CYTOSKELETON AND CELL REGULATION

Organizers: Kermit Carraway, Elias Lazarides and Ron Vale

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Plenary Sessions Page

Poster Sessions

Molecular Biology of the Cytoskeleton-I

B 001 BIOCHEMISTRY AND GENETICS OF MICROFILAMENT REGULATORY PROTEINS IN YEAST, Anthony Bretscher, Elizabeth Chen, Bradley Nefsky and Haoping Liu, Section of Biochemsitry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853.

Although the yeast Saccharomyces cerevisiae is known to contain a single essential actin gene, the proteins associated with actin and the function of microfilaments in this organism are largely unknown. We have initiated a combined biochemical, genetic and cell biological analysis of proteins believed to be involved in microfilament organization and regulation in yeast. So far we have focussed on yeast actin, tropomyosin and monomeric actin binding proteins.

Yeast actin has been purified and characterized: it has properties indistinguishable from those of higher cell actins. Yeast tropomyosin also has properties very similar to tropomyosins from higher cells[1]. The single tropomyosin gene of yeast predicts a protein of 23.5kD that has sequence characteristics typical of all tropomyosins. Disruption of the yeast tropomyosin gene is not lethal, but results in a slower growth rate and heterogeneity of cell size. In vivo, tropomyosin is associated with actin cables in yeast, but not with the cortical actin 'dots'. In tropomyosin disrupted strains, actin cables are no longer evident[2]. Strains carrying the conditional actin mutation *act1-2* also lack actin cables; over-expression of tropomyosin in these strains partially restores actin cables[2]. These results suggest that tropomyosin interacts with filamenous actin in vivo and may play an important role in assembling or stabilizing actin cables in yeast. Further cell biological and genetic analyses of tropomyosin function in yeast are underway.

Two approaches are being used to study monomeric actin binding proteins and their genes from yeast. First, we have developed an assay to detect monomeric actin binding proteins from crude cell extracts. We have purified and characterized yeast profilin and have identified another potential actin monomer sequestering activity. We are in the process of purifying this protein(s). Purified yeast profilin, like its higher cell counterparts, can sequester monomeric actin from polymerization. We have also developed a method to directly isolate genes coding for monomeric-actin binding proteins. Here, a lambda gt11 yeast genomic library is screened with 125-I-actin and clones expressing a fusion protein that bind the labeled actin are recovered. We have isolated a number of such clones, and selected one for study. Antibodies to the fusion protein identify a single 54kD protein by immunoblotting. We are currently in the process of isolating and sequencing the gene for this putative monomeric-actin binding protein.

[1] Proc. Natl. Acad. Sci. U. S. A. 86, 90-93 (1989)
[2] Cell 57, 233-242 (1989)

B 002 MICROTUBULES AND NEUROFILAMENTS: DETERMINANTS OF AXONAL STRUCTURE AND FUNCTION. D.W. Cleveland[§], M.J. Monteiro[§], H.C. Joshi[§], J. Gearhart[*] and P.N. Hoffman[‖]. Departments of Biological Chemistry[§], Physiology[*] and Neurology and Ophthamology[‖], The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Neurons are highly asymmetric cells whose cytoarchitecture is established principally by two components: microtubules and neurofilaments. Microtubules are rigid polymers that both support neurite elongation and form the linear tracks for particle transport into the axon. The principal components of microtubules, α and β tubulin, are each encoded by a small multigene family comprised of about 6 functional genes. For β tubulin, five of these (which encode 5 different β tubulin isotypes) are expressed in neurons. These isotypes are not biochemically equivalent since two are preferentially assembled and one is partially excluded from neurites. What role, if any, such isotype distinctions play in microtubule function is still unsettled, but double label immunogold electron microscopy reveals that axonal microtubules are assembled as nearly random co-polymers of the axonal isotypes.

Neurofilaments (NF), the intermediate filament proteins of axons, are synthesized and assembled primarily after neurite elongation is completed. We have found that in myelinated axons there is a linear correlation between axonal cross sectional area and NF number. Further, following crushing (axotomy) of a peripheral nerve, a reduction in diameter that progresses from the cell body outward is accompanied by a reduction both in NF number and in newly synthesized NF subunits transported into the axon. This reduction in NF synthesis and accumulation is the consequence of axotomy-induced changes in NF mRNA levels, as demonstrated by RNA blots and in situ hybridization. In view of these findings we have proposed that NF are intrinsic determinants of axonal diameter, which it turn specifies conduction velocity. As a further test of this proposal, we have cloned the NF-L and NF-M genes and have used DNA transfection to identify domains of the mouse NF subunits necessary for filament assembly. Not surprisingly, the 310 amino acid conserved α helical rod domain is essential for assembly and mutations into this domain can disrupt assembly both of mutant and co-expressed wild type subunits. We have used these gene constructs to prepare transgenic mice that express additional wild type or mutant NF-L genes. We find that NF-L can be expressed and assembled at high levels (2% of cell protein) in non-neuronal cells without major effect, except in lens where NF accumulation produces cataracts. Surprisingly, however, expression of a 20 fold excess of transgene RNA in neurons does not yield any increase in NF-L protein. This may be due to a translational or post-translational mechanism that restricts NF synthesis or assembly in neurons.

B 003 MOLECULAR GENETICS OF CYTOSKELETAL PROTEINS IN *DROSOPHILA*, L.S.B. Goldstein, Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138. My laboratory and our collaborators have been working to define the structures and *in vivo* functions of several critical components of the cytoskeleton in *Drosophila melanogaster*. We have been studying two different microtubule proteins, the 205K microtubule-associated protein (205K MAP) and kinesin, and one protein that is a component of the membrane linked cytoskeleton called spectrin. These proteins are thought to play critical roles in cell division, intracellular transport, and cellular morphogenesis during development. We have analyzed these proteins biochemically, by sequence analysis, and by molecular genetic analysis. We have also been pursuing classical genetic analyses with the aim of generating and recovering mutations in which these proteins are defective so that we may study the resultant phenotypes and infer the *in vivo* functions. In my talk, I will discuss our recent work on the primary structures of 205K MAP, kinesin, and spectrin and describe how this information, combined with information derived from binding studies with wild-type and mutant versions of these molecules has led to models of these molecules organization. In the case of kinesin I will go on and describe our studies of the motility of various truncated and rearranged versions of the molecule that have begun to allow us to identify regions important for force generation. I will also discuss our ongoing genetic analyses that have given us strong candidates for mutations in these genes.

Molecular Biology of the Cytoskeleton-II

B 004 COMPONENTS OF THE DYSTROPHIN MEMBRANE CYTOSKELETON, Frederick M. Boyce III, Michel Koenig, Simon Watkins*, and Louis M. Kunkel, HHMI, The Children's Hospital and Dana Farber Cancer Center*, Boston, MA 02115.

Duchenne Muscular Dystrophy (DMD) results from the absence of dystrophin, the protein product of the DMD gene. Dystrophin is believed to function as a membrane cytoskeletal element based upon its sequence homology to the spectrin family and its immunocytochemical localisation to the plasma membrane. To understand the molecular mechanisms by which dystrophin functions, we have identified several proteins which may interact with, or serve similar roles as dystrophin.

One strategy we have used is to express portions of dystrophin in bacteria as probes for proteins which bind directly to dystrophin. Using the highly conserved C-terminal region of dystrophin as a protein probe in blot overlay experiments, we have identified a putative dystrophin-binding protein in muscle cells. Control experiments show that native forms of both dystrophin and the binding protein are necessary for proper interaction. This protein is found in both striated and smooth muscle cells but is absent in non-muscle cell types. This protein appears to bind equally well to the alternative isoforms of dystrophin.

Another strategy we have employed is to use anti-dystrophin antibodies to detect proteins that share common determinants with dystrophin. The C-terminus of dystrophin is unique in that it does not share homology with the spectrin family. Using an antibody directed against this unique region of human dystrophin, we have detected a cross-reactive protein in western blots of brain tissue. This protein also shares with dystrophin the property of large size (350kD, dystrophin=427kD); however, this protein is clearly distinct from dystrophin in that it is found only in brain tissue and is absent in mdx mice, which do not express dystrophin. This protein has been further localized by immunocytochemistry to perivascular cells in the brain.

B 005 MOLECULAR GENETICS OF DROSOPHILA CYTOSKELETAL PROTEINS, Eric Fyrberg, Department of Biology, The Johns Hopkins University, Charles and 34th Streets, Baltimore, MD 21218. I will summarize what is known concerning the chromosomal arrangement and tissue-specific expression of particular Drosophila contractile and cytoskeletal protein genes, and present our analyses of mutations within genes encoding the microfilament crosslinking protein alpha-actinin. I hope to have data relating to mutants of vinculin as well.

B 006 GENES IMPLICATED IN MUSCLE ASSEMBLY IN THE NEMATODE C. ELEGANS, R. Waterston, R. Barstead, J. Waddle and B. Williams. Department of Genetics, Washington University, St. Louis MO 63110

The nematode Caenorhabditis elegans allows the in vivo assessment of functions necessary for normal muscle assembly. The principal muscle of C. elegans is associated with the body wall and is necessary for movement and viability. This muscle can be readily observed in the whole animal with the light microscope, and a battery of specific monoclonal antibodies can be used to assay a variety of muscle components.

The nematode vinculin gene has been recovered through antibody screening of an expression library, and its position on the physcial map ascertained. Using this information, we have identified two mutations affecting vinculin, one resulting in the lack of the protein and the other apparently producing a truncated protein. The nature of the vinculin mutations and their effects on the assembly of other muscle components are currently being determined. We anticipate that the mutants will also be useful in identifying genes for other interacting proteins.

CapZ proteins bind the barbed end of actin filaments and have been postulated to be involved in nucleating or otherwise regulating the assembly of thin filaments in vertebrate striated muscle. Using the polymerase chain reaction we have recovered candidate genes for both CapZ subunits in the nematode. Localization of these genes on the physical map of C. elegans will permit a genetic dissection of their function in muscle assembly.

Although more than 40 muscle affecting genes have been identified in C. elegans, recent experiences with the myosin and vinculin genes shows that other genes remain to be identified. Mutants of the vinculin and myosin genes result in arrest at the same developmental stage, and cause an almost complete loss of the ability to move. We have begun looking for additional mutants which confer the same phenotype with the expectation of identifying other components essential for muscle development and assembly. Several of the mutants isolated are more severe alleles of previously known genes, and show that these genes are essential for muscle function. In addition, several new genes have been identified, and are candidates for genes playing critical roles in the assembly or function of striated muscle. The similarity in phenotype of these animals indicates that muscle function is required for the completion of normal embryogenesis.

Cytoskeleton and Intracellular Dynamics-I

B 007 MODIFICATION AND STABILIZATION OF MICROTUBULES DURING CELLULAR MORPHOGENESIS, J.C.Bulinski, G.G.Gundersen, S.Khawaja*, N.Modesti*, and D.Webster, Dept. of Anatomy & Cell Biology, Columbia Univ., NY, NY 10032 and *Department of Biology, UCLA, Los Angeles, CA 90024

Previous studies with pharmacological agents have demonstrated that microtubules (MTs) play a vital role in morphogenetic differentiation. In our previous work with proliferating cells in culture, we demonstrated a cyclic mechanism in which post-translational addition of tyrosine to monomeric alpha tubulin and its removal from polymeric tubulin results in distinct subsets of cellular MTs enriched in either detyrosinated (Glu) or tyrosinated (Tyr) tubulin. We showed that MTs enriched in Glu tubulin (Glu MTs) are less dynamic than those enriched in Tyr tubulin (Tyr MTs); in at least one cell type, Glu MTs persisted for virtually an entire cell cycle. We have not yet determined the cause of the extraordinary longevity of this minor subset of MTs; however, we do know that the enrichment in Glu tubulin is an effect, rather than a cause, of stabilization of the MT. We have found that in several instances, cells generate stable Glu MTs during morphogenetic events. For example, 3T3 fibroblasts induced to migrate into an experimental wound exhibit a polar array of stable MTs prior to the onset of cell migration. Similarly, in PC-12 cells treated with NGF, stable Glu MTs appeared in the newly extended neurites. In cultured rat myogenic cells, stable Glu MTs were detectable soon after the onset of myogenesis, as the myoblasts were elongating and aligning prior to fusion. Thus, both the timing of the appearance of stable Glu MTs and their position in cells undergoing morphogenesis suggests that they may play a role in changing cell shape and polarity. We are currently investigating the function of these MTs, the mechanism(s) by which they are stabilized, and the enzymes that carry out the post-translational modifications. [Supported by grants from the Muscular Dystrophy Association and the N.I.H.]

B 008 CELL CYCLE -DEPENDENT FORMATION OF TUBULAR MEMBRANE NETWORKS IN VITRO, Ronald D. Vale, Department of Pharmacology, University of California, San Francisco, CA 94143

Many intracellular membrane-bounded organelles are specifically arranged within the cytoplasm of eukaryotic cells. The Golgi apparatus, for example, is located near the microtubule-organizing center, while the endoplasmic reticulum (ER) extends membrane tubules towards the cell periphery. Such distributions are determined, in part, by motor proteins that attach to these membranes and move them along microtubules to the correct location in the cell. During mitosis, the organization of the Golgi and ER is dramatically perturbed; both membranes fragment into smaller components which, at least in some cells, become randomly distributed throughout the cytoplasm. To study how membranes are spatially and temporarily organized, we have examined ER membrane tubule formation in interphase and mitotic extracts from Xenopus oocytes. Membranes from interphase extracts fuse with one another, migrate out along microtubules, and form an anastomosing network of membrane tubules that resembles images of ER networks observed in living cells. Membranes from a mitotic extract, on the other hand, bind to microtubules but do not form tubular membrane networks. Thus, factors involved in the formation of ER-like tubular membrane networks appear to be under cell cycle control. The ability to study such events in vitro should permit a biochemical dissection of ER tubule formation as well as the control mechanisms that governs such processes.

Cytoskeleton and Intracellular Dynamics-II

B 009 INTERMEDIATE FILAMENT DYNAMICS, ORGANIZATION & FUNCTION IN XENOPUS

Michael W. Klymkowsky and colleagues. Molecular, Cellular & Developmental Biology. University of Colorado. Boulder, CO. 80309-0347.

While highly conserved and phyllogenetically wide spread, the functions of cytoplasmic intermediate filaments (cIFs) remain unclear. The antibody-induced disruption of cIF organization in cultured cells has no apparent effect on cellular behavior. This suggests that cIFs function primarily at the level of tissues and organs, rather than at the level of the single cell. To study cIFs in an organismic context we have begun an examination of cIF organization and function in the Xenopus oocyte and embryo. We have concentrated primarily on the vimentin and cytokeratin cIF proteins that appear first during vertebrate development. **VIMENTINS.** There are two distinct forms of vimentin in Xenopus: one of 55kDa form (V_S) reacts with the monoclonal antibodies RV202 and 14h7; the longer, 57kDa form (V_L) reacts preferentially with a rabbit antivimentin antibody and 14h7, but not with RV202. V_L and V_S are differentially regulated. Most strikingly, V_L is present in the oocyte and is associated with the mitochondrial mass and germ plasm. We are currently working to determine whether V_L plays a role in the structural integrity of the germ plasm. Towards this end, full length cDNA clones for both V_S and V_L have been isolated and characterized (in collaboration with D. Sakaguchi, G. Sharma & W. Harris. UCSD). Using these clones we are preparing dominant mutant versions of vimentin.
CYTOKERATINS. The cytokeratin filament system of the Xenopus oocyte is polarly asymmetric in its organization. During oocyte maturation, cytokeratin filaments break down; this breakdown is initiated by maturation promoting factor (MPF), but unlike MPF's effect on the nuclear envelope and nuclear IFs (lamins), the breakdown of cytokeratin filaments requires constant protein synthesis. Inhibition of protein synthesis, even after the complete breakdown of cytokeratin filaments, leads to their reassembly. The meiotic breakdown of cytokeratin filaments is accompanied by their acidification, and presumably reflects their phosphorylation. When protein synthesis is inhibited, this post-translational modification does not occur and cytokeratin filaments remain intact. The maternal mRNA Vg1 is localized to the vegetal cortex of the oocyte and is associated with the insoluble, cytoskeletal component. During oocyte maturation, Vg1 mRNA becomes soluble, even if cytokeratin filament breakdown is blocked using cycloheximide. To determine unambiguously whether Vg1 mRNA interacts with cytokeratins, we are constructing dominant mutant versions of both type I and type II cytokeratin cDNAs. Finally, we have found that the injection of the monoclonal anti-type II-cytokeratin antibody AE3 causes a specific and dramatic effect on normal gastrulation in Xenopus. Mutant of versions of cytokeratins should enable us to unambiguously determine whether cytokeratin filaments play a role in this major morphogenic event of vertebrate embryogenesis.

B 010 ROLE OF TROPOMYOSIN AND CALDESMON IN NONMUSCLE CELL MOTILITY, Jim J.-C. Lin, Theresa E. Hegmann, Elizabeth J. Davis-Nanthakumar, Robert E. Novy and Jenny L.-C. Lin, Department of Biology, University of Iowa, Iowa City, IA 52242.

Tropomyosin is a rod-shaped protein that binds along actin filaments; in muscle cells, its role in the regulation of contraction has been well characterized, but in nonmuscle cells, its function has not been completely determined. We have recently shown that a monoclonal antitropomyosin CG1 antibody recognized an epitope depending on the motility states.[1] Microinjection of this CG1 antibody specifically inhibited the instantaneous speed and saltation distance of intracellular granule movements of chicken embryo fibroblasts (CEF).[2] Control injection of other monoclonal antibodies against tropomyosin or preabsorbed CG1 antibody did not show any effect on granule movement. When CG1 injected cells were counter-stained to reveal the microtubule, microfilament and intermediate filament systems, no obvious differences from the patterns normally seen in uninjected cells were observed. These experiments suggest that nonmuscle tropomyosin may play a critical role in the granule movement. Since there is no troponin complex found in nonmuscle cells, the molecular mechanism that nonmuscle tropomyosin regulates granule movement is not understood. Recent studies on and characterization of nonmuscle caldesmon suggest a reasonable mechanism for nonmuscle tropomyosin and caldesmon working together in the regulation of granule movement. To test this possibility, we have prepared monoclonal antibodies C2, C9, C18, and C21 against gizzard caldesmon. These antibodies also crossreacted to a 66 kD nonmuscle CEF caldesmon.[3] Epitope mapping revealed that both C2 and C21 were anti-C-terminal fragment antibodies, whereas both C9 and C18 antibodies recognized the N-terminal fragment of gizzard caldesmon. In the in vitro reconstitution, only antibody C21 was able to inhibit not only the binding of Ca^{++}/calmodulin to caldesmon but also the binding of caldesmon to F-actin or F-actin-tropomyosin complex. Introduction of this C21 antibody into live CEF cells caused to stop the movements of intracellular granules. This inhibition was also reversible and specific. Therefore, tropomyosin and caldesmon may function in intracellular granule movement by regulating the contractile system in response to [Ca^{++}] concentration change inside nonmuscle cells.

1. Hegmann, T.E., J.L.-C. Lin, and J.J.-C. Lin. (1988) J. Cell Biol. 106:385-393.
2. Hegmann, T.E., J.L.-C. Lin, and J.J.-C. Lin. (1989) J. Cell Biol. 109:1141-1152.
3. Lin, J.J.-C., J.L.-C. Lin, E.J. Davis-Nanthakumar, and D. Lourim. (1988) Hybridoma 7:273-288.

B 011 DYNAMICS OF ACTIN IN LIVING CELLS, Yu-li Wang, Long-guang Cao and Mitchell C. Sanders, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Most non-muscle cells are known to contain a relatively high concentration of unpolymerized actin, probably as building blocks for structural assembly. The unpolymerized actin may be maintained either by sequestration of monomers, or by capping and inhibition of nucleation. In order to determine the mechanism, exogenous nucleation sites, prepared by sonicating fluorescein phalloidin-labeled actin filaments, were microinjected into living cells. The microinjection induced neither an extensive polymerization of endogenous actin off the nucleation sites, nor changes in the distribution of actin filaments. However, the nucleation sites maintained the ability to bind actin monomers, as indicated by a second microinjection with monomeric rhodamine-labeled actin. These observations suggest that actin polymerization is maintained primarily by the sequestration of monomers.

Time-lapse studies of cells microinjected with fluorescently labeled actin have indicated that actin-containing structures undergo constant reorganization in non-muscle cells. We have recently focused on the process of cytokinesis, which is known to involve the formation of a circumferential actin filament bundle (contractile ring) along the equatorial plane. To analyze the assembly mechanism of the contractile ring, we microinjected a small amount of rhodamine phalloidin or rhodamine actin into dividing NRK cells. Rhodamine phalloidin was microinjected during prometaphase or metaphase to label actin filaments that were present at that stage. As mitosis proceeded into anaphase, the labeled filaments became associated with the plasma membrane. During cytokinesis, rhodamine phalloidin was depleted from polar regions and became highly concentrated into the equatorial region. The distribution of total actin filaments, as revealed by staining the whole cell with fluorescein phalloidin, showed a much less pronounced difference between the polar and the equatorial regions. The sites of de novo assembly of actin filaments during the formation of the contractile ring were determined by microinjecting rhodamine actin shortly before cytokinesis, then extracting and fixing the cell during mid-cytokinesis. Injected rhodamine actin was even less concentrated in the cleavage furrow, as compared to the distribution of total actin filaments. Our results indicate that pre-existing actin filaments, probably through movement and reorganization, are used preferentially for the formation of the contractile ring. De novo assembly of filaments, on the other hand, appears to take place preferentially outside the cleavage furrow and is unlikely to be important in contractile ring formation.

Membrane-Cytoskeleton Interactions-I

B 012 REGULATION OF CELL SURFACE TOPOGRAPHY BY MEMBRANE-MICROFILAMENT INTERACTIONS, Kermit L. Carraway and Coralie A.C. Carraway, Departments of Cell Biology and Anatomy and Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL 33101

Membrane-microfilament interactions are widely believed to play an important role in the organization of molecules at cell surfaces. To investigate such interactions and their role in organizational control, we have used ascites sublines of a mammary carcinoma which differ in morphology and cell surface receptor mobility. MAT-B1 cells have straight microvilli and mobile receptors; MAT-C1 cells have branched microvilli and essentially immobile receptors. MAT-C1 microvilli are also highly resistant to effects of cytochalasins. Studies of microvilli isolated by shearing from the cells show that MAT-C1 cell microvilli contain a membrane- and microfilament-associated 58 kDa protein absent from MAT-B1 microvilli. Purified 58 kDa protein binds microfilaments and inhibits actin polymerization in a manner similar to capping proteins. It also binds to lipid vesicles. Proteolysis of 58 kDa protein by endogenous or exogenous proteases yields separate membrane- and microfilament-binding fragments of 30 and 28 kDa. When Triton-solubilized microvillar membranes are fractionated by gel filtration, the 58 kDa protein is isolated in a complex with actin and four membrane glycoproteins. If microvilli are prepared in the presence of calf serum, bovine IgM binds tightly to and is isolated with the complex. Microvillar microfilament cores, prepared by extraction of microvilli with Triton, also contain the four membrane glycoproteins as their major glycoprotein components, identified by phalloidin shift analyses of metabolically labeled microvilli. Gel filtration of high salt extracts of the microfilament cores suggests that three of the glycoproteins are associated in a high Mr complex; the fourth dissociates under these conditions. The glycoprotein-58K-microfilament interaction can be reconstituted from the high salt extracts by microfilament polymerization. The high salt-dissociated, microfilament-associated glycoprotein has been identified immunologically as a component of the sialomucin complex which is the major cell surface component of these ascites tumor cells, thus linking a major fraction of the cell surface receptors to the cytoskeleton. These studies suggest that one or more of these glycoproteins provides linkages, either directly or indirectly, to the microfilaments. Such linkages can be stabilized by the 58 kDa protein, thus stabilizing the microvilli against perturbation and restricting receptor mobility.

B 013 REMODELLING THE PLASMA MEMBRANE DURING DEVELOPMENT OF EPITHELIAL CELL POLARITY, W. James Nelson, Rachel W. Hammerton, Helen McNeill, Allan Wang, and Eileen M. Shore, Institute for Cancer Research, 7701 Burholme Avenue, Philadelphia, PA 19111 An understanding of mechanisms involved in the spatial organization of proteins in cells is central to the problem of how cells become structurally and functionally polarized. In polarized kidney (MDCK) epithelial cells, membrane proteins are localized to either the apical or basal-lateral membranes. We have shown that the Na^+,K^+-ATPase, a basal-lateral membrane protein, is linked to ankyrin and fodrin which are major components of the basal-lateral membrane-cytoskeleton (1, 2). The assembly of this protein complex on the basal-lateral membrane is induced by cell-cell contact, which is regulated by the epithelial cell adhesion protein uvomorulin/E-cadherin. Recently, we identified a complex containing uvomorulin, ankyrin and fodrin in MDCK epithelial cells (3). The identification of these membrane-cytoskeletal complexes provides evidence of a possible linkage between the cell adhesion protein uvomorulin and a basal-lateral membrane protein, Na^+,K^+-ATPase. These results support a model in which cell-cell contact, through uvomorulin, plays a direct role in inducing the assembly of the membrane-cytoskeleton and Na^+,K^+-ATPase at the contact zone between cells that lead to the reorganization and polarization of protein distributions on the plasma membrane. We are testing this model by analyzing the distributions of Na^+,K^+-ATPase and other proteins in fibroblasts transfected with different uvomorulin cDNA constructs.

1. Nelson, W.J., and Veshnock, P.J. Nature (Lond.) 328:533-536 (1987).
2. Nelson, W.J., and Hammerton, R.W. J. Cell Biol. 108:893-902 (1989).
3. Nelson, W.J., Shore, E.M., Wang, A.Z., and Hammerton, R.W. J. Cell Biol., in press (1990).

Membrane-Cytoskeleton Interactions-II

B 014 THE INTERACTIONS OF PROFILIN WITH ACTIN AND MEMBRANE PHOSPHOLIPIDS PROVIDES CONTROL OF ACTIN ASSEMBLY AND PHOSPHOINOSITIDE SIGNALLING, Thomas D. Pollard, Pascal Goldschmidt-Clermont, Laura M. Machesky and Karen Magnus, Department of Cell Biology and Anatomy, Johns Hopkins Medical School, Baltimore, MD 21205.
Profilins from Acanthamoeba and human platelets bind to both actin monomers and phosphoinositides. Platelet profilin binds to phosphoinositol 4,5 bisphosphate incorporated into either micelles or large unilamellar vesicles composed largely of other lipids such as phosphatidyl choline and phosphatidyl ethanolamine. One profilin binds to 5 PIP_2 molecules with a dissociation constant in the submicromolar range. Platelet profilin binds to phosphatidylinositol 4 phosphate in a similar fashion, but does not bind with high affinity to phosphatidylinositol, phosphatidyl serine, phosphatidyl choline or phosphatidyl ethanolamine. Platelet profilin inhibits PIP_2 hydrolysis by platelet cytosolic phosphoinoside-specific phospholipase-C (PLC). Since the dissociation constant and stoichiometry estimated from enzyme inhibition experiments were similar to those measured by direct binding assays, the mechanism presumably involves physical sequestration of the substrate by the profilin. The concentrations of these molecules in platelets and their binding characteristics are consistent with profilin being a negative regulator of the phosphoinositide signalling pathway. We speculate that this suppresses PLC activity in resting platelets and that activation of PLC by receptor mediated processes makes the enzyme a more effective competitor of profilin for access to the substrate. Acanthamoeba profilin-II interacts with PIP_2 much like platelet profilin, but profilin-I binds much less strongly. This suggests that profilin-II may be specialized for interaction with phospholipids. Structural comparisions of these profilins should provide some clues about the mechanism of lipid binding. Our preliminary model for the 3-dimensional structure of Acanthamoeba profilin has the cluster of basic residue near the C-terminus arranged on a surface where they could make ionic bonds with multiple acidic lipid head groups. Since at least one actin contact site, lysine-115, is included in this region, we suggest that simple steric competition explains how PIP_2 can dissociate actin and profilin. Since profilin has a higher affinity for PIP_2 pentamers than for actin monomers, membrane phosphoinositides are in a position to regulate actin polymerization in the cell, but the quantitative aspects of this multicomponent regulatory system are far from understood.

B 015 CYTOPLASMIC ACTIN MOVES PARTICLES ON A NEURONAL GROWTH CONE SURFACE, Stephen J Smith and Paul Forscher, Section of Molecular Neurobiology, Howard Hughes Medical Institute Research Laboratories, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510. Crawling movements by growth cones are involved in guiding growing axons to their proper targets during nervous system development. These crawling movements comprise alternating cytoplasmic spreading and retraction phases. Two divergent models for such movements have been proposed: one based on exocytic and endocytic membrane traffic and the other based on cytoskeletal actin dynamics. Observations that surface markers often flow rearward from leading edges of crawling cells have been taken as evidence in favor of a membrane traffic model: it has been suggested that cytoplasmic spreading might reflect localization of exocytosis to the leading edge. Evidence for the actin-based motility model, on the other hand, has come from experiments using cytochalasins, toxins that inhibit actin polymerization. These agents immediately arrest cytoplasmic spreading, suggesting that the process may reflect actin polymerization. Experiments to be reported address the observations fundamental to both proposals. Our experiments were carried out on growth cones of cultured neurons from the marine mollusc Aplysia californica. Movements of surface-marking particles on neuronal growth cones were observed by video microscopy before, during and after treatments with cytochalasin-B (CB). Our results seem most consistent with an actin-based motility model and indicate that rearward flow of surface markers may not require a rearward flow of bulk membrane.

B 016 CALCIUM AND PHOSPHOLIPID REGULATION OF ACTIN CYTOSKELETON, Helen L. Yin, Department of Physiology, University of Texas Southwestern Medical Center, Dallas, Texas 75235-9040

Receptor-mediated stimulation induces marked actin polymerization and cytoskeletal reorganization in a variety of cells. The activity of a potent vertebrate actin modulating protein, gelsolin, is activated by micromolar Ca^{2+} and inhibited by polyphosphoinositides. During agonist stimulation, gelsolin reversibly associates with the plasma membrane and actin, suggesting that it may be involved in the restructuring of the actin cytoskeleton. Structure-function analyses by proteolytic cleavage have identified domains important for binding to actin, Ca^{2+} and polyphosphoinositides. Results from deletional and site-directed mutagenesis further defined critical functional and regulatory domains, and provide insight into how gelsolin severs actin filament and how it is regulated by the cellular messengers. Our model for gelsolin function may be applicable to other related actin regulatory proteins found amoung a variety of eukaryotic species.

Junctional Complexes

B 017 STUDIES ON THE MECHANISM AND REGULATION OF CELL-SUBSTRATUM ADHESION. Mary C. Beckerle, Maria Bertagnolli, Aaron Crawford, Sarah Locke, and Diane Miller. Department of Biology, University of Utah, Salt Lake City, UT 84112.

Cells are capable of establishing transmembrane connections between the extracellular matrix and filamentous actin at sites of cell-substratum adhesion. We are trying to understand how the actin-membrane interactions that occur at these specialized regions of the plasma membrane are achieved and regulated. We recently identified a new adhesion plaque component by analysis of a non-immune rabbit serum. The adhesion plaque protein recognized by this serum is low in abundance and has an apparent molecular weight of 82,000 Daltons. We have isolated this 82kD protein from low ionic strength extracts of avian smooth muscle by anion exchange, hydroxylapatite, and gel filtration chromatography. The isoelectric point of the 82kD protein is 6.9. It fractionates with the aqueous phase in Triton X-114 phase separation experiments and it does not bind Con A. Detection of the 82kD protein by indirect immunofluorescence requires detergent permeabilization of the cells. Antibodies raised against the purified 82kD protein reveal that in fibroblasts the protein is found at adhesion plaques as well as along stress fibers near where they terminate at adhesion plaques. This interesting sub-cellular distribution raises the possibility that the 82kD protein may be part of the machinery that links actin filaments to the plasma membrane at sites of cell-substratum adhesion.

In order to approach the question of how cell adhesion is controlled, we have focused our attention on platelets, cells that are specialized for regulated adhesion. We found previously that the adhesion plaque component, talin, is very abundant in platelets. We have now compared the subcellular distribution of talin in resting (non-adhesive) and activated (adhesion-competent) platelets by immunofluorescence and immunoelectron microscopy. By these approaches, talin appears to be uniformly distributed within resting platelets. In contrast, in activated platelets, talin is concentrated in the peripheral cytoplasm underlying the plasma membrane. By quantitative morphometric analysis of immunogold-labelled thin sections, we have determined that, relative to resting cells, activated platelets exhibit at least a 3-fold increase in the percentage of talin localized within 40nm of the plasma membrane. Since platelets are anucleate cells with little active translational machinery, it is likely that post-translational modifications of proteins present in resting cells are responsible for the control of talin's subcellular distribution. Talin itself is known to be modified post-translationally by proteolytic cleavage and phosphorylation. We have examined the calcium-dependent proteolysis of talin in thrombin-activated platelets and have found that the activation-dependent redistribution of talin precedes any detectable talin cleavage.

B 018 CYTOSKELETAL SYSTEMS ASSOCIATED WITH CLUSTERED ACETYLCHOLINE RECEPTORS AND AT THE NEUROMUSCULAR JUNCTION. Robert J. Bloch, David W. Pumplin*, Paul W. Luther, and Jacqueline G. Krikorian, Departments of Physiology and Anatomy*, University of Maryland School of Medicine, Baltimore, MD 21201.

The clustering of acetylcholine receptors (AChR) is one of the earliest events in the formation of the neuromuscular junction (NMJ). The extracellular matrix and cytoskeleton have both been implicated in the process of AChR clustering. We have studied the AChR clusters of aneural rat muscle cells in vitro. These clusters form preferentially at sites of myotube-substrate adhesion, and are organized in distinct domains, some rich in AChR, others poor in AChR but more closely apposed to the substrate. We have identified 3 distinct membrane-skeletal structures in these clusters. Intracellularly, the AChR-rich domain is composed in part of actin, an unusual isoform of β-spectrin (but no α-spectrin), and the 43kD and 58kD proteins, which were originally identified in Torpedo electric organ. Semiquantitative fluorescence suggests that these proteins are present in a stoichiometric complex of approximately 4-7 β-spectrin: 1 58kD: 1 43kD: 1 AChR. (The stoichiometry of actin cannot yet be determined). Extraction of these proteins from clusters occurs in the following order: actin, spectrin, 58kD, 43kD, AChR (actin most readily extracted). On the basis of these experiments, we suggest that the AChR in AChR-rich domains of AChR clusters is bound to an ordered membrane skeleton that resembles, but is distinct from, that of the human erythrocyte. The other two domains of AChR clusters are not directly associated with clustered AChR, but instead are associated with sites of adhesion of the muscle cell to the substrate. Membrane-to-substrate distances are approximately 10 nm. Intracellularly, one of these "contact domains" resembles the focal contacts of fibroblasts, and contains β-1 integrin, talin, vinculin, α-actinin, filamin, and associated microfilament bundles. The other consists of large plaques of clathrin-coated membrane, which also serve as sites of high coated vesicle activity. These two "contact domains" are more stable than the AChR-rich domain and its associated intracellular proteins. Fluorescence and ultrastructural studies of NMJ formed in vitro suggest that similar membrane skeletal structures are present in the developing postsynaptic region. We propose that AChR clustering during synaptogenesis occurs by the sequential assembly of these structures, with regions of nerve-muscle adhesion developing first, and inducing the formation of AChR-rich domains in adjacent areas of the postsynaptic membrane.

Supported by the NIH and the Muscular Dystrophy Association.

Focal Contacts/Cell Junctions (joint)

B 019 CYTOSKELETAL ORGANIZATION AT ADHESIONS TO THE EXTRACELLULAR MATRIX, Keith Burridge, Glen Nuckolls, Carol Otey, Fredrick Pavalko and Christopher Turner, Dept. of Cell Biology and Anatomy, UNC, Chapel Hill, NC 27599.

We are interested in how the actin cytoskeleton is linked to the plasma membrane at sites such as focal contacts where cells adhere to the extracellular matrix. Microinjection of anti-talin antibodies into respreading cells results in the formation of abnormal focal contacts, containing little or no talin. Although these focal contacts reveal an abnormal morphology, they contain vinculin and continue to anchor bundles of actin filaments, arguing that talin is not necessary for the attachment of actin or for the localization of vinculin to these sites. To look for cytoskeletal proteins that bind to integrin, we have used affinity chromatography on synthetic peptides that correspond to the integrin β_1 cytoplasmic domain sequence. One protein that has shown a salt-dependent interaction with these peptide columns is alpha-actinin. Using a solid phase assay with peptide adsorbed to microtiter wells, we have found that ^{125}I-alpha-actinin shows a saturable binding to this peptide, that is competitively displaced by unlabelled alpha-actinin as well as by the free peptide. Scatchard analysis indicates a K_d of 1.6×10^{-8}M for this interaction. Use of shorter peptides in this assay has revealed that the alpha-actinin binding site in this sequence is close to the membrane. We have localized the peptide-binding site on alpha-actinin to a 53 kd fragment that does not bind actin. These results suggest that alpha-actinin may be one direct link between integrins and actin. Supported by NIH grant GM29860.

B 020 ALTERNATIVE MECHANISMS FOR THE MODULATION OF ADHERENS-TYPE JUNCTIONS. Benjamin Geiger, Tova Volberg, Talila Volk and Dorit Ginsberg, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Adherens-type junctions (AJ) are a family of cell contacts which are associated, at their cytoplasmic aspects, with microfilament bundles. The attachment of actin filaments to the junctional membrane occurs through an electron dense plaque which contain vinculin and several additional molecules. It had been shown that cell-matrix and cell-cell AJ display unique mechanical properties, largely contributed by the associated cytoskeleton. The resulting transcellular forces thus generated are, most likely, involved in cellular and tissue morphogenesis. Indeed, localization of specific cell adhesion molecules which partake in AJ formation indicated that the expression of these molecules is tightly regulated and closely correlated to major morphogenetic events in developing embryos. Recent studies have indicated that intercellular interactions in AJ are commonly mediated through A-CAM, an intercellular contact receptor of the cadherin family. Recent results indicate that A-CAM may be modulated by at least 3 alternative mechanisms. The first is a biosynthetic control of A-CAM formation; immunohistochemical staining and in-situ hybridization suggest that the levels of A-CAM closely correspond to the apparent levels of its mRNA. Another process which may affect A-CAM-mediated interactions is proteolysis. Evidence will be presented suggesting that A-CAM may be cleaved by endogenous proteinases and that such processes occur in developing chick embryos. Another mechanism which might be involved in the down regulation of A-CAM mediated interactions was observed in Rous Sarcoma Virus transformed chicken lens cells. When cultured under non-permissive temperature these cells form elaborate intercellular AJ. These junctions rapidly deteriorate following switch to the permissive temperature. This deterioration of the junctions did not involve significant changes in the amount or integrity of surface-associated A-CAM. Moreover, immunoprecipitation with anti-phosphotyrosine antibodies provided preliminary indications that the protein might undergo specific tyrosine phosphorylation in the transformed cells. The possible functional significance of the alternative regulatory mechanisms mentioned above will be discussed.

B 021 SIGNAL-TRANSDUCING PROTEIN KINASES AND THEIR TARGETS, Tony Hunter, Bill Boyle, Rick Lindberg, Dave Middlemas, and John Pines, Molecular Biology and Virology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92138

Protein phosphorylation is a major mechanism whereby signals are transduced from external stimuli into cellular responses. Many growth factor receptors are ligand-activated protein-tyrosine kinases (PTK), while other PTKs, such as $pp60^{c\text{-}src}$, located on the inside of the plasma membrane may also be involved in signal transduction. We have identified novel PTKs by screening cDNA libraries with oligonucleotide probes to consensus sequences. In this way we have isolated are 2 novel receptor-like PTKs. One of these, *eck*, is predominantly expressed in tissues containing proliferating epithelial cells (skin, lung and intestine). The predicted *eck* protein, has 976 residues, and is closely related to the *eph* and *elk* receptor-like PTKs. Antibodies raised against a TrpE-*eck* fusion protein immunoprecipitate a 125 kDa protein from epithelial cell lines. This protein is phosphorylated on tyrosine in an immune complex kinase assay, indicating that the *eck* protein is a PTK. A second putative PTK, *trk*-B, was isolated from a rat cerebellar library. The predicted *trk*-B protein has 810 amino acids, and it is closely related to but distinct from the *trk* receptor-like PTK. *trk*-B is exclusively expressed in brain, as a series of RNAs ranging from 13 to 0. 8 kb. The smaller RNAs are clearly too short to encode the intact protein. Analysis of additional *trk*-B cDNAs indicates that there are mRNAs which encode a protein truncated just downstream of the transmembrane domain, with a short novel C terminus. We are investigating the distribution of the two types of mRNA in the brain, and trying to identify their protein products, and determine if the full length protein has PTK activity.

Cyclin, is a highly conserved cell cycle-regulated protein that is required in early embryos for entry into mitosis. We have isolated cDNA clones for human cyclin A and cyclin B. The level of mRNA for both cyclins varies during the cell cycle, being highest in G2. For cyclin B, this increase is due to an elevated rate of transcription in G2. Using antibodies against cyclin B we find that the level of cyclin B fluctuates during the cell cycle, being high in G2 and M, and falling rapidly as cells enter anaphase. Immunofluorescence staining shows that cyclin B accumulates in the cytoplasm during G2, enters the nucleus at prophase, and is abruptly destroyed at anaphase. During G2 cyclin B associates with the hyperphosphorylated form of the cell cycle regulatory protein kinase, cdc2, and these complexes have histone H1 kinase activity.

To determine how PKs activated at the cell surface induce nuclear events, we are examining the phosphorylation of nuclear regulatory proteins, which could be targets for a surface-initiated PK cascade. $p48^{v\text{-}myb}$, the AMV oncogene product, and its cellular counterpart, $p75^{c\text{-}myb}$, are phosphorylated at 5 clustered Ser near their C-termini. Among several PKs tested only glycogen synthase kinase 3 (GSK3) phosphorylates these sites in vitro. The transcription factor c-*Jun*, which associates with c-*Fos*, is a phosphoprotein. c-*Jun* has 4 major sites of phosphorylation. Three of these, which show decreased phosphorylation following TPA treatment, are clustered in a region just upstream of the DNA binding domain. These sites are also phosphorylated by GSK3, and we find that GSK3 phosphorylation of bacterially-expressed c-*Jun* protein decreases its ability to bind to a TRE. We propose that c-*Jun* function is negatively regulated by phosphorylation, and that TPA activation of transcription from TRE-dependent genes may in part involve dephosphorylation of c-*Jun*.

Cytoskeleton Development-I

B 022 DIFFERENTIATION OF ADULT ENTEROCYTES: CYTOSKELETAL PROTEIN AND mRNA ACCUMULATION AND POTENTIAL ROLE OF CYTOSKELETAL-ASSOCIATED TYROSINE KINASE AND SUBSTRATES. David R. Burgess, Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL 33101

The mechanisms by which cells organize their actin cytoskeleton into supramolecular complexes are unknown. Moreover, many cells form highly organized and stereotyped actin cytoskeletons such as those of the red blood cell membrane and the enterocyte brush border. In the simple epithelium of the adult intestine there exists a progenitor stem cell population of undifferentiated dividing cells in the crypts. These crypt cells, which by EM have primitive microvilli and no brush borders, differentiate into enterocytes at the base of villi by forming brush borders possessing the full complement of microvillar and terminal web actin cytoskeletal elements and transport enzymes. Immunofluorescence on cryostat sections shows actin, villin, myosin, tropomyosin and fodrin, but not myosin I, concentrated in the luminal portion of crypt cells. Myosin I, which is diffusely distributed in crypt cells, does not concentrate to the luminal surface until distinct brush borders are formed. Methods were modified to prepare pure fractions of the intestinal epithelium yielding undifferentiated crypts, differentiating base of villus cells, and differentiated villus tip enterocytes. Quantitation of mRNA levels for the above cytoskeletal proteins and calmodulin in isolated crypts, base of villus and villus tip cells shows that only villin, calmodulin and tropomyosin mRNA levels significantly increase as cells differentiate. ELISA of crypt, base of villus and tip cell lysates show no change in cytoskeletal protein levels; moreover, the actin G/F ratio does not change. Brush border cytoskeleton formation in the adult must involve the reorganization of pre-existing pools of cytoskeletal proteins triggered by some unknown signal. To approach possible factors regulating differentiation of the enterocyte cytoskeleton, we assayed for proto-oncogene tyrosine kinase activity since such activities have been shown to affect cytoskeleton organization and cell shape in transformed cells. Tyrosine kinase activity is rare in differentiated cells but occurs in transformed cells and in embryonic, mitotic, undifferentiated cells. Crypt stem cells were found to contain two major phosphotyrosine-containing proteins of 36kD (likely calpactin) and 17kD. The levels of P-tyr in these polypeptides were at least 15 times higher in crypt cells than that present in non-mitotic, differentiated enterocytes. All phosphotyrosine-containing protein was associated with the cytoskeleton. Phosphotyrosine kinase activity was associated with the cytoskeleton which was competent to phosphorylate in vitro the 36kD and 17kD proteins among others. Assays for src tyrosine kinase specific activity showed 7-10 fold higher activity in the crypt cytoskeleton than in differentiated enterocyte cytoskeletons, however corresponding protein and mRNA levels did not change. These results suggest that cytoskeleton-associated tyrosine kinase(s) and their substrates may play a role in brush border differentiation. Supported by NIH grant DK31643.

B 023 REGULATION OF KERATIN GENE EXPRESSION DURING EPIDERMAL DIFFERENTIATION AND DEVELOPMENT. Elaine Fuchs, Robert Vassar, Marjorie Rosenberg, Andrew Leask and Raphael Kopan. Howard Hughes Medical Institute, Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637. Keratins are the intermediate filament proteins predominant in epithelial cells. They can be subdivided into two distinct groups, type I (keratins K9-K19) and type II (keratins K1-K8), which are coexpressed as specific pairs. The expression of keratins is tightly linked to the particular differentiation state of an epithelial cell, and as such, keratins can be useful biochemical markers to track epithelial development and differentiation. In epidermis, keratins are the major structural proteins. K5 and K14 are the pair expressed in the mitotically active basal cells. As cells commit to terminally differentiate, they downregulate the expression of this pair, and switch on the expression of a new pair, K1 and K10. During wound-healing, in epidermal diseases associated with hyperproliferation, and in tissue culture, differentiating keratinocytes downregulate expression of K1 and K10, and switch on expression of K6 and K16. Vitamin A has a profound negative effect on epidermal differentiation, and it can also inhibit expression of both normal (K1/K10) and abnormal (K6/K16) differentiation-specific keratins. To examine the molecular mechanisms underlying the differentiation-specific and retinoid-mediated changes in keratin expression, we have isolated and characterized a number of human epidermal keratin genes. Using gene transfection in vitro and transgenic mice in vivo, we have investigated the sequences important in directing the expression of the human K14 gene in basal keratinocytes. Using a cell culture system optimized for epidermal differentiation, we have examined the mechanisms underlying the switches in keratin expression that take place during normal and abnormal differentiation.

Cytoskeleton Development-II

B 024 MATERNAL mRNAs AND THE CYTOSKELETON IN XENOPUS OOCYTES AND EMBRYOS, Mary Lou King and Marc D. Pondel, Departments of Cell Biology and Anatomy and REPSCEND Labs, University of Miami School of Medicine, Miami, FL 33101

The protein products of localized maternal mRNAs appear to function as cytoplasmic determinants during early embryogenesis. The mechanism through which the spatial distribution of these mRNAs is established and maintained is unknown, but several lines of evidence indicate that the cytoskeleton is involved. The maternal mRNA, Vg1, is progressively localized to the vegetal pole during oogenesis beginning at stage 3, and is lost from its cortical location in stage 6 oocytes at ovulation (1). We have isolated a detergent insoluble pellet (DIP) from stage 6 Xenopus laevis oocytes which is highly enriched in cytokeratins and contains a small amount of RNA (1% of total). Vg1 RNA is 50-100 fold more concentrated in the DIP than in the detergent soluble fraction (DSF). Upon ovulation, Vg1 RNA distribution in the egg radically changes. Vg1 RNA is now almost exclusively found in the DSF (2). This shift in the solubility of Vg1 RNA coincides with the loss of a 56-kDa cytokeratin from the DIP. The loss of Vg1 RNA from the DIP can be triggered by progesterone and inhibited by the phosphodiesterase inhibitor, theophylline. In contrast to these findings, histone H3 RNA always partitions with the DSF. In stage 2 oocytes at a time preceding Vg1 RNA localization, Vg1 RNA is not enriched in the DIP. By stage 3 however, Vg1 RNA localization has begun and coincidentally, Vg1 RNA is now found to be enriched some 10 fold in the DIP. Our results are consistent with a role for cytokeratins in anchoring Vg1 RNA to the vegetal cortex. In other studies we have found that mRNAs for cytoskeletal proteins are also highly enriched in the DIP. These results suggest a model for cytoskeletal assembly.

1. Melton, D.A (1987) Nature 328:80-83.
2. Pondel, M. and King, M.L. (1988) PNAS 85:7612-7616.

B 025 **"FUNCTIONS OF ACTIN BINDING PROTEINS IN DIFFERENTIATION MORPHOGENESIS OF INTESTINAL CELLS",** ***Daniel LOUVARD***

Unité de Biologie des Membranes, Département de Biologie Moléculaire, Institut Pasteur, 25, rue du Docteur Roux 75724 Paris cedex 15, France.

Epithelial cells display an asymetric organization of plasma membrane proteins and intracellular microfilaments structures. Precursors of polarized cells are not polarized.
Cell polarity is achieved during terminal differentiation. Transport and secretory functions of epithelial cells can be achieved only if cells develop and maintain an assymetric distribution of cell surface and cytoskeleton components.
We focused our research on the differentiation of intestinal cells. Normal adult or embryonic cells as well as malignant cells originating from intestine have been used in our studies. The brush-border is a specialized cell surface organelle found at the apical faces of enterocytes; it develops during terminal differentiation of intestinal cells. The molecular organisation of brush border cytoskeleton is well understood.
We observed that villin, an actin binding protein found in brush-border cytoskeleton, is a differentiation and cell linage marker of intestinal cells. Primary structure villin has been established, our data emphasize its homologies with other acting binding proteins. The pattern of expression of mRNA encoding for villin is restricted to cells derived from primitive endodermic intestinal cells. Villin is encoded by a single gene that is regulated during intestinal differentiation at the transcriptional level .
We also showed that villin plays a direct and key function in microvilli assembly since it induces microvilli growth and actin redistribution in fibroblasts transfected with villin.

B 026 DOMINANT MUTATIONS IN CYTOSKELETAL PROTEIN IN DEVELOPING *XENOPUS* EMBRYOS, Moon, Randall T., Christian, Jan L., Kelly, Greg M., Wolda, Sharon and Eib, Doug W., Department of Pharmacology, University of Washington, School of Medicine, Seattle, WA 98195. We have employed complementary approaches to test the functions of both cytoplasmic and membrane-associated structural proteins in *Xenopus* embryos. Two approaches were used to test the hypothesis that vimentin filaments play physiologically significant roles in embryonic development. First, wild-type vimentin was overexpressed during early development by microinjection of synthetic *Xenopus* vimentin mRNA. In uninjected embryos, vimentin is first detected in a subset of cells during the neural stage of development. In embryos injected with synthetic mRNA, this pattern of vimentin expression is deregulated, and vimentin is detected at high levels in most cells types during early development. This ectopically expressed vimentin does assemble into filaments, yet there is no overt effect on early development or cell morphology. However, later in development embryos overexpressing vimentin display increased mortality at times when endogenous vimentin expression is first detectable, and surviving tadpoles develop abnormal retinae. These data suggest that ectopic expression of vimentin has no deleterious action in cells not normally expressing this gene, but may interfere with vimentin functions in differentiating cells which normally express vimentin. A second approach we have used to analyze vimentin function was to construct dominant negative mutations in the full-length vimentin cDNA. Expression of this mutant vimentin in embryos blocks assembly of wild-type vimentin filaments, but does not noticeably affect development, nuclear positioning or muscle formation.

Data will also be presented on experiments utilizing antisense RNA to reduce endogenous *de novo* synthesis of nonerythroid α-spectrin, and protein 4.1.

Late Addition

B 027 COMPONENTS AND ASSEMBLY OF THE TIGHT JUNCTION IN CULTURED EPITHELIAL CELLS, James M. Anderson, Christina M. Van Itallie, Michelle D. Peterson, Bruce R. Stevenson, Matthew Heintzelman, and Mark S. Mooseker, Yale University, New Haven, CT 06510

The tight junction forms the demarcation between apical and basolateral membrane domains and seals the paracellular space of polarized epithelial cells. ZO-1 and cingulin have been previously described as large proteins peripherally associated exclusively with the cytoplasmic surface of junction contacts. We, in collaboration with Sandra Citi (Dept. of Cell Biology, Cornell Medical School, New York, NY) demonstrate by immunoblot analysis that ZO-1(225kDa) and cingulin(140kDa) are immunologically unrelated and co-localize, at the light microscope level, at the tight junctions in all tissues and cultured cell types examined. At the ultrastructural level, immunogold particles localize cingulin about three times farther from the membrane bilayer than ZO-1. ZO-1 protein sequence deduced from cDNAs reveals a multidomain protein, one large portion of which is 15% proline. We have examined the effects of cell contacts on ZO-1 localization and protein and mRNA expression in cultured Caco-2, human colonic carcinoma, cells. When cells are grown in calcium-free spinner culture junction complexes are lost and by immunofluorescence microscopy ZO-1 appears dispersed throughout the cell. Within hours of replating in calcium-containing media, ZO-1 relocalizes on the membrane at cell-cell contacts. mRNA levels for ZO-1 are highest in spinner-cultured cells and when replated fall by 90% after 2 weeks. In contrast ZO-1 protein levels rise 5- to 8-fold over the same period. Cell-cell contacts appear to trigger localization of ZO-1 to the membrane and influence long-term expression of ZO-1 protein and mRNA levels.

Molecular Genetics of Cytoskeletal Proteins

B 100 FILAMIN cDNA CLONES PREDICT A NOVEL 100 AMINO ACID REPEATING STRUCTURE, Catherine P. Barry[1], Vance Lemmon[2] and Anthony P. Young[3], [1]Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60680, [2]Center for Neuroscience, Case Western Reserve University, Cleveland, OH 44106, [3]Biotechnology Center, Ohio State University, Columbus, OH 43210.
Overlapping cDNA clones encoding the actin cross linking protein filamin have been isolated from a lambda gt11 expression library using two monoclonal antibodies directed against a filamin isoform expressed in chicken retinal glial cells. The clones span 2.64 kb, encode a single 880 amino acid open reading frame, and detect an approximate 13 kb transcript in Northern blots. Western blotting of the purified protein demonstrates that the determinant for one of the monoclonal antibodies resides within a 55 kd polypeptide liberated by cyanogen bromide cleavage. The existence of this peptide is predicted by the spacing of methionine residues within the open reading frame, thus linking the purified filamin protein to the cloned cDNA. The primary structure can be arranged as a series of tandem 100 amino acid repeating units, with any two repeats sharing approximately 35% sequence similarity. Interestingly, the filamin repeats are not similar to the repeating structures of the cytoskeletal proteins spectrin, alpha-actinin, and dystrophin. Moreover, computer analysis of the filamin repeats predict a structure devoid of alpha helical character. These data suggest that filamins have evolved independently to yield structural proteins with a repeating secondary structure distinct from the triple helical units that characterize other accessory cytoskeletal proteins.

B 101 CELL POLARITY AND MATING IN SACCHAROMYCES CEREVISIAE, Janet Chenevert and Ira Herskowitz, Department of Biochemistry and Biophysics, UC San Francisco, CA 94143.
We are interested in how cells orient towards extracellular signals. When budding yeast mate, cells of opposite mating types polarize towards one another: cytoskeletal components such as microtubules, actin, and the spindle pole body are directed towards the site of fusion. The mating factors are implicated in this chemotactic behavior, since purified factors will cause cells to put out a projection of localized growth, called a "shmoo tip". We hypothesize that a mating cell recognizes and orients towards a gradient of factor secreted by its partner.
In order to identify the components involved in polarizing towards a mating partner, we have taken a genetic approach and have isolated mutants which may be defective in this process. These mutants were isolated by virtue of the fact that they are unable to mate to a strain which itself has a defect in polarity. Although normal for vegetative growth, many of our mutants exhibit aberrant morphologies in response to mating factor. One interesting class is unable to form a shmoo tip, but instead enlarges in a uniform manner. Polarity indicators such as actin and chitin, a cell wall component, are delocalized throughout the cell surface, as evidenced by immunofluorescence. Such a mutant may be unable to determine the location of its mating partner, or may be defective in linking this site to the cytoskeleton.

B 102 GENETIC LOCALIZATION OF DROSOPHILA VINCULIN, Jennifer P. Macke[1] and Clarissa M. Cheney[2], [1]Department of Biology, The Johns Hopkins University, Baltimore, MD and [2]Department of Genetics, Washington University School of Medicine, St. Louis, MO. Adult Drosophila ovaries synthesize a protein with MW of approximately 110 kD and pI of 6.2-6.4. This protein appears to be synthesized at a high rate in ovaries, but synthesis is extremely low in thoracic muscle. On the basis of its electrophoretic properties, we tentatively identified this protein as vinculin. Partial proteolytic cleavage of this protein gave a digest pattern remarkably similar to purified chicken vinculin, confirming this identification. In Drosophila, there appear to be two genetic variants of vinculin that can be separated electrophoretically. Using these electrophoretic variants as markers, the genetic location of the Drosophila vinculin gene was determined by recombination and deletion mapping. The vinculin electrophoretic polymorphism maps as a single gene between ct and sn on the X chromosome, in polytene region 7C4-9. We are currently examining lethal and female sterile mutations in this region to determine whether any of these may be a possible candidate for a vinculin mutation.

B 103 ***DICTYOSTELIUM DISCOIDEUM*** **MYOSIN LIGHT CHAINS: STRUCTURE, EXPRESSION AND FUNCTION.** Rex L. Chisholm, Richard S. Pollenz, Patricia J. Moore and Bruce D. Ostrow. Dept. of Cell Biology and Anatomy, Northwestern University Medical School. Chicago, IL 60611

Dictyostelium discoideum has been shown to contain at least two different myosins: Myosin I, a 100 kd "mini-myosin" which localizes to the leading edge of migrating cells, and Myosin II, a more traditional myosin which has been shown to be important for cytokinesis, and which localizes to the posterior cortical regions of migrating cells. The myosin II molecule consists of two copies each of a 240 kd heavy chain, an 18 kd "regulatory" light chain which is phosphorylated, and a 16 kd "essential" light chain whose function remains unclear. We have isolated and characterized full length cDNAs for both of the *Dictyostelium* myosin II light chains. These cytoplasmic light chains display approximately 30 percent amino acid sequence homology with muscle myosins from vertebrates and avian cells. Although both myosin light chains are expressed throughout growth and development, there appears to be a transient increase in the levels of both light chain mRNAs during the period of maximal chemotaxis early in development. We have used our light chain probes to examine expression of the light chains in a *Dictyostelium* myosin II heavy chain null mutant (Manstein, et al. EMBO J.). Both light chains are expressed at both the mRNA and polypeptide level, despite the absence of heavy chain expression, suggesting that Dictyostelium cells are capable of maintaining pools of free myosin light chains. Using the cDNAs we have isolated genomic DNA encoding portions of both genes. To study MLC function we have employed the cloned genomic DNA and the cDNAs in constructs to disrupt expression of the light chains, using both expression of antisense RNA and targeted gene disruptions.

B 104 THE EFFECTS OF ACTIN ASP_{10} MUTATIONS ON YEAST VIABILITY. Ruth K. Cook and Peter A. Rubenstein, Department of Biochemistry, University of Iowa College of Medicine, Iowa City, IA 52242.

The actin N-terminal region may play an important role in the control of actin function. Mutation of the conserved Asp_{11} in this region of muscle actin inhibits actin N-terminal processing, the binding of actin to DNAse I, and anomalous behavior on native polyacrylamide gels. To study the role of the actin N-terminal region in vivo, we mutated Asp_{10} in a centromeric plasmid containing yeast actin cDNA adjacent to the yeast actin promoter. Asp_{10} is the homologous position for this residue in yeast actin. A diploid yeast strain with one disrupted actin gene was transformed with these plasmids. We assessed the effect of the mutant actins both in diploid cells and in haploids to determine if the mutation was compatible with cell viability. The mutant actin carrying the Asp10->Glu mutation caused no observable inhibitory effect on the disrupted diploid. We obtained viable spores carrying the mutated actin cDNA as the only actin coding sequence. These cells produced mini colonies on agar plates and had a 2.5 fold longer generation time in liquid cultures in comparison with control cells. However, no viable spores were obtained with the Asp_{10}->Asn mutation. The disrupted diploid cells grew when transformed with this actin cDNA. Mini colonies were observed, though, on agar plates and a 2 fold longer generation time was observed for these cells. Haploid cells carrying presumptive extragenic suppressors of the Asp-->Asn mutation have been isolated and are being characterized. These results demonstrate the importance of this conserved residue in proper actin function. (Supported by grants from NSF, NIH, and MDA).

B 105 DYNAMICS OF KERATIN FILAMENT ASSEMBLY: EXPRESSION OF MUTANT EPIDERMAL KERATIN CDNAS IN VIVO AND IN VITRO. Pierre Coulombe, Kathryn Albers, Anthony Letai, Yiu-mo Chan and Elaine Fuchs. Department of Molecular Genetics and Cell Biology, Howard Hughes Medical Institute, The University of Chicago, Chicago, IL 60637. There are ~30 different keratin proteins which constitute the intermediate filaments found in most epithelial cells. There are two distinct sequence classes: acidic type I (40-56.5K) and basic type II (53-67K), both of which are essential for filament formation. Each type has a 310 central α-helical domain which is 55-99% homologous within members of the same type, flanked by non-helical end domains of variable length and sequence. The helical sequences contain heptad repeats of hydrophobic residues, indicative of their ability to form coiled-coil dimers. The process of filament assembly involves ~20-30,000 polypeptides, which organize into a hierarchy of higher ordered structures in the apparent absence of auxiliary proteins and factors. To begin to elucidate (a) the nature of the molecular interactions that lead to filament formation and (b) the dynamics of this process in epithelial cells, we have introduced deletions and point mutations in the coding segments of epidermal keratin cDNAs, tagged these cDNAs with a C-terminal sequence encoding the antigenic portion of the neuropeptide substance P, and added a promoter/enhancer to drive the expression of these mutant constructs in various epithelial and non-epithelial cells. Using a monoclonal antibody against substance P, we have conducted both immunofluorescence and immunoelectron microscopy to examine the behavior of these mutant keratins in the assembly process. Our results reveal interesting insights into the dynamics and mechanisms of filament assembly.

B 106 IDENTIFICATION OF A NEW MEMBER OF THE SPECTRIN SUPERFAMILY OF PROTEINS, Ronald R. Dubreuil, C. Todd Stewart, Timothy J. Byers, Daniel Branton, Daniel P. Kiehart. Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138
We have identified and partially characterized a novel member of the spectrin superfamily of proteins. This protein, isolated from *Drosophila* tissue culture cells, resembles and in fact appears to share a common α subunit with the $\alpha\beta$ spectrin isolated from the same cells. The second subunit of this protein, which we term β_H, is unusually large and is antigenically distinct from the conventional *Drosophila* β spectrin. But like β, the β_H subunit cofractionates in a 1:1 complex with the α subunit and together with the α subunit it appears as a double stranded rod-shaped molecule by electron microscopy. Using an antibody against the β_H subunit, we isolated a 5 kb cDNA from a *Drosophila* head cDNA expression library in λgt11. We sequenced the cDNA and found extensive sequence similarity between the β_H sequence and other members of the spectrin superfamily. Specifically, the β_H chain shares a highly conserved domain that is implicated in the actin-binding activity of spectrins, α actinins, gelation factor, and perhaps dystrophin. The β_H cDNA hybridizes with a 13 kb mRNA in Northern blots of fly head and tissue culture cells, which is consistent with its unusually large size. By virtue of its association with α spectrin and its partial amino acid sequence, β_H appears to be a spectrin subunit. By virtue of its size, β_H is similar to dystrophin. Further analysis of the C-terminal sequences of β_H will reveal whether this protein contains dystrophin specific sequences. β_H may represent an evolutionary link between vertebrate dystrophins, of which relatively little is known, and spectrins. Supported by GM33830 and GM39686, an MDA research grant to DPK and an ACS postdoctoral grant to RRD.

B 107 INTERACTING MUTATIONS IDENTIFY GENES INVOLVED IN MICROTUBULE FUNCTION IN DROSOPHILA: THE *haywire* LOCUS. M.T. Fuller, L. Mounkes, R. Jones*, W. Gelbart*, N. Wolf, and C.L. Regan, University of Colorado, Boulder, and * Harvard University, Cambridge, MA. We have identified a network of interacting mutations that fail to complement mutant alleles of the gene encoding the testis-specific β2-tubulin of *Drosophila*, but do not map to the *B2t* locus. The genes identified by these second-site non-complementing mutations could encode proteins that interact with tubulin at the structural level and are important components of microtubule-based organelles. The product of one of these interacting genes, *haywire* (*hay*), is a candidate for an accessory protein important for function of several structurally different microtubule arrays. The original *haywire* mutation causes defects in flagellar elongation and shaping of the spermatid nucleus during spermatogenesis. More extreme *haywire* alleles are lethal, consistent with a role for *haywire* in general mictrotubule function. In addition, both the structure and the function of the meiotic spindle is defective in males heterozygous for certain combinations of *haywire* alleles. Asters form, but a full metaphase spindle was never observed and the paired homologues neither separated nor segregated to the poles. We have isolated DNA corresponding to the the *haywire* region by molecular cloning. The best candidate for the gene corresponding to *haywire* is expressed throughout several stages of development, as expected from the phenotypic effects of the different *haywire* mutants. We have begun sequence analysis of a near full-length cDNA in order to determine the nature of the *haywire* gene product.

B 108 *whirligig*: A GENE IMPORTANT FOR STRUCTURE OF THE FLAGELLAR AXONEME IN *DROSOPHILA*, Larry L. Green, Nurit Wolf and Margaret T. Fuller, Dept. of M.C.D. Biology, University of Colorado, Boulder, CO 80309-0347. Two different kinds of genetic interactions implicate the product of the *whirligig* gene in microtubule function during spermatid differentiation in *Drosophila*. First, the wrl^{nc4} allele fails to complement α- or β-tubulin mutations, even though it fails to map near any of the known α- or β-tubulin genes of *Drosophila*. Second, loss of function mutations at *wrl* are dominant male sterile, indicating that the level of the *whirligig* product is important for normal spermatogenesis. Surprisingly, the dominant male sterility is suppressed by mutations in α- or β-tubulin, or other mutations that affect microtubule function, indicating that it is the level of *wrl* product relative to tubulin or microtubule function that is important. Both types of genetic interaction, extragenic failure to complement and extragenic suppressors, often reflect underlying structural interactions between the gene products. Thus, the *wrl* gene product and α- and β-tubulin may interact structurally in the axoneme. The *wrl* product is most likely a component of the axoneme. Males homozygous for wrl^{nc4} are sterile and exhibit defects in post-meiotic spermatid differentiation. The most striking defects are missing central pair and accessory microtubules in the axoneme.

B 109 DIFFERENTIAL GENE EXPRESSION OF CONTRACTILE PROTEINS SUPPORTS AN ISOFORM COMPETITION - AUTOREGULATORY COMPENSATION CONTROL MECHANISM, P. Gunning R. Wade, D. Hailstones, M. Gordon, C. Sutherland, C. Lloyd, G. Schevzov, E. Hardeman, Muscle Genetics Unit Children's Medical Research Foundation, P.O. Box 61, Camperdown, NSW 2050, Australia. During mammalian myogenesis 3 major programs of contractile protein gene expression can be considered to exist. 1. Expression of non-muscle isoforms is eliminated. 2. Fast muscle isoforms are coordinately expressed to generate fast myofibers. 3. Slow muscle isoforms are coordinately expressed to generate slow muscle fibers. We have isolated DNA probes for essentially all these contractile proteins and evaluated the extent to which these 3 myogenic programs exist in humans and mice. In general, we find that no two mRNAs encoding contractile proteins are precisely coregulated. In particular, the down-regulation of non-muscle actins, tropomyosins and myosin light chains during myogenesis varies kinetically and quantitatively between different isoforms. Tropomyosin 4 is constitutively expressed at similar levels in adult muscle and non-muscle cells whereas Tm's 1,2,3 are the most strongly repressed of all the non-muscle isoforms. Similarly, no two fast and no two slow muscle isoforms are identically regulated in myogenic cultures where the fast: slow ratio between 2 gene families can vary up to 700 fold. However, the total amount of mRNA encoding each type of contractile protein is quite tightly coordinately regulated for all the protein families. We propose that the net amount of mRNA encoding each protein type is fixed and that competition exists within each family for utilization of the various isoforms at the protein level. This presumes that net mRNA levels for different contractile protein families is set by an autoregulatory mechanism and that the competitive performance of each isoform produces parallel regulation of the corresponding mRNA. In order to evaluate the extent to which autoregulation exists in myogenic cells, we have introduced normal and mutant actin genes into C2C12 cells and have observed that expression of an exogenous actin gene does indeed result in decreased accumulation of the endogenous actin mRNAs.

B 110 CYTOSKELETAL PROTEINS AND THEIR RELATIONSHIP WITH DNA METABOLISM, J.R. Jenkins, M.J. Pocklington, M. Csukai, G. Shiels, F. Sweeny, and E. Orr, Department of Genetics, University of Leicester, Leicester, LE1 7RH, UK.

The drug novobiocin inhibits DNA replication, transcription, transposition and sporulation in prokaryotes, the target of the drug being DNA gyrase. DNA metabolism and gene expression in eukaryotes are also affected by novobiocin. No target or mechanism of action has been identified, as yet. We have purified 5 yeast proteins which have great affinity for novobiocin. Three of these have been identified as cytoskeletal proteins; myosin II, a 200KD contractile protein; the product of NOV1, a protein that confers novobiocin resistance and is associated with the microtubular organiser; and a protein that forms a novel yeast filamentous network. The respective genes of these proteins have been cloned, along with those of the two other novobiocin binding proteins. Further work with MYO1 mutants has enabled us to isolate several other cytoskeletal proteins.

B 111 **PROTEIN ENGINEERING ON ACTIN AND PROFILIN.** ROGER KARLSSON AND PONTUS ASPENSTRÖM, Department of Developmental Biology, Uppsala university, Biomedical center, Box 587, S-751 23, Uppsala, Sweden. Previously chicken beta-actin was expressed in the yeast Saccharomyces cerevisiae which proved to be a suitable host for the production of such recombinant actin (Karlsson, R. Gene 68 249-257, 1988). This system where the expression of the cloned gene is induced by a down-shift in the temperature of the growing culture is now being used for the production of actin mutants and the actin binding protein profilin. The recombinant actin is isolated from homogenized yeast cells in a two-step procedure; first total actin is purified by affinity chromatography on DNAase I Sepharose and then the recombinant beta-actin is separated from endogeneous yeast actin by hydroxylapatite chromatography. During this later step beta-actin elutes after the more alkaline yeast isoform. Several mutants of actin, primarily localized at its N-terminal region, have now been constructed and one of these, with two aspartic residues (D_2 and D_3) replaced by alanines, has been produced on a large scale. In contrast to the wild type protein, this actin mutant elutes from the hydroxylapatite column before yeast actin reflecting the charge-shift in the recombinant protein and preliminary data suggest that it has a higher critical concentration (C_c) when induced to polymerize in the presence of KCl compared to that observed with the wild type actin. This and other actin mutants are now in the process of further characterization with respect to polymerization and interaction with profilin, and will hopefully increase our knowledge of structure/function relationships in the actin and profilin molecules.

B 112 ISOLATION OF A GENOMIC CLONE FOR A MOUSE TYPE I HAIR KERATIN, Paul S. Kaytes[1], Nicole T. Hatzenbuhler[2], Arthur P. Bertolino[3], and Gabriel Vogeli[1], [1]Molecular Biology Research and [2]Biopolymer Chemistry, The Upjohn Company, Kalamazoo, MI 49007. [3]Dept. of Dermatology, NYU Medical Center, New York, NY 10016.
In order to study the regulation of gene expression during the mammalian hair cycle, we have analyzed a genomic clone for a mouse Type I hair keratin. This clone was isolated by screening a mouse genomic library with an oligonucleotide probe derived from a mouse Type I hair keratin cDNA. Nucleotide sequence analysis verifies the correspondence of the genomic and cDNA clones. Comparison of the two sequences shows that the coding sequence is divided into at least 7 exons; these exons do not correspond to the domain borders of the protein, in agreement with studies of epithelial keratins. The 6 identified introns range in size from 96 to 840 nucleotides. In addition, the location of the introns is similar, but not identical, to their location in epithelial keratins and other intermediate filaments. A "TATA" box, characteristic of eukaryotic promoters, is found at the 5' end of the gene. The isolation of this clone, including several kilobases of 5'-upstream sequence presumed to contain control regions, will allow us to study the activity of this gene during the hair cycle, and to use its regulatory regions to express reporter genes in a hair-specific manner.

B 113 ISOLATION OF A PUTATIVE ALPHA-ACTININ GENE IN *C. ELEGANS*, AND CHARACTERIZATION OF A *C. ELEGANS* ALPHA-ACTININ cDNA, Lawrence Kleiman[1], Robert Barstead[2] and Robert Waterston[2], [1]Lady Davis Institute for Medical Research, Sir Mortimer B. Davis - Jewish General Hospital, Montreal, Quebec, Canada H3T 1E2; [2]Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110.
The actin binding protein α-actinin is a major protein component of the dense-body in nematode body wall muscle. We have isolated and sequenced an α-actinin cDNA from a *C. elegans* cDNA library. The 3.5 kb cDNA recovered is smaller than a 3.6 kb mRNA detected. The complete sequence of the cDNA showed that it does not include the initiator ATG codon, but a comparison with a previously reported chick α-actinin cDNA sequence indicates that greater than 95% of the coding sequence is present, representing a polypeptide of 908 amino acids (MW=105,535 daltons). Computer alignment of the nematode α-actinin sequence with chick α-actinin shows an overall amino acid homology between these proteins of 68%, with a 50% amino acid identity. The first 250 amino acids, which contain the putative actin-binding region of the molecule, is the most conserved region, showing an 80% amino acid homology, with a 68% amino acid identity. The central region of the nematode sequence contains 3-113 amino acid repeats which show weak and variable homology to each other and to similar internal repeats found in human erythroid spectrin and human dystrophin. The number of similar repeats found in the α-actinins from chick fibroblast and *Dictyostelium discoideum* are 4 and 2 respectively, and we have compiled a consensus repeat sequence based upon the 9 repeat units found in the α-actinins of these three species. The carboxy terminal region of the nematode protein has two putative EF hand motifs which are indicative of calcium binding sites. However, these sites appear non-functional, suggesting that this α-actinin is of muscle origin.

B 114 DELETIONS OF THE 3'UNTRANSLATED AND FLANKING REGIONS OF THE γ-ACTIN GENE CAN ALTER MYOBLAST MORPHOLOGY AND DIFFERENTIATION, Catriona M. Lloyd and Peter Gunning, Muscle Genetics Unit, Children's Medical Research Foundation, P.O. BOX 61, Camperdown, N.S.W. 2050, Australia. During myogenesis, the levels of mRNAs encoding the two non-muscle actins, β and γ, are substantially decreased. In order to define the sequence(s) necessary for this down-regulation, plasmids containing the human γ-actin gene with deletions in the 3'UTR and 3' flanking regions were transfected into C2C12 cells. Pooled transfectants carrying the full length γ-actin gene showed normal down-regulation of the γ-actin mRNA whereas transfectants carrying deletions of the 3'UTR or 3'flanking region showed poor regulation. A high proportion of transfectant cells carrying various deletions of the 3'UTR and 3'flanking region showed aberrant morphology and were no longer capable of normal differentiation. The cells which showed normal morphology, however, were able to differentiate properly and regulate the non-muscle actin isoforms appropriately. Aberrant regulation of γ-actin by transfectants with abnormal morphology would account for the lack of down-regulation observed with the pooled cultures. In addition, very few clones survived carrying the deletion of the entire 3'UTR and 3'flanking region, suggesting the creation of a lethal phenotype. We propose that the γ-actin 3'UTR and 3'flanking region can influence cell morphology, differentiation and cell viability. In contrast, cells transfected with the β-actin gene lacking the entire 3'flanking region and most of the 3'UTR showed neither aberrant morphologies nor poor differentiation ability. These results indicate that the 3'UTR and 3' flanking region of the γ-actin gene may have functional significance and that this differs from that of the β-actin gene. However, the 3'UTR and 3'flanking regions of the γ-actin gene do not appear to be necessary for down-regulation in contrast to the β-actin gene which contains the myogenic regulatory sequence in its 3'UTR (DePonti-Zilli et al., 1988, PNAS 35, 1389-1393).

B 115 IDENTIFICATION AND CHARACTERIZATION OF A PARTIAL cDNA ENCODING HUMAN α-ACTININ.
Masaki Nishiyama , Jack Wands, Mehmet Ozturk, Molecular Hepatology Laboratory, MGH Cancer Center, 149 13th Street, 7th Floor, Charlestown, MA 02129. We have isolated a 2.4 kbp clone from a human hepatocellular carcinoma (HCC) cDNA library. This cDNA clone hybridized to a 3.5 kb transcript found in low abundance in normal human liver. The expression was high in hepatoma tissues as well as in human fibroblast, macrophage and monocyte cell lines. This cDNA also hybridized to a similar transcript in various rat tissues except in the case of skeletal muscle and heart; a smaller transcript of approximately 3.0 kb was observed in these tissues. The 2,345 nucleotide cDNA was sequenced; it contained 1,788 nucleotides in an uninterrupted open reading frame followed by 557 non coding 3' nucleotide sequences which included a polyadenylation signal and a poly A tail. Our cDNA nucelotide sequence was found to have 85% identity to a chicken fibroblast α-actinin cDNA in the coding regions (Eur. J. Biochim., 1988; 177; 649-655). The deduced 596 amino acid sequence derived from human cDNA showed a 95% identity to the sequence derived from the chicken cDNA. Although the great majority of α-actinin sequences have been conserved between human and chicken in the coding region, we found substantial sequence diversion following the stop codon and extending to the poly A tail. By Northern blot analysis with the 3' non coding region of our cDNA, we have detected the same size transcripts in the FOCUS HCC cell line; as well as HCC, human fibroblast, macrophage and monocyte cell lines but not in rat tissues. Thus, we are led to believe that the discrepancy in the 3' untranslated regions was not caused by a cloning artifact but indeed was the result of a divergence in the α-actinin cDNAs between the two species. In conclusion, these results suggest that we have cloned a partial human cDNA encoding human α-actinin.

B 116 A DISCREPANCY BETWEEN VIMENTIN mRNA AND PROTEIN LEVELS EXPRESSED IN PROLIFERATING T HELPER CELLS: IS INCREASED PROTEIN TURNOVER INVOLVED IN FILAMENT REORGANIZATION DURING THE CELL CYCLE? Patricia L. Podolin, Daniel E. Sabath and Michael B. Prystowsky, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104
A murine vimentin cDNA clone was isolated by differential hybridization from a cDNA library, which was constructed using poly(A)+ RNA from IL2-stimulated T helper cells. Comparison of the coding region of this cDNA to the coding regions of human and hamster vimentin cDNA's revealed high sequence homologies (91% and 94%, respectively). Vimentin mRNA levels are nearly undetectable in unstimulated L2 cells; following 24 hr of IL2 stimulation (S phase of the cell cycle), mRNA levels increase 10- to 20-fold, after which they decline, reaching baseline levels as the cells return to a resting state. In contrast, vimentin protein levels increase only 1.3- to 3-fold following IL2 stimulation. This increase in vimentin protein content is not associated with a particular phase of the cell cycle. One possible explanation for the discrepancy between changes in vimentin protein and mRNA levels is that reorganization of the filaments requires degradation of existing filaments and synthesis of new ones. Protein synthesis studies indicate that following 24 hr of IL2 stimulation, the rate of vimentin synthesis increases 15-fold, after which it declines. We are currently assessing the changes in vimentin half-life during the cell cycle.

B 117 **THE *SUP-PF-1* LOCUS IN *CHLAMYDOMONAS* IS THE STRUCTURAL GENE FOR THE DYNEIN β HEAVY CHAIN.** **Mary E. Porter*, David E. Johnson#, and Susan K. Dutcher#, *Department of Cell Biology and Neuroanatomy, University of Minnesota Medical School, Minneapolis, MN 55455, and #Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO, 80303.**.
Genetic and molecular analyses indicate that the *sup-pf-1* locus in *Chlamydomonas reinhardtii* encodes the β heavy chain of outer arm dynein. *Sup-pf-1* is an extragenic suppressor that restores flagellar motility to paralyzed radial spoke and central pair mutants (Huang et al., 1982, Cell 28:115). Two additional defects cosegregate with the suppressor phenotype, a reduced flagellar beat frequency and an altered β heavy chain polypeptide (Huang et al., 1982; Brokaw et al., 1982, J. Cell Biol. 92:722). Dikaryon rescue experiments have suggested that the *sup-pf-1* locus might be the structural gene for the β heavy chain (Huang et al., 1982). We have obtained the following new evidence in support of this hypothesis: (1) The coexpression of wild type and mutant phenotypes in diploid strains suggests that the modification in the *sup-pf-1* β heavy chain is the result of an alteration in its primary sequence rather than a defect in post-translational modification. (2) Restriction fragment length polymorphism (RFLP) mapping techniques indicate that the *sup-pf-1* flagellar phenotype is tightly linked (<1 Centimorgan) to a molecular marker for the β heavy chain gene (*Dhl*, Williams et al., 1986, J. Cell Biol. 103:1-11) . (3) The β chain probe detects a mutant specific DNA sequence polymorphism. (4) Biochemical characterization of the wild-type and mutant dynein polypeptides has revealed that the site of the *sup-pf-1* defect is in the amino terminal half of the β heavy chain and that the presence of the mutation does not inhibit the *in vitro* microtubule binding or translocating activity of the outer dynein arm. These results indicate that the *sup-pf-1* mutation identifies a regulatory domain within the polypeptide backbone of the dynein heavy chain. The mechanism by which the β heavy chain locus regulates dynein activity and flagellar motility will be the subject of future investigations.

B 118 Alterations in cell ploidy in *Dictyostelium* are induced by mutations selected for by non-adhesion to plastic.

D.R. Waddell. Fachbereich 9, Biochemie, Bergische Universität, Wuppertal, West Germany. Present address: Department of Biochemistry and Molecular Biology, Mayo Foundation, Graduate School of Medicine, Rochester, Minnesota, USA.

Previous work has shown that pleiotropic mutations (*phg*) which affect non-specific phagocytic recognition, adhesion, cell-cell adhesion and cytokinesis can be selected for by their adhesive properties. Most non-adhesive strains have a large percentage of large multinucleate cells. This is due to the inefficiency of cytokinesis in mononucleate cells in the cell population. Five new mutant strains have been isolated using a new stronger selection protocol that spontaneously become diploid. Haploid segregants from one of these strains, DV212 which were induced by the microtubule inhibitor nocodazole, are unstable. Haploid clones eventually form rapid growing areas which contain diploid cells.

Revertants of DV212 were selected for by their adhesion properties. In the strongest revertant , DV214, adhesion was restored to 75% of wild-type levels and the other aspects of the mutant phenotype were similarly affected. Stable haploid segregants of DV214, have been isolated. These results support earlier results which indicated that revertants were due to dominant suppressor mutations.

The cell surface to ploidy ratio appears to be critical for the penetrance of the mutant phenotype. Higher ratios (found in haploid cells and in axenically-growing cells) increase the penetrance of the mutations. Lower ratios (found in diploid, bacterially-grown and multinucleate cells) result in lower penetrance. This relationship could be explained if the concentration of a limiting cell membrane component involved in all of the mutant processes were reduced in the mutants. According to this model, the strains become diploid because this is a way to partially suppress the mutant phenotype.

B 119 TROPOMYOSIN EXPRESSION IN EMBRYONIC STEM CELLS, David Wieczorek, Steven Hughes, Laura Pajak, Phil Howles, and Tom Doetschman, Dept. Molecular Genetics, Biochemistry, and Microbiology, Univ. Cincinnati Medical Center, Cincinnati, Ohio 45267.

Tropomyosin (TM) is a coiled-coil dimer that in association with other proteins contributes to both the cytoskeletal architecture and motile activities of nonmuscle cells. In striated and smooth muscle, TM is an essential component of thin filaments and acts to regulate muscle contraction. The rat α-TM gene generates cytoskeletal, smooth, and striated (skeletal and cardiac) muscle isoforms through alternative splicing of 5', middle, and 3' exons. Studies were initiated to monitor α-TM expression in the in vitro embryonic stem (ES) cell model of organogenesis and embryoid body development and differentiation. Results demonstrate that cytoskeletal mRNA isoforms of α-TM are expressed in all stages of embryoid body development, from undifferentiated ES cells through cystic embryoid bodies which contain blood islets, visceral yolk sac, and primitive myocardium. The incorporation of striated specific exons in α-TM mRNA transcripts is restricted to the cystic embryoid bodies and correlates with the appearance of myofiber formation and contractions in the developing myocardium. Smooth muscle α-TM isoforms are not detected in ES cells or the differentiated embryoid bodies. These results confirm that the α-TM gene exhibits a "housekeeping" pattern of cytoskeletal isoform expression and demonstrate the muscle specific TM isoforms are initially detected during the development and differentiation of the myocardium. Furthermore, the trans-acting factors which regulate TM isoform production must themselves be present and functional during this early embryonic period of organogenesis.

B 120 THE HUMAN β SPECTRIN GENE, John C. Winkelmann and Bernard G. Forget, Department of Medicine and Institute of Human Genetics, University of Minnesota, Minneapolis MN 55455; Departments of Medicine and Human Genetics, Yale University, New Haven CT 06510

The most abundant molecule of the erythrocyte membrane skeleton is spectrin. Spectrin consists of α and β subunits associated to form a 100nm rod-like heterodimer. Dimers self-associate into tetramers that, in turn, assemble with other proteins to become the lattice-like erythrocyte membrane skeleton. The spectrin-based membrane skeleton is also important in cell types other than the red cell. To facilitate understanding of spectrin functional interactions, erythrocyte β spectrin primary structure has been deduced from 6773 bp of contiguous nucleotide sequence of overlapping cDNA clones. The 2137 amino acid β spectrin molecule has a calculated Mr of 246 kDa. The peptide can readily be divided into three domains. Domain I, 270 amino acids at the N-terminus, does not adhere to the spectrin repetetive motif. Instead, this region displays high homology to the N-terminal "actin binding" domains of α actinin and dystrophin. Domain II consists of 17 spectrin repeats. These repeats show, in places, marked sequence deviations from prototype spectrin repeats. α Actinin homology extends well into domain II. Domain III, the C-terminus, also does not have spectrin repeat structure. We also have examined transcripts of the erythroid β spectrin gene in nonerythoid tissues. A 4.3 kb 3' partial β spectrin cDNA isolated from human skeletal muscle has also been completely sequenced. The nucleotide and derived amino acid sequences are identical to red cell β spectrin until the 3' end, where there is complete divergence. The result of this divergence in nucleotide sequence is a change in the derived amino acid sequence of the C-terminal domain of β spectrin. The muscle C-terminus is longer, has no obvious homology to known proteins, and is missing a possible casein kinase phosphorylation site. The intron/exon organization of the 3' β spectrin gene, including red cell and muscle coding sequences, has been determined. These data confirm that the red cell and muscle isoforms are generated by tissue-specific differential processing of β spectrin pre-mRNA. The sequence of possible regulatory splice sites has been examined. The functional consequences of this tissue-specific differential splice is unknown.

B 121 MOLECULAR CLONING OF NESTIN: A NOVEL INTERMEDIATE FILAMENT RESTRICTED TO CNS STEM CELLS, Lyle Zimmerman, Urban Lendahl, and Ron McKay, Department of Biology, MIT, Cambridge, MA 02139.

The monoclonal antibody Rat.401, which has previously been shown to mark neural precursor cells in the developing rat CNS (Frederiksen & McKay, J. of Neurosci., 1988, v.8, #4) has been used to generate a complete cDNA sequence with significant homology to the conserved alpha-helical core domain of the intermediate filament gene family. The cDNA encodes an 1805 amino acid protein with a predicted molecular weight of 200 kD. Northern blots probed with this cDNA yield a 6.2 kb band present in the mitotic neuroepithelium but absent from all other tissues examined, with the exception of developing muscle which displays traces of the message. The name nestin (neuroepithelial stem cell) was selected based on this restricted pattern of expression.

Cytoskeletal Organization and Dynamics

B 200 IMMUNOCYTOCHEMICAL CO-LOCALIZATION OF GLUCOCORTICOID RECEPTOR WITH CYTOPLASMIC MICROTUBULES AND Mr 90,000 HEAT SHOCK PROTEIN IN NORMAL HUMAN FIBROBLASTS. Gunnar Akner, Karl-Gösta Sundqvist, Marc Denis, Ann-Charlotte Wikström and Jan-Åke Gustafsson, Departments of Medical Nutrition and Clinical Immunology (K.-G.S), Karolinska Institute, Huddinge University Hospital F-69, S-141 86 Huddinge, Sweden. The cellular distribution of the glucocorticoid receptor (GR), various intracellular- and plasma-membrane structures and the Mr 90,000 heat shock protein (hsp90) in human fibroblasts was studied using indirect immunofluorescence techniques with monoclonal and polyclonal antibodies. During interphase, G R was located predominantly in the cytoplasm, showing a similar pattern as tubulin and hsp90. In mitotic cells, GR and tubulin but not hsp90 were localized in mitotic spindles and in post-mitotic midbodies. Colchicine and vinblastine induced a similar redistribution of GR, tubulin and hsp90 to the cell periphery. This redistribution was reversible for colchicine but not for vinblastine. Vinblastine also induced paracrystals containing GR and tubulin. Hsp90 was not redistributed to the paracrystals.

These results support the hypothesis that GR interacts in vivo with cytoplasmic microtubules and hsp90.

B 201 TAU HETEROGENEITY IN CLIAL CULTURES FROM MOUSE CEREBELUM

Helena M.M. Araujo, Regina P. Keller, Carlos Chagas Filho and Vivaldo Moura Neto, Department of Molecular Biophysics, IBCCF, Federal University of Rio de Janeiro, Rio de Janeiro, Brasil, 21944.

The low molecular weight microtubule associated proteins are know as a highly heterogenous family named Tau proteins. Mouse brain Tau isoforms can be analised by submitting microtubular preparations to two dimensional gel electrophoresis in a non-equilibrium pH gradient (NEPHGE). In an earlier report we have shown that Tau heterogeneity is modified during development. A higher degree of Tau heterogeneity was seen in 15-day old mouse brain than in brains of the other ages analised. Also the number of phosphorylated Tau isoforms is greater at this age (Araujo et al.,1989). Here we present the two-dimensional image obtained when submitting proteins from primary glial cultures and astrocytic clones to NEPHGE. The results suggest that glial lineages present only few Tau isoforms. May the neuronal population and other cell types present a different pattern of heterogeneity ? Studies in this respect are being realised at the moment.

B 202 MAPs-DEPENDENT COLCHICINE-STABILITY OF ACETYLATED COLD-LABILE BRAIN MICROTUBULES FROM THE ATLANTIC COD, *Gadus morhua*, Martin Billger, Elisabet Strömberg and Margareta Wallin, Comp. Neurosci. Unit, Dept. Zoofysiology, University of Göteborg, PO Box 25059, S-400 31 Göteborg, Sweden. Isolated cold-labile cod brain microtubules were found to exhibit unusual properties, in that they are not broken down by calcium and have a specific composition of MAPs. In addition, they are insensitive to colchicine in concentrations that completely inhibit assembly of bovine brain microtubules. Furthermore, axonal transport in cod sciatic nerves *in vivo* persists colchicine treatment, and the ultrastructure of such nerves reveals the presence of microtubules. In spite of this, cod brain tubulin binds colchicine in a manner similar to that of bovine tubulin, the dissociation constants, K_d, being in the same range (8,4 and 7,6 micromolar respectively). Cod brain tubulin was also found to be acetylated, in contrast to bovine tubulin. However, the assembly of cod tubulin in the absence of cod MAPs, is inhibited by colchicine, indicating that the composition of MAPs rather then the state of posttranslational modifications of tubulin is the reporter for colchicine stability.

B 203 REGULATION OF ACTIN FILAMENT POINTED END STABILITY BY TROPOMYOSIN, Kay O. Broschat & David R. Burgess, Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL 33101. The formation of stable actin filaments requires regulation of elongation and depolymerization at both filament ends. The barbed filament end can be capped to elongation or depolymerization by the abundant capping proteins which bind to that end with high affinity. An experimental model utilizing villin, a barbed end capping protein which stops polymerization at that filament end, has been used with fluorescence assays to study the regulation of pointed end stability. We show that nonmuscle and muscle tropomyosins (TMs) lower the critical concentration for elongation at the pointed filament end. Neither TM inhibited pointed end elongation. Skeletal muscle TM differs from nonmuscle TM in that it stops filament depolymerization from the pointed end at G-actin concentrations far below the critical. Filaments did not elongate at concentrations below 0.2 uM G-actin. Since filaments did not depolymerize in the presence of skeletal TM and 25 nM G-actin, the critical concentration for elongation differs experimentally from the concentration required to prevent depolymerization. This data suggests that TM lowers the off rate constant for the pointed end to nearly 0, but cannot drive polymerization of actin to completion as does the mushroom toxin phalloidin. We present a model in which stable actin filaments can be formed by capping the barbed end and saturating the pointed filament end with TM. Therefore, TM's presence at the pointed end may regulate actin filament length and stability.

B 204 MECHANISM FOR OSCILLATORY ASSEMBLY OF MICROTUBULES, Michael Caplow and John Shanks, Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27599-7260. Dampened oscillations of microtubule assembly can accompany polymerization at high tubulin subunit concentrations. This presumably results from a synchronization of dynamic instability behavior, which generates a large population of rapidly disassembling microtubules, that liberate tubulin-GDP oligomers. Subunits in oligomers cannot assemble until they dissociate, to allow GDP-GTP exchange. To determine whether rapidly disassembling microtubules generate oligomers directly we measured the rate of dilution-induced disassembly of tubulin-GDP microtubules, and the rate of dissociation of GDP from the so-formed tubulin-GDP subunits. The rate of GDP dissociation from liberated subunits was found to correspond to that of tubulin-GDP subunits ($t_{1/2}$ = 5 s), rather than tubulin-GDP oligomers. This indicates that tubulin-GDP subunits are released from microtubules undergoing rapid disassembly. Oligomers apparently form in a side reaction from the high concentration of tubulin-GDP subunits liberated from the synchronously disassembling microtubules population. GDP dissociation from oligomers follow the path: $(\text{Tubulin-GDP})_n$ -> nTubulin-GDP ->Tubulin + GDP, rather than a path in which GDP dissociates directly from oligomer. The rate of subunit dissociation is 0.11 s^{-1} with oligomers formed by concentrating tubulin-GDP subunits and 0.045 s^{-1} with oligomers found by cold-induced microtubule disassembly. This difference provides evidence that the conformation of tubulin-GDP subunits released from rapidly disassembling microtubules differs from tubulin-GDP subunits that were not recently in the microtubule lattice.

B 205 PRIMATE NONNEURONAL 210Kd MICROTUBULE-ASSOCIATED PROTEIN (MAP4) CONTAINS A DOMAIN HOMOLOGOUS TO THE MICROTUBULE BINDING DOMAIN OF NEURONAL MAPS, S.Chapin and J.C.Bulinski, Dept. of Anat. & Cell Biol., Columbia Univ. Coll. of P & S, New York, NY, 10032

MAP4 is a microtubule-associated protein that exhibits Mr ~200Kd, species-specific antigenic sites, and widespread tissue distribution. Antisera raised against HeLa cell MAP4 (210Kd MAP), labeled microtubules of primate cells by immunofluorescence. In western blots of primate cell extracts these antisera reacted with a 210Kd species, and often, a less prominent 255Kd MAP. We showed, using peptide mapping, that the 210Kd and 255Kd polypeptides are closely related. In order to understand the relationship between the structure and function of these primate MAPs and those of the known neuronal MAPs, we have isolated cDNA clones from a human fetal brain lambda gt-11 library using our polyclonal MAP4 antiserum. Five strongly immunoreactive clones were shown to be related, but not identical, as determined by restriction mapping, partial sequencing, and expression of fusion proteins. Antibody affinity-purified using the 220Kd ß-gal fusion protein encoded by one clone recognized both 210Kd and 255Kd polypeptides on western blots and stained microtubules. We sequenced a 4Kb clone encoding a 245Kd ß-gal fusion protein. Preliminary analysis of this sequence revealed a region that contains three-18 amino acid repeats highly homologous to repeats found in the microtubule binding domain of the neuronal MAPs, tau and MAP2 [1]. Immediately outside of the 90 amino acid region containing the repeats, no obvious homology between MAP4 and the neuronal MAPs was observed.

[1] Lewis, S.A., Wang, D., and Cowan, N.J. Science 242, 936-939 (1988).

B 206 ROLE OF VENTRAL CARPET-ASSOCIATED GLYCOPROTEINS IN CYTOSKELETAL ORGANIZATION: INDUCTION BY CELL SHAPE-MODULATING AGENTS, Panna R. Chaudhari, Michael P. Ryan, and Paul J. Higgins, Laboratory of Cell & Molecular Biology, VA Medical Center, Albany, NY 12208

There is a direct relation between the flattened morphology, generation of well-developed actin microfilaments, substratum adhesion, and expression of the matrix-associated glycoproteins mag50 and p52 in hamster (FF) and rat (NRK) cells, respectively. These proteins distribute to the saponin-resistant cytomatrix fraction where they mediate changes in cell shape and cytoskeletal organization. Synthesis of both proteins is itself regulated by agents which modulate cell shape. Sodium butyrate (NaB), which increases cell-to-substrate adhesion and microfilament bundling, augments mag50 synthesis in an actinomycin D-sensitive manner. Cytochalasin D (CD), which promotes rounding of NRK cells, produces a similar selective hyperinduction of p52 synthesis. p52 augmentation is due to a CD-associated increase in p52 gene transcription and concomitant increase in the cytoplasmic steady-state level of p52 mRNA. Northern/slot blot analysis and nuclear runoff transcription showed that CD stimulated the p52 gene exclusively at the transcriptional level, while having no significant affect on stability of the p52 mRNA. CD-mediated increases in matrix-associated p52 levels correlated with increases in the cytoskeletal framework-deposited p52 mRNA transcripts. These data further suggest that cytoskeletal organization plays a role in regulation of expression of particular genes encoding major matrix-associated cellular glycoproteins.

B 207 TRANSLOCATION OF ACTIN FILAMENTS BY 110K/CALMODULIN *IN VITRO*: CALCIUM REGULATION OF MOTILITY, ATPase ACTIVITY AND STRUCTURE, Kathleen Collins, James Sellers* and Paul Matsudaira, Department of Biology, Massachusetts Institute of Technology, and Whitehead Institute for Biomedical Research, Cambridge, MA 02142 *Laboratory of Molecular Cardiology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20205

A complex of 110K-protein and calmodulin directly connects actin to apical and basolateral membranes of intestinal epithelial cells. Because the DNA sequence of the 110K gene and the structure and enzymatic activity of the protein suggest homology to myosin, we examined the potential of the complex to function as a mechanoenzyme. 110K/calmodulin, when immobilized on nitrocellulose-coated coverslips, translocates actin filaments at 37°C at a maximal rate of 0.08 to 0.1 μm/second, similar to the rate of actin translocation by non-muscle myosins. In our preparations of the 110K complex, actin activates MgATPase activity greater than 40-fold, with a K_m of 20- 40μM and V_{max} of 0.86/second. Micromolar calcium increases both rate of translocation and rate of actin-activated MgATP hydrolysis. Higher concentrations of calcium completely inhibit motility but not actin-activated ATPase activity, correlated with dissociation of calmodulin from the complex in a velocity sedimentation assay. The calmodulin of the complex can be phosphorylated *in vitro* by a kinase endogenous to brush borders, but not by myosin light-chain kinase. Rate of 110K/calmodulin-mediated motility is dependent on temperature but independent of time, actin filament length, amount of enzyme, or ionic strength. These properties suggest that myosins I and II share a molecular mechanism of motility, yet are uniquely regulated by calcium.

B 208 REORGANIZATION OF THE ACTIN CYTOSKELETON DURING CHEMOTACTIC STIMULATION, John Condeelis, A. Hall, A. Bresnick, S. Dharmawardhane, M. Demma, Fan Yang, V. Warren, Anatomy and Structural Biology, Albert Einstein Coll. of Med., Bronx, NY 10461.

In Dictyostelium amoebae, chemotaxis is regulated by cell surface receptors through G proteins. Agonist stimulation leads to actin polymerization and incorporation of various actin binding proteins (ABP) into the cytoskeleton causing a reorganization of cell polarity. We have characterized an actin nucleation activity (NA) and its inhibitor (INA) in cytoskeletal and cytosolic compartments, respectively, which are regulated by agonist to control actin polymerization. The INA has been purified and further analysis is in progress. Using the signal transduction mutants synag 7 and fgd A, and treatment with pertusis toxin (PT) we have demonstrated that neither adenylate cyclase activation nor a PT sensitive G protein are involved in signal transduction to NA while expression of the G protein that regulates phospholipase C is required for signal transduction to NA. We have identified two ABPs (ABP-120 and ABP-50) whose associations with the cytoskeleton are regulated by agonist and are involved in filament cross-linking during pseudopod extension. Amino acid sequence and domain analysis of the ABP-120 and cDNA sequencing of ABP-50 has been done to identify regulatory and actin binding sites on these proteins. Based on this analysis and the properties of NA and INA, we will present a model for the role of actin filament assembly and cross-linking in the development of polarized pseudopod extension by cells in gradients of chemoattractant. (Supported by T32 cA09475 and GMS).

B 209 DIFFERENTIAL BEHAVIOR OF NEURON-SPECIFIC ISOTUBULINS DURING AXONAL TRANSPORT IN SCIATIC MOTOR-AXONS, Philippe Denoulet, Ghislaine Fillia treau*, Béatrice de Néchaud, Bernard Eddé and Luigi Di Giamberardino*, Biochi mie Cellulaire, Collège de France, 75005 Paris, and (*) Service Hospitalier F. Joliot, C.E.A. 91406 Orsay, France.

Most of the 20 isoeclectric variants of tubulin from nervous tissues are spe cific of neurons and are produced by posttranslational modifications (PTM). They appear progressively throughout development, their expression being tri ggered at crucial steps of neurogenesis. We show here that these isotubulins are differentially conveyed along the sciatic motor-axons. Most of tubulin is carried as stable microtubules (MT) by both waves (SCa and SCb) of the slow axonal transport: 2/3 with SCa (with neurofilaments) and 1/3, faster, with SCb. In addition to this differential velocity, SCb MT can be distinguished on the basis of their isoform composition: compared to SCa, SCb tubulin is significantly enriched in a neuron-specific β-tubulin subcomponent (β'). Moreover, when soluble vs cytoskeletal tubulin were compared, two groups of tubulin isoforms, the most acidic α (α"') and β', were exclusively found as sociated with the cytoskeletal fraction, whereas another group (α") was reco vered only in the soluble compartment. These data indicate that PTM's of tu bulin are involved in MT dynamics, the events of polymerization and modifica tion being linked one to the other. These data also suggest that distinct MT subsets co-exist within the axon where they could play distinct roles.

VILLIN, Don S. Doering and Paul T. Matsudaira, Whitehead Institute and Department of Biology, M.I.T, Cambridge, MA 02139.

Villin is a 93 kD calcium-dependent actin binding protein found in the microvilli of absorptive epithelial cells. In calcium concentrations lower than μM free calcium, villin assembles f-actin *in vitro* into bundles that resemble the actin bundle that supports the microvillar membrane. Proteolysis with V8 protease divides villin into an amino terminal 84 kD "core" domain and an 8.5 kD domain. The 8.5 kD "headpiece" domain binds f-actin and calcium, and is required for bundling activity. The headpiece sequence is the most conserved region among human, mouse, and chicken villins. We have over expressed and purified chicken headpiece from E.Coli (rHP) in order to study the properties of this small actin and calcium binding domain. rHP is a monomer in solution and binds to F-actin with a Kd of ~8 μM. Circular dichroism spectroscopy shows the Tm of the domain to be greater than 75 °C. The fusion of villin headpiece cDNA to E. Coli. β-galactosidase confers f-actin binding and bundling activity to the purified, enzymatically active (tetrameric) fusion protein. We have micro-injected villin into confluent MDCK cells and examined the distribution of micro-injected proteins by immunofluorescence confocal microscopy. Micro-injected villin is localized to the apical microvilli. Micro-injected rHP is sufficient to localize to the microvilli and also localizes to the circumference of these cells. These results support the hypotheses that the headpiece peptide; 1) comprises a stable structural domain, 2) functions to target the severing activity of villin to the microvilli, and 3) contains one of villin's f-actin bundling sites. In addition, mutagenesis of the recombinant headpiece and the headpiece-β-galactosidase fusion protein will be a powerful method with which to study the structure and functions of an actin binding domain.

B 211 GLUTAMYLATION, A NEW POSTTRANSLATIONAL MODIFICATION OF ALPHA-TUBULIN IN NEURONS, Bernard Eddé, Jean Rossier*, Jean-Pierre Le Caer*, Elisabeth Desbruyères and Philippe Denoulet, Biochimie Cellulaire, Collège de France, Pl. M. Berthelot, 75005 Paris, and (*) Physiologie Nerveuse, C.N.R.S. 91198 Gif sur Yvette, France.

Tubulin heterogeneity is very high in neuronal cell types. We previously showed that among the eight α isoforms present in cultured mouse brain neurons, six were posttranslationally labeled by ^{3}H-acetate (Eddé *et.al.*1989, Biol. Cell, *65*, 109). We show here that this labeling is mainly due to a new modification of proteins: glutamylation. α-tubulin peptides carrying the radioactive moiety were purified by HPLC. Edman degradation sequencing showed that these peptides (1) have the same primary sequence which matches that of Mα1 and Mα2 tubulin genes from aa 440-450 and (2) have a modified Glu-445 residue. Mass spectrometry and biochemical analyses revealed that these peptides carry 1 to 5 glutamyl units linked to the γ-carboxyl group of Glu-445. Pulse-chase experiments indicated that α-tubulin is progressively glutamylated, probably during its transport along the nerve processes. The different glutamylated forms were estimated to represent 50% of the total α-tubulin present in neurons. Thus, glutamylation is a quantitatively major modification of neuronal α-tubulin. Localized within a region involved in the interactions of tubulin with Map's and Ca++, glutamylation could play a crucial role in regulating microtubule dynamics and functions.

B 212 MICROTUBULES AND INTERMEDIATE FILAMENTS IMAGED BY THE SCANNING TUNNELLING MICROSCOPE, Stuart R. Hameroff[1], Yovana Simic-Krstic[1], Lawrence A. Vernetti[1], Y.C. Lee[3], Dror Sarid[3], Robert McCuskey[2], Departments of Anesthesiology[1] and Anatomy[2], College of Medicine, and Optical Sciences Center[3], University of Arizona, Tucson, Arizona, 85724.

Scanning tunnelling microscopy (STM) can image atomic surfaces of metals and semiconductors. Biological STM applications have been somewhat limited by poor conductivity, adsorbate layers, elasticity and poor stability of biomolecules. However, recent improvements in preparation techniques have resulted in useful biomolecular imaging. For direct STM observation of microtubules (MT) isolated from pig brain by standard techniques of differential ultracentrifugation (Shelanski, 1973), we determined optimal preparation conditions: fixation with 0.1% glutaraldehyde and solution in 0.8 M glycerol reassembly buffer (Mes, EGTA, GTP, $MgCl_2$). Both freeze dried and hydrated MT prepared in this way were reproducibly imaged in air at room temperature on graphite with a Nanoscope II STM (Digital Instruments, 135 Nogal Drive, Santa Barbara, CA 93110). The presence of MT was verified by electron microscopy. STM probing showed semiflattened structures 25 nm in width, consisting of 5 to 7 longitudinal filaments of about 4 nm width: top views of MT which show about half of their 13 component protofilaments. Inverted image scans revealed 4x8 nm individual tubulin subunits within protofilaments. Intermediate filaments (IF) were isolated from cell culture in urea. STM images of IF in 4 M urea showed flattened, parallel 10 nm filaments comprised of coiled chains of 6 to 12 nm "tetramer" subunits. IF in 9 M urea showed depolymerized monomers in an "angled" array. STM images of IF treated with monoclonal antibodies showed antibody molecules arrayed on each monomer. STM and related techniques (atomic force microscopy, scanning near field optical microscopy, scanning ion microscopy) offer unique opportunities for the study of biomolecular structures in general, and the cytoskeleton in particular.

B 213 REGULATION OF F-ACTIN CONTENT BY VASOPRESSIN (AVP), PROSTAGLANDIN (PGE) AND OTHER AGONISTS IN THE TOAD BLADDER EPITHELIAL CELL, Richard Hays, Guohua Ding, Nicholas Franki, and John Condeelis, Departments of Medicine and Anatomy - Structural Biology, Albert Einstein College of Medicine, Bronx, NY 10461

Hormones and other stimuli produce rapid changes in the F-actin content of target cells. In many instances, the change is an increase in polymerization, and initiates motility, phagocytosis or adhesion. We have determined by a rhodamine-phalloidin labeling technique the F-actin content of the toad bladder epithelium (as a % of untreated controls), in response to AVP and other agents that stimulate or inhibit water flow. In this system, AVP, via cAMP, promotes fusion of cytoplasmic vesicles (aggrephores) carrying water channels with the apical cell membrane. AVP, 8-Br-cAMP and forskolin depolymerized F-actin as early as 1 minute. Forskolin was more potent than AVP in initiating depolymerization (71±4 vs 84±5% of controls respectively; $p<0.01$). Depolymerization, like water flow, was PGE-inhibited: 1mU/ml AVP, a low concentration ordinarily incapable of stimulating water flow or depolymerization, stimulated both when PGE synthesis was blocked by naproxen. Depolymerization did not require external calcium. F-actin polymerization, rather than depolymerization, was seen 15 min after administration of $d(CH_2)_5Tyr(Me)AVP$, an AVP analogue which has no hydroosmotic effect. Here, F-actin content was 110±2% of control ($p<0.01$). Thus, F-actin depolymerization, like water flow, is a cAMP-mediated, PGE-inhibited response. The F-actin content can be shifted in opposite directions by AVP and an AVP analogue. The cytoskeletal and hydroosmotic responses to AVP therefore have common features, and actin depolymerization may contribute to aggrephore fusion.

B 214 POTENTIAL ROLE OF EXTRACELLULAR MATRIX COMPONENTS ON CYTOSKELETAL ALTERATIONS DURING MYOFIBRILLOGENESIS, L.L. Hilenski, L. Terracio and T.K. Borg, Departments of Pathology and Anatomy, University of South Carolina, Columbia, SC 29208. As cells spread and interact with extracellular matrix (ECM) components, the internal cytoskeleton is altered in response to these external signals. Alterations in the topological distribution of three cytoskeletal proteins, vinculin, α-actinin, and F-actin, were visualized by immunofluorescence microscopy in neonatal rat myocytes cultured on the ECM components laminin (LN) and collagen I + III (C I/III). Cells were processed for immunofluorescence microscopy after various time intervals in culture. A major difference in cytoskeletal organization on LN and C I/III was the increased appearance on C I/III of rosettes which consisted of an F-actin core surrounded by α-actinin and vinculin rims. By 4-8 hours, 50% of cells on C I/III had clusters of these rosettes compared to 18% of cells on LN. Assembly of these rosettes did not require new protein synthesis. Initially these rosette clusters on both matrices were found beneath the nuclear area. In later cultures, the intracellular location shifted to paramarginal areas and trailing edges. The rosettes gradually disappeared in cells on both matrices. The rosettes in spreading myocytes were similar in appearance and composition to specialized adhesive structures previously reported in virus-transformed fibroblasts, myotubes treated with a tumor-promoting agent and in highly migratory cells of monocytic origin. The increased incidence of rosettes on C I/III suggests that these transitory specialized cytoskeletal complexes are involved in recognition and interaction with ECM components. Supported by grants from the NIH (#HL37669, HL42249), NSF and the SC and GA Affiliates of the American Heart Association.

B 215 DYSTROPHIN IN MYOGENESIS: PATTERNS OF TRANSCRIPTIONAL AND TRANSLATIONAL EXPRESSION. Henry J. Klamut, Lucy O. Bosnoyan, Ronald G. Worton, and Peter N. Ray, The Genetics Department and Research Institute, The Hospital for Sick Children, Toronto, Ontario, M5G 1X8, Canada.

Duchenne (DMD) and Becker (BMD) muscular dystrophy result from molecular genetic defects which either abolish (DMD) or modify (BMD) dystrophin assembly into the subsarcolemmal (and possibly t-tubular) cytoskeleton of skeletal muscle tissue. In order to begin to approach a definition of dystrophin function in the maintenance of muscle cell integrity, the pattern of accumulation of dystrophin at both the transcriptional and translational levels has been examined as normal clonal human myoblasts differentiate into multinucleated myotubes *in vitro*. Quantitative PCR analysis reveals a 30-fold elevation in the levels of dystrophin transcripts upon myogenic differentiation, with a further 3-fold elevation in mature skeletal muscle. These results are in accordance with a concomitant increase in DMD gene muscle promoter activity. Furthermore, indirect immunofluorescence analysis indicates that dystrophin appears at the myotube surface very early in myogenesis, a process which continues with myotube maturation. These results will be discussed in relation to the patterns of accumulation of other, more well-defined cytoskeletal proteins.

B 216 **THE EFFECTS OF GAMMA CRYSTALLIN ON ACTIN POLYMERIZATION** *Charles Lee Kuckel,* Peter K. Lambooy# and Patricia N. Farnsworth** * Departments of Ophthalmology and Physiology, University of Medicine and Dentistry, New Jersey Medical School, Newark, New Jersey 07103 # Eli Lilly Corporation, Indianapolis, Indiana 46285

The focus of this investigation is to determine the role of the 20 kDa lens protein family, the gamma crystallins, in the maintenance and pattern of the lens fiber cell cytoskeleton. Experimental results established that gamma crystalin produces: (1) a decreased critical concentration, i.e. the minimum concentration of actin monomer required to produce polymerization as assayed by fluoresence measurements with the probe 1-N-pyrene iodoacetamide covalently linked to actin; (2) an increased apparent viscosity as monitored by falling ball viscometry; (3) an altered apparent rate of polymerization and final specific viscosity as monitored by capillary viscometry; and (4) a crosslinked and bundled network of polymerized actin filaments when visualized by transmission electron microscopy. The pattern of fibers formed resemble the polygonal arrays (geodesic domes) previously described in a variety of embryonic cells *(Lazarides, J. Cell Bio., 1975 and 1976)*. Carbamylation of the lysine residues of gamma crystallin by methylisocyanate produced a loss of bundling and crosslinking properties and no alterations in the final specific viscosity of actin filaments. The results indicate that gamma crystallin functions as an isotropic crosslinking protein for actin polymerization. The mechanism for gamma crystallin's effect is most likely the binding of actin filaments on its two lysine groups. *(Supported by NIH grant #565921 [National Eye Institute] and Lions Eye Research Foundation of New Jersey).*

B 217 CYCLIC BIAXIAL STRAIN OF PULMONARY ARTERY ENDOTHELIAL CELLS CAUSES AN INCREASE IN CELL LAYER ASSOCIATED FIBRONECTIN. Edward J. Macarak, Stephen F. Gorfien, Pamela S. Howard and Jeanne C. Myers. Connective Tissue Research Institute, Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.
Bovine pulmonary artery endothelial cells were cultured on an artificial compliant substrate and were strained biaxially at a frequency of 1 sec^{-1} for 2,4,6,7 or 24 hours. Total protein synthesis, determined by estimating the incorporation of radiolabeled precursors into non-dialyzable protein, was increased in cultures that had been biaxially strained for 6,7 or 24 hours, with differences more apparent in the cell layer fraction than in the medium fraction. Medium and cell layer-associated fibronectin were quantitated by ELISA and by densitometric analysis of the autoradiograms of electrophoresed protein. Fibronectin levels in the medium of biaxially strained cells were initially depressed in comparison to non-strained controls, but with time, began to approach control values. Cell layer associated fibronectin of biaxially stained cultures was significantly elevated at 24 hours and a 140 kD cell layer protein was diminished whereas DNA synthesis was not altered. Immunohistochemical localization of fibronectin and factor VIII-vonWillebrand antigen revealed a more intense staining pattern in strained cultures. Distribution of stress fibers containing fibrous actin was visualized by staining with rhodamine-phalloidin and was altered in strained cultures. These observations indicate that cells respond to cyclic biaxial strain by selectively enhancing structural components associated with cell adhesion.
Supported by NIH Grants HL34005 and HL41882.

B 218 THE FILAMENTOUS NETWORK AROUND MAST CELL SECRETORY GRANULES, Ellen H. Nielsen, Institute of Anatomy and Cytology, Odense University, 5230 Odense M, Denmark.
Freezing-thawing of mast cells was found to be an excellent way to obtain isolated mast cell granules still surrounded by an intact membrane to which a coat of filaments is attached. In SEM the filaments are seen to form a basket-like structure surrounding each granule. The filaments are of equal diameter and some are connected to the granule membrane. Only a few filaments are passing the gaps between the granules. Also platinum replicas reveal filaments connected to the granule surface and radiating in all directions. Using specific anti-actin antibody and labelling with gold particles the filaments on the granule surface are heavy loaded with gold particles indicating that the filaments are actin. This filament system might have a secretory role as it has been described for the subplasmalemmal actin network.

B 219 "IN SITU" TRANSLATION: USE OF THE CYTOSKELETAL FRAMEWORK TO DIRECT CELL-FREE PROTEIN SYNTHESIS, Joel S. Pachter and Diane Biegel, Department of Physiology, University of Connecticut Health Center, Farmington, CT 06032
While many examples of translational control are evident throughout the literature, such as alterations in either translation initiation rate, polypeptide elongation rate, translation termination rate and/or stability of translating mRNAs, there is little data illuminating the molecular mechanisms by which any of these are achieved. Given that most translating mRNAs as well as certain translational cofactors are associated with the cytoskeletal framework, we have developed a cytoskeletal-based protein synthesizing system from cultured mouse cells to aid in examining the molecular bases for such control. Devoid of membraneous barriers, yet morphologically intact, this system allows high access to a relatively unperturbed translational machinery, i.e.; cytoskeletal-bound polyribosomes, and thus permits experimental variables to be manipulated under nearly "in situ" conditions. Preliminary data indicate that when this system is supported by various cofactors from a reticulocyte lysate, it is capable of sustaining the translation of endogenous mRNAs for a period of at least one hour. SDS-PAGE analysis of the translation products reveals that their pattern mirrors that produced from unextracted cells, with proteins of molecular weight greater than 200Kd being synthesized. Additionally, a continued increase in intensity of almost all protein bands during this one hour period further suggests that translational reinitiation is taking place. This system should prove useful in examining the role played by the cytoskeletal framework in regulating gene expression.

B 220 ACTIVATION OF HUMAN T-LYMPHOCYTES VIA THE T-CELL ANTIGEN RECEPTOR (TCR) CAUSES A PROTEIN KINASE C (PKC) MEDIATED INCREASE IN CELLULAR F-ACTIN. PD Phatak, CH Packman, MA Lichtman. Univ. of Rochester School of Medicine, Rochester, N.Y. 14642.

We have demonstrated that activation of the PKC arm of the phosphatidyl inositol (PI) pathway in human T-lymphocytes by phorbol myristate acetate, mezerein or oleoyl acetyl glycerol results in a rapid (1-2 min) increase in cellular F-actin content (1·5-2 fold). Monoclonal anti-CD3 activates T-lymphocytes through the TCR via the PI pathway, activating PKC and increasing intracellular Ca^{2+}. We studied the relative contribution of the two arms of the PI pathway to the F-actin increase using the F-actin specific probe NBD-phallacidin and flow cytometry. Anti-CD3 caused a rapid dose dependent increase in cellular F-actin (1·5 fold at 2 min) and a 2-3 fold increase in intracellular Ca^{2+}. Anti-CD2 which blocks sheep E-rosetting but does not activate T cells, caused no change in F-actin content or intracellular Ca^{2+}. The calcium ionophore, ionomycin 400 nM, caused a 1.15 fold increase in F-actin and a 10-fold rise in intracellular Ca^{2+}. Ionomycin 100 nM caused a Ca^{2+} increase similar to that caused by anti-CD3 but had no effect on F-actin content. The PKC inhibitor, H7, blocked the F-actin increase by both anti-CD3 and ionomycin 400 nM but not the Ca^{2+} increase. Cells leached of Ca^{2+} with EGTA and ionomycin exhibited no Ca^{2+} increase in response to anti-CD3 or ionomycin; however, they retained the F-actin increase caused by anti-CD3 but not that caused by 400 nM ionomycin. We conclude that activation of T-lymphocytes via the TCR causes an early rapid increase in F-actin content that is mediated by PKC. The concomitant calcium increase is neither sufficient nor necessary for the F-actin increase. The role of the F-actin increase in mediation of events that follow T-lymphocyte activation remains unclear. The measurement of actin polymerisation may be useful to examine signal transduction pathways involved in early T-lymphocyte activation.

B 221 MODIFICATION OF CYTOSKELETON ORGANIZATION UNDER CRYOPROTECTIVE TREATMENT, Prulière G. and Nguyen E., U. 310 INSERM, Institut de Biologie Physico-chimique, Paris, FRANCE.

We show that the good survival rate observed for some cells submitted to freeze-thaw exposure can be correlated to the modification of cytoskeleton organization during cryoprotective treatment. Thus propanediol which is currently employed for the cryoprotection of early-stage embryos induces the depolymerization of cortical actin filaments within these cells. Propanediol exerts also a significant influence on the interactions between actin and several actin binding proteins such as α-actinin, filamin and fodrin. Since the actin-linked cytoskeleton is thought to influence the cell water mobility within the cell, we suggest that the changes observed in cytoskeleton architecture under propanediol effect might allow the vitrification of the cytoplasm and avoid the lethal effects due to water cristallization.

B 222 AUTOMATON MODELS OF MICROTUBULE "LEARNING" AND ASSEMBLY/DISASSEMBLY, Steen Rasmussen[1], Hasnain Karampurwala[2], Rajesh Vaidyanath[2], Klaus Jensen[4], Stuart Hameroff[3], CNLS & T Division, Los Alamos National Labs[1], Electrical and Computer Engineering[2], and Department of Anesthesiology[3], University of Arizona, and Department of Anesthesiology, University of Colorado[4].

We have developed computer simulations based on automaton theory for dynamic conformational interactions among microtubule (MT) subunits, and among MT bridged by MT-associated proteins ("MAPs"). This model has previously demonstrated capabilities for information processing in MT (Hameroff, Rasmussen, Mansson, "Molecular Automata in Microtubules: Basic Computational Logic of the Living State?" in Artificial Life, Ed. C. Langton, Addison-Wesley, 521-553, 1989; Hameroff, Smith and Watt, "Automaton Model of Dynamic Organization in Microtubules". Ann NY Acad Sci 466, 949-952, 1986; Hameroff and Watt, "Information Processing in Microtubules", J Theor Biol 98, 549-561, 1982.) Recently, we have modelled MT connectionist "networks" (MT linked by "MAPs") through which patterns of MT subunit conformation ("information") can flow. Using variation, selection, and recursive iteration, we have shown "learning" in these MT networks similar to adaptive "neural networks". However, unlike neural networks which rely on high level algebraic operations on incoming signals, MT networks demonstrate distributed computation governed simply by low level, local interactions. Such processes within cells could help regulate various processes, neuronal synaptic plasticity being but one example. We have used the same model construct to model MT polymerization. We define 5 possible states for each MT subunit site: GTP-polymerized, GDP-polymerized, GTP-unpolymerized, GDP-unpolymerized, and "absent" (no subunit available). By defining probabilities and rate constants for interconversion among these states, assembly/dissassembly of MT fragments may be modelled. Our results show both growth and shrinking of MT, and are thus consistent with the model of "dynamic instability" (Kirschner and Mitchison, Cell 45, 329-342, 1986). Modelling of MT subunit interactions, as well as other cytoskeletal elements, may prove useful for evaluating dynamic cytoskeletal activities.

B 223 IDENTIFICATION OF A NOVEL NUCLEOTIDE-SENSITIVE MICROTUBULE-BINDING PROTEIN IN HELA CELLS, Janet E. Rickard and Thomas E. Kreis, European Molecular Biology Laboratory, Meyerhofstrasse 1, 6900 Heidelberg, FRG.

A protein of M_r 170,000 (170K protein) has been identified in HeLa cells, using an antiserum raised against HeLa nucleotide-sensitive microtubule-binding proteins. Affinity purified antibodies specific for this 170K polypeptide have been used for its characterization. *In vitro* sedimentation of the 170K protein with taxol-microtubules polymerized from HeLa high speed supernatant is enhanced in the presence of an ATP depleting system, but unaffected by the nonhydrolysable ATP analogue AMP-PNP. In addition, it can be eluted from taxol-microtubules by ATP or GTP, as well as NaCl. Thus it shows microtubule-binding characteristics distinct from those of the previously described classes of nucleotide-sensitive microtubule-binding proteins, kinesin and cytoplasmic dynein, homologues of which are also present in HeLa cells. Immunofluorescence localization of the 170K protein using affinity-purified antibodies shows a patchy distribution in interphase HeLa cells, often organized into linear arrays which correlate with microtubules. However, not all microtubules are labelled, and the antigen appears to be localized predominantly at the peripheral ends of microtubules. In mitotic cells, the microtubules of the spindle are labelled, but there is also dotty labelling of the cytoplasm. In addition, after depolymerization of microtubules by nocodazole, the staining pattern is patchy but not organized in linear arrays, suggesting that the protein may be associated with some other intracellular structures in addition to microtubules. In vinblastine-treated cells, there is strong labelling of tubulin paracrystals, and random microtubules induced *in vivo* by taxol are also labelled by the antibody. These immunofluorescence labelling patterns are stable to extraction with Triton X-100, further suggesting an association of the protein with the cytoskeleton. *In vivo*, therefore, the 170K protein appears to be associated with a subset of microtubules at discrete sites. Its *in vitro* behaviour suggests that it belongs to a novel class of nucleotide-sensitive microtubule-binding proteins.

B 224 A MORPHOLOGICAL TRANSITION CORRELATED WITH INCREASED ADHESIVE PROPERTIES IN RBL-2H3 CELLS, Schneebeck, M.C., J.R. Pfeiffer, R.F. Stump, J.M. Oliver, Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM 87131

In the RBL-2H3 mast cell model, IgE receptor crosslinking stimulates a rearrangement from an apparently random microvillous surface to a lamellar one. Within about 30 seconds after stimulation, the microvilli have formed rough lines on the cell surface. About 2 minutes later, lamellae with periodically spaced microvillous structures projecting from their crests have appeared. This "fringed" lamellar intermediate suggests that the aligned microvilli are acting as scaffolding for lamellar formation. By 7 minutes after antigen stimulation, the cells have conventional lamellae, which proceed to coalesce and produce a "honeycombed" cell surface. RBL-2H3 cells also become more adhesive after antigen stimulation. Using agents which selectively induce the cells to either secrete without ruffling or to ruffle without secreting, we have shown that this property is associated with lamellar formation. Thus, the microvillous to lamellar transition is correlated with, and may mediate, increased adhesion of antigen-stimulated cells.

B 225 IDENTIFICATION OF ACTIVATORS FOR DYNEIN-DRIVEN ORGANELLE MOVEMENT. T.A. Schroer and M.P. Sheetz, Dept. of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110.

Cytoplasmic dynein drives the movement of membranous organelles toward minus ends of microtubules in vitro. However, soluble accessory factors are also required. We have identified three distinct activating factors that stimulate dynein to move organelles. The "organelle motor activity" of dynein plus factors was assayed in vitro using a quantitative video microscope assay. A mixture of microtubule-binding proteins including kinesin, dynein and other proteins was isolated from chick embryo brain by microtubule-affinity chromatography; the preparation had significant organelle motor activity (≈ 80% minus-end-directed, ≈ 20% plus-end-directed) indicating that it contained all the components required for motility. 20S dynein (further purified by velocity sedimentation) had very low organelle motor activity as previously described. Its activity was stimulated by adding back a 7-9S sucrose gradient fraction containing kinesin and other proteins. The 7-9S and 20S sucrose gradient pools were subjected to Mono-Q ion-exchange chromatography to isolate activator(s) of dynein-driven organelle transport. The 20S pool contained an activator that was separated from dynein on the Mono-Q column. A second activator was found in the 7-9S sucrose gradient pool. Both activators stimulated only minus-end-directed movement. A third dynein activator (in the 7-9S pool) was found in a fraction that contained kinesin. This fraction by itself had plus-end-directed organelle motor activity and therefore appeared to contain activator(s) of both motors. We are currently purifying the activators further and studying their mechanism of action.

B 226 MATURATION ASSOCIATED CHANGES IN REGULATION OF ACTIN CONFORMATION IN HL60 CELLS. R.L. Sham,C.H. Packman, C.N. Abboud, M.A. Lichtman. Hematology Unit, Department of Medicine, University of Rochester Medical Center, Rochester, NY, 14642.

Maturation of human myeloid cells is associated with both an increase in chemotactic receptors for f-Met-Leu-Phe (fMLP) and actin content. We have studied the effects of fMLP and protein kinase C (PKC) activators and inhibitors on actin polymerization in HL60 cells undergoing neutrophilic maturation induced with 1% DMSO. The polymerization state and distribution of F-actin was determined by flow cytometry and fluorescent microscopy using the specific probes NBD phallacidin and rhodamine phalloidin. 1 μM fMLP caused no change in F-actin in uninduced cells. Activators of PKC, 0.1 μM PMA, 50 μM OAG, and 10 nM bryostatin resulted in a 15-30% decrease in F-actin in uninduced cells by 5 minutes. The PKC inhibitor, H7, mildly accentuated the decrease in F-actin in uninduced cells treated with PMA and bryostatin. 24 hours following DMSO addition, 1.0 μM fMLP resulted in a 40% increase in relative F-actin at 1 minute and 0.1 μM PMA resulted in no response. 48 hours following DMSO addition, the fMLP response persisted and 0.1 μM PMA now resulted in a 20-30% increase in F-actin. The effect of fMLP and PMA on F-actin in mature HL60 cells was similar to that seen in normal neutrophils. In matured cells, 200 μM H7 partially inhibited the F-actin increase caused by PMA and bryostatin. Fluorescent microscopy showed F-actin rich pseudopod formation upon fMLP or PMA stimulation of mature HL60 cells; immature HL60 cells had no detectable change. Scanning electron microscopy of the uninduced HL60 cells stimulated with PMA showed dramatic loss of their surface ruffles coincident with the observed decrease in F-actin. Multiple pseudopods appeared in the induced HL60 cell upon stimulation with fMLP and PMA. In summary: neutrophilic maturation of HL60 cells is associated with the ability to form pseudopods and to polymerize actin in response to fMLP and PMA. The PMA induced F-actin decrease and shape and surface change in uninduced HL60 cells may reflect immaturity in the protein kinase C isoenzyme pattern or in the actin regulatory mechanism.

B 227 NUCLEATION OF SPECTRIN SELF ASSEMBLY. David W. Speicher and Kevin S. Beam, Wistar Institute, Philadelphia, PA 19104.

Spectrin, a long flexible rod-like actin crosslinker, is comprised primarily of many repetitive segments about 106 residues in length. The basic conformational unit is probably the same size as the repeat unit and hinge regions between slightly overlapping adjacent conformational units produce the observed molecular flexibility. Assembly of erythrocyte spectrin monomers to form $\alpha\beta$ dimers and assembly of dimers to form tetramers have been further investigated using resin-based HPLC gel filtration and related techniques. Experiments using intact monomers have shown that monomers reassemble to heterodimers quite rapidly and with complete fidelity, even at low protein concentrations. Native dimers and reconstituted dimers are indistinquishable using several criteria. Also, dimers are more rigid than the individual subunits. Attempts to reassemble intact monomers with domain peptides and other fragments suggest that a nucleation site involving the carboxy-terminal of the α subunit and a corresponding site near the amino-terminal of the β subunit is absolutely required for assembly of dimers. In contrast, the actin binding domain is not involved in heterodimer assembly. The putative nucleation region contains a number of somewhat atypical repeats which have several insertions and deletions relative to most spectrin repeats, and these segments show the strongest homology to spectrin-like repeats in α-actinin, another actin crosslinking protein. It is likely that this slightly altered type of repeat, which is responsible for dimer formation in α-actinin, imparts specialized structural features responsible for nucleation of spectrin heterodimer assembly. While nucleation occurs at a single discrete site, many additional repeat units are involved in stabilizing the assembled heterodimer. Since fragments lacking the nucleation site can not be forced to associate with the complementary subunit even at high concentrations, it is likely that subunit-subunit assembly at the nucleation site induces a slight conformational change in the remainder of the molecule that is required to complete heterodimer assembly.

B 228 CONTROL OF ACTIN POLYMERIZATION IN FIBROBLASTS, Marc Symons, Rong Li and Tim Mitchison, Dept. Pharmacology, University of California, San Francisco, CA 94143-0450.

We are investigating the role of actin dynamics in cell translocation. We have studied the incorporation of actin monomers into the cytoskeleton by injecting biotinylated actin into locomoting NIH-3T3 fibroblasts, followed by cell permeabilization/fixation at various times post-injection and visualization of the polymerized biotin-actin by successive labelling with streptavidin and a rhodamine-biotin-dextran complex (J Cell Biol 107, 451a). We have shown that the injected actin first incorporates at the very tip of the leading edge and is subsequently transported in a centripetal fashion at a rate of about 1.1 μm/min. Furthermore, actin incorporation appears to be proportional to the extent of protrusion of the edge, implying that actin polymerization is strictly correlated to leading edge extension. In order to study the mechanisms which control the site and kinetics of actin polymerization in fibroblasts, we have studied the nucleation of actin in saponin-permeabilized cells, in which the concentration of polymerization-competent monomeric actin can be controlled. We observed that actin polymerizes predominantly at the tip of the leading edge, as was found in the injected cells. This nucleation is strongly inhibited by cytochalasin D. In conclusion, our results indicate that actin polymerization in fibroblasts is controlled by the presence of nucleating sites (provided by either free filament ends or by nucleating proteins) which are located at the protruding tip of the leading edge.

B 229 ACTIVATION OF HUMAN NEUTROPHILS (PMN) ADHERING TO ARTIFICIAL SURFACES AS DETECTED BY FLOW CYTOMETRY. W. Van De Meerendonk, T. Beugeling, J. Feijen, A. Bantjes and W.G. Van Aken. Department of Chemical Technology, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands.

Because the in vivo application of biomaterials may influence the activation of PMN we studied the expression of granule derived proteins after adhesion of PMN onto Tissue Culture Polystyrene (TCPS), compared to PMN in suspension. Therefore we applied ta monoclonal antibody (B13.9) which binds to a protein that is upregulated from secondary granules after activation. After adhesion onto TCPS, cells were detached from the surface and antigen expression was determined by means of flow-cytometry. Mean fluorescence signals (mean fl) for B13.9 antigen expression of adhering PMN were significantly higher then mean fl of PMN in suspension (Mean fl adh. PMN: 136 $\pm$ 51, mean fl susp. PMN: 100 $\pm$ 24; $p \leq 0.025$). Stimulation of adherent PMN with 10^{-8} M FMLP resulted in a strong increase of antigen expression (mean fl 533 $\pm$ 151). This increase reached a maximum within 15 min. after stimulation and did not change significantly after an additional incubation period of two hours. The increase of antigen on suspended PMN after stimulation with FMLP was less (mean fl. 317 $\pm$ 85; $p < 0.0005$). The moment PMN were stimulated proved to be important. PMN, first stimulated in suspension and allowed to adhere afterwards, did not show significant differences in antigen expression compared to PMN stimulated in suspension. This experiment was also used to show that the removal of adhering cells from the surface did not cause a significant upregulation. We also studied if adhering PMN were activated at lower FMLP concentrations than PMN in suspension. In both cases, significant upregulation of antigen expression caused by the stimulus can be noticed at concentrations from 10^{-9} M FMLP. Flow cytometry can be used to study the activation of PMN adhering to artificial surfaces. Our results prove that PMN adhering to TCPS have a stronger degranulation of secondary granules and can upregulate antigen to a stronger degree after stimulation with FMLP compared to PMN in suspension.

B 230 Ca^{2+}-DEPENDENT REGULATION OF ACTIN BUNDLING BY LIPOCORTIN-85, David M. Waisman and N. Wayne Ikebuchi, Department of Medical Biochemistry, University of Calgary, Calgary, Alberta, Canada, T2N 4N1. Lipocortin-85 (L-85) is a Ca^{2+} and phospholipid binding protein which consists of two 36 kDa subunits and two 10 kDa subunits. This protein has been shown to be an in vivo substrate of both tyrosine protein kinases and protein kinase C. Using a high speed cosedimentation assay (353,000 x g, 20 min) we have shown that L-85 binds to F-actin with a $K_{0.5}$ of 0.18 μM and saturates at 0.58 mol L-85/mol actin. Positive cooperativity in the binding event was suggested from the Hill coefficient of 1.40. The binding of L-85 to actin was totally dependent on Ca^{2+}. Using light scattering or low speed cosedimentation (15,600 x g,10 min) we have quantitated the Ca^{2+}-dependent bundling of F-actin by L-85. Bundling occurred with a K_d (L-85) of 0.27 μM, saturated at 0.63 mol L-85/mol actin and was totally dependent on Ca^{2+}. The K_d (Ca^{2+}) varied with the molar ratio of L-85/actin, typically a K_d (Ca^{2+}) of 2 μM was determined at equimolar L-85/actin. At 1.2 mol L-85/mol actin and 6.7 μM Ca^{2+}, bundling of F-actin was extremely rapid ($t_{0.5}$ of 6 sec) and reversable by chelation of Ca^{2+} ($t_{0.5}$ of 4 sec). The presence of large anisotropic F-actin bundles was confirmed by electron microscopy. These results therefore document the first example of an actin bundling protein whose activity is stimulated by micromolar Ca^{2+}.

B 231 **SPECIFIC DISRUPTION OF INTERMEDIATE FILAMENTS AND THE NUCLEAR LAMINA BY THE 19-KILODALTON PRODUCT OF THE ADENOVIRUS E1B ONCOGENE, Eileen White and Ralph Cipriani,** Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724. The E1B 19K protein is essential for proper regulation of viral gene expression in adenovirus infected cells and for oncogenic transformation. Expression of the 19K protein and the E1A gene products is sufficient for transformation of primary rodent cells, long term growth of transformed cell lines, and for anchorage independence. The 19K protein resides in the cytoplasm and the nuclear envelope where it is associated with the nuclear membranes and the lamina in transfected, infected and transformed cells. Studies with 19K mutant viruses have demonstrated that localization of the 19K protein to the nuclear envelope is required for 19K protein function. Biochemical fractionation has demonstrated the 19K protein copurifies with the insoluble lamina and cytoplasmic vimentin filaments. Transient expression of the 19K protein results in the specific disruption of cytoplasmic vimentin filaments and the nuclear lamina. Vimentin filaments become detached from their anchoring points on the plasma membrane and the nuclear envelope and form large aggregates in the cytoplasm. The nuclear lamina is reorganized such that large areas of the nuclear envelope become devoid of lamins. Microtubules and microfilaments were unaffected by 19K expression. Intermediate filament and lamina disruption was a normal occurrence in adenovirus infected cells but did not occur upon infection with an E1B 19K mutant virus. Furthermore, transformed cells which express the 19K protein also showed perturbations in the organization of intermediate filaments where those cell lines transformed by other oncoproteins did not. Therefore, we have identified two related cellular protein structures (vimentin filaments and the lamina) which are targets for functional modification by a unique viral transforming protein. This suggests a role for intermediate filaments and the nuclear lamina in modulation of viral gene expression and the process of oncogenic transformation.

Membrane-Cytoskeleton Interactions

B 300 THE Rh POLYPEPTIDES ARE A FAMILY OF MEMBRANE BILAYER SPANNING PROTEINS LINKED TO THE ERYTHROCYTE CYTOSKELETON, Peter Agre and Barbara L. Smith, Departments of Medicine and Cell Biology/Anatomy, Johns Hopkins University School of Medicine, Baltimore, MD 21205

The structural site of the Rh antigens is a non-glycosylated integral membrane protein of M_r 32,000 which is associated with the cytoskeleton. Human Rh c, D, and E polypeptides were isolated by monoclonal immunoprecipitations. Although nonhuman erythrocytes lack Rh immunoreactivity, analogs to the Rh polypeptide were isolated from monkey, cow, cat, and rat erythrocytes by hydroxylapatite chromatography. In addition, a M_r 28,000 protein with biochemical similarities was isolated from erythrocyte and kidney membranes. These proteins were compared by 2-dimensional iodopeptide analysis after chymotryptic digestion. The iodopeptide labeled at the extracellular surface was restricted to the human D, c, and E polypeptides, while an intracellular iodopeptide was conserved amongst all of the proteins. N-terminal amino acid sequencing demonstrated that the human Rh polypeptides and the M_r 28,000 protein each contained a leucine zipper motif which were distantly homologous. The presence of these related proteins in both erythrocyte and kidney membranes from diverse species suggest a fundamental role in membrane architecture, whereas the immunologic nature of the Rh antigen is a phenomenon related to a surface domain expressed only in human erythrocytes.

B 301 ALTERATIONS IN BAND 3 IN CHRONIC MYELOGENOUS LEUKEMIA (CML), Joyoti Basu, Manikuntala Kundu and Parul Chakrabarti, Department of Chemistry, Bose Institute, Calcutta 700 009, and Madan Mohan Rakshit, NRS Medical College and Hospital, Calcutta 700 014, India. CML is a hematologic malignancy characterized by excessive growth of myeloid cells and their progenitors. Our previous studies have shown that CML erythrocytes contain a decreased proportion of spectrin tetramers and show a reorganization of other cytoskeletal components and enhanced Con A agglutinability(1). The latter suggests alterations in the properties of band 3, the Con A receptor. We have now studied the alterations in the structural domains of band 3. $^{35}SO_4^{2-}$ self-exchange efflux was found to be the same in normal and CML cells. The extent of inhibition by the anion transport inhibitor DIDS, was also comparable, suggesting no alterations in the extracellular DIDS-binding domain. Alterations in the cytoplasmic domain of band 3 were evident from the altered binding of ^{125}I-ankyrin to CML erythrocytes. Estimates of high-affinity ankyrin-binding sites in normal membranes ranged from 39-45 ug/mg membrane protein, whereas for the CML patients estimates were consistently lower, ranging from 9-15 ug/mg membrane protein. It is tempting to speculate that the increased thermal sensitivity of CML erythrocytes may be due to partial loss of anchorage of the skeleton to the membrane. The long-range translational mobility of band 3 is restricted by spectrin tetramers. Since the proportion of spectrin tetramers and the number of ankyrin-binding sites is reduced in CML erythrocytes, both these factors may lead to an increased lateral mobility of band 3 in this case, and lead to clustering of band 3. The rearrangement of band 3 into clusters provides the recognition site for antibodies against senescent cells. It may therefore be speculated that the clustering of band 3 in CML erythrocytes leads to their removal from circulation, accounting in part for the anemia associated with the disease.

1. Basu et al. Biochim. Biophys. Acta (1988) 945, 121 - 126

B 302 TENSIN, A 150kD VINCULIN-BINDING PROTEIN AT THE ADHESION PLAQUE, MAY PLAY A ROLE IN THE REGULATION OF MEMBRANE-ATTACHMENT OF ACTIN FILAMENTS, James A. Butler and Shin Lin, Department of Biophysics, Johns Hopkins University, Baltimore, MD 21218

A 150kD protein designated as tensin has been purified from chicken gizzard (Wilkins, Risinger, Coffey and Lin, 1987, J. Cell Biol. 105:130a). Antibodies against this protein stained adhesion plaques of fibroblasts as well as other locations of actin filament attachment in various tissues. In blot-overlay experiments, radioactively labelled tensin was found to bind with saturability to vinculin transferred from SDS gels to nitrocellulose. Tensin was also shown to have potent actin-capping activity. In pyrene actin assays, substoichiometric levels of the protein strongly inhibited monomer association and dissociation at the barbed end of F-actin. Tensin can be separated into three isoelectric forms on 2D gels, with actin capping activity associated with the most basic of the three forms. While the physiological significance of this study remains to be proven, existing data suggest an interesting role of tensin in the regulation of attachment of actin filaments to the plasma membrane and to other cellular structures. (Supported by research grants 87-955 from the American Heart Association and GM-22289 from NIH).

B 303 AN UNSTIMULATED PROTEIN KINASE AND A 58KDA SUBSTRATE AT THE MEMBRANE-MICROFILAMENT INTERFACE OF ASCITES TUMOR CELL MICROVILLI. Coralie A.C. Carraway, Shin-Hun Juang and Yuechueng Liu, Univ. of Miami School of Medicine, Miami, FL 33101

Isolated microvilli from ascites rat mammary tumor cells are a very useful model for studying microfilament-associated proteins and enzymes. They are a highly purified, minimally perturbed plasma membrane preparation retaining associations with microfilaments. The microvilli contain significant amounts of an unstimulated kinase activity which phosphorylates several discrete microfilament (MF)-associated proteins, including a major species of 58 kDa. The 58 kDa protein is also a major component of MF-depleted microvillar membranes, from which it has previously been isolated as part of a transmembrane complex containing actin and a cell surface glycoprotein. Phosphorylation of the 58 kDa protein occurs in the intact microvilli, as observed by metabolic labeling with $^{32}P_i$ and by in vitro labeling with $\gamma^{32}P$-labeled ATP. In vitro phosphorylation studies showed that 58 kDa protein was also phosphorylated in isolated microfilament cores and a crude transmembrane complex preparation. These studies show that an unstimulated protein kinase(s) and a 58 kDa substrate protein co-localize at the membrane-microfilament interface, associating with both microfilament cores and transmembrane complex. A possible role for phosphorylation of 58 kDa in modulation of membrane-microfilament interactions is being examined.

B 304 CORRELATION BETWEEN CELL POLARITY AND ANKYRIN STABILIZATION IN CANINE KIDNEY EPITHELIAL CELLS. Carol D. Cianci, and Jon S. Morrow, Department of Pathology, Yale University School of Medicine, New Haven, CT 06510. The red cell membrane spectrin skeleton has provided a testable model for the analysis of complex cell systems. The MDCK epithelial cell line, which polarizes in the presence of cell-cell contact after the establishment of junctional complexes, also displays a similar spectrin (fodrin)-based cortical cytoskeleton. During the formation of tight cell-cell contacts in these cells, fodrin undergoes a dramatic change in its stability and distribution, and associates tightly with the membrane. Ankyrin a protein possibly involved in linking fodrin to the upperlying lipid bilayer, displayed a somewhat more diffuse distribution in tightly associated cells. By reducing the extracellular calcium concentration to 5 um (LC medium) (versus the normal 1.8 mM) cell-cell contacts were disrupted. The disruption of the tight cell-cell contract by this mechanism profoundly altered the distribution of fodrin. In normal high calcium (HC) medium, fodrin was localized to the cell periphery and exhibited a slow (t1/2 >14 hours) rate of turnover. In LC medium, fodrin redistributed to the cytoplasm, and its turnover rate increased to less than 5 hours. In normal (HC) medium, erythroid type ankyrin was localized to the nuclear, peripheral, and cytoplasmic regions of the cell. This pattern was not as dramatically affected by reduced calcium in the media, nor was there an effect of calcium on ankyrin's overall rate of turnover. These results suggest that only a relatively small pool of ankyrin may be involved with the binding of fodrin to the membrane, and raise the possibility that ankyrin-independent mechanisms may play a larger role in stabilizing fodrin at the membrane surface. One candidate for an ankyrin-independent fodrin binding site at the membrane is the cell adhesion molecule, L-CAM, which was always found to colocalized with fodrin at the cell periphery. The direct role of L-CAM in stabilizing fodrin at points of cell contact is under investigation.

B 305 MEMBRANE FRAGILITY IN SKELETAL MUSCLE MEMBRANES LACKING THE CYTOSKELETAL PROTEIN, DYSTROPHIN. Barry J. Cooper and Owen P. Hamill, Departments of Veterinary Pathology and Neurobiology and Behavior, Cornell University, Ithaca, NY 14853. Duchenne muscular dystrophy is the most common and most severe form of muscular dystrophy in man. It is inherited as an X-linked recessive trait and the gene which is defective has recently been identified. Most patients with Duchenne muscular dystrophy lack dystrophin, the product of this gene. Muscle cells of the dystrophic dog, *xmd*, also lack dystrophin and this mutant has been shown to be a genetic homologue of DMD. Dystrophin is a large (approximately 400 kD) protein thought, on the basis of sequence homology with α-actinin and spectrin, to function as a cytoskeletal component. It has been shown by immunohistochemical methods to be localized at the cytoplasmic face of the plasma membrane and is postulated to provide structural support for the sarcolemma. We have tested this hypothesis by using the patch clamp technique to measure the pressure required to rupture the plasma membrane in isolated intact canine skeletal muscle cells under two ionic conditions. Under both conditions dystrophic muscle cell plasma membrane ruptured at lower pressures than normal membrane. Both dystrophic and normal membranes ruptured at lower pressures in a calcium-free solution than in solution containing calcium, thus validating the ability of this system to measure perturbations of the plasma membrane. These differences were highly significant ($P<0.0001$). These results support the hypothesis that dystrophin functions *in vivo* to provide structural support to the sarcolemma.

B 306 PRIMARY SEQUENCE OF THE 110kD-CALMODULIN COMPLEX OF THE AVIAN INTESTINAL MICROVILLUS . STRUCTURAL AND FUNCTIONAL ANALYSIS. E. Coudrier*, A. Garcia*, J. Carboni**, J. Anderson**, J. Vandekerkhove+, M. Mooseker**, D. Louvard*, M. Arpin*. *Institut Pasteur, Département de Biologie Moléculaire, 3,25, rue du Docteur Roux, 75724 Paris cedex 15, France. ** Department of Biology, Yale University, New Haven, Connecticut 06511-8112. USA. +Laboratorium voor Genetica Rijksuniversiteit Gent, B-9000 Gent, Belgium.
The actin bundle within each microvillus of the intestinal brush border is laterally thethered to the membrane by bridges composed of the protein complex, 110kD-calmodulin. Previous studies have shown that avian 110kD-calmodulin shares many properties with myosins including mechanochemical activity. In the present study, a cDNA molecule encoding 1000 amino-acids of the 110kD protein has been sequenced, providing direct evidence that this protein is a vertebrate homologue of the tail-less, single-headed myosin I first described in amoeboid cells. The primary structure of the 110kD protein (or Brush Border myosin I heavy chain) consists of two domains, an amino-terminal "head" domain and a 35kD carboxy terminal "tail" domain. The head domain is homologous to the S1 domain of other known myosins, with highest homology observed between that of Acanthamoeba myosin IB and the S1 domain of the protein encoded by bovine myosin I heavy chain gene (MIHC; Hoshimaru and Nakanishi, 1987. J. Biol. Chem. 262:14625-14632). The carboxy-terminal domain shows no significant homology with any other known myosins except that of the bovine MIHC. This demonstrates that the bovine MIHC gene most probably encodes the heavy chain of bovine Brush Border Myosin I (BBMI). Northern blot analysis of various chicken tissues indicates that Brush Border Myosin I heavy chain is preferentially expressed in the intestine.

B 307 ANTIBODIES THAT DISCRIMINATE BETWEEN THE PROENZYME AND ACTIVATED FORMS OF EACH CALPAIN (CANP), D.E. Croall, C.A. Slaughter*, H.S. Wortham, Depts. of Physiology and *Biochemistry University of Texas Southwestern Medical Center, Dallas, Texas 75235. Many cytoskeletal proteins are susceptible to proteolytic cleavage in vitro by the intracellular, calcium dependent proteases (CDPs) known as calpains or CANPs. Proteins linking transmembrane proteins to the actin cytoskeleton (e.g. talin) or to the membrane skeleton (e.g. ankyrin, fodrin) are particularly good substrates. Several substrates are modified in only a limited way and current evidence suggests that such proteolytic cleavages may have regulatory significance. These proteases exist in cells predominantly in an inactive, proenzyme form consisting of an 80 kDa and 30 kDa protein. After binding to Ca^{2+} each enzyme subunit is proteolytically modified to produce an activated, heterodimer (78 kDa/17 kDa). Using immunoblot analysis to quantify covalent changes in the smaller subunit we have identified the activation of CDP-1 in erythrocytes treated with ionomycin and Ca^{2+} (Biochemistry 28, 6882). To develop antibodies specific for the proenzyme forms of each protease subunit the N-terminal sequences of the activated CDP-1 (bovine) were determined by automated Edman degradation. These sequences; LGRHENA and ANESEEV were aligned with published sequences derived from various mammalian cDNA libraries. Peptides were synthesized to correspond to sequences surrounding the activation-cleavage sites, conjugated with KLH and used as antigens for raising polyclonal antisera. Characterization of these anti-peptide antisera by immunoblotting demonstrates significant specificity of each antiserum for the proenzyme form of its respective subunit. By comparison with antisera that recognize both the proenzyme and activated forms we will be able to identify cellular conditions that induce activation of each CDP.

B 308 T LYMPHOCYTE INTERACTION WITH anti-T CELL RECEPTOR (TCR) ANTIBODIES: ROLE OF CYTOSKELETAL COMPONENTS. K.E. DeBell, M.S. Taplits, T. Hoffman, and E. Bonvini. Laboratory of Cell Biology, DBBP, CBER, Bethesda, MD 20892. The conditions and requirements for the interaction of cloned murine helper T lymphocytes (Th) with immobilized anti-TCR antibodies (Ab) have been studied using a novel assay system. Radiolabeled, polystyrene beads coated with either the hamster monoclonal Ab (MoAb) 145-2C11 (which recognizes the CD3 epsilon chain of the TCR), the murine MoAb F23.1 (directed against the $V_{\beta 8}$-encoded determinant of the alpha/beta heterodimer of the TCR), or a proper control Ab were incubated with Th cells. Bead-to-cell contact was promoted by centrifugation, and cell-associated beads were separated from unbound beads on a Percoll gradient. Conjugate formation between anti-TCR Ab-coated beads and Th cells was specific, stable, temperature- and energy-dependent, and was inhibited by pretreating the cells with cytochalasins, but not vinca alkaloids. Conjugate formation, therefore, required microfilament assembly, but not the microtubule apparatus. Perturbation of the TCR by either immobilized anti-TCR Ab or soluble anti-TCR Ab (aggregated with a second anti-hamster or anti-mouse Ab) results in the generation of signals, including inositol phospholipid (InsPL) hydrolysis. Notably, InsPL hydrolysis induced by soluble anti-TCR Ab was substantially lower than that produced by immobilized Ab. Pretreatment with cytochalasin B did not affect InsPL hydrolysis induced by immobilized anti-TCR Ab, but enhanced, in a dose dependent manner, that induced by aggregated, soluble Ab, approaching the level obtained with immobilized Ab. Treatment with cytochalasins in absence of TCR perturbation had no significant effect on InsPL hydrolysis. These data suggest that signal transduction occurs independently of conjugate formation. Microfilament assembly may be a regulatory mechanism which controls the extent of signal generation.

B 309 IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF VINCULIN AND TALIN IN CHICKEN EMBRYO FIBROBLASTS, Constance A. Feltkamp, Ruud Brands and Ed Roos, Department of Cell Biology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands.
Models of the spatial distribution of interacting molecules concentrated at adhesion plaques are based mainly on chemical data and immunofluorescence studies. With the "wet-cleaving" method, labeling of these components with gold particles for immunoelectron microscopy is facilitated. We studied the distribution of talin and vinculin in normal adhesion plaques, during cell spreading and after transformation by ts-RSV. In fully developed adhesion plaques both molecules were concentrated in a thin layer of moderately electron-dense material which was quite strongly attached to the inner face of the membrane. This layer showed a substructure of interconnected parallel bands that followed the same direction as overlaying bundled microfilaments. On stereomicrographs no difference in distance between the inner face of the membrane and either vinculin or talin was found. In early spreading cells vinculin and talin were colocalized at fine radiating streaks covering the ventral membrane at the rather large domains of close contact with the substrate. These were overlayed by an isotropic network of actin filaments, of which only the outermost were attached to and followed the direction of the streaks. Studies on ts-RSV infected cells showed that a high concentration of vinculin was not a prerequisite for attachement of bundled microfilaments to adhesion plaques. Early after the onset of transformation, seemingly intact adhesion plaques with practically no vinculin but a normal amount of talin were found. At later stages, also talin disappeared and microfilaments were no longer attached to the membrane.

B 310 REDISTRIBUTION OF SPECTRIN (FORDIN) DURING MITOSIS IN CHINESE HAMSTER OVARY CELLS, Velia M. Fowler and Ermone J.H. Adam, Department of Molcular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037. Spectrin in non-erythroid cells is proposed to influence the generation of plasma membrane domains, membrane stability and architecture, as well as cell-cell and cell-substratum attachment. Chinese hamster ovary (CHO) cells express an isoform of spectrin that is recognized by affinity purified antibodies prepared against bovine brain spectrin (fodrin) and comigrates with authentic bovine brain spectrin α (M_r 240,000) and β (M_r 235,000) subunits on SDS-polyacrylamide gels. Immunofluorescence staining of substrate-attached, well-spread interphase CHO cells reveals a fibrillar, plasma membrane-associated localization of spectrin as has been described previously for other fibroblastic cell types. In contrast, immunofluroescence staining of spectrin in round mitotic cells reveals a uniform cytoplasmic localization. Biochemical analysis of spectrin solubility in Triton X-100 extracts of interphase and mitotic cells demonstrates that there is about a 1.5-fold increase in the amount of soluble spectrin during mitosis, as determined by immunoadsorption of spectrin from ^{35}S-methionine labelled cells. This increase in spectrin solubility is correlated with a 1.5-fold increase in the level of phosphorylation of the β-subunit of spectrin. We hypothesize that phosphorylation of spectrin in mitosis may regulate the association of spectrin with the plasma membrane and contribute to the morphological and functional changes in the plasma membrane that occur during mitosis.

B 311 ANTIBODIES TO THE CYTOSKELETON PROTEINS TUBULIN, ACTIN AND TROPOMYOSIN BIND TO THE SURFACE OF HUMAN CAPILLARY ENDOTHELIAL CELLS AND HUMAN LUNG FIBROBLASTS, Peter French, Kim Pryor, Christine Hicks and Ronald Penny, Centre for Immunology, St Vincent's Hospital, Sydney, NSW, 2010 Australia. Antibodies to a range of cytoskeleton proteins have been used to probe the surface of glutaraldehyde-fixed and living normal human fibroblasts and endothelial cells in culture. Antibodies to actin, tubulin and tropomyosin bind to the surface of both cell types, whereas antibodies to spectrin, alpha-actinin, vinculin and vimentin do not bind. The addition of anti-cytoskeletal antibodies to cultures of fibroblasts demonstrably increases the uptake of ^{3}H-thymidine. It appears, therefore, that actin, tubulin and tropomyosin are present on the surface of normal human cells and may act as targets *in vivo* for circulating anti-cytoskeletal auto-antibodies which arise in a number of disease states.

B 312 DESMOPLAKINS: POSSIBLE CONNECTING LINKS BETWEEN INTERMEDIATE FILAMENTS AND THE CELL SURFACE, Kathleen J. Green, David A.D. Parry , Ma. Luisa A. Virata, Rita M. Wagner, Brigitt D. Angst, and Laura A. Nilles, Dept. of Pathology, Northwestern Univ. Med. School, Chicago, IL 60611 and Dept. of Physics and Biophysics, Massey University, Palmerston North, New Zealand . Desmoplakins (DPs) I and II are related proteins localized in the innermost portion of the desmosomal plaque. Although it has been suggested that the DPs may mediate an interaction between intermediate filaments (IF) and the plaque, relatively little is known regarding their structure or relationship with other proteins. Human cDNA clones encoding two major structural domains of DP were isolated from human foreskin λgt11 libraries using previously characterized bovine DP cDNA clones as probes. The predicted amino acid sequence obtained so far for DPI appears to comprise two major domains. Computer-aided analysis indicates that the central domain contains a series of heptapeptides written as $(a,b,c,d,e,f,g)_n$ with the *a* and *d* positions usually occupied by apolar residues. This pattern strongly predicts a left-handed coiled-coil dimer ~130nm in length. The number of possible interchain ionic interactions was determined to be greatest for two chains oriented parallel and in register. The periodicity of acidic and basic residues in this central rod suggests that DPI will form higher-order filamentous structures either by self-aggregation or aggregation with similar molecules. The rod domain of DPII is predicted to be significantly shorter than that of DPI presumably due to the splicing out of ~1.8Kb of this domain, a fact which has important implications for stability of the DPII dimer *in vivo*. The C-terminus contains three highly homologous regions each of which comprises almost five repeats of a 38 residue motif. It is likely that these regions each fold into a compact globular conformation stabilized by intrachain ionic interactions. Significantly, the periodicity in acidic and basic residues of these domains is precisely that found in the 1B rod domain of IF proteins. This suggests the possibility that the C-terminal domains of DPs might interact with IF via their common periodicity of charged residues.

B 313 INTERACTION OF THE E-CADHERIN CELL ADHESION MOLECULE WITH CYTOPLASMIC PROTEINS, Barry Gumbiner and Pierre McCrea, Department of Pharmacology, University of California, San Francisco, CA 94143. The epithelial cell-cell adhesion protein E-cadherin is believed to interact with the membrane-cytoskeleton. We have investigated the nature of the interaction of E-cadherin with cytoskeletal proteins in monolayers of polarized epithelial cell lines. In both confluent and subconfluent monolayers of the *Xenopus* A6 kidney cell line and the canine MDCK kidney cell line E-cadherin is highly extractable by a variety of isotonic buffers containing mild nonionic detergents. In confluent A6 cells, fodrin remains largely in the detergent insoluble residue under these same conditions. Also, we were unable to detect fodrin in anti-E-cadherin immunoprecipitates of A6 cell extracts. These results suggest that little of the E-cadherin in polarized epithelial cells is associated with the spectrin-based detergent insoluble membrane-skeleton, unless the interaction is extremely labile. E-cadherin is extracted from these cells as a large oligomeric protein complex in association with a set of cytoplasmic polypeptides, named catenins (Ozawa, M., et al. *EMBO J.* **8**, 1711-1717, 1989). We have been able to purify one of these polypeptides of MW = ~100kD because it interacts most strongly with E-cadherin under stringent buffer conditions. By criteria of antibody crossreactivity this 100 kD protein does not seem to be alpha-actinin, vinculin, or adducin, proteins of similar MW.

B 314 CHARACTERIZATION OF HUMAN ERYTHROCYTE PROTEIN 4.1-CALMODULIN (CaM) INTERACTIONS, Alan S. Harris, Carol D. Cianci, Sheenah M. Mische and Jon S. Morrow, Department of Pathology, Yale University School of Medicine, New Haven, CT 06510. Human erythrocyte protein 4.1 (4.1) potentiates the interaction between spectrin and f-actin forming the major infrastructure of the erythrocyte cytoskeleton. Additionally, 4.1 links the cytoskeleton to the membrane via its interaction with the integral membrane proteins band 3 and glycophorin A. These interactions have been demonstrated to be regulated by several second messenger systems. Protein 4.1, purified by ion exchange chromatography of a high ionic strength extract of human erythrocyte inside out vesicles, bound to a CaM-agarose column in a calcium dependent manner, and was eluted with either EGTA or the CaM antagonist W-7. Non-denaturing PAGE in the presence of calcium demonstrated that 4.1 and ^{125}I-CaM formed a complex that migrated faster than unbound 4.1. Sedimentation velocity of 4.1 + CaM in calcium containing sucrose gradients yielded a complex of 3.5S, while in the absence of CaM, 4.1 migrated at 3.2S, similarly, 125I-CaM migrated at 1.8S in the absence of 4.1 and in the presence of 4.1 at 3.5S. Crosslinking experiments of 4.1 + ^{125}I-Cam with a homobifunctional N-hydroxysuccinimide ester yielded radioactive species of 120 and 100 kDa on SDS-PAGE. Estimates of the CaM:4.1 stoichiometry in the 120 and 100 kDa ^{125}I-labeled species were 1.8:1 and 1.4:1 respectively. The CaM binding site on 4.1 is hypothesized to be near the chymotrypsin cleavage site that generates the characteristic 30 and 16 kDa fragments since inclusion of Ca^{2+}/CaM prevents only this cleavage when 4.1 is digested with chymotrypsin. The data presented here introduces another site for regulatory control of the cytoskeleton by CaM.

B 315 POTENTIAL INVOLVEMENT OF ACTIN AND MYOSIN IN BULK PACKAGING AND RETROGRADE REDISTRIBUTION OF AXOPLASM IN GROWING AXONS, Edward Koenig, Sarah Finnegan Sloan and Vance Lemmon, Department of Physiology, SUNY at Buffalo, Buffalo, NY 14214 and Center for Neurosciences, Case Western Reserve Univ., Cleveland, OH 44106

Monoclonal antibody 8A2 (mouse IgM) consistently triggers an evacuation of axoplasm from distal growing axons, involving formation of a proximally moving aggregate mass which may also include a contiguous column of axoplasm of varying length. The process appears to involve three overlapping phases: (1) retrieval of axoplasm from filo/lamellipodia, (2) aggregation of retrieved axoplasm into a varicose pleomorphic mass, and (3) bulk retrograde movement. The mass often exhibits dynamic protrusive activity in all directions which appears to "drive" the retrograde movement. Cytochalasin D rapidly blocks each of the phases of the process, and may trigger a partial disaggregation. Phalloidin fluorescence is strong and appears as discrete aggregate forms in the active pleomorphic mass while otherwise appearing as filamentous and strand-like in contiguous regions. Myosin immunofluorescence co-localizes with phalloidin fluorescence suggesting a role for myosin in powering the second and third phases of the response.

B 316 DEGRADATION OF SPECTRIN FROM THE EYE LENS BY CALCIUM ACTIVATED NEUTRAL PROTEASE, Julia M. Marcantonio, Roger J.W. Truscott* and George Duncan, School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, U.K. and *Department of Chemistry, University of Wollongong, Wollongong, NSW 2500, Australia.

Opacification of the eye lens in man is usually accompanied by an increase in lens calcium. Incubation of animal model lenses in conditions which promote increased intracellular calcium results in opacification of the outer cortex. Comparison of the protein profiles of the cytoskeletal fractions of rat lenses by SDS PAGE shows a marked decrease in several polypeptides in high calcium lenses. These include vimentin (58 kDa) and a triplet (230, 235, 245 kDa) which cross reacts with monospecific polyclonal antibody to sheep erythrocyte spectrin. Degradation occurs via a number of transient immunoreactive polypeptides of approx. 150 kDa. Cytoskeletal proteins isolated from clear bovine lenses were incubated with the 80 kDa subunit of calcium activated neutral protease (CANP. EC 3.4.22.17) in the presence of calcium. A number of polypeptides were rapidly lost, and spectrin was again degraded via 150 kDa polypeptides. Lens transparency depends on a high degree of structural regularity and ordered packing of the lens-specific proteins (crystallins) within the fibre cells. Since CANP is known to be present in the epithelium and cortical fibres, disruption of the cytoskeletal-membrane complex by CANP may be an important mechanism whereby increased calcium leads to cataract formation.

B 317 IMMUNOCYTOCHEMICAL TOPOGRAPHY OF INTEGRINS AND MICROFILAMENT BUNDLES IN CULTURED HUMAN KERATINOCYTES, Pier Carlo Marchisio°, Michele De Luca*, Sergio Bondanza*, Richard N. Tamura^, Ranieri Cancedda* and Vito Quaranta^, °Dip. di Scienze Biomediche e Oncologia, Univ. di Torino, 10126 Torino, Italy, *IST, Ist. Naz. per la Ricerca sul Cancro, 16132 Genova, Italy, ^Dept. of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

The distribution of integrins in colonies of human keratinocytes *in vitro* was studied using indirect immunofluorescence. In agreement with biochemical studies, antibodies to the β_1 and β_4 chains, but not to the β_2 and β_3 chains, were found to react with keratinocytes. Distinct localization patterns were observed with anti-$\alpha_E\beta_4$ (S3-41) and anti-β_1 (A-1A5, a gift of M. Hemler) antibodies. S3-41 stained keratinocytes intensely on the basal surface in those areas of the plasma membrane in direct contact with the substrate with a pattern of large connected patches. An identical pattern was observed with an anti-α_6 antibody (GOH3, a gift of A. Sonnenberg) supporting the idea that it is in fact the heterodimer $\alpha_E\beta_4$ that is expressed at the basal surface. No anti-β_4 reactivity was observed in the cellular contour, i.e., in areas of cell-to-cell contacts, or in the apical zones. In keratinocytes double-stained for F-actin the patches of $\alpha_E\beta_4$ had mutually exclusive location with microfilament bundles adjacent to the inner face of the ventral membrane. A sharply different pattern was obtained with the anti-β_1 mAB A-1A5, which stained only the periphery of cells, i.e., the areas of plasmamembrane involved in cell-to-cell contact in the growing keratinocyte colonies. No β_1 staining was seen in the basal surface areas found reactive with anti-β_4. Similarly, cell footprints that were readily reactive with anti-β_4 were not stained with anti-β_1. Antibodies to α chains known to associate with β_1 were tested and found to be negative except those to α_2 and α_3 (12F1 and J143, gifts of V. Woods and A. Albino) which stained sites of intercellular contact, in a fashion essentially identical to anti-β_1. These results show that the $\alpha_E\beta_4$ integrin is restricted to the basal membrane and α_2-$\alpha_3\beta_1$ to the lateral membrane of keratinocytes, in a rather mutually exclusive distribution.

B 318 THE HELICAL STRUCTURE OF ERYTHROCYTE SPECTRIN: IMPLICATIONS FOR RED CELL ELASTICITY, Amy McGough and Robert Josephs, Department of Molecular and Cell Biology, University of Chicago, Chicago, IL 60637

The human erythrocyte's ability to elastically deform during its turbulent passage in the vasculature is generally believed to derive from the membrane skeleton. The skeleton is comprised of dodecamers of actin which are interconnected by spectrin molecules to form a network on the cytoplasmic face of the membrane. Because spectrin is a flexible molecule capable of reversible changes in structure in vitro it is commonly thought to be responsible the red blood cell's elasticity.

Fourier analysis of electron micrographs of negatively stained spectrin reveals that it is a two-start helix with approximate two-fold rotational symmetry. A three-dimensional reconstruction of the mass distribution of the particle (using the filtered back-projection method) supports this interpretation of spectrin's structure. We have found that the pitch and diameter of spectrin are inversely related and can vary from molecule to molecule. These data suggest that the elastic properties of the red cell are the result of extension or contraction of the helical spectrin molecule.

B 319 STIMULUS-DEPENDENT ASSOCIATION OF HUMAN NEUTROPHIL GLYCOPROTEIN 140 WITH THE CYTOSKELETON, William M. Nauseef, Kurt B. Stevenson, and Robert A. Clark, Department of Medicine, College of Medicine, University of Iowa, Iowa City, IA 52242

Polymorphonuclear neutrophils (PMNs) are motile cells which are the major effector cells in host defense against pyogenic organisms. The predominant surface protein radiolabelled by NaB^3H_4 is a glycoprotein of 140-kDa (gp140) which binds wheat germ agglutinin and is thought to be important for normal PMN chemotaxis. In order to identify associations of gp140 with the cytoskeleton, we compared the distribution of gp140 in Triton X-100 soluble (TxS) and insoluble (TxI) preparations of resting and stimulated PMNs. In unstimulated PMNs, gp140 was in the TxS fraction but shifted to the TxI pellet after stimulation with the chemotactic peptide formyl-methionyl-leucyl-phenylalanine (FMLP, 10^{-8}M). In contrast, immunoblots demonstrated no change in the TxS-distribution of CD11b, the α-chain of the leukocyte CD11/18 integrin complex. In addition, immunochemical analysis of TxI pellets from isolated PMN plasma membrane vesicles demonstrated the presence of fodrin, 4.1 and small amounts of actin, whereas a myeloid analogue of erythrocyte 4.2 was associated with TxS fraction from these vesicles. Taken together, these data suggest that with stimulation, gp140 becomes associated with elements of a cytoskeleton which appear to be associated with the PMN plasma membrane. We believe that the interactions of functionally important membrane proteins with these cytoskeletal proteins may be critical for signal transduction in PMN locomotion, degranulation, and initiation of the respiratory burst.

B 320 TALIN ASSOCIATIONS IN LIVING CELLS, G. Nuckolls, C. Turner, and K. Burridge, Dept. Cell Biology and Anatomy, UNC Chapel Hill, NC 27599.

We have examined the functional domains of talin in living fibroblasts and epithelial cells. Proteolytic cleavage of talin resulted in a large fragment (200kD) and a small fragment (47kD) which were purified and labeled with fluorescent dyes. The large talin fragment, which contains a vinculin binding domain as shown by Western blot overlays, localized to focal adhesions (FA) when microinjected into cultured fibroblasts. The small talin fragment also expressed an affinity for FA, suggesting that talin has more than one site that contributes to its localization in FA. The microinjection of affinity purified polyclonal anti-talin antibodies into spreading fibroblasts immunoprecipitated talin in these cells. This impaired cell spreading and caused the formation of abnormal focal adhesions as judged by interference reflection microscopy. Despite the apparent absence of talin from these structures, vinculin remained associated with these sites of stress fiber termination. This suggests that actin can link to the membrane without talin, although talin appears necessary for normal FA organization. The zonulae adherentes (ZA) of epithelial cells contain vinculin but not talin even though both talin and vinculin are concentrated in FA in the same cells. However, the large talin fragment microinjected into epithelial cells became associated with both the ZA and FA. This suggests that the small talin domain may regulate the distribution of talin in epithelial cells. Supported by NIH grant, GM29860.

B 321 THE MOLECULAR CLONING AND SEQUENCE ANALYSIS OF CHICK EMBRYO FIBROBLAST TALIN, Vasken Ohanian, Jasper Rees*, Richard Hynes* and David Critchley, Department of Biochemistry, University of Leicester, Leicester, England & *Department of Biology, MIT, Boston, USA.

Talin is a major cytoskeletal protein localised at focal adhesion sites. By virtue of its ability to bind the transmembrane fibronectin receptor:integrin and vinculin, talin links actin filaments to the membrane. Furthermore, it is a substrate for pp60v-src, and its phosphorylation by v-src is paralleled by a rapid loss of stress fibres and focal contacts. We report the successful isolation of several overlapping cDNA clones for talin. A chick embryo fibroblast library in λgt11 was screened with a polyclonal antibody against chick gizzard talin. The complete nucleotide sequence of the isolated clones was determined utilizing double stranded plasmid sequencing after generating unidirectional nested deletions with Exonuclease III. Partial amino acid sequence data obtained from CNBr treated talin was used to authenticate the clones. The cDNA's cover approximately 4.6kb of the 8.5kb talin transcript. Talin cDNA's were subcloned into a bacterial expression vector and the talin polypeptide expressed as a fusion protein. Using vinculin gel overlays on the expressed proteins, we have identified a region on talin which contains the vinculin binding site.

B 322 IgE RECEPTOR TOPOGRAPHY PREDICTS RECEPTOR ACTIVITY. Oliver, J.M. JC. Seagrave, J.R. Pfeiffer, B. Wilson, G.G. Deanin. Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM 87131

In RBL-2H3 rat tumor mast cells, crosslinked IgE receptors interact with a GTP-binding protein to stimulate Ca influx and secretion. Crosslinked receptors can also associate with the detergent-insoluble fraction (the "cytoskeleton") of RBL-2H3 cells, forming topographically-restricted cell surface clusters visible by gold-labelling and scanning electron microscopy. The G protein-dependent events of IgE receptor-mediated Ca influx and secretion are impaired in GTP-depleted cells and in cells incubated with high concentrations of crosslinking antigen; these same conditions enhance receptor aggregation and cytoskeletal association. Conversely, secretion is enhanced by treatment with dihydrocytochalasin B that delays the conversion of receptors to the detergent-insoluble form. It is proposed that crosslinked receptors can associate with G proteins to promote secretion or can aggregate in association with cytoskeletal elements to terminate secretion. Net secretion is determined by the balance of these alternative coupling pathways.

B 323 Cytoskeletal organization of contact, coated-membrane, and receptor domains of AChR clusters in cultured rat myotubes. D.W.Pumplin and J.C.Strong, Dept. of Anatomy, Univ. of Maryland at Baltimore, Baltimore, MD 21201. Acetylcholine receptor (AChR) clusters form at sites of attachment between ventral membranes of cultured rat myotubes and their substrate. Clusters are composed of three distinct non-overlapping domains differing in their cytoskeletal, intramembrane, and extracellular portions, as visualized by thin-sectioning, freeze-fracture, and quick-freeze, deep-etch rotary replication techniques. Contact domains are similar to focal contacts of fibroblasts, having overlying bundles of actin microfilaments that run parallel to the membrane and are attached to it by short orthogonal filaments. Membrane of both contact and clathrin-coated domains lies close to the substrate and is attached to it by short filaments. Clathrin-coated domains resemble those of cultured fibroblasts and are often prominent at the lateral edges of clusters. Receptor domains contain AChR IMP in an even distribution. Immunogold labeling shows that a beta-isoform of spectrin, 43K, and 58K proteins lie close to these IMP. The cytoskeleton of receptor domains contains a network of cytoplasmic projections linked by small diameter filaments. Longer filaments, resembling actin microfilaments, attach to this network. The three domains do not appear to be linked to each other by cytoskeletal elements, and pharmacological experiments suggest that they are independently stabilized. Supported by NIH and the Muscular Dystrophy Association.

B 324 LIGAND-INDUCED REDISTRIBUTION OF LYMPHOCYTE SURFACE ACTIVATION ANTIGENS ACCOMPANIED BY SELECTIVE CYTOSKELETAL REORGANIZATION, Stephen J. Rosenman, Amir A. Ganji, and W. Michael Gallatin, Department of Cell Biology and Immunology, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Circulating lymphocytes express the CD44 cell surface glycoprotein which is involved in recognition of high endothelial venules (HEV) of lymph nodes, gut-associated lymphoid tissues, and inflammatory synovia prior to extravasation to these tissues. Recent data have also implicated CD44 as an accessory molecule in T cell activation. Direct immunofluorescent examination of the cell surface distribution of CD44 using monoclonal antibodies (MAb) to distinct epitopes showed it to be uniformly distributed on peripheral blood lymphocytes (PBL) from the pig-tailed macaque (*M. nemestrina*). CD44 could be aggregated into patches and caps by indirect immunofluorescence using a multivalent second stage, and this process was inhibited at 0°C or by the presence of azide. CD44 redistributes under capping conditions independently of several other lymphocyte markers studied. Particularly striking is the formation of mutually exclusive patches by separately cross-linked MAbs to CD44 and CD2, a known activation molecule, as observed by 2-color confocal microscopy. Immunofluorescent examination of cytoskeletal proteins in these lymphocytes reveals dramatic reorganization of microtubules and intermediate filaments, and probably microfilaments. However, certain cytoskeletal components preferentially co-localize with only one patched surface antigen. We speculate that these observations reflect early events in transmembrane signal transduction, and that differential association of cytoskeletal components with lymphocyte activation antigens provides a mechanism to regulate T cell responses.

B 325 **ANKYRIN-PROTEIN 4.2 ASSOCIATIONS IN RED CELL MEMBRANES: EVIDENCE FOR DIRECT INTERACTIONS.** Anne C. Rybicki, Eric E. Bouhassira, Ronald L. Nagel, Lanping Amy Sung, Shu Chien and Robert S. Schwartz. Division of Hematology, Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY 10467, and Department of AMES-Bioengineering and Medicine, University of CA at San Diego, La Jolla, CA 92093.

Protein 4.2 constitutes ~5% of the protein mass of human red blood cell (RBC) membranes yet its function is still largely unknown. We have investigated the functional relationships between protein 4.2 and ankyrin by examining the membrane binding of these proteins in human protein 4.2 deficient (-4.2) or mouse ankyrin-deficient (-2.1) RBCs. We find that in -4.2 RBCs, ankyrin binding to the membrane is compromised as evidenced by a ~70% decreased content of ankyrin in inside-out vesicles (IOVs) prepared from these cells. Reconstitution of -4.2 membranes with purified protein 4.2 partially restores IOV ankyrin content. In -2.1 RBCs (normoblastosis homozygous), the amount of protein 4.2 bound to the membrane is reduced by ~80%. This decrease is apparent despite active protein 4.2 synthesis. These results suggest that protein 4.2 and ankyrin influence the membrane binding of the other protein. Recently, we have cloned and sequenced the cDNA for human RBC protein 4.2. The protein 4.2 sequence indicates the presence of structurally asymmetric domains; enriched in either cysteines, hydrophobic or charged amino acids. Furthermore, protein 4.2 exhibited strong homology with transglutaminases. Protein 4.2 sequence information should aid in the determination of the domains involved in ankyrin binding.

B 326 THE CA^{++}-DEPENDENT CELL ADHESION MOLECULE UVOMORULIN IN MDCK CELLS: BIOCHEMICAL STUDIES AND INTERACTION WITH THE MEMBRANE CYTOSKELETON.
Eileen M. Shore and W. James Nelson, Institute for Cancer Research, Philadelphia, PA 19111. The cell adhesion molecule uvomorulin (E-cadherin) appears to be important for the regulation of cell-cell contact in MDCK cells. Cell-cell contact induces the assembly and accumulation of elements of the membrane cytoskeleton (ankyrin and fodrin) and specific integral membrane proteins (such as Na^+K^+ATPase) in the regions of cell-cell contact at the lateral plasma membrane. Our observations of the co-localization of these proteins and uvomorulin suggest the possibility of direct molecular interactions that co-ordinate cell-cell contact with cytoskeleton assembly and polarization of the cell. Analyses of proteins solubilized from MDCK cells and fractionated in sucrose gradients and non-denaturing polyacrylamide gels show the presence of a protein complex that includes uvomorulin, ankyrin and fodrin. To investigate the formation and function of such complexes, the temporal and spacial regulation of uvomorulin synthesis, processing, and turnover in the presence and absence of cell-cell contact have been examined through biochemical studies.

B 327 STUDIES ON A NEW 68KD FOCAL ADHESION PROTEIN. Christopher E. Turner, Keith Burridge and John R. Glenney Jr*. Dept of Cell Biology and Anatomy, UNC-Chapel Hill, NC27599-7090 and *Dept. of Biochemistry, University of Kentucky Cancer Ctr, Lexington, KY 40536-0084.
It has previously been demonstrated that a protein of approximately 68-76Kd, which is heavily phosphorylated on tyrosine in RSV transformed CEFs localises to focal adhesions in normal cells (Glenney et al JCB 108 2401). We show here that, like many other focal adhesion proteins, the 68Kd antigen is present primarily in smooth muscle and to a lesser extent in skeletal and cardiac muscle. Interestingly, the protein is also present in mammalian platelets where it displays a molecular weight of 85Kd. Immunofluorescence of epithelial MDBK cells reveals that, like talin and vinculin, the 68Kd protein localises to the focal adhesions of these cells. However, it is absent from the zonula adherens cell-cell junctions where vinculin but not talin is found. We are currently trying to purify the 68Kd protein from chicken smooth muscle where half of the protein is water soluble and the remainder requires a high salt extraction. Although the protein often appears as a diffuse band on immunoblots, based on the solubility properties we conclude that it is not an integral membrane protein. Its low abundance in comparision to talin and vinculin suggests that the 68Kd protein may have a regulatory function in focal adhesions rather than a structural one. Supported by GM29860 to KB and GM32866 to JG.

B 328 PROTEOLYSIS OF CYTOSKELETAL PROTEINS IS NOT REQUIRED FOR COMPLEMENT-INDUCED VESICULATION OF THE PLATELET PLASMA MEMBRANE AND EXPOSURE OF THE FACTOR Va RECEPTOR, Therese Wiedmer, Sanford J. Shattil and Peter J. Sims, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104 and Department of Medicine, University of Pennsylvania, Philadelphia, PA 19104.
The role of calcium and intracellular calpains in the expression of platelet prothrombinase activity was investigated. Incubation of platelets with complement proteins C5b-9 resulted in α- and dense granule secretion, exposure of membrane binding sites for coagulation factors Va and Xa, and release of microparticles from the cell surface. Generation of these microparticles was dependent on extracellular calcium, and was accompanied by proteolytic degradation of the cytoskeletal proteins, actin binding protein (ABP), talin, and myosin heavy chain. Pre-incorporation of the calpain inhibitor, leupeptin, into the platelet cytosol blocked C5b-9-induced proteolysis of ABP, talin, and myosin. However, inhibition of this calpain-mediated proteolysis had no effect on platelet secretion, generation of microparticles, exposure of membrane sites for factors Va and Xa, or expression of prothrombinase activity. The microparticles that formed in the presence of leupeptin contained intact ABP, talin, and myosin heavy chain. Prior depletion of ATP with metabolic inhibitors did not affect C5b-9-induced microparticle formation or exposure of binding sites for factor Va. These data indicate that formation of microparticles and expression of platelet prothrombinase activity induced by C5b-9 are dependent upon an influx of calcium, but do not require metabolic energy or calpain-mediated proteolysis of cytoskeletal proteins.

Cytoskeleton in Development and Neoplasia

B 400 REGULATION OF VINCULIN GENE EXPRESSION DURING GROWTH ACTIVATION OF 3T3 FIBROBLASTS BY SERUM FACTORS, Avri Ben-Ze'ev, Department of Genetics, Weizmann Institute of Science, Rehovot 76100, Israel.
Changes in the organization of focal contacts are part of the rapid cellular response to stimulation with growth factors. We have therefore studied the expression of the gene coding for the adherens junction protein vinculin in Balb/c 3T3 cells following growth activation of density arrested monolayer cultures and of cells arrested by anchorage denial in suspension culture, which were subsequently allowed to reattach and spread. Under both conditions of growth stimulation, in the presence of serum, transcription of the vinculin gene was transiently induced (up by 30 to 45 min after stimulation, down by 2 h), followed by a transient increase in mRNA and protein synthesis levels (up by 2 h down by 5 h). PDGF and FGF could replace serum, but EGF was unable to produce this effect. Reattachment from suspension culture in the absence of serum on either plastic, fibronectin or extracellular matrix-coated substrates allowed cell adhesion and spreading, but no induction of vinculin expression was observed. Furthermore, in such cells vinculin was not found in adhesion plaques, but displayed a diffuse pattern by immunofluorescence. The results from both systems strongly suggest that serum growth factors can act to rapidly affect not only the organization of focal contact components, but also of genes coding for such proteins, as part of the regulatory program that occurs during transition from quiescence to a growth stage.

B 401 MESENCHYMAL-EPITHELIAL INTERACTIONS INFLUENCING CYTOKERATIN EXPRESSION DURING DEVELOPMENT OF A STRATIFIED EPITHELIUM, Robert M. Bigsby, Department of Obstetrics & Gynecology, Indiana University, School of Medicine, Indianapolis, IN 46223. Cytokeratin expression was studied using monoclonal antibodies that recognize cytokeratin polypeptides typical of simple epithelia (PKK1, K4.62) and antibodies which react with the cytokeratins found in keratinocytes and stratified squamous epithelia (K8.12, AE8, K8.60). During postnatal development in the rat, the vagina and uterine cervix are initially lined by a simple epithelium. Prior to stratification, the epithelia stained with PKK1 and K4.62, and with K8.12. At the time of initial stratification the epithelium stained with AE8 and K8.60, suggesting that these antibodies recognize cytokeratins whose expression is closely coordinated with differentiation of the stratified squamous phenotype. Once stratification was evident, only the suprabasal cells retained positive staining with PKK1 and K4.62. Thus, before stratification the keratinocytes lining the vagina and cervix possess cytokeratin proteins typical of both keratinocytes and simple epithelia and once these cells enter the process of stratification they preferentially synthesize cytokeratins typical of the stratified phenotype.

Tissue recombinant studies showed that vaginal mesenchyme induces stratified histogenesis and that certain stratified-type cytokeratins are expressed prior to actual stratification. When uterine epithelium was combined with vaginal mesenchyme and grown in a syngeneic host for 5 weeks the epithelium was induced to become stratified. These newly stratified epithelial cells expressed stratified-type cytokeratins, i.e., they stained positively with K8.12, AE8, and K8.60. When the tissue recombinants were harvested prematurely at 1-2 weeks after grafting there were epithelial cells that as yet did not show signs of stratification but which stained for both stratified-type and simple-type cytokeratins (positive with K8.12 & AE8, PKK1 & K4.62, respectively). These results suggest that expression of specific cytokeratins is one of the early events in the mesenchymally induced cytodifferentiation leading to epithelial stratification.

B 402 CHANGE IN CYTOSKELETAL PROTEINS IN RESPONSE TO CYCLIC MECHANICAL STRESS, Michael J. Buckley, Department of Oral and Maxillofacial Surgery, The University of Iowa Hospitals and Clinics, Iowa City, IA 52242

Cells isolated from avian calvaria were subjected to cyclic mechanical stress with the Flex-cell Culture apparatus. The cells were subjected to a stress regimen of 3 cycles per minute at a maximum of 24% elongation for four days. Triplicate cultures were then subjected to 2 dimensional gel electrophoresis autoradiography and protein spot identification. The following proteins were increased:

	kD	pI	Fold Change	DPM/Spot Cont	DPM/Spot Stress
∝Tubulin	57.4	5.21	*8.7	2516	21861+
Vimentin	61	5.16	*22.5	260	5838+
Actin	42	5.42	.500	17540	35055
Vinculin	140	6.07	*5.62	30.1	169+

*$p \leq 0.05$

This increase in critical focal contact and cytoskeletal proteins to mechanical strain and the concurrent changes in phenotypic markers (i.e. alkaline phosphatase, collagen and non-collagen proteins) gives further strength to the role of the cytoskeleton in modulating gene expression.

B 403 CYTOSKELETON-BOUND POLYSOMES IN PLANTS. DO THEY EXIST? Eric Davies, Shunnosuke Abe, Eric C. Comer, Becky D. Fillingham, Jack M. Lionberger, Jeremy P. Meyer, and Weimin You. School of Biological Sciences, Univeristy of Nebraska, Lincoln, NE 68588-0118.

When plant tissues (pea stems, pea roots, bean roots, corn endosperm) are ground in a low ionic strength buffer (CSB), specifically formulated to maintain the integrity of F-actin, yet containing 0.5% non-ionic detergent to release the membrane-bound polysomes (MBP), over 75% of the polysomes sediment during centrifugation for 20 min at 4,000 x g. With pea stem tissue, about 1/3 of these sedimentable polysomes are released from the pellet upon treatment with CSB containing 200 mM monovalent cation (Tris, K^+, Na^+, NH_4^+), an additional 1/3 is released by monovalent cation containing detergent, and the remaining 1/3 is released only after prolonged treatment with protease K. Western blots show that over 80% of the actin is in the pellet. Fluorescence microscopy of pellets stained with rhodamine-phalloidin reveals fragments of F-actin, and electron microscopy of negatively-stained samples reveals filamentous systems, sometimes bearing ribosomes. With corn endosperm, over 80% of the polysomes sediment at just 32 x g. Phase contrast microscopy of pellets obtained in the presence of detergent reveals many large starch grains and numerous smaller protein bodies clumped together. Fluorecence microscopy reveals few filaments, but each protein body appears to be coated with a layer of actin. Treatment of pellets with RNase or protease K causes little release of polysomes, indicating that the primary attachment is not through mRNA or nascent chains, treatment with low levels of monovalent cation releases just monosomes, whereas increasing amounts of cation cause the release of increasingly large polysomes, implying that the primary attachment is via the ribosomes.

B 404 INVESTIGATION INTO THE MECHANISM OF VINCULIN'S RECRUITMENT TO THE CORTICAL CYTOSKELETON DURING THE TRANSITION FROM OOCYTE TO EGG IN *XENOPUS LAEVIS*. Janice P. Evans and Brian K. Kay. Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280.

In our examination of the distribution of the cytoskeletal proteins talin and vinculin in the *Xenopus laevis* oocyte, we found that talin is present in the oocyte cortex with little or no accompanying vinculin. Vinculin becomes localized to the cortex of eggs, hence becoming co-localized with talin sometime during the transition from oocyte to egg. We have done protein overlay blots to examine protein-protein interactions *in vitro*. Chicken talin binds *Xenopus* oocyte and egg vinculin equally well. However, chicken vinculin binds *Xenopus* egg talin 4x better than it binds oocyte talin, indicating that talin is possibly modified in some way that facilitates its interaction with vinculin. We are also doing experiments to pinpoint the timing of vinculin's appearance in the cortex more precisely. It appears that oocyte maturation, in response to exposure to progesterone *in vitro*, is not sufficient to mobilize vinculin to the cortex. However, preliminary experiments with GRDGS pentapeptides indicate that exposure of oocytes to these compounds will stimulate vinculin localization to the cortex. We plan to perform similar experiments with extracellular matrix proteins and jelly water from eggs. Our present hypothesis is that oocyte contact with the jelly coat stimulates a re-organization of the cortical cytoskeleton so that vinculin and talin are co-localized in the ovulated egg. This would be analogous to the formation of adhesion plaques in a fibroblast in response to contact with fibronectin.

B 405 SECRETION OF A Mr 60,000 GLYCOPROTEIN BY BENOMYL-TREATED CELLS OF NEUROSPORA CRASSA. K. Hoang-Van, C. Rossier, and G. Turian. Laboratory of General Microbiology, University of Geneva, 1211 GENEVA 4/Switzerland. In the presence of the microtubule inhibitor benomyl at micromolar concentrations, cells of Neurospora crassa wild type strain St. Lawrence 74A were found to secrete high amounts of a Mr 60,000 protein into the culture medium (about 35 µg/ml after a 12 h treatment). The secretion also occurred after treatment with the other antitubulin drugs carbendazim (MBC), nocodazole, thiabendazole, griseofulvin, vincristin and vinblastin. This secretion is apparently induced by the specific action of benomyl on N. crassa ß-tubulin as no secretion of the Mr 60,000 protein could be detected after treatment of the benomyl-resistant mutant bml 511 (r), mutated in its ß-tubulin gene (Orbach et al. 1986). The secretion was abolished by 12 µM cycloheximide and by 5 µM monensin. The Mr 60,000 protein was shown to be a glycoprotein by concavalin A binding. In the presence of benomyl and of tunicamycin at 0.5 ug/ml, two glycoproteins of lower molecular weight (Mr 54,000 and Mr 52,000) were secreted. The Mr 60,000 protein could be separated into two main and four secondary components by two-dimensional gel electrophoresis (pIs = 6.67 and 6.52 and pIs = 6.93, 6.81, 6.44 and 6.32, respectively). The Mr 60,000 protein was not a major intracellular protein of benomyl-treated cells and could only be revealed by immunoblotting with polyclonal antibodies raised against the extracellular form. It was undetectable in untreated cells collected at various stages of vegetative growth or in their culture medium.

B 406 ADDITION OF EXOGENOUS MEVALONIC ACID IN LOVASTATIN TREATED SYNCHRONOUS BHK-21 CELLS RESULTS IN ISOPRENYLATION OF NUCLEAR LAMINS AND INCREASED DNA SYNTHESIS, Hughes-Fulford, M., Schmidt, J., Erickson, S. and R. Appel, Department of Medicine, University of California and V.A. Medical Center, San Francisco, CA 94904 Previous work from this laboratory (PNAS 76: 5056-5060, 1979) has shown that lovastatin treated cells are inhibited from entering S-phase and that mevalonic acid is an essential component needed for DNA synthesis. Others have demonstrated previously that lovastatin causes changes in morphology and addition of exogenous mevalonic causes isoprenylation of nuclear lamins. In this report we describe the effects of lovastatin on cell cycle progression in synchronous BHK cells. Lovastatin treated cells have decreased DNA synthesis accompanied with a reduced intermediate filament network. If mevalonic acid is added to lovastatin treated cells within 4 hours of peak S-phase, the majority of DNA synthesis is restored. Later addition of the mevalonate causes a similar delay in onset of DNA synthesis. Using immunofluorescence, we found that lovastatin induced changes in the vimentin cytoskeleton were reversed with addition of mevalonic acid. Using western blot analysis and radioautography of the lovastatin treated cells, we found that there is a increase of a 74 kD protein that is recognized by anti nuclear lamin A,B,C. When mevalonic acid is added to the cells for 4 hours before expected S-phase, the majority of the 74 kD protein disappears and the concentration of a second 72 kD protein doubles. When 2-^{14}C mevalonic acid is added to the lovastatin treated cells, the 72 kD protein is modified suggesting isoprenylation of nuclear lamins. These studies support the hypothesis that isoprenylation of nuclear lamins is required before initiation of S-phase DNA synthesis.

B 407 $p150^{c\text{-}abl(IV)}$ IS A CYTOPLASMIC TYROSINE KINASE THAT IS BOTH NUCLEAR AND ASSOCIATED WITH ACTIN STRESS FIBERS. Peter Jackson, R. Van Etten, and David Baltimore. Whitehead Institute, 9 Cambridge Center, Cambridge, MA 02142.
Investigation of the subcellular localization of $p150^{c\text{-}abl(IV)}$ by indirect immunofluorescence has revealed that the protein primarily associates with three structures: (i) the plasma membrane; (ii) the nucleus; and (3) actin stress fibers. Confocal laser scanning microscopy shows the protein is localized throughout the nucleus. Costaining with FITC-phalloidin establishes colocalization of abl and F-actin, as does sensitivity of the abl staining pattern to cytochalasin D. Mapping of nuclear and actin binding sites and the assessment of the effect of mutations in these sites of the latent transforming ability of abl are underway. Initial characterization of fluorescently-labeled fusion proteins containing domains of the c-abl(IV) protein suggest that a domain involved in negative regulation of the kinase and its transforming ability may indeed be an actin binding protein when microinjected in murine fibroblasts. The ability of this protein domain to directly bind actin in vitro is currently being evaluated.

B 408 MICROTUBULE DYNAMICS AND CELL DIVERSIFICATION IN THE EARLY MOUSE EMBRYO
B. Maro, Institut Jacques Monod, CNRS - Université Paris 7, 2 place Jussieu, 75005 Paris, France
During preimplantation development, the cells of the mouse embryo undergo both a major subcellular reorganisation (at the time of compaction) and, subsequently, a process of differentiation (as the phenotypes of trophectoderm and inner cell mass diverge). Microtubules (MT) redistribute during compaction at the 8-cell stage. As the blastomeres flatten upon each other and cytoplasmic and surface features polarize along an axis perpendicular to cell contacts, an apical-basal asymmetry in the MT distribution becomes progressively more marked. A less dynamic subpopulation of acetylated MTs exists in these cells with a distinct distribution: they did not redistribute in the apical part of the cytoplasm but rather concentrate in the basal cortex. Drug experiments indicate that MTs have a constraining role on various events of compaction: they inhibit flattening, control the distribution of cytoplasmic organelles and stabilize components of the surface pole although surface polarization can occur in the absence of MTs if blastomeres are allowed to flatten. Taken together, these observations suggest that the apical redistribution of dynamic MTs may coordinate the various changes taking place during compaction. If the process of flattening is prevented, polarization can still occur but without the normal contact-directed orientation and in a lower proportion of cells than for control population. This process involves a MT-mediated interaction between the nucleus and the cell cortex. We conclude that surface polarization of blastomeres can be accomplished by at least two alternative routes: one requires flattening but is independent of MTs, and another requires MTs and is independent of flattening. When the cells of a compact 8-cell embryo divide, the first two distinct cell types are formed. Outer cells have a polarized phenotype and tend to envelope the apolar inner cells during the 16-cell stage. Acetylated MTs accumulated preferentially in inside cells. Differences in nocodazole sensitivity between cell types did not relate to the level of acetylation, although within each cell, acetylated MTs were more stable. Although the basal accumulation of acetylated MTs at the 8-cell stage required cell contact, differences between cell types at the late 16-cell stage were maintained in the absence of cell contact.

B 409 ACTIVATION OF EXCITATORY AMINO ACID RECEPTORS INDUCES CALCIUM DEPENDENT PROTEOLYSIS OF SPECTRIN IN CULTURED CEREBELLAR NEURONS. Tamara C. Petrucci, Anna Maria M. Di Stasi, Marina Ceccarini and Vittorio Gallo*. Department of Cell Biology and *Department of Physiopathology, Istituto Superiore di Sanità, Rome, Italy.

The activation of excitatory amino acid (EAA) receptors has been implicated in the development of synaptic plasticity and in certain neurological diseases. Although the molecular mechanisms of the long-lasting physiological and pathological modifications induced by EAA in neurons are not known, it appears that calcium influx may play a critical role. Activation of a calcium dependent protease, calpain I, has been specifically linked to EAA-induced neuronal degeneration in rat hippocampus and the proteolysis of cytoskeletal proteins, such as spectrin, is thought to contribute to the disintegration of neuronal structure. We have utilized cerebellar primary cultures, 95% enriched in granule cells, to examine the relationship between EAA neurotoxicity and calcium dependent proteolysis of spectrin. Exposure of neuronal cells culture to 100 uM N-methyl-D-aspartate (NMDA), in the absence of Mg^{2+}, caused the appearance of a 150 kDa fragment of brain spectrin detected by immunoblots. The spectrin breakdown was dependent on extracellular calcium, inhibited by the NMDA antagonist 2-amino-5-phosphonovalerate (AP5), and by the addition of 3 mM Mg^{2+}, and by calpain I inhibitor in the culture medium. The EAA kainate, at the same dose of NMDA, was less effective and no degradation of spectrin was observed after KCl induced depolarization of cerebellar neurones. These results support the hypothesis that the influx of calcium and the subsequent calpain I dependent proteolysis of cytoskeletal proteins are critical events in glutamate neurotoxicity.
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B 410 THE EFFECT OF ESTRAMUSTINE ON MITOSIS IN SENSITIVE AND RESISTANT DU 145 HUMAN PROSTATIC TUMOR CELLS. V.R. Sheridan, L.A. Speicher. K.D. Tew, and M.E. Stearns. Department of Pharmacology, Fox Chase Cancer Center, Philadelphia, PA, 19111. The chemotherapeutic drug, estramustine (EM), has been shown to bind to microtubule associated proteins (MAPs) resulting in microtubule disassembly in vivo and in vitro. Using DIC and immunofluorescence microscopy we have shown that drug-sensitive, mitotic cells treated with 2.5-10 uM EM lose microtubule organization resulting in chromosome disorganization and the inhibition of mitotic progression. Electron microscopy studies indicate that microtubules associated with centrosomes, kinetochores, and midbodies are present at these drug concentrations. Preliminary investigation of EM's effect on several EM-resistant DU 145 cell lines, isolated in our laboratory, indicate that these lines are capable of forming mitotic spindles in drug concentrations up to 15 uM. In the presence of EM, drug-resistant cells form functional, albeit smaller, spindles than those formed in normal cells in the absence of drug. The EM-resistant lines do not exhibit cross-resistance to other anti-cytoskeletal drugs including vinblastine, cytochalasin B, and taxol. In both sensitive and resistant lines, EM exerts a greater effect on mitotic versus interphase microtubules.