfield, National Research Council of Canada, Ottawa, Canada K1A 0R6.

Extracellular calcium controls a late stage of prereplicative development in all non-neoplastic and pre-neoplastic cells studied so far. However, calcium does not control the proliferative development of any of the neoplastic cells tested to date. The PD-50 (the extracellular free calcium concentration which supports proliferation to 50% maximum) of non-neoplastic cells is 0.3 mM; for pre-neoplastic cells is 0.05 mM; and for neoplastic cells is less than 0.001 mM. Thus, extracellular calcium's control of proliferation is lost in a stepwise fashion as the cell progresses towards the neoplastic state. The restriction point imposed by extracellular calcium deprivation is different from the much earlier restriction point imposed by serum deprivation or by density-dependent factors because upon readdition of extracellular calcium a large proportion of cells enter S phase within two hours. The pre-DNA-synthetic rise in cyclic AMP, occurring in all normal and preneoplastic cells so far examined, may be linked to the calcium control system at the G1/S boundary. We propose that changes in cell surface calcium control the configuration of cell membrane organelles which bind a DNA synthetic regulator on the cytosolic side of the membrane. Thus, the pre-S phase cyclic AMP surge releases this regulator protein from their binding sites on the calcium-dependent membrane organelles. The released regulator then activates the appropriate DNA-synthetic system. Extracellular calcium deprivation would prevent the initiation of DNA synthesis by reversibly changing the configuration of the membrane organelles so that the regulator can no longer be released by cyclic AMP action. Calcium's proliferative control could be lost during neoplastic transformation by a permanent elimination of the calcium-dependent regulator-binding sites.

802 INDUCTION OF DIHYDROFOLATE REDUCTASE SYNTHESIS BY POLYOMA VIRUS IN METHOTREXATE RESISTANT 3T6 CELLS, R.E. Kellems, V. Morhenn,* F.W. Alt, R.T. Schimke, Department of Biological Sciences, Stanford University, Stanford, California 94305.

One of the major biochemical events resulting from polyoma infection of mouse fibroblasts is the induction of cellular DNA synthesis and numerous host enzymes. In order to understand the molecular parameters underlying viral regulation of host gene expression we have focused our attention on one viral induced enzyme, dihydrofolate reductase (DHFR). For these studies we isolated several lines of methotrexate(mtx)-resistant 3T6 cells having as much as a 200-fold increase in the cellular content of DHFR enzyme and mRNA, and a corresponding increase in the number of DHFR gene copies. Infection of confluent cultures of mtx-resistant cells with polyoma virus (100 PFU per cell) resulted in a 5-fold increase in the relative rate of DHFR synthesis as judged by specific immunoprecipitation of pulse labeled DHFR. The induction did not occur until 20 hours following infection, after which time the relative rate of DHFR synthesis continued to increase until cell lysis (70-80 hours). The addition of fresh serum to confluent cultures of mtx-resistant cells resulted in a 2-fold increase in DHFR synthesis beginning 10-15 hours after serum addition. Serum induction of DHFR synthesis was completely inhibited by dibutyryl-cAMP and theophylline, whereas polyoma induction was unaffected. These results indicate that mtx-resistant cells having several hundred copies of the DHFR structural gene are well suited for use in investigating the relationship between polyoma, serum, and cell cycle mediated control of DHFR gene expression. The effect of polyoma infection on DHFR mRNA metabolism is being investigated.

* Department of Dermatology, Stanford University Medical School

803 INTRACELLULAR SULFHYDRYL LEVELS, HISTONE REDUCTION, AND THE MAMMALIAN CELL CYCLE,Paul Todd and Zivile M. Khoury, The Pennsylvania State University, University Park,PA 16802

Several published investigations have pointed out that non-protein sulfhydryl and protein sulfhydryl levels do not coincide as a function of position in the mammalian cell cycle. One notable exception to this observation is the reversible reduction of histone H3 during periods of important nuclear activity in the cell cycle. Measurements of sulfhydryl levels in normally progressing HeLa cells and Chinese hamster (V79-793A and M3-1) cells resuming growth from plateau-phase cultures indicate that maximum amounts of reduced non-protein SH and histone SH coincide near the beginning of G1 phase and during the period of maximum DNA synthetic rate. Three methods of reduced thiol estimation were used: those of Ellman, Saville, and ^{14}C-N-ethylmaleimide binding. It was also found that cells were most sensitive to N-ethylmaleimide-induced reproductive lethality during the maximum rate of DNA synthesis.

804 ARREST IN G_1 OF BALB/c 3T3 CELLS CULTURED AS AGGREGATES, Howard Gershman and David A. Carrino, Dept Biochem, Case Western Reserve Medical School, Cleveland, OH, 44106

BALB/c 3T3 mouse fibroblasts were cultured as cellular aggregates containing about 1000 cells for one to 5 days. Previous studies have shown that with daily medium changes, the amount of ^{3}H-thymidine incorporated during 2 hour pulses was 23% that of the same cells in logarithmically growing monolayer cultures, but 16 times that of confluent monolayer cultures.The rate of DNA synthesis,as measured by isotope dilution, indicated no cell proliferation for 2 to 3 days after aggregate formation. Thereafter, a population doubling time of 76.4 hours was seen. To determine the point of arrest, cells were recovered from aggregates after 1 and 5 days using trypsin (Difco 1:250, 0.05%, 37°,10 min) and replated into dishes. Hourly pulses with ^{3}H-thymidine indicated a low percentage (11%) of cells entering S during the first 15 hours, then a peak of cells (67%) entering S 32 hours after replating. In contrast, cells replated from asynchronous log phase cultures gave 25 to 50% labeled cells at all times after replating,with no clearly defined peak. Cells from quiescent confluent cultures when replated showed a very low fraction (1.5%) of cells entering S during the first 15 hours, and a peak at 32 hours, coincident with the peak from aggregate arrested cells. These data were confirmed by flow microfluorometry of cells recovered from aggregates. Analysis of DNA content by this technique indicated that cells recovered from aggregates after one day of culture were 75.6% in G_1, 14.3% in S, and 10.0% in G_2+M. The distribution of cells after 5 days of aggregate culture was nearly identical (78.3%, 14.3%, and 7.4%). Therefore, within one day of aggregation, the bulk of the 3T3 cells are arrested in G_1.Thus aggregate culture is a simple method of synchronizing large numbers of cells without drugs.(We thank Dr. Harry Crissman for the flow microfluorometry)

805 PROPERTIES OF A DNA-UNWINDASE PURIFIED FROM RAT LIVER, Gordhan L. Patel, Dept. of Zoology, Univ. of Georgia, Athens, GA. 30602

A DNA-unwinding protein has been purified to homogeneity from 0.05M NaCl soluble and insoluble fractions of rat liver by affinity chromatography on native and denatured DNA-cellulose and by gel filtration. The protein has following characteristics: It has molecular weight of 29,000, and acidic/basic amino acids = 1.44, but pI = 8.6. It contains tryptophan and 43% of its amino acid residues are hydrophobic. It binds poly d(AT)·d(AT) cooperatively and denatures it, as measured by hyperchromicity. It has no measurable nuclease activity in vitro. The extent of poly d(AT) denaturation is dependent on protein concentration. Under identical conditions it does not denature poly d(GC)·d(GC). While free protein precipitates above 50°C, the DNA-protein complex is soluble at elevated temperatures. Preliminary data indicate that the proteins from soluble and insoluble fractions may be differentially phosphorylated. The pI and amino acid composition data suggest amidated acidic residues. These modifications may be important in regulating the activity and cyto-nuclear translocation of the protein in control of cell replication.

(Supported by USERDA contract # EY-76-S-09-0644)

806 EPIDERMAL MITOSIS IN VITRO IN PRECONDITIONED MEDIA. Kim Almodovar and Graciela C. Candelas, University of Puerto Rico, Rio Piedras, PR 00931

A series of experiments have been conducted to test the effects of tissue or cell products on the cell proliferative activity of mouse ear epidermis under in vitro conditions. The in vitro incubation system used has been the one described by Gelfant (Exptl. Cell.Res. 21:603,1960).
Preconditioning of the incubating media was acieved by a number of ways; by preincubating the media with either strips, macerates or homogenates of ear epidermis for four hours. At the end of the preincubation period, the media received aeration and adjustments for pH where these were necessary.
Mouse ears stimulated to enter into mitosis by cutting into strips were now incubated in these media for five hours as previously described. Mitotic acitvity was analyzed in these strips in histological sections.
Inhibition of mitosis was evidenced in the experimental strips which were incubated in the media which had been preconditioned using ear strips and macerated ear tissues. No inhibition was obtained when the media had been preconditioned with tissue homogentes. The inhibiting factor (s) in the preconditioned media exhbit thermolability.

807 THE CLOCK IN ANIMAL CELLS IS A LIMIT CYCLE OSCILLATOR, Robert R. Klevecz, Department of Biology, City of Hope Medical Center, Duarte, CA 91010

A periodicity of four hours duration has been observed to occur within the cell cycle of animal cells. It has been found to be manifested in enzyme activity maxima, in the rate of DNA synthesis within S phase, and in mitotic frequency, in all cells and by a variety of synchronization techniques. These periodicities fall midway between the very short period oscillations observed by Chance et al. and the circadian period and may be an expression of the cellular clock. Several clock criteria including temperature compensation, phase dependence, and temperature entrainment have been established for this rhythm. The time of median cell division in V79 Chinese hamster cells following high serum pulses was determined for two synchronous cell generations following mitotic selection. Differences in cell cycle time for each pair of pulse and control cultures were computed and plotted as a function of time of serum pulse. The phase response of hamster cells with an 8.5 hour cell cycle shows a characteristic biphasic curve. Beginning at the time of mitotic selection early pulses generate increasing delays which give way abruptly to advances at 2.5 hours. The amount of phase advance decreases more gradually with a crossover and increasing delays beginning at 5 hours of the cell cycle. Delays again give way abruptly to advances at 6 hours and again the amount of advance decreases through the late portion of the cycle. Tests of the notion that the underlying timekeeping mechanism can be modeled using a limit cycle oscillator are proposed. Explanations of G_0 and G_1, the heterogeneity of cell cycle times and of entry into S, and the quantizement of generation time are made using the resulting model.

809 A STOCHASTIC MODEL GENERATING A CONSTANT RATE OF CELL PRODUCTION FROM A POPULATION OF DIVIDING CELLS. W.D. Stein and S. Shall, Biochemistry Laboratory, University of Sussex, Brighton, England.

Smith and Martin (1973) proposed a probabilistic hypothesis for cell proliferation, generating an exponentially growing cell population. For tissues such as epithelia and for secondary cell cultures, an arithmetic rate of cell production is more appropriate. We wish to extend the Smith-Martin hypothesis by proposing that in these situations, each cell, following division, has an equal chance either (i) of dividing in the future with a certain probability as described by Smith and Matin, or (ii) of never dividing again. We suggest that there are two stochastic events, the signal to division given by the transition probability of Smith and Martin or the signal to mortalization, the mortalization probability. When the two stochastic events have an equal probability they yield the observed arithmetic progression of population growth. The molecular mechanisms for these two stochastic events may be coupled, since the occurrence of mortalization precludes the transition to replication. The validity and application of this model is tested on published population growth curves (I.E. Hayflick, 1961; Todaro and Green, 1963) and good agreement is observed.

810 THE EFFECT OF GRISEOFULVIN ON CHROMOSOME CONDENSATION AND DNA-SYNTHESIS IN SEA URCHIN EGGS. H. Schatten and D. Mazia. Department of Biological Science, The Florida State University, Tallahassee, Florida, 32306, and Department of Zoology, University of California, Berkeley, California, 94720.

The effect of Griseofulvin on chromosome condensation in sea urchin eggs (Strongylocentrotus purpuratus) has been examined by light microscopy. DNA-synthesis in these treated cells has been determined by ^{3}H-Thymidine incorporation and by autoradiography.

When eggs were incubated in 4 x 10^{-5}M or 1 x 10^{-4}M Griseofulvin in sea water at fertilization, no spindle formed at metaphase. The examination of fixed eggs (fixed in Ethanol-Acetic Acid; 3:1), which were stained in Orcein, demonstrates that the chromosomes are scattered throughout the cytoplasm and undergo a prophase and metaphase-like cycle of condensation. Later the chromosomes appear even more condensed than those at metaphase. Remarkably these supercondensed chromosomes undergo cycles of DNA synthesis. Control and Griseofulvin treated cells were incubated in ^{3}H-Thymidine and the incorporation into TCA precipitable material was measured at various time points. Although the synchronous cycles of DNA synthesis in the Griseofulvin treated cells lagged slightly behind that of the control cells, the drug did not significantly inhibit the extent of Thymidine incorporation. Control and Griseofulvin treated cells were prepared for autoradiography at various times. Both samples had grains over the chromosomal regions, indicating DNA synthesis.

We wish to thank Dr. D. Nishioka for assistance with the autoradiography.

Regulation of Developmental Events in Embryogenesis

811 DIRECT MEASUREMENT OF INTRACELLULAR pH OF THE SEA URCHIN EGG, Sheldon S. Shen and Richard A. Steinhardt, Dept. of Zoology, Univ. of California, Berkeley, CA 94720

Sea urchin eggs are ovulated in a metabolically repressed state and are derepressed by fertilization. It has been postulated that part of the derepression is accomplished by raising the intracellular pH. We measured intracellular pH by means of the Thomas-type, pH-sensitive micro electrode. Unfertilized eggs of Lytechinus pictus were placed in natural sea water, penetrated with microelectrodes and inseminated with sperm. Unfertilized eggs maintained a steady intracellular pH of 6.84 ± 0.02 (n=44). Upon fertilization, the intracellular pH rapidly becomes alkaline and within 5-6 min the intracellular pH of fertilized eggs stabilized at 7.26 ± 0.06 (n=8) and remained close to those values throughout the 30 min recording periods. We also examined the intracellular pH changes during ammonia activation. Unfertilized eggs of L. pictus were exposed to different concentrations of NH_4Cl. The higher concentrations of NH_4Cl acted more quickly in raising the intracellular pH as expected from the kinetics of H^+ generation. However, the H^+ generation was finished well before the change in intracellular pH and given enough time, the different NH_4Cl concentrations achieved the same extent of an increase in pH (0.72 ± 0.01, n=8). Apparently, the intact egg can regulate its intracellular pH. Further evidence of more complex regulation of intracellular pH is seen when a NH_4Cl stimulus is washed out. The intracellular pH only partially recovers and then remains steady at a level well above the control values for unstimulated eggs. We can conclude that both normal fertilization and ammonia activation raise intracellular pH throughout the cytoplasm of sea urchin eggs and therefore this increase in pH is a leading candidate in further investigations of the mechanisms of derepression of protein and DNA syntheses. Supported by NSF Grant 77-04260

812 MACROMOLECULAR SYNTHESIS IN A CLEAVAGE-ARRESTED MOUSE DEVELOPMENTAL LETHAL, Susan E. Lewis, Dept Human Genet, The University of Michigan, Ann Arbor, MI 48109

Embryos homozygous for the radiation-induced lethal mutation c^{25H} at the albino locus of the mouse become arrested during early cleavage, at the 3-6 cell stage. Histological and cytological analysis demonstrate an apparent disturbance of the mitotic process in c^{25H} homozygotes. Most of the blastomeres of c^{25H}/c^{25H} embryos have multiple extra interphase nuclei which vary considerably in size. In addition, abnormal mitotic figures are found in many mutant embryos. Studies were undertaken to determine if the early lethality of this homozygote might be related to disturbances of the cell cycle or macromolecular synthesis in such embryos. Autoradiographic studies show that DNA synthesis of mutant embryos is comparable to that of control embryos at 2 1/2 days of development but is reduced relative to normal embryos at 3 1/2 days of development. Analysis of protein synthesis reveals that both uptake and incorporation of ^3H-amino acids are normal in mutant embryos at 2 1/2 days of development, although they fail to undergo the significant increase in synthetic levels which normal littermate and control embryos exhibit at 3 1/2 days of development. Studies of RNA synthesis in mutant embryos are in progress. The analysis of such mutations which affect mitotic phenomena in early embryos could further our understanding of the genetic control of normal cleavage and the mammalian cell cycle.

813 ROLE OF PROTEASES IN MYOBLAST PROLIFERATION AND DEVELOPMENT. Stephen J. Kaufman, Department of Microbiology and School of Basic Medical Sciences, University of Illinois, Urbana, Illinois 61801.

The development of skeletal muscle is marked by a shift from a proliferating population of myoblasts into a non-proliferating one. This change in proliferative capacities precedes the spontaneous fusion of myoblasts into multinucleated myotubes, and it appears to be an obligate requirement for the further differentiation of these cells. Myoblasts which have lost their capacity to cease replication do not develop into skeletal muscle, but acquire many characteristics of transformed cells [Kaufman and Parks, P.N.A.S. 74:388 (1977)]. Since proteases may be intimately involved in promoting cell proliferation, and since elevated levels of plasminogen activator have been associated with the uncontrolled proliferation characteristic of transformed cells, we have proposed that this protease activity may also maintain the proliferative stage of normal myoblast development. In support of this, we have found that the protease inhibitors, leupeptin and antipain, and plasminogen deprivation promote premature and more extensive skeletal muscle differentiation. Furthermore, elevated levels of plasminogen dramatically inhibit myotube formation. 12-O-tetradecanoyl-phorbol-13-acetate (TPA), a potent cocarcinogen and an inducer of plasminogen, maintains myoblasts in their proliferative stage and thereby inhibits differentiation. Leupeptin and antipain counter this effect of TPA on myoblast development. Thus, cessation or inhibition of a specific protease activity may be essential for the termination of myoblast proliferation and the subsequent development of skeletal muscle.

814 EMBRYONIC (AND OTHER) DEVELOPMENTAL TEMPERATURE SENSITIVE MUTANTS OF CAENORHABDITIS ELEGANS: PHENOTYPIC & GENETIC CHARACTERIZATION. Randall Cassada, E. Isnenghi, E.Schierenberg, J Miwa, & G von Ehrenstein, MaxPlanckInstitute(ExpMed),Göttingen, W Germany

Using ethylmethanesulfate mutagenesis, several hundred independent temperature sensitive developmental mutants of the nematode C. elegans have been isolated by replica plating and characterized. Methods are related to those described for such C. elegans mutants by Hirsh et al (e.g.,see Dev.Biol.49,220,1976). However, we have mutagenized newly hatched juveniles so as to have a highly synchronized target population with well-defined germline state. Also such mutagenized "hatchees" can be stored frozen indefinitely and survive thawing in high proportion , thus providing many batches from one standard mutagenesis. The mutants can be classified according to the developmental stage(s) blocked following various temperature shift regimes. Pleiotropy was frequent. Some 50 mutants showing specif. block in embryogenesis and thus accumulating eggs have been identified and further characterized genetically and phenotypically. The eventual blocked stage and uniformity thereof (% penetrance and expressivity) as well as relative "brood size"(egg production) were determined. Cellular aspects of embryogenesis have been studied on liviing and Feulgen-stained embryos to learn more about the processes defective in the mutants. Clustering of the blocked stages early and late suggests early and late gene classes. This is consistent with our picture of wildtype embryogenesis; at the beginning there is a long cell-cleavage period without much differentiation, followed by a late, strictly morphogenetic phase. Comparison of our set of embryological mutants to independent, related sets (e.g. from Hirsh's laboratory) will be made, i.e., discussed.

815 STUDIES ON LOW-MOLECULAR WEIGHT COMPOUNDS IN LIVER THAT REGULATE CHICK EMBRYO HEPATOCYTE AGGREGATION, L. Sankaran, John R. Petersen, and Burton M. Pogell. St. Louis University, St. Louis, Missouri 63104.

Two major salt-free fractions have been obtained from $CHCl_3$:CH_3OH (2:1) insoluble extracts of boiled chicken liver supernatant by Sephadex G-10 gel filtration - one promoting (HAP) and the other inhibiting (HAI) hepatocyte aggregation. One active component of HAP was identified as taurine, $^{+}NH_3CH_2CH_2SO_3^{-}$ (PNAS 74: 4486, 1977). Excess taurine increased the rate of aggregation to only 50% of that seen with crude HAP. Another component of HAP was completely separated from radioactive taurine by G-10 fractionation. It was much more active than taurine, slightly cationic upon TLE at pH 5.2, and contained ninhydrin-positive material. Conditioned medium obtained with crude HAP promoted significantly more rapid aggregation than seen with fresh medium and HAP. The active material also eluted in the same region as HAP on a G-10 column. Crude HAI inhibited the uptake of radioactive taurine and N-methylaminoisobutyric acid by hepatocytes. By TLC in phenol:H_2O(8:2), one compound (R_f=0.52) was separated which only inhibited taurine uptake. It was identified as β-alanine, a known inhibitor of taurine uptake, by TLC and automated amino acid analysis. Another fraction (R_f=0.16) inhibited the uptake of both amino acids. This effect was completely eliminated by preincubating cells for 1 hr at 37° in the absence of the inhibitor. However, HAI as well as puromycin still completely inhibited aggregation when added after 1 hr. Thus, although it appears that a simple repair of membranes or other components of the amino acid uptake systems may be affected by HAI, continued synthesis of protein and other specific cell components is still required for hepatocyte aggregation.

816 A SHIFT IN TOTAL HISTONE GENE ACTIVITY ASSOCIATED WITH HATCHING IN THE SEA URCHIN, LYTECHINUS PICTUS, Michael Grunstein, Molecular Biology Institute and the Department of Biology, University of California, Los Angeles, California 90024

Just after fertilization of the sea urchin embryo, the histone genes are selectively activated. Virtually all the mRNA of 9S size made is histone messenger RNA. These RNAs can be fractionated on acrylamide gels into individual classes of mRNA for each of the histone proteins, H1, H3, H2B, H2A and H4.

At 11-11½ hours of development the embryo hatches from its fertilization membrane and starts to swim. Associated with this event is the disappearance of the histone mRNAs present in early (morula) development. A new class of histone mRNAs appears. Each of the (hatching) histone mRNAs is shorter in length than its morula counterpart. We present evidence for the activation of a new class of histone genes at hatching stage.

Microtubule Assembly In Vitro and In Vivo

817 MICROTUBULE ASSEMBLY COMPETENCE OF GDP-TUBULIN, Robert V. Zackroff and Richard C. Weisenberg, Temple University, Philadelphia, PA 19122

GDP completely prevents assembly, but causes only partial disassembly of microtubules. This apparent irreversible behavior may be explained by three general models: (1) Microtubule assembly is a non-equilibrium process, and GDP-tubulin, although capable of stabilizing microtubules, cannot participate in elongation; (2) GDP-tubulin which has undergone the GTP-binding/hydrolysis/assembly cycle can participate in a microtubule-subunit equilibrium, while GDP-tubulin which has not undergone this cycle is not competent to assemble; or (3) GTP is required for nucleation, and GDP-tubulin can participate in an equilibrium elongation process. Addition of excess GDP to microtubules at early times during assembly in GTP results in cessation of elongation and a final plateau turbidity which is lower than controls not receiving GDP until plateau in GTP was attained. Dilution of samples inhibited at early times by GDP results in a biphasic dilution curve: microtubules do not depolymerize until a dilution is reached such that the total tubulin concentration equals the critical concentration of controls which were not inhibited by early GDP addition. When microtubules were directly mixed with microtubule-free GDP tubulin, net assembly or disassembly was not detectable by turbidity. These results argue against model (2), and demonstrate that GDP-tubulin is equally competent to stabilize microtubules regardless of GTP binding or assembly history. Although undetectable by turbidity, electron microscopy revealed rapid, bidirectional, but limited elongation of GDP-tubulin. These data support model (3), and demonstrate at least limited assembly competence of GDP-tubulin.

818 LOCALIZATION OF THE HIGH MOLECULAR WEIGHT COMPONENT OF MICROTUBULE PROTEIN IN PtK_1 CELLS. Judith A. Snyder and J.R. McIntosh. MCD Biology. Univ. Colo., Boulder, CO.

An antibody has been prepared against the high molecular weight component (HMW) of microtubule associated proteins (MAPS) isolated from porcine neurotubulin. This component copurifies with tubulin stoichiometrically during successive cycles of assembly-disassembly and can be visualized in TEM as the filamentous projections found along neurotubules polymerized in vitro. The antibody was prepared by immunizing rabbits with antigen purified by SDS acrylamide gel electrophoresis. Twice cycled MTP was electrophoresed on 10% thick gels and the entire HMW region was removed. The protein was eluted electrophoretically, briefly treated with glutaraldehyde and then prepared for immunization following the method of Lazarides (J. Supramol. Struct. 5:531, 1976). The antibody was purified from rabbit sera using affinity chromatography. Indirect immunofluorescence was used to localize the antibody in PtK_1 cells. In interphase cells numerous fluorescent filaments are seen to radiate from the centrosphere toward the peripheral regions of the cell, a pattern similar to that seen with tubulin antibody. These fluorescent filaments are sensitive to cold and Colcemid treatment, suggesting that the location of the HMW component is primarily in association with microtubules. Mitotic cells also show faint fluorescent staining, mainly in the spindle domain.

819 IDENTIFICATION OF MICROTUBULE-ASSOCIATED PROTEINS IN THE MEIOTIC SPINDLE, D.B. Murphy, Kansas State University, Manhattan, KS and Marine Biological Laboratory, Woods Hole, MA

The chromosomal fibers and astral rays of the mitotic apparatus (MA) consist of microtubules that are organized into discrete bundles, perhaps by specific crossbridge proteins. To determine if spindle-associated proteins could be identified, meiotic spindles were isolated from KCl-activated oöcytes of Spisula solidissima in 30° lysis medium (5 mM MES, pH 6.2, 5 mM EGTA, 0.1 mM $MgCl_2$, 0.5% Triton X-100 and 1.0 M glycerol). The isolated MA, stable at 22° but labile at 0°, were resuspended in tubule assembly buffer (0.1 M Pipes, pH 6.94, 0.1 mM $MgCl_2$, 1.0 mM GTP) and centrifuged to prepare a cold-soluble extract. When warmed to 30°, the extract formed tubules which were purified by two cycles of in vitro assembly. Although the yield of tubulin after two cycles of assembly (13%) was comparable to that for brain, the efficiency in forming microtubules was less. Both the purified tubules and the extract contained a dynein-like component and a high molecular weight doublet species that migrated closely to sperm tail axonemal dynein and beef brain HMW protein respectively. Both proteins appear to be significant spindle components: (1) The MA extract is enriched for these components and their stoichiometries to tubulin are nearly same in the extract as in the MA pellet and (2) are also the same for MA isolated in glycerol and hexylene glycol; (3) The same components copurify with spindle microtubules during in vitro assembly; (4) Analysis of tubule surfaces in thin sections by EM reveals lateral projections that resemble other tubule crossbridges. Thus, several classes of microtubule-associated proteins are present in the spindle, and these proteins may be similar to the tubule accessory proteins found in cilia and neuronal cells. (Supported by N.I.H. grant GM24208 and M.B.L. Steps Towards Independence Award to D.B.M.)

820 PURIFICATION OF A TUBULIN ASSEMBLY PROTEIN FROM BRAIN AND CULTURED CELLS
Arthur H. Lockwood, Dept. of Cell Biology, NYU Medical School, New York,N.Y.10016.

A tubulin assembly protein (TAP) which retains full biological activity has been purified to homogeneity by a novel procedure. The enzymology involves heat precipitation of either isolated microtubules or total brain tissue homogenate, phosphocellulose chromatography, gel filtration, and affinity chromatography on a tubulin-agarose column which has the properties of native tubulin. An advantageous byproduct of the purification is the recovery of the microtubule associated HMW proteins in pure form. A monospecific antibody against the 67,000 dalton TAP inhibits microtubule assembly with kinetics which indicate that TAP represents at least 80% of the assembly promoting activity in isolated microtubules. This conclusion has been confirmed biochemically. Hence, TAP may well be the major functional species among the tau proteins. TAP antibody does not recognize the HMW proteins or their proteolytic fragments. Nor does anti-HMW recognize TAP or significantly inhibit microtubule assembly.

A functional TAP has been isolated from cultured human Hela and WI38 cells: Polymerization competent microtubules were isolated from either cell line (Lockwood,ICB 1976). After heat precipitation or salt dissociation, a TAP-like activity was recovered by immunoabsorption on a column of anti-bovine brain TAP IgG. Isolated Hela or WI38 TAP has a molecular weight of 66-68,000. The protein promotes microtubule formation from phosphocellulose purified brain tubulin. Immunoflourescence has been used to localize TAP in the cytoplasmic microtubules and mitotic spindle of Hela and WI38. These results support a ubiquitous biological function for TAP in microtubule assembly. (Supported by NIH Grant AG00378).

821 BINDING OF ANTI-MITOTIC DRUGS TO CYSTEINE RESIDUES OF TUBULIN , Raphaël Kram and Henri Schmitt, Molecular Biology Dept., Université Libre de Bruxelles , Belgium

Rat brain tubulin was purified by one cycle of polymerisation andreacted with (^{3}H)bromocolchicine, an affinity label derived from colchicine (Schmitt Atlas ,I976,J.Mol.Biol.I02,I6§1.
Acid hydrolysis ot the labelled protein yielded two labelled products : carboxymethylcysteine and another, not yet identified, derivative of cysteine.
We found, on the other hand, that colchicine and vinblastine inhibit the reaction of tubulin with DTNB (Ellman's reagent) , a specific reagent for cysteine residues.
In conclusion , two independent experimental approaches allow us to conclude that colchicine binds around a cysteine residue on the tubulin polypeptide chain, through the interaction of its aromatic rings with hydrophobic domains of the tubulin molecule.
Rebhun and Nath Rebhun (J.Cell.Biol.70, 43a, 44a, I976) reported that oxidation of some free sulfhydryl of tubulin inhibits its polymerisation into microtubules; Organic sulfhydryl-blocking agents and di- and trivalent cations also inhibit the polymerisation of microtubules in vitro (Larsson et al.,Exp.Cell Res.IOO,I04, I976). Clearly our results provide a link between these observations and the anti-mitotic activity of colchicine and vinblastine

822 EFFECTS OF TAXOL ON CELL GROWTH AND IN VITRO MICROTUBULE ASSEMBLY. Peter B. Schiff, Jane Fant, Lori A. Auster, and Susan B. Horwitz. Depts. of Cell Biol. and Mol. Pharm. Albert Einstein College of Medicine, Bronx, New York 10461.

Taxol, isolated from the plant Taxus brevifolia (western yew) has antitumor activity when tested in experimental animals (Wani et al., (1971) J. Am. Chem. Soc. 93:9 2325-2327). We find that 0.25 μM taxol completely inhibits division of an exponentially growing suspension culture of HeLa cells (S_3) 90 minutes after addition of the drug; this inhibition is independent of drug concentration (25 and 250 nM) and suggests that taxol blocks cells in G_2 approximately 90 minutes prior to division. Flow microfluorometric studies of HeLa cells incubated with 0.25 μM taxol demonstrate a time dependent accumulation of cells with a G_2 or M DNA content. Neither DNA, RNA, or protein synthesis is inhibited during a 90 minute incubation of HeLa cells with 1.0 μM taxol. Taxol exhibits unusual effects on the in vitro assembly of calf brain microtubules. Assembly was monitored by turbidimetric measurements and electron microscopy, with 1 mg/ml tubulin in 0.1 M MES buffer (pH 6.6), 1.0 mM EGTA, 0.5 mM $MgCl_2$, and 1.0 mM GTP at 37°. Taxol (50 nM to 50 μM) causes a dose-dependent decrease in the lag time for assembly and an apparent decrease in the length of the microtubules polymerized. Microtubules assembled in the presence of taxol are resistent to depolymerization by $CaCl_2$ (4.0 mM) or by cold (4°). Ribbon structures are observed when polymerization is carried out at 4° for 30 min in the presence of 50 μM taxol. Our observations provide a possible basis for the G_2 arrest and reported antitumor properties of taxol. (Supported in part by ACS, Grant No. CH-86,)

823 RELATIONSHIP OF MICROTUBULES AND HEART CELL CONTRACTION, A.P. Bollon, J. Fuseler, and J.W. Shay, The Univ. Texas Hlth. Sci. Ctr., Dallas, TX 75235

Utilizing primary heart cell cultures, we have found that myocyte contraction is inhibited by 0.2 mM - 2 mM dibutyryl cyclic AMP (dibu cAMP) and that the addition of 10 mM colchicine reverses this inhibition, but lumicolchicine does not. (Nath, Shay, Bollon PNAS, in press). In this report we extend these studies to a morphological examination of normal contracting heart cell cultures and dibu cAMP treated cultures. Scanning electron microscopy reveals that cells of the dibu cAMP treated cultures and are more elongated than cells from untreated cultures. To clarify if the altered morphology found in dibu cAMP treated cultures is due to a change in microtubule organization, transmission electron microscopy was done with cells from dibu cAMP treated and untreated cultures. In dibu cAMP treated heart cell cultures, the microtubules are aligned in a parallel arrangement whereas in cells of nontreated cultures, the microtubules are randomly aligned. In addition to these morphological changes the inhibition of contraction by dibu cAMP is cell density dependent - cultures with densities lower than 5×10^5 cells/ml of medium are not inhibited by dibu cAMP. Due to this relationship of cell density and dibu cAMP inhibition and the presence of nonmyocytes in our primary heart cell cultures, techniques have been developed to distinguish between myocytes and nonmyocytes. We have found by immunofluorescent studies which are not apparant in myocytes. The arrangement of fibrous proteins in cells from dibu cAMP treated cultures and nontreated cultures utilizing actin and tubulin antibodies as immunofluorescent probes will be presented.

824 **FORMATION OF SE URCHIN SPINDLE ASTERS IN VITRO, Richard C. Weisenberg, Temple University, Philadelphia, Pa. 19122.**

Formation of mitotic spindle asters has been obtained in crude supernatents of eggs from the Hawaiian species Tripneustes gratilla. Aster formation requires the presence of a particulate component which is pelleted in 15 min. at 39,000 g and of soluble material which is not pelleted. The particulate fraction contains the centriole and associated material which forms the microtubule organizing center (MTOC) while the soluble fraction contains tubulin and possibly associated proteins. Both the soluble and particulate fractions are present and active in eggs by 5 min. post fertilization (metaphase in this species is at 1 hr. 30 min.) indicating that regulation of spindle formation does not involve either changes in the MTOC, tubulin or required accessory proteins. Aster formation was sensitive to low concentrations of Ca and relatively high concentrations of EGTA were required for assembly. No GTP was required, although this may have been present in the crude supernatents used. Thin section electron microscopy demonstrated the presence of one or two centrioles in the MTOC. Microtubules insert primarily into a thin layer of electron dense material around one centriole, but some microtubules apparently connect directly with one end of the centriole. Aster microtubules tend to form bundles and apparent cross bridges between microtubules were observed in negatively stained preparations. Sea urchins may provide a valuable system for studies of MTOC and microtubule function and regulation.

825 SULFHYDRYLS AND THE ASSEMBLY OF MICROTUBULES: IN VIVO AND IN VITRO STUDIES, Lionel I. Rebhun, Margaret Mellon and Chris Amy, Univ. of Virginia, Charlottesville, Va. 22903.

Diamide causes oxidation of sulfhydryls on low molecular weight thiols such as glutathione. It also oxidizes adjacent thiols on some proteins. When applied to tubulin, it causes disappearance of rings, inhibition of polymerization and loss of 4 of the 8 thiols per monomer. These effects are readily reversed with disulfide reducing agents. When diamide treated tubulin is analyzed on SDS gels (after binding remaining thiols with N-ethyl maleimide) the MAP function and some tubulin disappear and a large aggregate appears which does not enter the running gel. When this aggregate is removed, reduced and rerun on SDS gels in the presence of mercaptoethanol, only tubulin and the MAP fraction are present. When rings isolated on phosphocellulose columns are treated with diamide, all of the material, tubulin and MAPs disappears into the aggregate and reappears on reduction. Polymerized tubulin can be depolymerized by NEM with loss of 2 of the 8 thiols per monomer. Diamide then no longer causes the aggregate to form. Finally, while diamide rapidly inhibits polymerization of tubulin, it causes intact microtubules to slowly depolymerize. We suggest that thiol groups are part of a site, important for polymerization, which is also involved in MAP binding and which is less accessible following polymerization. In vivo experiments involving loss of birefringence of MAs of eggs on application of diamide, t-butyl hydroperoxide (which forms oxidized glutathione via glutathione peroxidase) and phenyl glyoxal which decreases reduced glutathione (without affecting oxidized glutathione or protein bound glutathione) through the glyoxylase system suggest that reduced tubulin is necessary for in vivo polymerization in accord with the in vitro results described above.

826 KINETOCHORE COMPONENTS IN MITOTIC AND MEIOTIC CHROMOSOME STRUCTURE, M. J. Moses, Department of Anatomy, Duke University Medical Center, Durham, NC, 27710.

Preferentially stained whole mount spreads (Counce and Meyer, 1973) of meiotic prophase and mitotic chromosomes reveal a common axial structure that is continuous with the kinetochore; the latter may function as chromosome backbone as well as microtubule organizing center. Proportional chromosome lengths are constant and equivalent in both stages (Moses et al, 1977); the axial organization may play a part in this regulation. -- The meiotic prophase chromosome consists of a transient axial core from which attached chromatin fibrils loop in the fashion of lampbrush chromosomes. The axis assembles prior to synapsis, forms the lateral element of the synaptonemal complex (SC) at synapsis, and disassembles at diplotene. The kinetochore is represented by a localized differentiation of the axis. Intact SCs isolated by differential centrifugation and DNase digestion are continuous with a major kinetochore component and confirm the proteinaceous nature of both structures, suggesting that they contain components in common. Thus, the axis becomes in effect an extension of the kinetochore. The absence of microtubules associated with the kinetochore at this time in vivo and in vitro reflects its immaturity as an organizing center. -- Protected-dried (Moses and Solari, 1976), spread mitotic chromosomes show an axial organization similar to that at diplotene. The kinetochore is preferentially stained and often extends along the chromatin as a filamentous axis from which loops of beaded chromatin fibrils radiate. In isolated metaphase chromosomes, microtubules assemble from these kinetochores (Telzer, Moses and Rosenbaum, 1976), but not from the axial material, implying a localized competency of the metaphase kinetochore. [Research supported by grants from the NSF (PCM 76-00440) and the USPHS (CA-104236)].

827 BEHAVIOR AND DISASSEMBLY OF MICROTUBULE AGGREGATES IN VITRO - A FILM, A. Bajer, C. Cypher and R. Hard, Dept. of Biology, Univ. of Oregon, Eugene, OR 97403

Microtubule (Mt) aggregates from reconstituted porcine brain tubulin can be followed in the light microscope. The film shows the behavior of the aggregates and their disassembly when exposed to various precisely controlled conditions (UV, low temperature, colchicine, etc.). The technique permits precise measurements of disassembly in strictly controlled conditions. At the time of writing of this abstract (Nov. 1977), precise numerical data are not yet available.

Micrograph of Mt aggregates in dark field.

Cell Motility: Microtubule-Based

828 FACTORS AFFECTING FLAGELLAR LENGTH IN Chlamydomonas reinhardii, Alvin Telser, Dept. of Anatomy, Northwestern University Medical School, Chicago, Il. 60611

When non-deflagellated cultures of Chlamydomonas reinhardii are placed in distilled water, their flagella elongateby 10-20% when compared to comparable cells placed in growth medium. Elongation is first evident after ca. 90 min; flagella do not lengthen significantly for the next 3 hrs. Cells can then be taken from H_2O and placed in medium and vice versa; in 1-2 hrs the flagella of cells that were in H_2O (i.e. those with elongated flagella) will shorten while those that had been in medium (i.e. those with normal length flagella) will elongate their flagella. Based on studies of flagellar regeneration, one would predict that cycloheximide (CH, 2-5 µg/ml) would have no effect on elongation, while colchicine (COL, 1 mg/ml) would inhibit it. The exact opposite was observed; in fact, CH caused flagella to shorten and COL permitted elongation, although COL at 2 mg/ml did prevent lengthening. Cells were pulse labeled with ^3H-acetate for 2 hrs before being placed in H_2O, medium, CH, or COL. After the cells were left in each of the test solutions for 3 hrs, the flagella were isolated, and the amount and distribution of radioactive proteins was examined by SDS-polyacrylamide gel electrophoresis. As expected, flagella from cells in H_2O had the most radioactivity. Flagella from cells left in medium had a lesser amount of radioattive protein. Flagella from cells in CH had more radioactivity than cells in medium even though their flagella were shorter. Lastly, flagella from cells in COL had the least amount of radioactivity. In all four cases, the great majority of labeled protein was found in the tubulin region of the gels. Components of the growth medium are being studied to determine the basis for these observations. Supported by USPHS Grant HD-09108.

829 CHROMOSOME MOVEMENT IN LYSED MITOTIC CELLS, W. Zacheus Cande, Department of Botany, University of California, Berkeley, CA 94720.

Mitotic PtK_1 cells, lysed at anaphase in a solution that contains 100 mM Pipes, pH 6.94, 2.25 mM $MgSO_4$, 1.25 mM ATP, 1 mM EGTA, 10 mM TAME, 0.1 mM DTT, 3% carbowax 20 M, 0.1% Brij 58, continue to move chromosomes to the spindle poles and move the spindle poles apart. Chromosome movement usually persists for 8-10 minutes after lysis. Typically, kinetochore-kinetochore distances increase from 5-6 μ at the time of lysis to 15 μ after lysis. Chromosomes move at 70% in vivo rates while spindle elongation occurs at 40% in vivo rates. Cells lysed late in anaphase display extensive spindle elongation (>5 μ). The cells are lysed by the following criterion; addition of the lysis brew prevents hypotonic swelling of cells, spindle birefringence fades rapidly when 5 mM $CaCl_2$ is added, or spindle birefringence increases when neurotubulin is added to the lysis brew. Chromosome movement can be inhibited by adding metabolic inhibitors (CCCP, KCN, DNP) at the time of lysis and inhibition of movement is partially reversed by adding ATP to the lysis medium. AMPPCP or ADP do not reverse this inhibition. Polymerizable neurotubulin added at the time of lysis decreases the rate of chromosome movement to the spindle poles but does not alter the extent of spindle elongation. This result suggests that microtubule depolymerization is a rate limiting step in chromosome to pole movement. The effect of adding sea urchin dynein or heavy meromyosin to the lysed cell preparation will be described.

830 DYNEIN CROSSBRIDGES AND CILIARY MICROTUBULE SLIDING, Fred D. Warner and David R. Mitchell, Department of Biology, Syracuse University, Syracuse, New York 13210.

Tetrahymena cilia slide apart in the presence of ATP-Mg, which enables negative contrast imaging of the dynein crossbridges responsible for force production and tip-directed microtubule sliding in these cilia. Both bridged (+ATP) and unbridged (-ATP) arms tilt opposite to the direction of force generation or toward the ciliary base. This orientation is maintained under all observed conditions, including both Mg- and Ca-activation, suggesting that the orientation represents the equalibrium state for the crossbridge. Although Ca-activation also results in tip-directed sliding, the concomitant level of motility-coupled ATPase activity is substantially reduced (Ca, 0.44 μmoles Pi/mg-min; Mg, 0.66 μmoles Pi/mg-min). Isolated 14S (monomeric) and 30S (polymeric) dynein have ATPase activities of 1.55 and 0.75 μmoles Pi/mg-min respectively. When the enzyme is activated by ATP-Mg in the presence of either solubilized 6S subfiber B tubulin or intact doublets, the specific activities (μmoles Pi/ml-min) of the 2 dynein fractions are enhanced by about 30%, indicating that tubulin apparently stimulates the activity of the enzyme. The dynein crossbridges of intact *Unio* cilia can also be preserved, but for thin-section microscopy, by reactivating the cilia and keeping them in ATP-Mg throughout the fixation process. Sectioned cilia retain their bridged arms and show a related 14% decrease in axoneme diameter and interdoublet spacing. The orientation of the dynein bridges, their retention in the presence of ATP-Mg, and the decreased interdoublet spacing indicate that ciliary dynein differs markedly from muscle myosin in terms of its functional behavior.

Supported by NIH research grant GM 20690.

Regulation of Early Developmental Events

831 PARTIAL LOSS OF DEVELOPMENTAL INFORMATION UPON REFEEDING STARVED TETRAHYMENA. William R. Wellnitz, Genetics & Devel., Cornell Univ., Ithaca, New York, 14853

If starved Tetrahymena cells of a single mating type are refed, much of the information for the formation of pairs is lost within 15 min of refeeding. There seems to be no further loss of information during the next 12 hr of refeeding. Cells resume exponential growth about 3.5 hr after refeeding and have doubled at least twice by 12 hr. Cells refed in the presence of cycloheximide behave identically to those refed without cycloheximide. Starved cells treated with cycloheximide and not refed behave as if they have been refed. Since recovery from cycloheximide is rapid, these results suggest 1) that protein synthesis is necessary for maintaining this information, and 2) a quantum of new information must be resynthesized once the information is lost. A mutant which is shown to exhibit a biphasic mode for the loss of information is described. A comparison of proteins made by refed cells, cycloheximide treated starved cells, and refed mutant cells is presented.

832 CHANGES IN TRANSCRIPTION OF MRNA DURING EARLY DICTYOSTELIUM DISCOIDEUM DEVELOPMENT, Carl Mathew Palatnik, Carol Wilkins, Cheryl T. Mabie and Allan Jacobson, Department of Microbiology, University of Massachusetts Medical School, Worcester, MA 01605
Using thermal elution from poly (U)-Sepharose, we have been able to separate most newly synthesized poly (A)-containing RNAs of Dictyostelium discoideum from steady state "background" transcripts, including mitochondrial poly (A)-containing RNAs. Translation of these RNAs in mRNA dependent wheat germ extracts and subsequent analysis on SDS polyacrylamide gels, has enabled us to demonstrate changes in transcription as early as the first five minutes of development in this organism. Analysis of translation products has also led to the following observations: (1) poly (A) minus RNA has a very low complexity with respect to translation activity, (2) there are at least two classes of poly (A)-containing RNA and (3) there are two or three classes of actin mRNA. The metabolism of these classes of actin mRNA during early development has been analyzed and will be discussed with respect to changes in the differential rate of actin synthesis which occur during early Dictyostelium development.

833 SWITCHES IN HISTONE SYNTHESIS DURING EMBRYOGENESIS, K. M. Newrock*, R. V. Nardi*, M. B. Hendricks†, E. S. Weinberg†, and L. H. Cohen*, *The Institute for Cancer Research, Philadelphia, PA 19111 and †Johns Hopkins University, Baltimore, MD 21218
There are several subtypes of histones H1, H2A, and H2B in the sea urchin embryo, resolved by Triton-acid-urea gel electrophoresis. Different subtypes are encoded in different mRNA sequences, as shown by in vitro translation of mRNA from appropriate stages. Subtype synthesis is stage specific: CS subtypes are synthesized during the cleavage stage, α-subtypes during preblastula stages and β-, γ- and δ-subtypes during gastrulation. CS subtypes begin to be synthesized by the second S-phase after fertilization and are the principal subtypes found in chromatin at the 2-cell stage, at which stage α subtypes are barely detectable. At the next S-phase α subtype synthesis is increased markedly, such that these are the principal subtypes being synthesized. Actinomycin D does not block the onset of synthesis of CS or α-subtypes, but does prevent the cessation of synthesis of CS subtypes, which normally occurs at about the 64-cell stage. The onset of synthesis of β, γ and δ subtypes coincide approximately with the first appearance of mRNAs for these subtypes, as detected by in vitro translation. These results reveal a complex sequence of chromatin remodeling beginning early in development and influenced by both maternally stored and newly transcribed information. Early synthesized subtypes (CS and α) are retained in chromatin in later cell generations. This raises the possibility of mechanisms for their non-random distribution without base sequence recognition of DNA by histones.

834 THE DEVELOPMENTAL CHANGES IN MYOSIN AND THE MYOSIN LIGHT CHAIN KINASE DURING MYOGENESIS IN RAT MYOBLASTS IN VITRO, Stylianos P. Scordilis and Robert S. Adelstein, Section on Molecular Cardiology, N.I.H./N.H.L.B.I., Bethesda, Maryland 20014
Myosin and the myosin light chain kinase (MLCK), which phosphorylates a specific light chain of mysoin, were isolated from proliferative myoblasts (PMB), early myotubes without sarcomeres (EMT), and myotubes containing sarcomeres (MTS). These cell types were obtained from a cloned cell line of rat myoblasts (Yaffe L5-810), which divide continually in a growth medium containing 10% serum. When the serum level is decreased to 1%, the cells cease division and fuse. The myotubes then go on to form sarcomeres. These MTS beat spontaneously. This developmental sequence was investigated at the ultrastructural level. It appears to proceed normally, except that the MTS appear to contain little or no Z-line material in their sarcomeres. Myosin and the MLCK were isolated as described previously (Nature 268: 558-560, 1977). The myosin from each of the three cell types has a heavy chain which migrates on SDS-polyacrylamide gels at 200,000 daltons. The light chain migration patterns showed differences: PMB, 20,000 and 15,000; EMT, 20,000; MTS, 23,000, 19,000 and 16,000 daltons. The MTS light chains co-electrophorese with adult rat thigh muscle light chains. The MLCKs were also different The PMB MLCK is a Ca^{2+} independent enzyme. The MTS MLCK activity is approximately 75% Ca^{2+}-dependent. If the EMT cells have any MLCK, it must be present in very low amounts, since it has not been detected. No inhibitor has been demonstrated in these cells either. The P-light chains (19,000-20,000 daltons) of each of these three myosins can be phosphorylated. (S.P.S. is a Post-doctoral Fellow of the Muscular Dystrophy Association of America).

835 ACCURATETRANSCRIPTION AND TRANSLATION OF EUKARYOTIC GENES INJECTED INTO AMPHIBIAN OOCYTES, Laurence D. Etkin and Robert Maxson,Dept. of Zool.Univ. of Calif.Berkeley,CA. Recombinant plasmids provide a unique opportunity to study control mechanisms involved in the regulation of gene expression. I will report on experiments involving a linked transcriptional- translational system in which one obtains accurate expression of eukaryotic genes. The system involves the injection of a plasmid pSp2 which contains the sea urchin histone genes H1, H2b, and H4 into the germinal vesicle(oocyte nucleus) of Xenopus laevis oocytes. The oocytes are labeled with H^3 lysine for 24-48 hours. Histones are extracted and examined by 2D acid urea- SDS polyacrylamide gel eleectrophoresis. The gels are dried and fluorographed. The results indicate that one may detect newly synthesized histones which co-migrate with purified cold sea urchin H1 and H2b histones(sea urchin H4 migrates similarly to endogenous Xenopus H4 and therefore could not be distinguished). The newly synthesized histones also co-migrate with H1 and H2b sea urchin histones in control oocytes which received injections of purified sea urchin 9S mRNA. These results suggest that this system may provide a means by which one may directly aalyze the mechanisms controlling the transcription of eukaryotic genes.

Cell Motility: Microfilament-Based

836 CAPPING, ENDOCYTOSIS, AND SHEDDING: A UNIQUE CELLULAR EXCRETORY SYSTEM? [1,2]R. Rajaram, [3]B. Westermark, [3]J. Pontén, [4]A. Vaheri, [1,2]R.A. Fox, and [2]J.M. MacSween, Dept [1]Microbio and [2]Med, Dalhousie Univ, Halifax, N.S., Canada; [3]Wallenberg Lab, Univ Uppsala, Uppsala, Sweden; and [4]Dept Virology, Helsinki Univ, Helsinki, Finland.

We have earlier proposed that capping is caused by abortive adhesion and successful adhesion results in the cell detaching itself from the substratum by spurning it in a manner analogous to capping only to repeat the same process resulting in locomotion. Thus, capping and locomotion may be two phases of the same process i.e., cell rejecting the foreign and unwanted materials as part of the cellular transport system.

We report some experimental observations that substantiate this interpretation. Human glia cells, after harvesting by trypsin, extrude vesicular material during spreading on the substratum. This phenomenon is increased if the cells are exposed to serum or to anti-fibronectin. Glia cells seem to discard the trypsin-damaged molecules and the cell surface fibronectin-anti-fibronectin complexes by capping endocytosis, and subsequent shedding. Since fibronectin may be the surface molecule mediating cell adhesion, blockage of this site by anti-fibronectin results in abortive adhesion inducing capping, endocytosis and shedding. This process is inhibited by cytochalasin-B and colcemid, suggesting the involvement of microfilaments and microtubules in these events. The neoplastic glioma cells are inefficient in performing this process. These events may be related to regulation of cell division and lectin-induced agglutination of transformed cells.

837 Ca^{++}-DEPENDENT PROTEASE (PLATELET CAF) IN HUMAN PLATELETS: SPECIFIC HYDROLYSIS OF ACTIN BINDING PROTEIN, David R. Phillips, Milica Jakabova and Harold H. Edwards, Dept. of Biochem., St. Jude Children's Research Hospital, Memphis, Tennessee 38101

During platelet stimulation, nonfilamentous actin is rapidly converted into actin filaments at the same time the concentration of intracellular Ca^{++} increases. Recently, we described a Ca^{++}-dependent protease (platelet CAF) within human platelets [J. Biol. Chem., *252*: 5602 (1977)]. When this protease is activated, either by adding Ca^{++} to lysed platelets or by adding Ca^{++} plus the ionophore A23187 to intact platelets, two high Mr proteins (band 1, Mr = 255,000 and band 2, Mr = 230,000) are rapidly cleaved. Ba^{++} and Mn^{++} only caused slight hydrolysis when added to the lysed platelets while Mg^{++} and Zn^{++} were totally ineffective. Platelet CAF was found not to be bound to any membraneous structures. The two protein substrates, however, were found in both soluble and membrane-bound forms. Platelets were extracted with 1% Triton-X100 which almost instantly solubilized the platelets. When solubilized at 0-2°, the solution remained clear, but upon warming, a precipitate formed. Analysis of the precipitate showed that only a few polypeptides were present (Mr = 45,000 similar Mr to actin; Mr = 95,000; Mr = 200,000, similar Mr to myosin; and 255,000 similar to band 1). Electron microscope analysis of the precipitate after negative staining showed that the predominant structures present were actin filaments. Extraction of the precipitate with 0.6 M KCl solubilized band 1; addition of platelet CAF to this extract caused specific cleavage of this high Mr polypeptide. The molecular weight and solubility characteristics of band 1 indicate that it is actin binding protein. Thus, the data suggest that a possible function of platelet CAF is to regulate actin polymerization through hydrolysis.

Regulation of Cell Growth

838 CONTROL OF ORNITHINE DECARBOXYLASE INDUCTION IN A TEMPERATURE-SENSITIVE CHINESE HAMSTER CELL CYCLE MUTANT, Fyllis Otsuka and Immo E. Scheffler, Dept. of Biology, University of California, San Diego, La Jolla, CA 92093

Recent studies from our laboratory have centered around the characterization of a temperature-sensitive Chinese hamster cell cycle mutant (Tenner et al., J. Cell Physiol. 90:145, 1977). This mutant grows at the permissive temperature of 34°C but not at 40°C. Flow microfluorometric analysis showed this mutant to be blocked in the G1 phase of the cell cycle.

Two dimensional gel electrophoretic analysis of proteins synthesized during the G1 period showed that most of the proteins being made at 34°C were also being made at 40°C. Further analysis showed that at least one enzyme, ornithine decarboxylase, was not being synthesized at the nonpermissive temperature. At 34°C, in cells released in synchrony by the addition of serum after serum starvation, synthesis of ornithine decarboxylase peaked before the onset of DNA synthesis. There was a 10-fold stimulation of ornithine decarboxylase activity after this release. At 40°C no ornithine decarboxylase activity was observed. Temperature shift experiments revealed that the ornithine decarboxylase activity quickly disappeared after shifting the cells from 34°C to 40°C. Experiments using cycloheximide, α-amanitin, and actinomycin D are now in progress to determine if the control of ornithine decarboxylase is at the transcriptional or translational level.

839 CYCLIC AMP-ARRESTED G_1 CELLS SHOW RANDOM-ORDER EXIT FROM THE BLOCK, Philip Coffino, Univ. Calif., San Francisco 94143, and Joe W. Gray, Lawrence Livermore Lab. 94550

S49 mouse lymphoma tissue culture cells accumulate in G_1 when treated with dibutyryl cAMP (dbcAMP) (Proc. Natl. Acad. Sci. 72:878, 1975). The kinetics of this process have been studied using fluorescent flow cytometric analysis and sorting methods. When dbcAMP is removed from arrested cells, they exhibit a delay of 6-8 hours before resuming DNA synthesis and then exit from G_1 into S phase with first-order kinetics. To determine whether exit from the block occurs randomly or in a manner dependent on the order of arrival in the block, exponentially growing cells were treated with dbcAMP and the last cohort of cells to enter the block labelled with ^{3}H-thymidine. The dbcAMP was removed, aliquots taken periodically, fixed and stained, and sorted to select cells in early S phase. Each sample sorted in this way was subjected to autoradiography to determine the fraction of labeled cells in early S. This fraction was constant with respect to time, indicating that the rate of exit of the G_1 arrested cells from the block is equal for early- and late-arriving cells. These observations are consistent with models that postulate cell growth to be regulated primarily by a randomly-occurring event in G_1, the probability of which is subject to external manipulation.

840 Ribosome conformation differences in growing and starved Tetrahymena, Richard L. Hallberg and Claudia A. Sutton, Cornell University, Ithaca, NY 14853.

When ribosomal subunits are isolated in the absence of Mg^{++} from exponentially growing and 18-h starved Tetrahymena cells, the proteins associated with the ribosomal subunits are not identical (Hallberg and Sutton, J. Cell Biol. 75, 268-276). However, if subunits are isolated in the presence of Mg^{++}, the subunit-associated proteins are the same. The differences arise, in part, by differential partitioning of certain proteins between the two subunits upon dissociation in the absence of Mg^{++}. We tested the possibility that these observations could be explained by the fact that ribosomes have different conformational states in growing and in starved cells. The change in ribosomal protein profile in starved cells occurs at 6-7 hours after starvation is initiated. This change was correlated with a) a change in the turnover rate of ribosomes; b) a change in the cycloheximide sensitivity of ribosomes; c) a change in the electrophoretic mobility of isolated ribosomal subunits. Analysis of a mutant with altered cycloheximide sensitivity, altered turnover rates of ribosomes, and altered electrophoretic mobilities of ribosomal subunits will also be presented to support the hypothesis that ribosomes undergo a conformational alteration in starved cells which is reversible upon refeeding.

841 CONTROL OF ^{35}S PROTEOGLYCAN SYNTHESIS OF CHONDROCYTES. R.Paul Miller and Susan Lohin. St. Mary's Hospt. and U. of Rochester Sch. Med. Dent., Rochester, N.Y. 14611

We probed the relationship between replication and the 24h synthesis of sulfated proteoglycans of chondrocytes grown in monolayer culture with vasopressin (LVP) and $N^{6}O^{2'}$ dibutyryl adenosine 3'5' monophosphate (dbcAMP). LVP (0.5-10 ng/ml) in 2% calf serum (C.S.) stimulated replication and enhanced both medium soluble and cell layer ^{35}S proteoglycans. However, the label incorporation was directly related to cell number. When 5 X 10^{-4}M dbcAMP in 2% C.S. was utilized, no replication occurred and synthesis of medium and cell layer ^{35}S proteoglycans was enhanced. If LVP and dbcAMP were added together, hormone stimulated replication was suppressed and no enhancement of ^{35}S proteoglycans was noted above that caused by dbcAMP. Therefore, LVP is mitogenic but fails to enhance ^{35}S proteoglycan synthesis except for that which appears obligatory to cell division. Stimulation with 16% C.S. and dbcAMP inhibited replication, slightly suppressed medium ^{35}S proteoglycans and dramatically enhanced cell layer ^{35}S proteoglycans. When dbcAMP and graded amounts of C.S. were added, the lower concentrations of serum failed to increase ^{35}S incorporation to that of 16% C.S., suggesting dbcAMP was enhancing C.S. components, presumably serum hormones. The dose response noted with graded amounts of C.S. when cultures were sacrificed at 24h was not present when cultures were sacrificed at 6h, indicating a delayed cellular event. Pulse addition of label for 30m localized the onset of dbcAMP effect to 8-12h after C.S. stimulation, a time prior to thymidine incorporation, confirming a localized event late in the G_1 compartment of the cell cycle. We conclude that chondrocytes synthesize 2 groups of proteoglycans, one which is obligatory to cell division and a second type, which is late in the G_1 compartment and under control of adenylate cyclase.

842 VISIBLE LIGHT INHIBITS GROWTH OF CHINESE HAMSTER OVARY CELLS. GEORGE B. BODER, RICHARD J. HARLEY, DANIEL C. WILLIAMS AND WALTER J. KLEINSCHMIDT. LILLY RESEARCH LABORATORIES, INDIANAPOLIS, IN 46206.

Time lapse cinematographic and video analysis are frequently used to monitor mitotic events and cell functions. Until recently, little attention has been paid to the effect of visible light on medium constituents and cell growth. Utilizing a video tape time lapse system to study the effect of various inhibitors of mitosis, we have found that visible light (23 W/m^2) is completely inhibitory to Chinese hamster ovary cells. Reduction in irradiance to 14 W/m^2 is permissive for cell growth as is red light (650 nm) at 23 W/m^2. Growth inhibition of white light at 23 W/m^2 is prevented by inclusion of 5 x 10^{-8}M selenium or 1 x 10^{-7}M α-tocopherol phosphate to the medium. Combined low doses of selenium and α-tocopherol phosphate are synergistic. Mitosis appears to be the most sensitive part of the cell cycle. A role of heat in the inhibitory process was excluded. In addition, precautions were made to minimize heat generation of light by several techniques. Pre-conditioning of the medium by exposure to visible light reduces sensitivity of cells exposed to light in this preconditioned medium. Various lots of fetal calf serum provided concentration related protection. These studies suggest that subtle effects of visible light on exposed cells may be factors in mutagenesis,senescence, and differentiation of isolated and cultured cells.

843 The Effect of Cyclic AMP on the Growth Properties and Surface Morphology of XC Sarcoma Cells in Vitro, William M. Mitchell, Shirley S. Schuffman, Josef K. Korinek, and Harold L. Moses*, Vanderbilt University School of Medicine, Nashville, TN 37232, and The Mayo Clinic*, Rochester, MN 55901

The intracellular concentration of cyclic AMP has been associated with a regulatory role in cell division, cell morphology, and physical properties of the plasma membrane. We have shown previously that temperature induced modulation of cAMP concentration in NRK cells transformed by temperature sensitive mutants of the Kirsten murine sarcoma virus resulted in an increased generation time and a decrease in surface microvilli during G1 as a function of increasing cAMP content (J. Supramolecular Structure 5:309, 1976). In this study we have observed directly by scanning electron microscopy the effect of cAMP on cell shape and surface morphology in the rat XC sarcoma cell in vitro. $^{3}(H)$-cAMP is bound to the cell in a temperature dependent process with an abrupt cessation of cell division. XC cells cultivated in cAMP and theophylline rapidly loose their surface microvilli and their ovoid shape becomes flattened and elongated, resembling normal fibroblasts in culture. Removal of cAMP from the culture medium reverses the growth inhibitory and morphological effects. Potent inhibitors of phosphodiesterase (1 methyl-3-isobutylxanthine) mimic the cAMP induced changes.

844 RNA METABOLISM AND CONTROL OF CELL DIVISION IN SACCHAROMYCES CEREVISIAE, Gerald C. Johnston and Richard A. Singer, Dalhousie University, Halifax, Nova Scotia, Canada

Although many treatments can lead to G1 arrest in Saccharomyces cerevisiae, it would be useful to have procedures with known molecular effects causing cell division arrest within G1. We have found compounds which reversibly arrest cell division. Cells of S. cerevisiae rapidly accumulated in the G1 phase of the cell cycle when exposed to the chelating agents o-phenanthroline (OP) or 8-hydroxyquinoline (HQ). During treatment with these compounds, there was limited RNA accumulation and little RNA degradation. Rates of RNA synthesis were drastically reduced by low concentrations of these compounds, whereas rates of protein synthesis were initially unaffected. Even during this initial period cells were accumulating in G1 as evidenced by an increase in the proportion of cells without buds. Zinc salts fully reversed the inhibitory effect of both OP and HQ. We feel that the initial alteration in RNA metabolism is the major influence in blocking cells in G1, and that the eventual cessation of protein accumulation we observed was a consequence of prolonged inhibition of RNA synthesis.Previous work by others has focused on protein synthesis, or some process directly related to protein synthesis, as one signal sensed by the cell to regulate cell division. Our results make it unlikely that protein synthesis is the only process monitored for cell cycle control. RNA metabolism also plays a key role in the regulation of the cell cycle.

845 SYNERGISTIC INTERACTION OF GLYCYLHISTIDYLLYSINE AND TRANSITION METALS AS MODULATORS OF SERUM-INDUCED EFFECTS ON CELL GROWTH, Loren Pickart and M.Michael Thaler,Univ. of California,San Francisco,CA 94143

A peptide from human serum,glycylhistidyllysine (GHL),modulates cultured growth of several cell types. Added in nanomolar amounts to media low in serum,GHL stimulates growth of hepatoma cells and neurons,enhances survival of hepatocytes,and inhibits growth of glial cells and fibroblasts (Experientia 33:324 (1977)). Physicochemical and cell culture experiments indicate that GHL functions as a chelator of transition metals such as Co,Cu,Fe, and Zn,and in this manner modulates cell growth patterns. Hepatoma (HTC_4) cells (2×10^5),cultured in monolayer (Medium = 90% BME,10% S-77,0.7% fetal calf serum) under 92.5% air and 7.5% CO_2,grow slowly in a spherical configuration. Addition of Co,Cu,Fe and Zn,each at 2.5×10^{-7}M,increases cellular adhesion to the substratum and induces cellular flattening. Addition of 2×10^{-6}M GHL plus the metals causes cells to grow more rapidly in multicellular clusters. Cell numbers at 72 hrs ($\times 10^{-5}$): control 3.1±0.4;metals 2.4±0.3;metals plus GHL 7.4±1.2. Cu and Fe are the primary mediators of this effect. Among analogs,his-lys-gly is equivalent to GHL, while gly-lys-his is 20% as active. Spectrophotometric titrations indicate each mole of GHL binds 0.8 mole of Cu at pH 7.4 in PBS containing Ca and Mg. These results suggest many growth effects attributed to small peptides from serum may be due to their chelatory properties. (Supported by HD 03148 and P50 AM18520).

846 THE INTERACTION OF A 6-MERCAPTOPURINE DERIVATIVE (NBMI) WITH THE URIDINE UPTAKE SYSTEM OF QUIESCENT, SERUM ACTIVATED AND TRANSFORMED HAMSTER FIBROBLASTS
Ruth Koren, Nechama Kanner and Esther Shohami, The Institute of Life Sciences, The Hebrew University, Jerusalem, Israel.

The uptake of uridine by mammalian cells is composed of two consecutive steps: transport across the cell membrane and metabolic trapping (e.g. phosphorylation). At short uptake times (up to 30 seconds) transport is the rate limiting process while phosphorylation is rate limiting at longer times. Two different modes of inhibition of uridine transport across the plasma membrane of transformed NIL-8 cells are exhibited after their exposure to NBMI (nitrobenzyl-mercapto-inosine). When NBMI is present at the transport phase, linear Dixon plots are obtained up to 100 nM of inhibitor, and 85% inhibition. The apparent inhibition constant (K_i) is $\sim$15 nM. On the other hand when the cells are pretreated with NBMI prior to the experiment a curved Dixon plot is obtained and the apparent affinity of the probe increases. Normal NIL-8 cells can be rendered quiescent by serum starvation. Uridine uptake is one of the early events activated upon the addition of serum. While transport is the rate limiting step in the uptake of uridine by serum activated cells, metabolic trapping is rate limiting in the quiescent cells (above one minute of uptake). Pretreatment of cells with NBMI abolishes the serum activation of uridine uptake by these cells. Transport is now rate limiting both for quiescent and serum activated cells thus indicating that metabolic trapping, rather than transport across the cell membrane, is concerned with the serum activation of the uridine uptake process.

847 Cell Culture Model of Epithelial Growth Regulation: Misfeldt, D.S., and Nicoll, H.: Stanford University and Palo Alto Veterans Hospital.

In vivo proliferative activity of many epithelial tissues can be characterized as renewing cell populations which maintain a constant cell number adjusting proliferation to cell loss. A previously characterized transporting epithelial cell line (MDCK) retains asymmetrical polarity and intercellular tight junctions (Misfeldt et al PNAS 73: 1212, 1976) and can be considered a stem cell population. After plating, the cell density increases to a plateau of 4-6 X 10^5 cells/cm^2 which is a dynamic steady state, proliferative activity matching cell loss 2.5% per day. The steady state is maintained 3-4 weeks before foci of cells become multilayered. There was no effect on cell density related to the frequency of feeding (0.5,1,2d intervals) or percentage fetal bovine sera (0,1,1,10) in the media. A discontinuity or wound of the monolayer stimulated DNA synthesis (^{3}H-TdR autoradiography), with synchronization in space and time of cells in relation to the cell layer edge caused by the wound. The cell edge migrates into the wound as a sheet and between 28-32h DNA synthesis begins in the midpoint of the migrated width. By 36h the labelling index at the cell edge has increased from 2.5 to 15% and to over 50% by 40h, and falling to 5% by 80h. The labelling cycle is repeated with a periodicity of 36-40h. There is a related synchronization of cells with regard to the cell edge throughout the width of the migrating cell sheet yet the cell layer 75-150μ from the original wound is uneffected either in density or labelling index. The culture model of epithelial growth regulation is similar in vivo and may operate via identical mechanism. (Supported by The American Cancer Society-California Division #806, The Cobb Foundation, and The Dufrense Foundation).

848 KINETIC CHARACTERIZATION OF THE UPTAKE OF AMINO ACIDS BY SV40 TRANSFORMED AND NON-TRANSFORMED 3T3 MOUSE FIBROBLASTS, Thomas L. McCarthy, Katharine Muirhead, Jeffrey Roth, and Arnold I. Meisler, University of Rochester Medical Center, Department of Microbiology, Rochester, New York, 14642.

A method has been devised to look at initial rates of amino acid uptake in surface adherent cell cultures, involving a minimum perturbation of physiological growth conditions. All work is done in a humidified 37°, 10% CO_2 environment in a system which allows multiple replicates at very short time intervals for a large number of substrate concentrations within a single experiment. Uptake is studied by incubation in buffer approximating growth medium with the exception of serum and all but the amino acid of interest to avoid competition between amino acids for common transport systems. Both cell plating and amino acid uptake techniques developed by us for this protocol allow excellent kinetic data reproducibility in and among experiments. Results for several amino acids have shown that pool turnover is rapid and equilibration is virtually instantaneous. Initial uptake of methionine, for example, has been characterized for intervals between 10 and 60 seconds; such very short exposure times to radio-labelled amino acid are necessary due to rapid equilibration and avoidance of problems associated with efflux and changes in specific activity. A linear relationship is seen following classic Lineweaver-Burk analysis. While Km's remain fairly constant from confluency through very high density cell cultures, the apparent Vmax drops, which may indicate only partial exposure of the multi-lamellar cultures. Experiments are in progress to study the kinetic parameters of growing and contact inhibited non-transformed fibroblasts and the effects of amino acid deprivation on the major transport systems as a means of growth regulation in eukaryotic cells.

849 IS CELL GROWTH INHIBITION DENSITY DEPENDENT? Philip Skehan and Susan J. Friedman, Department of Pharmacology, University of Colorado Medical Center, Denver, 80262

Cell growth inhibition in culture is traditionally assumed to result from density dependent cell interactions. Attempts to validate this theory, however, have largely failed. We have re-examined this question for two cell lines, mouse 3T6 and rat C6. The simpelist formulation of density dependent growth inhibition is that inhibition begins when some critical threshold density is reached. However, with both cell lines the inhibition density shows no such threshold, but instead increases with seeding density and can vary by a full order of magnitude. C6 growth inhibition is independent of density, occurs at a fixed time after plating, and is caused by an endogenous timing mechanism. Regardless of population density, 3T6 growth inhibition begins only after global confluency is reached, and probably reflects an anchorage dependence involving a shift from a substratum suitable for growth (plastic) to one unsuitable (cell layer). Although density dependent cell interactions do not appear to cause the growth inhibition of either line, they do contribute to other aspects of cell growth regulation. Such interactions promote the viability of both lines at sparse density, regulate C6 growth rate amplitude at all times after lag phase, regulate 3T6 growth rate amplitude at all densities above confluency, and serve to lock C6 cells into a state of growth inhibition that has already been established by the endogenous timer.

850 QUANTITATIVE ASPECTS OF THE NUCLEAR PORE COMPLEXES, Gerd G. Maul, The Wistar Institute, Philadelphia, Pennyslvania 19104

The nuclear pore complex in the nuclear envelope is one of the most conservative structures in the eukariotic cell. Aside from its function in nucleocytoplasmic exchange, it may have other functions, as implied by the presence of pore complexes in annulate lamellae and RER. Analysis of the number of pores per nucleus under various conditions was used to find determinants of pore formation. Pore complexes can form and disappear during interphase. Pore formation is biphasic during the cell cycle, doubling from a G1 plateau to G2. The turnover of pore complex material must be slow since up to 4 hr after mitosis the same number of pores is reconstructed in control and protein synthesis-inhibited cells. Among proliferating cells the pore number is independent of surface and volume, but dependent on DNA content, when mammalian heteroploid cell types are compared. However, when additional (36%) DNA is heterochromatin as in the case of two diploid mouse lines, the number of pores is the same. Suprisingly, the nuclear surface volume and pore number were the same for diploid mammalian cells as for amphibian and avian cells with the same or substantially less than the mammalian DNA content. Excluding nuclear surface, volume and DNA content leaves nuclear activity as a determinant of pore number which raises the question of control of pore formation. A higher pore number was found in the less active nucleus (i.e., proliferating Xenopus cells grown at 30° C had a lower pore number than when grown at 20° C) indicating a compensatory mechanism. On the other hand, if the activity under normal conditions decreases, as in differentiating RBC of chickens, one finds pore formation to only half the pore number after each successive mitosis during the last few cell cycles.

851 SIDEROPHORE-LIKE GROWTH FACTOR SYNTHESIZED BY SV40-TRANSFORMED CELLS ADAPTED TO PICOLINIC ACID STIMULATES DNA SYNTHESIS IN CULTURED CELLS, J.A. Fernandez-Pol Nuc. Med. lab., VA Hosp. & St. Louis Univ. St. Louis, Missouri 63125.

The results of previous studies demonstrated that picolinic acid arrests the growth of cultured cells at a specific phase of the cell cycle and that these effects are transformation dependent (Proc. Natl. Acad. Sci. U.S. 74:2884-2893, 1977). Picolinic acid may preferentially exert its effects by iron deprivation and the selectivity of the agent may be due to different trace metal ion requirements by normal and transformed cells (Biochem. Biophys. Res. Commun 78:136-143, 1977). To investigate the mechanism of action of picolinic acid, mutant cell lines of SV40-transformed BALB/3T3 (SVT2) cells adapted to grow in picolinic acid have been isolated. From ultrafiltrates of medium conditioned by contact with those cell lines, we have identified and partially prufied a low-molecular-weight iron-binding ligand, termed Siderophore-like Growth Factor (SGF). Experiments have indicated that SGF has a molecular weight of approximately 1500 and is ninhydrin postive. In submicrogram amounts, this factor solubilizes and binds Fe^{3+} accumulation preceded the stimulation of DNA synthesis induced by the factor in cells maintained at low serum concentration. SGF could not be separated into Fe^{3+} binding and DNA synthetic activity. These results strongly suggest that the adaptation to grow in picolinic acid may be conferred by the synthesis of a siderophore-like molecule which competes for iron with picolinic acid. In additon, these observations provide evidence for the existance of mammalian siderophores which may be important in the initiation of DNA synthesis in transformed cells.(Supported by VA Funds MRISS 657/2620-01).