FUNCTIONS OF THE CYTOSKELETON IN CELL GROWTH, ORGANIZATION AND DIFFERENTIATION

Organizers: Jeannette Chloe Bulinski and Kathleen Green

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Molecular Diversity (Joint)

J4-001 DYNEIN HEAVY CHAINS: FUNCTIONAL AND STRUCTURAL DIVERSITY. David J. Asai, Peggy S. Criswell, Kimberly A. Kandl, Hendri Tjandra, and James D. Forney[1], Departments of Biological Sciences and [1]Biochemistry, Purdue University, West Lafayette, IN 47907.

The translocation of dynein along microtubules is the basis for a wide variety of essential cellular movements. The current view is that specific heavy chain isoforms, derived from separate genes, are targeted to various places in the cell in order to perform specific tasks. The functional specialization of dynein heavy chains is most evident in the axoneme where each of eight or more isoforms combines with specific proteins and localizes to a specific place along the outer doublet microtubules; each axonemal dynein, depending on its location, contributes uniquely to the overall production of propagated bending [Asai and Brokaw, TICB 3:398-402 (1993)]. The examination of the regulation of expression and the structural complexity among dynein isoforms will lead to a better understanding of dynein functional diversity.

Paramecium is a single-celled ciliated organism that expresses at least twelve dynein heavy chains. The genes for two dynein heavy chains, DHC-6 and DHC-8, have been isolated and characterized. Site-directed antibodies demonstrate that DHC-6 encodes ciliary beta chain and DHC-8 encodes cytoplasmic dynein. DHC-8 epitopes are not detected in cilia, indicating the spatial segregation of the DHC-8 isoform. In response to deciliation, the steady state level of DHC-6 mRNA, but not DHC-8 mRNA, rises significantly in a pattern similar to the increase in tubulin mRNA. Nuclear run-on experiments reveal that the DHC-6 gene, but not DHC-8, is transcriptionally activated during reciliation.

A second study focuses on the complexity of cytoplasmic dynein. In addition to the MAP1C gene [Mikami et al., Neuron 10:787-796 (1993); Zhang et al., PNAS 90:7928-7932 (1993)], the expression of a second putative cytoplasmic dynein has been detected in tissues that do not have cilia. This isoform appears to be the same as the cyto1b isoform previously detected in sea urchin embryos [Gibbons et al., Mol. Biol Cell 5:57-70 (1994)]. Sequence specific antibodies are being applied to cytoplasmic dynein in order to determine whether or not the second gene is expressed at the protein level, and, if so, whether the two cytoplasmic isoforms are distributed differently in the cell.

Subcellular Targeting: Role of Microtubules and Microfilaments

J4-002 THE INTEGRAL MEMBRANE PROTEIN, CAVEOLIN, CYCLES BETWEEN PLASMA MEMBRANE CAVEOLAE AND THE GOLGI COMPLEX BY MICROTUBULE-DEPENDENT AND MICROTUBULE-INDEPENDENT STEPS, Patricia A. Conrad, Eric J. Smart, Richard G.W. Anderson, and George S. Bloom. University of Texas Southwestern Medical Center, Dallas.

Caveolae are small plasma membrane invaginations that mediate cellular uptake of ions and small molecules, such as folate, from the extracellular environment. Recent work has demonstrated that treatment of normal human fibroblasts with cholesterol oxidase (CO) reversibly induces the movement of caveolin, a 22 kDa integral membrane marker protein for caveolae, from caveolae to the Golgi complex (Smart et al. 1994. *J. Cell Biol.* 127: in press). The present study was undertaken to determine whether microtubules (MTs) are required for reversible, CO-induced transport of caveolin between these two membrane compartments, and to assess whether transport reflects a normal cycling pathway for caveolin. In the presence of nocodozole, CO-induced movement of caveolin into the ER and then into a peripherally distributed ER/Golgi intermediate compartment (IC) occured with normal kinetics, but caveolin was unable to reach the centrally located Golgi. In contrast, nocodozole did not inhibit the rapid redistribution of caveolin from the Golgi to caveolae, which was observed after removal of CO and apparently occured via vesicular intermediates. Bidirectional movement of caveolin between the plasma membrane and the Golgi was also seen in cells that had never been exposed to CO. For example, incubation of cells for 2 hours at 16° followed by elevation of the temperature to 37°resulted in transient localization of caveolin at the Golgi, after which caveolin became localized once again in plasma membrane caveolae. Finally, when cells were treated with nocodozole alone, caveolin quickly became concentrated in the IC. Collectively, these results indicate that MTs are essential for maintaining the localization of caveolin in caveolae at steady state in normal human fibroblasts, and suggest a detailed model for the constitutive cycling of caveolin between plasma membrane caveolae and the Golgi complex in unperturbed cells. This model incorporates the following features. **1)** Movement of caveolin from the plasma membrane to the ER and then to the IC is rapid and does not require MTs. **2)** Transport of caveolin from the IC to the Golgi requires MTs. **3)** Transport of caveolin from the Golgi back to the plasma membrane is apparently MT-independent.

J4-003 INTERACTIONS BETWEEN CYTOPLASMIC DYNEIN AND INTRACELLULAR ORGANELLES, Christine A. Collins, Sharron X.H. Lin, Kristina L. Ferro, Susan L. Gotlieb, and Ameet R. Kini, Department of Cell and Molecular Biology, Northwestern University Medical School, Chicago, IL 60611-3008.

Cytoplasmic dynein is a microtubule-associated ATPase whose proposed functions include retrograde transport of membranous organelles, and assembly and positioning of the mitotic spindle. As an organelle translocator, dynein is likely to interact in a specific and regulated way with both its cargo and the microtubule tracks in order to carry out unidirectional transport. Though from subcellular fractionation experiments using brain tissue cytoplasmic dynein appears to be largely soluble, in other cell and tissue types similar fractionation studies reveal a significant membrane-associated population of cytoplasmic dynein. Immunofluorescence localization studies using cultured cells have also demonstrated that dynein is present in both diffuse cytoplasmic and membrane-bound pools. In normal rat kidney fibroblasts as well as other cell lines, dynein has been localized primarily to large cytoplasmic organelles identified as lysosomes. We have found that the intracellular distribution of dynein is modulated under various culture conditions. We have experimentally altered the dynein immunofluorescence staining pattern in fibroblasts by serum starvation, calcium depletion, and treatment with okadaic acid, a phosphoprotein phosphatase inhibitor. Under these conditions dynein redistributes from a membrane-bound pool to one that is more diffuse within the cytoplasm. Cytoplasmic dynein has been found to be phosphorylated in fibroblasts under control conditions, and an increase in phosphorylation occurs upon either brief serum starvation or okadaic acid treatment concomitant with the observed change in cellular distribution of the protein. We speculate that the diffuse form of dynein is functionally active, in that cellular activities suspected to rely on dynein, such as positioning of the lysosomes and Golgi apparatus, assembly and function of the mitotic spindle, and endocytic movement (rates of endocytosis) are not inhibited under conditions when dynein does not appear to be concentrated on specific intracellular organelles. Our model suggests that phosphorylation of cytoplasmic dynein through an as yet undefined signaling pathway in response to changes in the intracellular calcium levels and/or presence or absence of serum factors is involved in the regulation of dynein function, by altering its intracellular distribution and interaction with specific organelle populations. The regulation of this signaling pathway appears to be somewhat cell specific, in that neuronal cells do not significantly alter their dynein distribution in response to serum starvation, and the response of virally transformed fibroblasts to serum starvation and recovery are significantly altered compared with their non-transformed counterparts. The elucidation of this regulatory pathway and the effects of modification of the dynein molecule on transport functions are the focus of continuing investigations in our laboratory.

J4-004 CLIPs, A NOVEL CLASS OF MICROTUBULE-ORGANELLE LINKER PROTEINS, Thomas E. Kreis, Philippe Pierre, Janet Rickard, Olaf Rosorius, Jochen Scheel, Caterina Valetti, University of Geneva, CH-1211 Geneva 4, Switzerland.

Intracellular membrane traffic and the spatial arrangement of membrane-bounded organelles depends on microtubules. The interactions of these components is regulated by cytoplasmic linker proteins (CLIPs). They may lead to anchoring of organelles to a specific region of the cell, or to their motor protein-driven movement along microtubules. Using in vitro binding assays, we have identified and characterized CLIP-170, a protein which links endosomal vesicles to microtubules. CLIP-170 colocalizes with a subset of transferrin receptor-positive endocytic membranes. It is a homodimeric protein containing a nonhelical C- and N-terminus connected by a long coiled coil. We have identified a novel motif present in a tandem repeat in the globular N-terminus which is involved in binding of the protein to microtubules. One of these motives is also present in the rat DP150, Drosophila Glued and yeast BIK1 proteins, which are all similar in overall structure to CLIP-170. Phosphorylation of serines present in clusters downstream of the microtubule-binding repeats regulates the binding of CLIP-170 to microtubules; hyperphosphorylated protein is released from microtubules. The extent of phosphorylation of CLIP-170 may regulate its targeting to the peripheral microtubule plus-ends. By transient expression of wild-type and mutant CLIP-170 in primate cells we have established that the C-terminal domain of the protein is involved in its interaction with and targeting to patchy structures at the cell periphery. Most likely, these patches contain endosomal membranes. These features of CLIP-170, and its very elongated structure, suggest that it belongs to a novel class of proteins mediating specific interactions of organelles with microtubules.

J4-005 REGULATED DEPLOYMENT AND RETRIEVAL OF LEUKOCYTE ADHESION RECEPTORS, Frederick R. Maxfield[1], Moira Lawson[1], Richik Ghosh[1], John Mandeville[1], Eugene Marcantonio[1], and Bill Hendey[2], [1]College of Physicians & Surgeons, Columbia University, New York, NY 10032 and [2] Rush Medical College, Chicago.

Continued migration of cells requires adhesion to a surface, application of force against the adhesion site, release of adhesion, and formation of new attachments farther forward (1). When human neutrophils migrate on surfaces containing vitronectin, they use at least two types of integrins for attachment. One of these is the leukocyte-specific ß2 integrins which have broad binding specificity, and the other is an αvß3 integrin which has restricted binding specificity. We have found that in neutrophils the binding of αvß3 to vitronectin is regulated by $[Ca^{2+}]_i$ levels. When $[Ca^{2+}]_i$ is clamped at low levels by intracellular buffering, the cells are unable to detach from adhesion to vitronectin even though they continue to generate pseudopods and generate force. The motility of $[Ca^{2+}]_i$ -buffered cells on vitronectin can be rescued by monoclonal antibodies that specifically block αvß3 integrin binding. We have examined the deployment of αvß3 integrins in neutrophils migrating on vitronectin. Immunofluorescent confocal imaging shows that the αvß3 is present on the adherent surface and in intracellular organelles. On the adherent membrane, αvß3 is heavily concentrated towards the leading edge of the cell in proximity to high concentrations of F-actin as detected by rhodamine phalloidin staining. Using HL-60 cells (differentiated to neutrophil-like properties), we have shown that many of the intracellular organelles are endocytic recycling compartments that also contain endocytosed transferrin receptors. Transferrin enters cells by receptor-mediated endocytosis and is recycled rapidly to the cell surface via a recycling compartment that consists mainly of narrow diameter tubules. The organization of this recycling compartment is dependent on microtubules since it is rapidly disrupted by nocodazole. When $[Ca^{2+}]_i$ is buffered, the amount of αvß3 in recycling endosomes is decreased, and the αvß3 on the adherent membrane is concentrated towards the *rear* of migrating cells. We suggest that recycling αvß3 integrins are preferentially recycled to the leading edge of migrating cells where they bind and assemble into actin-associated adhesion plaques. As the cell moves forward, these plaques are exposed to $[Ca^{2+}]_i$ transients which release the αvß3 binding and allow these integrins to be internalized and recycled to the front of the cell. When $[Ca^{2+}]_i$ is buffered, the αvß3 remains attached to vitronectin as the cell moves forward.

1. Maxfield, F.R. (1993) Calcium regulation of leukocyte locomotion. *Trends in Cell Biology* **3**: 386-391

Intermediate Filaments in Differentiation and Cell Adhesion

J4-006 DYNAMIC PROPERTIES AND POTENTIAL FUNCTIONS OF INTERMEDIATE FILAMENTS (IF), R.D. Goldman[1], Y.-H. Chou [1], F.W. Flitney[3], A. Goldman[1], S. Khuon[1], P. Loomis[1], M. Montag-Lowy[1], R. Moir[1], P. Opal[1], V. Prahlad[1], O. Skalli[1], A. Trejo-Skalli[1], T. Spann[1], P. Steinert[2] and K. Straube-West[1], [1]Northwestern University, Chicago, [2]NIH/NIAMSD, Bethesda, and [3]St. Andrews University, Scotland.

IF and their associated proteins are major cytoskeletal components of the cytoplasm and karyoskeletal components of the nucleus in many cell types. In the four major cytoskeletal IF systems, we have determined through use of microinjection techniques, that each of these systems is capable of incorporating new or additional subunits at the post-translational level. The mode and pattern of incorporation of each of the microinjected proteins varies according to cell and protein type. Furthermore, by varying the concentration of injected protein, we have been able to add sufficient amounts of subunit proteins to drive partial disassembly or reorganization of the endogenous network, indicating the existence of a state of dynamic equilibrium *in vivo* between subunits and polymer. Based on these findings we have attempted to inject high enough concentrations of subunit proteins in order to selectively disrupt IF in live cells which would provide a means of dissecting their functions. However, we have been unable to inject sufficiently high concentrations for this purpose, due to solubility problems. This has led us to the use of much more soluble low molecular weight 1A peptides (derived from the highly conserved N-terminal region of the central rod domain) to specifically disrupt IF *in vivo*. These peptides can be injected to achieve catastrophic disassembly of IF *in vivo*. In the case of cultured fibroblasts containing vimentin IF, the result of such disassembly is the complete loss of cell shape over a very short time period. This effect is totally reversible. We are also studying the role of phosphorylation in regulating subunit exchange and states of assembly of vimentin-IF, using mitotic BHK cells which disassemble their IF in mitosis. These studies involve the isolation and characterization of kinases responsible for the mitotic specific hyperphosphorylation of vimentin at both N- and C-terminal sites, the sequencing of these sites, and the use of site directed mutagensis and transfection to demonstrate the functional significance of specific phosphorylation events. The results indicate that specific phosphorylation in the head domain, but not in the tail domain, is responsible for IF disassembly in mitosis. The Type V IF, the nuclear lamins appear to have properties which are similar to those seen for the cytoskeletal IF systems. They are dynamic throughout interphase and changes in their organization within the nucleus are correlated with specific stages of the cell cycle, including in S-phase, the association of lamin B with DNA replication foci. Overall, our results indicate that both cytoplasmic and nuclear IF systems are dynamic constituents of the cell's molecular architecture and that they are implicated in important functions ranging from cell shape maintenance to DNA replication. This work has been supported by NIHGM, NCI, the Les Turner ALS Foundation and the British Heart Foundation.

J4-007 ADHERENCE JUNCTIONS, EPITHELIAL MORPHOGENESIS & THE SIGNALS INVOLVED IN MESODERM INDUCTION.
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A supracellular cytoskeletal network composed of intermediate filaments, microfilaments and their respective membrane attachment junctions, desmosomes and adherens junction, underlies the mechanical and motile properties of epithelia. To study the role of membrane-cytoskeletal interactions in the morphogenetic behavior of epithelia, we use the *Xenopus* embryo. Based on the observations of Kintner (1992. *Cell* **69**:229) and Troyanovsky *et al* (1993. *Cell* **72**:561), we constructed RNA synthesis plasmids encoding epitope-tagged forms of the cytoplasmic tail domain of the desmosomal cadherins desmoglein-1 (DsgTail) and desmocollin-1b (DscTail). When injected into the fertilized egg DscTail, and to a lesser extent DsgTail, induced a defect in gastrulation similar to that seen previously in anti-keratin antibody injected embryos (Klymkowsky *et al* 1992. *Proc. Natl. Acad. Sci. USA* **89**:8723). It is thought that the tail domains of cadherins act as dominant mutants by binding to, and sequestering, cytoplasmic accessory proteins involved in cell-cell adhesion and cadherin-cytoskeletal linkage. To test whether this was the case for the desmosomal cadherins, we constructed a plasmid that encodes a myc-tagged, full length form of plakoglobin (mycPLKG), which is known to associate with the tail domain of desmoglein. Over-expression of mycPLKG induced a duplication of the anterior portion of the embryo. This axis duplication was suppressed by the co-expression of DsgTail. Immunocytochemical analysis indicates that mycPLKG accumulates in the nuclei in many embryonic cells and that this nuclear accumulation is suppressed by the co-expression of the DsgTail. In *Xenopus*, anterior axis duplication has been linked to the induction of excess dorsal mesoderm. Such an anterior axis duplication is found in embryos injected with RNA encoding molecules related to *wingless/int-1* (*Wnts*)(McMahon & Moon, 1989. *Cell* **58**:1075; Sokol et al. *Cell* **67**:741; Smith & Harland, 1991. *Cell* **67**:753). Using the DsgTail polypeptide as an "anti-plakoglobin" reagent, we found that DsgTail can partially suppress, but not abolish, the phenotype induced by expression of *Xwnt8*. This suggests that plakoglobin plays a role in *Wnt* signalling; the work of Barry Gumbiner and colleagues suggests that β-catenin is also involved. Based on these data, we propose that free, *i.e.* non-cadherin-associated, plakoglobin/β-catenin are second messengers in embyronic cells, and may act within the nucleus during dorsal mesoderm induction. We are currently studying the relationship between plakoglobin levels, cell adhesion, desmosome/adherens junction formation, and dorsal mesoderm induction. *Support for this project was provided by the American Cancer Society.*

J4-008 DEVELOPMENTAL EXPRESSION, ASSEMBLY AND REGULATION OF NEURONAL INTERMEDIATE FILAMENTS.
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During the past few years, the discovery of previously unknown intermediate filament proteins, the elucidation of the developmental expression of these proteins, as well as their in vivo assembly characteristics have changed our understanding of the way that intermediate filaments are organized in developing and adult neurons. The major neuronal intermediate filament proteins in the adult central and peripheral nervous systems are the neurofilament triplet proteins, NF-L, NF-M and NF-H. Adult peripheral neurons also contain peripherin, another neuronal-specific intermediate filament protein. Expression of a fifth neuronal IF protein, α–internexin occurs earlier in the developing mammalian central nervous system than the expression of the neuronal triplet proteins. Neuroepithelial stem cells transiently express yet another IF protein, nestin along with the more ubiquitously expressed vimentin. By in situ hybridization and immunocytochemical studies, we have shown that in the rat telencephalon, vimentin and nestin are absent from post-mitotic neurons, while internexin mRNA and protein can readily be detected as soon as the migrating neurons have reached the cortical plate. NF-L and NF-M are not expressed in the rat central nervous system until several days later, while the expression of NF-H is even further delayed.

In order to study the assembly of these neuronal IFs in vivo, we have used the SW13 vim- cell line, which lacks any endogenous cytoplasmic intermediate filaments. By transfecting fully encoding cDNAs for each of the neuronal IFs into these cells, we have shown that only internexin and peripherin can self-assemble into a filamentous network in the absence of other IF proteins. The neurofilament triplet proteins co-assemble with vimentin, internexin or peripherin, but do not self-assemble in vivo. Mixtures of NF-L with NF-M or NF-H can form filamentous networks in the transfected cells. Deletion analyses show that the carboxyl-terminal tails of the neurofilament triplet proteins can be removed without compromising their ability to co-assemble with other intact intermediate filament proteins. The amino-terminal head regions are however more important for co-assembly of different neurofilament triplet proteins. By co-transfecting mutant NF-H, NF-M and NF-L constructs into vimentin positive and negative cells, we can show that NF-H interacts more strongly with NF-L than with either NF-M or vimentin.

These studies suggest that during development, the α–internexin filament system is first expressed and then used by NF-L and NF-M as a scaffold, while later NF-H interacts specifically with NF-L.

Tau Protein Function and Its Relation to Alzheimers' Disease

J4-009 *TAU PHOSPHORYLATION, STRUCTURE, AND FUNCTION. E.-M. Mandelkow, J. Biernat, G. Drewes, N. Gustke, B. Trinczek, O. Schweers, E. Mandelkow. Max-Planck-Unit for Struct. Mol. Biol., Notkestr. 85, D-22603 Hamburg, Germany.*

Alzheimer paired helical filaments consist of aggregated tau, and this tau is abnormally phosphorylated. One question is therefore, are these two features causally related or merely accidental? Moreover, tau interacts with microtubules which are the tracks for axonal transport whereas tau might only serve as ties. Thus another question is: Does tau really stabilize microtubules in the axon, and if so, does phosphorylation really matter? To anser these questions one has to dissect the tau molecule in terms of its phosphorylation sites, the corresponding kinases and phosphatases, and one has to find assays to detect the phosphorylation and the interaction with microtubules (or possibly other cell components). Several recent studies (for review see TIBS 18:480-483, 1993) can be summarized as follows:

-- Sites: Many kinases phosphorylate tau, mostly without clear effect on functions. Of special interest are the Ser/Thr-Pro motifs because their phosphoration can be detected by antibodies that also distinguish between normal tau and PHF-tau. Other sites cannot be detected this way but may be interesting for functional reasons (e.g. Ser 262). Of particular relevance are sites found directly in Alzheimer PHFs which includes both Ser262 and several Ser/Thr-Pro motifs.
-- Kinases: PKA, PKC, CaMK phosphorylate tau but only to a low extent. Kinases that phosphorylate Ser-Pro or Thr-Pro motifs include MAP kinase, GSK-3, cdk2, and cdk5 (a brain-specific cdk). Of these, MAP kinase is the most efficient one in vitro. Another kinase phosphorylates Ser 262 and its equivalent serines in the other repeats (Ser 293, 324, 356).
-- Phosphatases: All phosphates on tau can be removed by calcineurin and PP-2A while PP-1 shows little effect. Calcineurin appears to be more efficient with respect to Ser/Thr-Pro motifs while PP-2A is more efficient with Ser 262.
-- Structure: In the EM tau appears as a elongated molecule. However, unlike other "amyloid" fibers and their subunit proteins, neither tau nor PHFs show evidence for secondary structure. Tau has a tendency to form antiparallel dimers, and these in turn assemble into synthetic PHFs in vitro. The self-association is based on the repeat domain of tau.
-- Function: Tau binds to microtubules with Kd values in the μM range and a stoichiometry of 1:2 (tau:tubulin dimer). Most phosphorylation sites have little effect on these parameters, except Ser 262 whose phosphorylation weakens the binding several-fold and lowers the stoichiometry about 3-fold (to 1:6). Thus the Ser262-kinase could be a potential cause of microtubule destruction by inactivating the ties of tau protein. - Supported by DFG and BMFT.

Transgenic Mice - Models for the Study of Differentiation and Disease

J4-010 FUNCTIONS OF SIMPLE EPITHELIUM KERATINS: GASTROINTESTINAL DISEASE IN KERATIN 8 DEFICIENT FVB/N MICE.

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Although keratin 8 (*mK8*) gene disruption causes mid-gestational lethality in (C57Bl/6 X 129/Sv) mice, with a penetrance of 94%, the same mutation in FVB/N mice allows the majority of homozygotes to survive into adulthood. However, they develop a colorectal hyperplasia and acute inflammmation (colitis). The intestinal lesions affect uniformly the cecum, colon and rectum, but not the small intestine. The crypts are elongated, and at a later stage, an inflammation of the lamina propria and submucosa is observed. Hepatic transaminases are elevated, and at the late stages of the disease, pericholangial fibrosis with inflammation is also observed. In contrast, renal and pancreatic functions tested in clinical assays were within non-pathological range. No homozygous mouse line has been established, because $mK8^{-/-}$ FVB/N females fail to carry pregnancy to term. These results emphasize the importance of using several inbred mouse strains to reveal the polygenic contribution to mutant phenotypes. Our results demonstrate that genetic modifiers of K8/K18 filament functions, with profound effects on embryogenesis and gut functional integrity, are differentially active in the FVB/N and C57Bl/6 genetic backgrounds.

J4-011 MYOSIN MUTATIONS AND HEART DISEASE: TRANSGENIC MOUSE MODELS. L.A. Leinwand, A.J. Straceski, S. Factor and K.L. Vikstrom, Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461.

Mutant cardiac myosin heavy chain (MHC) genes have been identified in a number of families with the autosomal dominant disease, familial hypertrophic cardiomyopathy (FHC). This disease is characterized by myofibrillar disarray and sudden death. We have developed a cell culture model system for examining myosin thick filament assembly and for studying how the process may be disturbed by myosin mutations. Rat α cardiac MHC, when transfected into nonmuscle cells (COS) assembles into arrays of thick filaments which appear as spindle shaped structures by light microscopy. The formation of these structures is dependent on the presence of an intact MHC rod. We have also determined the ability of myosin with different FHC missense mutations to assemble into these structures. Although all of the mutations examined are found in the myosin head, up to 29% of COS cells transfected with FHC mutations failed to demonstrate spindle shaped structures compared with 2% of cells transfected with wild type MHC. Electron microscopic analysis suggests that the mutant MHC still assembles, but frequently into smaller structures. One of the most prevalent mutant alleles, Arg403Gln, has been shown to result in a molecule with impaired actin binding. To examine the effects of cardiac MHC mutations in an experimental animal model, a transgene was constructed encoding a rat αMHC mutated in the putative actin binding domain. Five independent transgenic lines were established. RNase protection analysis determined that the transgene is found at high levels in the heart. Histological analysis of hearts from all five lines revealed nuclear hypertrophy, degeneration focal or multifocal cellular disarray and vessel hypertrophy. These findings are consistent with hypertrophic cardiomyopathy. Indeed, the two lines selected for further studies have significantly increased heart weights compared to normal mice. Experiments are underway to analyze development of hypertrophy and the basis for cellular disarray in these animals.

Cell Motility: Mechanisms and Extracellular Signaling

J4-012 MOLECULAR GENETIC ANALYSIS OF MYOSIN LIGHT FUNCTION IN VIVO, Rex L. Chisholm, Pengxin Chen, Tung-Ling L. Chen, Guyu Ho, Patricia A. Kowalczyk and Bruce D. Ostrow, Northwestern University Medical School, Chicago.

In non-muscle cells, myosin II plays an important role in a variety of motility processes including cytokinesis, cell locomotion, chemotaxis, receptor capping and morphogenesis. *Dictyostelium discoideum* has been an important experimental system for the investigation of cell motility, particularly the contributions of the acto-myosin system to non-muscle cell motility. *Dictyostelium* myosin II is a hexameric molecule consisting of two copies each of a 240 kd heavy chain (MHC), an 18 kd phosphorylatable or regulatory light chain (RLC) and a 16 kd alkali or essential light chain (ELC). We have employed a molecular genetics to investigate the contribution of light chains to myosin function. Gene targeting has been used to produce *Dictyostelium* cell lines in which the endogenous ELC gene was disrupted, creating ELC null mutants (mlcE-). Southern blot analysis has confirmed the disruption of the EMLC gene and Northern and western blot analysis are unable to detect ELC mRNA and protein. These mutants show a severe cytokinesis defect when cultured in suspension. Expression of wildtype ELC in the null mutant rescues the cytokinesis defect, confirming that the absence of ELC is responsible for this phenotype. Myosin isolated from these cells has significant calcium ATPase, but lacks significant actin-activated ATPase. To investigate the regions of the ELC important for interaction between the ELC and MHC, we have created a series of ELC mutations and expressed them in both wildtype and mlcE- backgrounds. Removal of as few as 11 amino terminal and 15 carboxyterminal residues dramatically decreases the ability of the ELC to associate with the MHC. In contrast, insertions or deletions of four amino acids in the central region domain does not dramatically alter MHC - ELC association. Site directed mutation has been used to modify charged residues found in the loop domains of the ELC. When expressed in the mlcE- cells mutants in three of the four loops produce ELCs which only partially correct the phenotypic defects of the mlcE- cells. Phosphorylation of the RLC has been shown to regulate the ATPase and motor function of smooth muscle and non-muscle myosins. To investigate the role of the RLC *in vivo* we have also used gene targeting to create *Dictyostelium* cell lines which no longer express RMLC polypeptide (mlcR- cells). These cells are also defective in cytokinesis and fail to undergo normal multicellular development suggesting they exhibit motility defects. Surprisingly, they appear capable of capping cell surface receptors in response to crosslinking by Con-A. Reintroduction of wildtype RMLC into the cells rescues the observed phenotypes. The *Dictyostelium* RMLC is phosphorylated in response to chemoattractant, leading to increased actin-activated ATPase. We determined the site of phosphorylation on the RMLC by peptide sequencing of purified phosphorylated and unphosphorylated protein. We have produced mutations in this phosphorylation site and demonstrated that the modified RMLC is no longer a substrate for purified myosin light chain kinase *in vitro*, and is not phosphorylated when expressed in wildtype or RMLC null mutant cells. When the phosphorylation site mutation is expressed in mlcR- cells, all of the observed phenotypic defects are corrected. Myosin purified from cells expressing the mutant myosin shows actin-activated ATPase levels similar to dephosphorylated myosin. These results suggest that phopshorylation is not required for normal cytokinesis or multicellular development. Supported by NIH grant GM39264.

J4-013 THE ROLE OF TUBULIN DETYROSINATION IN THE POLARIZATION OF INTERMEDIATE FILAMENTS , ER AND MITOCHONDRIA IN MOTILE FIBROBLASTS.

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In many cell types there are at least two distinct populations of microtubules (MTs); tyrosinated (Tyr) MTs, which are usually dynamic, and detyrosinated (Glu) MTs, which are generally stable. Glu MTs arise by the post-polymerization detyrosination of Tyr MTs and are thought to be important for the development of cellular asymmetries and differentiation. Nonetheless, a specific functional role for detyrosination of tubulin has not been established. We have found that vimentin IFs preferentially colocalize with Glu MTs in polarized 3T3 fibroblasts migrating into an in vitro wound. The normal distribution of IFs in 3T3 cells was disrupted by conditions that selectively depleted Glu MTs from the cells (serum starvation or okadaic acid treatment). Rapid formation of stable, Glu MTs, by taxol-treatment of serum-starved cells, resulted in a rapid coalignment of IFs with MTs. IFs are known to collapse around the nucleus in cells treated with nocodazole to breakdown MTs; when we released 3T3 cells from such a nocodazole treatment, we found that the re-extension of IFs into the lamella was temporally correlated with the reappearance of Glu MTs rather than Tyr MTs. To test directly whether codistribution of IFs with stable, Glu MTs was dependent upon the Glu tubulin epitope, we microinjected affinity-purified antibodies (Abs) against Glu tubulin (a rabbit polyclonal and a mouse monoclonal) and Tyr tubulin (a rabbit polyclonal and a rat monoclonal) into 3T3 cells at the edge of wounded monolayers. Injection of either Glu Ab resulted in collapse of the IF network to a perinuclear location. The injected rabbit Glu Ab labeled a subset of the MTs which corresponded to Glu MTs detected by immunoflourescence. Thus, injected Glu Ab induced IF collapse even though only a subset of the MTs were labeled by the injected Ab. Injection of non-specific IgG or breakthrough IgG from the Glu Ab purification had no effect on IF organization. Injection of Tyr Abs into cells resulted in the labeling of Tyr MTs, but did not cause significant collapse of the IF array. We have also found that the ER and mitochondria, organelles known to colocalize with MTs in vivo, are dependent upon the Glu epitope for their localization. In 3T3 cells injected with the rabbit Glu Ab, the ER and mitochondria (detected by *DiOC6* staining), were found collapsed around the nucleus. In contrast, in cells injected with the rabbilt Tyr Ab, the organelles exhibited distributions similar to those in uninjected cells. Our results show that the IF-MT codistribution and the normal extended distributions of ER and mitochondria in motile cells can be disrupted by antibodies against Glu, but not Tyr tubulin. This suggests that tubulin detyrosination may function as a signal to direct the interaction of IFs (and perhaps ER and mitochondria) with MTs. The possibility that disruption of the interaction between MTs and IFs (or ER and mitochondria) may interfere with cell locomotion is under investigation.

J4-014 REGULATION OF ACTIN POLYMERIZATION BY GTPγS IN PERMEABILIZED NEUTROPHILS, Sally H. Zigmond, Sherry Huang, Marianne Tardif, Tim Redmond. Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6018

To understand how agonists regulate actin polymerization, we have examined the ability of GTPγS to stimulate actin polymerization in streptolysin-O (SO) permeabilized neutrophils. Addition of GTPγS to SO-permeabilized neutrophils, like addition of chemoattractant to intact cells, induced a doubling of the F-actin, assayed by TRITC-labeled phalloidin binding. In both intact and permeabilized cells, the increase in F-actin was blocked by cytochalasin. Permeabilized cells maintain their basal F-actin. Permeabilization in the presence of actin-monomer binding proteins (DNase I, thymosinb4 (Tβ4) and profilin) caused a dose-dependent depolymerization of basal F-actin. The presence of DNase I and Tβ4 decreased the extent of polymerization induced by GTPγS. Addition of profilin at concentrations that reduced basal F-actin levels did not reduce the extent of polymerization induced by GTPγS.

Since DNase I, which is not a physiological G-actin binding protein, had a similar effect on both basal and GTPγS-induced F-actin as did Tβ4, it is unlikely that GTPγS acts by inactivating Tβ4. While addition of profilin can increase the F-actin level in mixtures of actin and Tβ4 in vitro, the addition of profilin decreased cellular basal F-actin indicating that the polymerizing effect of GTPγS can not be due merely to freeing profilin from a sequestered or inactive pool. Together these results suggest that GTPγS does not induce actin polymerization by modification of these monomer binding proteins. Rather, the ability of profilin to decrease basal but not GTPγS induced F-actin fits with the known property of profilin to sequester G-actin from the pointed but not the barbed-ends of filaments, supporting the hypothesis that GTPγs acts by shifting the actin steady state from one defined by the filament pointed ends to one set by barbed ends. The data can be fit with a model in which GTPγs shifts the affinity of F-actin for G-actin from 0.5 to 0.1 μM and Tβ4 serves as a passive G-actin buffer

The induction of F-actin in permeabilized cells occurred in the presence of EGTA and did not require the addition of ATP. Neither addition of apyrase to deplete residual ATP nor addition of ADP or UDP to compete with residual endogenous ATP inhibited significantly the GTPγS-induced polymerization. ATP on its own caused no increase in F-actin and did not affect the time course or concentration dependence of GTPγS-induced F-actin. The presence of ATP did increase the maximal amount of F-actin induced by GTPγS by about 20%. These data help restrict the possibilities for signal transduction leading to actin polymerization.

Function and Regulation of Microtubule and Microfilament Associated Proteins

J4-015 EZRIN AND MOESIN IN THE CORTICAL CYTOSKELETON. Anthony Bretscher, Mark Berryman and Ron Gary. Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853.

Ezrin and moesin are two members of the closely related ERM family of proteins, that are themselves members of the band 4.1 superfamily. Most cultured cells express both ezrin and moesin, where they are predominantly associated with the cortical cytoskeleton. Immunoelectron microscopy with ezrin antibodies reveals that ezrin is specifically associated with actin-containing cell surface structures, such as microvilli and membrane ruffles. This localization, together with an analysis of the sequence of these proteins, suggests that they are membrane cytoskeletal linking proteins. To identify proteins that associate with ezrin and moesin in vivo, we have used a co-immunoprecipitation approach with ezrin-specific and moesin-specific antibodies. This revealed that ezrin and moesin can each form homodimers and heterodimers when expressed in the the same cell. Remarkably, ezrin purified from human placenta can exist as stable monomers or dimers, with no detectable conversion between the species. Analysis of domains of ezrin expressed in bacteria reveal that monomeric soluble ezrin can bind tightly to a C-terminal domain of unfolded and immobilized ezrin. This C-terminal domain is not accessible in soluble ezrin. Additional experiments reveal that an N-terminal domain in soluble ezrin is responsible for the interaction with the immobilized C-terminal domain. Thus, ezrin contains two domains that have the ability to bind to each other in vitro. The relationship of this finding to ezrin monomers, dimers and the function of this protein will be discussed. To begin to characterize the associations ezrin makes in it's natural setting, we have begun to analyze the protein composition of microvilli isolated from human placental syncytiotrophoblasts. This analysis reveals that ezrin is a major component of these structures, and that most of the ezrin is present as oligomers rather than monomers. Possible binding partners for ezrin will be discussed.

J4-016 MAP4, A MODULATOR OF MT DYNAMICS DURING PROLIFERATION AND DIFFERENTIATION, J. C. Bulinski[1], S. J. Chapin[2], C.-M. Lue[1], S. Chan[1], D. Gruber[1], T. Itoh[3], H. Hotani[3], and S.-I. Hisanaga[4], [1] Department of Anatomy & Cell Biology, Columbia University, College of Physicians. & Surgeons, NY, NY 10032, [2] Department of Anatomy, UCSF, San Francisco, CA 94143, [3]Department of Bioscience, Teikyo University, Utsunomiya, Japan, and [4]Laboratory of Cell & Developmental Biology, Tokyo Institute of Technology, Yokohama, Japan.

MAP4 is a microtubule (MT) associated protein expressed in every mammalian cell type except differentiated neurons. Like the nervous tissue MAPs, tau and MAP2, MAP4 is capable of binding to and stabilizing MTs in vitro. Molecular cloning of MAP4 by our group and others has revealed that the MT binding domain of MAP4, like those of tau and MAP2, is located within the C-terminal one-third of the molecule, and contains within it several imperfectly repeated segments of 18-amino acids. We isolated cDNA clones encoding several isoforms of MAP4, containing three, four, and five repeated elements within their MT-binding domains. A different four-repeat isoform was encoded by bovine cDNA clones isolated by Aizawa et al. ([1990] J. Biol. Chem. 265, 13849). We determined that alternative splicing from a single gene gives rise to MAP4 isoforms. Using the RNase protection technique to investigate the expression of MAP4 isoforms in mammalian cell lines and rat tissues, we established that the five-repeat isoform (form IV) was the only form detectable in proliferating cells, and was the most abundant form in most tissues. However, in brain, heart, and skeletal muscle, a four-repeat isoform (form III), which is most homologous to the developmentally regulated four-repeat form of tau, was also expressed and its expression increased during development. The three-repeat isoform (form I) was also detectable in several tissues, while the four-repeat isoform cloned by Aizawa et al. (form II) was not found in any cells or tissues we examined. Our results suggested that differential expression of MAP4 isoforms could be involved in changes in MT dynamics that accompany differentiation. To test this, we prepared stable mouse cell lines inducibly expressing human MAP4 form III. MAP4-expressing cells were inhibited in their growth, and their interphase MTs showed heightened resistance to depolymerization by nocodazole. However, MTs in MAP4-transfected cells were not as severely altered in stability or organization as MTs in cells transfected with tau or MAP2 cDNAs, suggesting that cultured cells regulate the MT stabilizing capacity of exogenous MAP4, while they fail to regulate that of tau or MAP2. This regulation probably involves phosphorylation, since we determined that exogenous MAP4, like endogenous MAP4, is phosphorylated at the G_2/M transition of the cell cycle. Because of the timing of MAP4 phosphorylation, we explored the possible role of the kinase complex, MPF ($p34^{cdc2}$/cyclin B). We determined that MAP4 is a substrate for phosphorylation by MPF in vitro, that MPF phosphorylation of MAP4 abrogates its MT-stabilizing capacity in vitro, and that MPF may be targeted to MAP4 in vivo, by virtue of direct binding of cyclin B to MAP4. Thus, MAP4 appears to play a role in stabilizing MTs in vivo, and that role may be modulated by differential expression of isoforms during differentiation, and differential phosphorylation during the cell cycle. [Supported by a grant from the American Cancer Society]

Microtubule Dynamics and Organization during the Cell Cycle and Differentiation

J4-017 AN ACTIVE ROLE FOR CHROMOSOMES IN SPINDLE ASSEMBLY, Eric Karsenti[1], Søren Andersen[1], Jos Raats[2], Chris Wylie[2] and Isabelle Vernos[1], [1]EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany, and University of Minnesota, Institute of Human Genetics, 420 Delaware Street S.E., Mn 554550392.

Assembly of the mitotic spindle is thought to occur through the stabilisation of highly dynamic microtubules by kinetochores and possibly by a local effect of the whole chromosomes. Positioning of the chromosomes on the metaphase plate results from the action of forces pulling the chromosomes towards the poles and of other forces acting on the chromosome arms to push them away towards the spindle equator.

We have identified a 230 KDa Xenopus Microtubule Associated Protein (XMAP230) that binds to interphase microtubules and spindle microtubules but not to astral microtubules in prophase and during metaphase. This MAP suppresses catastrophes, reduces the microtubule shrinking rate but has little effect on microtubule growth rate. Phosphorylation by the cdc2 kinase or by MAP kinase inhibits the binding of this MAP to microtubules. We propose that the specific binding of XMAP230 to spindle microtubules is due to its local dephosphorylation around the chromosomes, where it may provide a relative stabilisation of microtubule plus ends allowing their preferential growth towards the chromosomes.

We have also identified a kinesin-like protein (XKLP1) with a predicted molecular weight of 139 KDa present in nuclei during interphase and on chromosomes during mitosis. This protein has an N-terminal motor domain, a stalk containing 4 Nuclear Localization Signals and a tail containing potential zinc fingers. The localization of this protein suggests that it may be involved in the polar ejection forces that help to position the chromosomes on the metaphase plate. This is supported by the finding that in mitotic eggs depleted of XKLP1, spindles are disrupted and nuclear division strongly impaired.

These results indicate that chromosomes may be much more active in driving spindle assembly and their own localization in the spindle than previously thought.

J4-018 KINESIN-LIKE MOTOR MOLECULES PRESENT IN INTERPHASE CENTROSOMES/NUCLEI, AND MITOTIC SPINDLES IN CULTURED MAMMALIAN CELLS, Ryoko Kuriyama[1], Sasa Dragas-Granoic[1], Matthew Kofron[1], Russell Essner[1], Takako Kato[1,2], Charlotte Omoto[3], Takami Maekawa[1,4], Alexey Khodjakov[1,5], [1]University of Minnesota, Minneapolis, MN 55455, [2]University of Tokyo, [3]Washington State University, Pullman, WA 99164, [4]Hokkaido Red Cross, Sapporo, Japan, [5]Moscow State University, Russia.

In order to analyze the macromolecular composition of microtubule-organizing centers (MTOCs), we prepared monoclonal anti-centrosomal antibodies (CHO1 to CHO7) using mitotic spindles isolated from CHO cells as immunogens (Sellitto *et al.*, 1992). The CHO1 antibody recognized 95/105 kDa polypeptides present in the centrosome and nucleus in interphase cells. During mitosis, however, the antigen displayed a unique subcellular localization, shifting from diffuse spindle staining at metaphase to an increasingly narrow bands of microtubules within the midzone during anaphase. The 66 kDa CHO2 antigen is localized in interphase centrosomes and nuclei, and mitotic spindle poles. cDNAs coding for these antigens were cloned by screening a λUni-Zap CHO expression library with the antibody probes. Analysis of the full-coding nucleotide and deduced amino acid sequences revealed the presence of 340 amino acid residues that have significant identity with the motor domain shared among kinesin-like proteins. While the motor domain is in the N-terminal half of the CHO1 antigen, which is in good agreement with the CHO1 human clone as reported by Nislow *et al.* (1992), it is localized in the C-terminal domain of the CHO2 clone. Both the proteins are composed of a central α-helical portion with globular domains at both N and C termini. The epitope to the monoclonal CHO1 and CHO2 antibodies was shown to reside in the central stalk, indicating that the α-helical coiled-coil regions display a strong antigenicity. Full as well as truncated polypeptides were expressed in both bacteria and insect Sf9 cells using the baculovirus expression vector. Recombinant/fusion proteins of the CHO1 and CHO2 antigens supported microtubule-gliding activity *in vitro*. Using asymmetrically labeled fluorescent microtubules, the CHO1 antigen was shown to be a plus-end-directed motor, whereas the CHO2 antigen with a C-terminal motor domain moved microtubules towards the minus end. These results are consistent with many other recent reports describing a number of microtubule based motor proteins present in the spindle and playing an important role in formation and maintenance of the mitotic spindle structure. Northern and western blot analyses showed the antigens accumulated in nuclei during interphase. The CHO1/CHO2 motors could be essential either for mitosis, thus sequestered inside nuclei prior mitosis, or for nuclear functions in interphase cells, or both.

Mitosis (Joint)

J4-019 KINESIN- AND DYNEIN-RELATED MOTORS COOPERATIVELY ACCOMPLISH ANAPHASE B IN *S. CEREVISIAE*, M. Andrew Hoyt, John R. Geiser, Tami Kingsbury and William S. Saunders, The Johns Hopkins University, Baltimore, MD 21218.

We have examined the roles of three putative microtubule-based motor proteins during anaphase in *S. cerevisiae*. Previously we demonstrated that kinesin-related Cin8p and Kip1p redundantly perform an essential function prior to the onset of anaphase; their action is required for spindle pole separation and the maintenance of bipolar spindle structure (1). Dyn1p, a relative of the heavy chain of cytoplasmic dynein, is not essential for viability but is required for normal nuclear migration and spindle positioning during mitosis (2). Various combinations of *cin8*-temperature-sensitive and *kip1-Δ* and *dyn1-Δ* alleles were constructed. Cells were allowed to enter anaphase and tested for their ability to complete anaphase following a shift to a non-permissive temperature. Loss of any single gene-product or the loss of Kip1p and Dyn1p together had little or no effect on continuation of anaphase. *cin8*-ts combined with either *kip1-Δ* or *dyn1-Δ* caused a severe delay in anaphase, but did not completely block. Triple mutant cells, however, were blocked in anaphase when shifted. This finding indicates that all three motor proteins contribute to anaphase B pole separation. We suggest that anaphase B may be accomplished by Cin8p and Kip1p pushing out from between the poles combined with Dyn1p pulling from outside the poles.

Genetic and biochemical experiments are being performed to better understand the roles of the Cin8p and Dyn1p motors. Like *cin8-Δ kip1-Δ*, *cin8-Δ dyn1-Δ* form an inviable combination. This finding is consistent with the proposed overlap in function for the products of these genes. Among new mutants collected with a perish in absence of *cin8* (*pac*) phenotype were found thirteen alleles of *kip1* and seven alleles of *dyn1*. In addition, mutations in at least seven other genes caused *dyn1*-like phenotypes (frequent nuclear migration failures and cold-sensitivity). Our analysis suggests that the products of these gene act in a common pathway with Dyn1p, perhaps as accessory chains.

(1) Hoyt et al., *JCB* 118:109; Roof et al., *JCB* 118:95; Saunders and Hoyt, *Cell* 70:451.
(2) Eshel et al. *PNAS* 90:11172; Li et al., *PNAS* 90:11096.

J4-020 KINESINS PURIFIED FROM EGGS AND EARLY EMBRYOS, Jonathan M. Scholey, Section of Molecular and Cellular Biology, University of California, Davis, CA 95616.

We have been purifying members of the kinesin superfamily from eggs and early embryos using AMPPNP-induced microtubule affinity binding, and ATP-induced desorption of motors from these microtubules, followed by various combinations of gel filtration fractionation, ion-exchange FPLC and sucrose density gradient centrifugation. The presence of kinesins in the resulting fractions is monitored by immunoblotting with pankinesin peptide antibodies. The primary function of microtubules in eggs and embryos is cell division, so it is not surprising that three of the purified kinesins appear to associate with mitotic spindles. The first of these, Drosophila Melanogaster KRP_{130} behaves as a homotetramer consisting of four 130 kDa polypeptides that transports particles to the "plus ends" of microtubules at a slow rate (~ 0.04 $\mu m.sec^{-1}$), similar to the rates of many mitotic motions. KRP_{130} is thought to be a member of the BimC subfamily of kinesins that serve to push spindle poles apart during spindle morphogenesis, and we are testing the hypothesis that KRP_{130} is a bipolar tetramer that crosslinks and slides apart antiparallel microtubules, thereby separating the poles that are attached to the minus ends of the microtubules. Two other kinesins, kinesin itself and $KRP_{(85/95)}$ from Strongylocentrotutus Purpuratus are "fast" kinesins that transport particles to the plus ends of microtubules at ~ 0.6 μm/sec and ~ 0.4 μm/sec, respectively. Kinesin is a homotetramer of 2x130 Kd kinesin-related polypeptides and 2x80 Kd polypeptides, whereas $KRP_{(85/95)}$ is a heterotrimeric complex consisting of 85 Kda and 95 Kda kinesin-related polypeptides and an uncharacterized 115 Kda polypeptide. Both these kinesins appear to associate with vesicles that accumulate in different regions of the sea urchin mitotic spindle. Kinesin is associated with vesicles in the asters, and is thought to deliver vesicles out to the cell surface in response to membrane damage, supplying new membrane that reseals the damaged cell and minimizes the leakage of intracellular components. $KRP_{(85/95)}$ appears to bind vesicles that concentrate in the half spindles during metaphase and in the spindle interzone during anaphase. We hypothesize that $KRP_{(85/95)}$ may deliver membrane to the site of membrane deposition during cytokinesis.

Interaction of the Cytoskeleton with Cell Surface Adhesive Junctions

J4-021 THE ASSEMBLY AND FUNCTION OF ADHESION-SIGNALING COMPLEXES, Benjamin Geiger, Oran Ayalon, Tova Volberg and Alexander D. Bershadsky, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, 76100, Israel.

The adhesion to external surfaces, such as the extracellular matrix or neighboring cells, triggers, within cells, both short- and long-range responses. The former include the local assembly of junctional structures composed of transmembrane adhesion molecules, actin filaments and anchor proteins which link the two. The long-range processes triggered by cell adhesion affect cell growth and viability, regulation of gene expression as well as cell shape and motility.

In order to elucidate the molecular-structural basis for these-long range responses, the physical interaction of different signal transduction enzymes with cell-cell and cell-matrix adhesions was investigated. In epithelial and endothelial cells, intercellular junctions were the major sites of tyrosine-specific phosphorylation, triggered either by growth factors or by inhibition of the respective phosphatases. Mesenchymal cells, on the other hand, were phosphorylated mainly at their focal contacts. The effect of phosphorylation on cell-cell adhesion in endothelia was further investigated, showing, initially a marked increase in the size and cytoskeletal interactions of junctions, followed by deterioration of these structures and the acquisition of a fibroblastoid morphology. Quantitative analysis of this process, using digital microscopy, pointed to cyclic changes in junction assembly. It was further shown that additional factors, such as actomyosin-generated tension and the integrity of other cytoskeletal systems, may affect both tyrosine phosphorylation at adhesion sites and their state of organization. An immunofluorescence microscopic survey indicated that a variety of signal transduction enzymes are associated with cell adhesions (some constitutively and others- only after stimulation), suggesting that these cellular regions may be involved in the generation and regulation of long-range trans-membrane signals.

J4-022 **ASSEMBLY AND REGULATION OF DESMOSOMES**, Kathleen J. Green, Elayne A. Bornslaeger, Andrew P. Kowalczyk, Jennifer A. Lamb, Suzanne Norvell, Helena L. Palka, Thaddeus S. Stappenbeck, Dept. of Pathology, Northwestern University Medical School, Chicago, IL 60611.

Desmosomes are adhesive intercellular junctions that serve as cell surface attachment sites for intermediate filaments (IF). The transmembrane adhesion complex consists in large part of the desmosomal glycoproteins, desmogleins (Dsg) and desmocollins (Dsc), recently assigned to a new subclass of the cadherin family of calcium-dependent adhesion molecules. Closely associated with the cytoplasmic tails of the desmosomal cadherins is the plaque protein, plakoglobin (Pg), and located in the innermost portion of the desmosomal plaque are the most abundant desmosomal components, the desmoplakins (DP). Together these molecules play a critical role in organization of the IF cytoskeleton throughout a tissue and their proper assembly and regulation are critical for maintaining its integrity. In order to characterize assembly of the adhesion complex, we generated stable L cell fibroblast lines expressing: Dsg1, Dsg1 and Pg, Dsc3a and Pg, and Pg only. Cell lines expressing Pg exhibited barely detectable levels of steady state protein with a half life of ~10 min. However, in cell lines expressing both Pg and either Dsg or Dsc, Pg formed a co-immunoprecipitable complex with its desmosomal cadherin partner, a process which may represent the first step in assembly of the adhesion complex. Furthermore, Pg exhibited a 15-20 fold increase in metabolic stability suggesting that association with Dsg or Dsc protects Pg from rapid degradation, posttranslationally regulating its expression. It is believed that the desmosomal cadherins mediate calcium-dependent cell-cell adhesion, but there is little experimental evidence to support this contention. Adhesion assays carried out using these desmosomal cadherin-expressing L cells suggest that neither Dsg or Dsc alone or with Pg are sufficient to convey strong adhesion when expressed in normally non-adherent L cell fibroblasts. Both Dsg and Dsc may be required, perhaps in conjunction with other plaque proteins, to form an adhesion-competent complex. The integrity and assembly of cell junctions may be regulated in part by the phosphorylation state of individual components. Our recent studies have focused on the regulation of Pg and DP by phosphorylation. In the case of DP, we previously demonstrated that DP.ΔN, a truncated DP molecule lacking the N-terminal cell surface targeting domain, aligns with IF networks in transfected cells. A serine residue located 23 residues from the C-terminus of this molecule is in a perfect consensus site for phosphorylation by cAMP-dependent protein kinase (PKA). In cultured HeLa cells, association of DP.ΔN.serC23 with keratin IF networks is inhibited when cells are incubated in forskolin to activate PKA. In contrast, DP.ΔN.glyC23 which has a glycine in this position still associates with keratin IF after forskolin treatment. Phosphopeptide mapping of ectopically expressed DP domains indicates that serC23 is phosphorylated. We propose that phosphorylation of this residue may negatively regulate the association between DP and IF networks in cells.

J4-023 THE CADHERIN/CATENIN COMPLEX AND MECHANISMS OF CELL-CELL ADHESION, W. James Nelson, Brigitte Angres, Tsu-Shuh Jou, Inke Näthke, Daniel Stewart, James A. Marrs, Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305-5426.

Cadherins comprise a large family of membrane glycoproteins that mediate Ca^{++}-dependent cell adhesion during development and in the adult. Although the extracellular domain controls homotypic recognition and binding between cadherins on adjacent cells, proteins that bind to the cytoplasmic domain also regulate cell adhesivity. Three cytosolic proteins, termed α–, β– and γ– (plakoglobin) catenin, form a complex with cadherin. The assembly of the complex is temporally and spatially regulated during transport to the cell surface, and, in polarized epithelial cells, different cadherin/catenin and catenin complexes have specialized sub-cellular distributions. Changes in the levels of expression, dynamics of assembly, and phosphorylation of catenins directly affect cadherin function. Levels of β– and γ– catenin expression, and dynamics of assembly are regulated in mammalian cells by Wnt-1, which results in up-regulation of cell adhesion. Beta- catenin is also the target for phosphorylation by tyrosine kinases associated with oncogenes (eg. *src*), and growth factor receptors (eg. EGF receptor, *c-met*), which results in down-regulation of cell-cell adhesion. In addition to their interactions with cadherins, catenins have been shown recently to interact with APC (a tumor suppressor gene involved in colo-rectal carcinoma), growth factor receptors and cytoskeletal proteins. Members of the cadherin superfamily differ in their capacities to induce specialized epithelial cell phenotypes. *In situ*, the retinal pigment epithelium (RPE) forms cell-cell contacts within the monolayer, and at the apical membrane with the overlying neural retina; Na/K-ATPase and membrane-cytoskeleton distributions are restricted to the RPE apical membrane domain, and desmosomes are not formed on lateral membranes. The RPE cell line (RPE-J) expresses endogenous cadherin and forms a tight cell monolayer, but Na/K-ATPase is localized to both apical and basal-lateral membrane domains. Expression of E-cadherin in RPE-J cells results in the restriction of Na/K-ATPase distribution to sites of cell-cell contact, and the reorganization and accumulation of the membrane-cytoskeleton to cell-cell contacts. Significantly, E-cadherin expression induces the accumulation of a different ankyrin isoform and the expression and assembly of desmosomes. These results demonstrate that plasticity in the structural organization and functional phenotype of one epithelial cell-type can be directly regulated by the expression and distribution of different cadherin superfamily members.

Molecular Diversity; Subcellular Targeting: Role of Microtubules and Microfilaments

J4-100 αB-CRYSTALLIN IS CHAPERONE FOR MICROTUBULE ASSEMBLY (Y. Atomi, H.Arai and Y. Hashimoto) Department of Life Sciences, College of Arts and Sciences, University of Tokyo, Tokyo 153

In our previous studies, we identified 22-kDa protein which specifically decreased in muscle atrophy and localized at Z-bands, as αB-crystallin (αB). The mRNA and protein expression of αB increased with mechanical stimulus of muscle. Although recent *in vitro* studies showed that αB not only played a role as molecular chaperone for proteins denatured by heat stress, but also had a autokinase activity, the role of it in non-lenticular tissues and cultured cells were unknown yet. Associated proteins with αB (L6E9etc.) were examined by biochemical and immunocytochemical analysis. Anti αB C-terminal peptide polyclonal antibodies were produced and purified by peptide affinity chromatography. Analyzing by confocal microscopy, it was surprisingly found that αB colocalized microtubule network as well as intermediate filament (vimentin) in immunofluorescent stained L6E9 cells. Immuno-precipitates for cell and muscle lysates were a large heteropolymer composed of tubulin, vimentin and some other proteins in addition to αB analyzed by immunoblotting. *In vitro* binding analysis of purified tubulin and αB showed that it increased microtubule assembly, especially under the existence of calcium ions. Further immunostaining against anti-αB antibody was seen both at the nucleus and cell attachment regions in cultured cells except around mitotic phase. Taken together, αB plays a role of shaperones for cytoskeletal networks as well as seems deeply associated with mechanical signal transduction from cell membrane to nucleus, relating to calcium ions and/or possible phosphorylation. In conclusion, αB-crystallin unexpectedly functionates associating with microtuble networks as well as intermediate filament as cytoskelton stabilizing protein like as previously suggested.

J4-101 EXPRESSION OF mRNAs ENCODING TWO MEMBERS OF THE ADF/COFILIN FAMILY PRODUCED FROM DIFFERENT GENES DURING XENOPUS DEVELOPMENT, J.R. Bamburg, H. Abe, L. Minamide and T. Obinata, Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO 80523 and Department of Biology, Chiba University, Japan

A Xenopus embryo (stage 28) cDNA library was screened with cDNAs encoding chick cofilin and actin depolymerizing factor (ADF). Eight clones selected with the ADF probe and seven clones selected with the cofilin probe were partially sequenced and fell into two groups designated A9 and A11. One cDNA from each group was sequenced completely. Both encode proteins of 168 amino acids, differing in 14 residues spread throughout the sequence. Both proteins are about 77% homologous to chick cofilin and 66% homologous to chick ADF. Each protein was bacterially expressed as a GST-fusion protein, purified, and shown to interact with actin identically to chick cofilin. Monoclonal antibodies, raised against the GST-fusion proteins, or rabbit antiserum raised against synthetic decapeptides containing 3 amino acid differences between the two proteins, specifically recognized each isoform. The antipeptide antibodies are inhibitory to the actin depolymerizing activity of the GST-fusion proteins. Western blots of tissue extracts with affinity purified antibodies showed tissue specific expression of isoforms, with the A9 species being the major one expressed in brain, spinal cord and stomach. Two species of the A9 isoform appeared on 2D-gel immunoblots of tissue extracts, one of which has the pI expected for a phosphorylated form. This presumptive phosphorylated species is expressed in highest amounts during early embryonic development. Northern blots showed both probes hybridize to single bands of about 2 kb in RNA extracted from adult Xenopus tissues. A maternal mRNA of about 2.5 kb in oocytes also hybridized to both probes. This species was replaced with the 2 kb species between the 8 cell and morula stage of development. *In situ* hybridizations with digoxigenin-containing antisense riboprobes are in progress. (Supported in part by NIH grants GM35126 and NS28338 to JRB, and grants from the Ministry of Education, Science and Culture, Japan to TO)

J4-102 INDUCTION OF MICROTUBULE CATASTROPHE BY FORMATION OF TUBULIN-GDP AND APOTUBULIN SUBUNITS AT MICROTUBULE ENDS. ((Michael Caplow and John Shanks)) Dept. of Biochem., Univ. of N. Carolina, Chapel Hill NC 27599.

The recent discovery that GTP linked to microspheres binds to microtubule ends suggested that nucleotide interactions at this site may play a role in regulating microtubule (MT) dynamics. Evidence for this was sought using DIC microscopy to compare MT dynamics in the presence of minimum equilibrium concentrations and of excess concentrations of free GTP and GDP. We found that the rate of the transition from growth to rapid disassembly (catastrophe, k_c) at the microtubule plus-end was increased from 0.001 s^{-1} in the presence of 15 uM tubulin-GTP subunits (TuT) and 100 uM GTP, to 0.054 s^{-1} with 17.1 uM TuT and 0.5 uM free GTP. The increased rate with low free GTP suggests that formation of nucleotide-free (apotubulin) subunits at ends destabilizes microtubules. Excess free GDP also had a profound effect on microtubule dynamics, presumably because this exchanges into terminal subunits. With a mixture of 36.2 uM TuT and 53.8 uM Tu-GDP k_c was reduced when the free GDP concentration was decreased from 175 to 1.6 uM: k_c at the plus end went from about 0.05 to 0.0036 s^{-1}, and at the minus end from 0.006 to 0.0006 s^{-1}; the rate of elongation of the MT minus-end was increased 4-fold by reducing the free GDP concentration to 1.6 uM. Our evidence for destabilization of microtubules by formation of apotubulin and by nucleotide exchange to form terminal TuD subunits suggests that microtubule dynamics can be regulated in cells by an exchange factor that generates apotubulin subunits, or by a GTPase activating protein that forms TuD subunits at microtubule ends.

J4-103 IDENTIFICATION OF A NOVEL KINESIN-LIKE PROTEIN FROM RAT MUSCLE, Faire, K. and Bulinski, J.C., Department of Anatomy and Cell Biology, Columbia University, New York, NY, 10032.

Microtubules participate in requisite changes in myoblast cell shape, organization of myofibrillar assembly and structure, as well as transport and positioning of nuclei and organelles throughout muscle differentiation. Kinesin is a microtubule based mechanoenzyme that has been implicated in the movement of vesicles, organelles, nuclei and chromosomes. In order to elucidate the mechanisms of nuclear and organelle motility in muscle cells, we have begun to identify kinesin or kinesin-like molecules that are expressed during muscle differentiation.

We examined the expression of traditional kinesin and related proteins in differentiating rat skeletal muscle cells using a PCR based strategy. Using degenerate oligonucleotide primers corresponding to several amino acid sequences in the motor domain of the kinesin heavy chain we amplified related sequences from rat skeletal muscle cDNA. Our strategy yielded several products that fall within the size range of the products expected from such amplifications. We have cloned and sequenced one PCR product from differentiating rat muscle cells that bears significant sequence identity to the putative motor domains of murine KIF3a, the 85kDa and 95kDa subunits of sea urchin $KRP_{(85/95)}$, and Xenopus XKLP3. We have designated this clone, Rat Muscle Kinesin-Like Protein (RMKLP). The expression of RMKLP is not restricted to muscle tissue as transcripts can also be detected in rat brain using RNase protection assays.

We have also used an immunological approach to examine the expression of kinesin-like proteins in muscle and found an immunoreactive polypeptide of 85kDa in rat muscle cell extracts using antibodies raised against murine KIF3a and the 85kDa subunit of sea urchin $KRP_{(85/95)}$. Further studies are underway to examine the role of RMKLP in myogenesis. [Supported by a grant from the MDA (JCB) and an NIH Postdoctoral Fellowship (KF)]

J4-104 THEORETICAL ASPECTS OF MICROTUBULE ASSEMBLY AND CELL MORPHOGENESIS,
Timothy E. Holy, Henrik Flyvbjerg, and Stanislas Leibler, Departments of Physics and Molecular Biology, Princeton University, Princeton, NJ 08544

Dynamic instability plays a role in the morphogenesis of cells. The spatial organization of cells seems to depend in many cases (e.g. during mitosis) on the ability of microtubules to find "targets" within the cell. We build a simple model of this search process. The model shows that dynamic instability plays a large role in the search: compared to an "equilibrium" polymer, dynamic instability reduces the search time by several orders of magnitude. The model can be used to determine the choice of dynamic parameters (e.g. catastrophe and rescue rates) which minimize the search time; this choice is similar to the observed values during mitosis. Finally, the model shows quantitatively that random searching can, in many cases, account for the rapidity with which the chromosomes are captured by microtubules.

In addition, we introduce and solve a phenomenological model for the so-called catastrophes of dynamic instability. The model may explain existing experimental results and resolve some long-standing apparent contradictions. In particular, the model reproduces observed catastrophe rates and waiting times for catastrophes upon sudden dilution. It may also explain why recent experiments fail to measure the GTP content in growing microtubules and provides a mechanism for so-called coupled hydrolysis.

J4-105 TUBULIN CHANGES IN *APOPTOSIS*.
C.M.Ireland, A.E.Peaston and S.M.Pittman.
Children's Leukaemia and Cancer Research Centre, Prince of Wales Children's Hospital, Randwick, NSW, 2031, Australia.

We have shown that tubulin structures correlating with apoptotic morphology occur in a variety of normal and neoplastic cells undergoing apoptosis induced by a range of cytotoxic drugs. Depolymerisation of the tubulin structures at a late stage in apoptosis caused necrosis, suggesting that they play a functional role in the morphological changes of apoptosis. The tubulin structures were also observed at doses of vincristine and colchicine which caused depolymerisation of the mitotic spindle, implying that their properties differ from those of mitotic MTs. We reasoned that there may be quantitative changes in total cellular tubulin, in the expression of different isotypes or the occurrence of post-translational modifications which could account for the observed increased stability of apoptotic MTs. We induced apoptosis in the human T-cell leukaemia line CCRF-CEM using a dose of 17mM etoposide and investigated the expression of different tubulin isotypes and the occurrence of post-translational modifications with monoclonal antibodies using flow cytometric (FCM) analysis. Cells were harvested at times up to 6 hours following treatment and fixed for dual colour FCM. Cells were stained with antibodies to α-,β- and acetylated α-tubulin, or the appropriate isotype controls, and indirectly labelled with FITC. DNA was labelled with propidium iodide. Mean FITC channel number of the treated samples was compared to that of the controls to detect relative immunofluorescence changes. Samples were also prepared for immunocytochemistry and DNA electrophoretic analysis. Apoptosis was confirmed by observation of apoptotic morphology, occurrence of DNA 'ladders' on gel electrophoresis and the appearence of an hypodiploid peak on the DNA histogram. DNA fragmentation was observed at 3 hours following treatment. Acetylated α-tubulin levels were higher in treated cells from 1-3 hours (mean channel number ratio treated/untreated>1.2), falling from 4-6 hours. Mean immunofluorescence of other tubulin antibodies showed variable changes up to 3 hours, decreasing at later time points. Acetylated α-tubulin is a marker for MTs stable to depolymerisation treatments; these results thus support our observations that the MT structures seen during apoptosis are relatively stable and suggest that these structures may play a role in the morphological changes of apoptosis.

J4-106 NEURONAL CELL-SPECIFIC VERTEBRATE MYOSIN II,
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Previous work has demonstrated that unique isoforms of nonmuscle myosin heavy chain-B (MHC-B) are expressed in chicken and human neuronal cells (Takahashi et al., J. Biol. Chem. 267: 17864, 1992). These isoforms, which are generated by alternative splicing of pre-mRNA, differ from the MHC-B isoform present in a large number of nonmuscle cells in that they contain inserted cassettes of amino acids near the ATP binding region and/or near the actin binding region. The insert near the ATP binding region begins after amino acid 211 and consists of either 10 or 16 amino acids and contains a phosphorylation site for a proline-directed kinase. The insert near the actin binding region begins after amino acid 621 and consists of 21 amino acids. We have studied the distribution and expression of the inserted MHC-B isoforms. In the developing chicken brain, mRNA encoding the 10 amino acid insert gradually increases after fertilization, peaks in the 10-14 day embryo and then declines. In contrast, the mRNA encoding the 21 amino acid insert appears just before birth and is abundantly expressed in the adult chicken cerebellum. There is a marked species difference between the distribution of the inserted isoforms in adult tissues. The mRNA encoding the 10 amino acid insert near the ATP binding region is very poorly expressed in the chicken brain (ibid.), but makes up most of the MHC-B mRNA expressed in the human cerebrum and retina. It is also expressed in neuronal cell lines. The mRNA encoding the 21 amino acid insert is abundantly expressed in the chicken cerebellum and human cerebrum, but is absent from the retina and cell lines. Using a variety of neuronal derived cell lines, we studied agonists that increase the expression of the 10 amino acid insert present near the ATP binding region. Employing human retinoblastoma (Y-79) and neuroblastoma (SK-N-SH) cell lines, we are able to increase the expression of mRNA encoding the inserted isoform using a number of agonists or serum deprivation. In each case, expression of the inserted isoform correlates with cell differentiation and inhibition of cell division. Using a rat pheochromocytoma cell line (PC12), we show that prior to treatment with nerve growth factor (NGF), there is no evidence for either inserted isoform. However, treatment with NGF results in the appearance of mRNA encoding the 10 amino acid inserted isoform, concomitant with neurite outgrowth. Both the inserted isoform and neurites disappear following withdrawal of NGF.

J4-107 **IDENTIFICATION OF BINDING PARTNERS FOR THE F-ACTIN BINDING PROTEIN CORTACTIN**
Trudy D. Marshall and J.T. Parsons, Department of Microbiology, University of Virginia, Charlottesville, VA, 22908

Submembranous enrichment of cortactin to cortical structures (such as membrane ruffles) is indicative of a role for this filamentous actin-binding protein in forging microfilament-membrane interaction(s) or in relaying signalling events which occur at the membrane/cytoskeleton (Wu and Parsons, 1993, JCB, 120: 1417-1426). To elucidate the function(s) for cortactin, we are characterizing the binding partners for this p80/85 kDa protein. We have identified several specific bands on a "Far-Western" blot; several of which are enriched in brain (chicken and rat) and has appparent molecular weights of 70-80 kDa. We have localized the binding domain to the c-terminal portion of cortactin [GST fusion] and are presently further delineating this domain(s) in cortactin. The fact that this binding component is highly enriched in the adult brain relative to the developing chick (day 10, 13 or 15) brain indicates a putative role for this association in the development and function of adult brain processes. Identification of the protein-protein interactions involved in the targeting and function of cortactin will be instrumental in elucidating the role for cortactin in signal transduction at the membrane/ cytoskeleton.

J4-108 TEMPERATURE-SENSITIVE MUTATIONS IN THE MYOSIN TAIL: INSIGHTS INTO THE CONTROL OF FILAMENT ASSEMBLY, Sheri L. Moores, Bruce Patterson, and James A. Spudich, Department of Biochemistry, Stanford University, Stanford, CA 94305

Conventional myosin participates in multiple activities of nonmuscle cells including cytokinesis, cell migration, and shape changes associated with development. Although the importance of myosin in a wide spectrum of cellular functions is clear, the *in vivo* regulation of myosin filament formation is not well understood. The cell must not only assemble myosin into bipolar filaments, but also must control when and where these filaments are made. We would like to identify regions of the myosin molecule that affect filament assembly and understand the mechanism by which filament assembly is controlled. Mutants have been isolated in *Dicytostelium discoideum* that cannot divide or complete development at low temperature (13°C), but regain most wild-type functions at normal temperature (21°C). We have mapped and sequenced two mutations in the myosin tail that give rise to this cold-sensitive phenotype. Both mutations alter residues within a region of the tail known from previous experiments to be involved in regulation of filament assembly. One mutation results in a change from an arginine to a proline, and the other causes a four amino acid deletion including this same arginine. We are analyzing these mutant myosins biochemically to determine whether these changes affect filament assembly by structurally inhibiting monomer packing, or by disrupting interactions between regulatory proteins and the myosin tail.

J4-109 ABNORMAL SUBCELLULAR DISTRIBUTION OF MYOSIN AND TALIN IN WISTAR FURTH RAT PLATELETS, Tamara I. Pestina, Carl W. Jackson, and Paula E. Stenberg, Department of Hematology/Oncology, St. Jude Children's Research Hospital, Memphis, TN, 38105, and Department of Pathology, Oregon Health Sciences University, Portland, OR, 97201

The roles of most cytoskeletal proteins in platelet formation and function remain largely undefined. We earlier detected megakaryocyte membrane blebbing and a unique antigenic determinant associated with a missense mutation in the cytoskeletal protein, talin, in an animal model of hereditary macrothrombocytopenia, the Wistar Furth (WF) rat, which led us to examine the distribution of talin and other cytoskeletal proteins in resting normal and WF rat platelets. In contrast to the conclusions of an earlier ultrastructural analysis, our biochemical and ultrastructural immunogold studies indicate a significant membrane-association of talin in both resting normal and WF rat platelets as found earlier for rat megakaryocytes. Talin was associated with plasma membranes, membranes of the surface-connected canalicular system, and with α-granule membranes of both normal and WF rat platelets, but as in WF megakaryocytes, talin was absent from the large membrane complexes of WF platelets. An even more striking difference was seen in the distribution of myosin in subcellular fractions of normal and WF rat platelets separated in density gradients, in which the proportion of myosin in the least dense WF rat platelet membrane fraction was one-half that in the same normal platelet fraction. This difference was balanced by a four-fold increase in myosin in the most dense WF rat subcellular fraction, which is highly enriched for α-granules. These results support our hypothesis that the platelet abnormalities of the WF rat are related to defects in the megakaryocyte-platelet cytoskeleton.

J4-110 MULTIPLICATION AND MIGRATION OF THE CENTROSOME IN APOPTOSIS. S.M.Pittman, A.E.Peaston and C.M.Ireland, Children's Leukaemia and Cancer Research Centre, Prince of Wales Children's Hospital, Randwick, NSW, Australia.

Apoptotic cell death is an ordered process in which the cell breaks up into bodies which are removed from the system by phagocytic cells. During apoptosis the cell digests its own nuclear DNA. Tubulin reorganisation was observed in cells undergoing apoptosis and we hypothesise that microtubules (MTs) may play a role in the morphological changes that occur during this process. In the untreated mammalian cell tubulin nucleates from the centrosome and we wished to determine whether centrosomes were required for the tubulin reorganisation we had observed. Apoptosis was induced in CCRF-CEM T-cell leukaemia cells using VP-16. In order to investigate the sequence of events during the process treated and control cells were harvested a 0,.5,1,2 and 3 hours. Comparisons were made using flow cytometry (FCM) and immunocytochemistry with monoclonal antibodies which recognise a centrosomal antigen[1] and β-tubulin. DNA was isolated at each time point in order to identify when the nucleosomal fragments which are characteristic of apoptosis were first seen on an agarose gel. We found that an early change detected by FCM and immunocytochemistry was an increase in the expression of the centrosomal antigen. Analysis showed increasing levels of relative immunofluorescence from 1 to 3 hours. Cell preparations showed that the centrosomes had multiplied and migrated to the nucleus. β-tubulin decreased at 1 hour, followed by an increase with maximum expression at 2-3 hours. The increase in β-tubulin in cell preparations could be seen as cells were recruited into morphologically recognisable apoptosis. This included cells with invagination of the nucleus associated with tubulin bands surrounding the nucleus and single or multiple apoptotic bodies with strong tubulin staining. The ladders which confirm these cells were apoptotic were detected at 3 hours. Thus the process of apoptosis appears to be coordinated in VP-16 treated CCRF-CEM cells with multiplication and migration of the centrosome to multiple nuclear sites, associated with increased expression of β-tubulin, the nucleation of microtubules and reorganisation into the structures we have found to be characteristic of apoptosis. As the cells break-up the DNA laddering is detected.

[1]MAbIIa138kindly provided by K.A.O.Eilem, QIMR, Brisbane.

J4-111 2.0 Å STRUCTURE OF BOVINE PROFILIN REVEALS AN ACTIN-INDUCED CONFORMATIONAL CHANGE, Michael D. Rozycki, Nalin C. W. Goonesekere, Eila Cedergren-Zeppezauer, Zbigniew Dauter, James C. Myslik, Uno Lindberg and Clarence E. Schutt, Department of Chemistry, Princeton University, Princeton, NJ 08544, USA, Department of Zoological Cell Biology, Stockholm University, S-10691 Stockholm, Sweden, and European Molecular Biology Laboratory, DESY, D-2000 Hamburg, Germany

Bovine profilin crystals (space group C2; a = 69.15 Å, b = 34.59 Å, c = 52.49 Å; $\alpha = \gamma = 90°$, $\beta = 92.56°$) were grown from a mixture of poly(ethylene glycol) 400 and ammonium sulfate. X-ray diffraction data was collected on an imaging plate scanner at the DORIS storage ring (DESY, Hamburg), and was phased by molecular replacement, using a search model derived from the 2.55 Å resolution structure (Schutt et al., *Nature* **365**, 810-863) of profilin complexed to β-actin. The refined model of bovine profilin has a crystallographic R-factor of 16.5 % in the resolution range 6.0 - 2.0 Å and includes 128 water molecules, several of which form hydrogen bonds to stabilize unconventional turns.

The structure of free bovine profilin is similar to that of profilin complexed to β-actin, and Cα atoms from the two structures superimpose with an r.m.s. deviation of 1.25 Å. However, a rotation by approximately 140° about the Cα-C bond of Gly 2 causes Ala 1 and the N-terminal acetyl group of profilin to be placed in almost diametrically opposed positions in the actin-free and actin-bound structures. These residues lie immediately adjacent to the poly(L-proline) binding site of profilin, and they form part of an interface with actin in profilin:β-actin crystals. Thus, the N-terminus of profilin could act as a switch to mediate interactions with actin, perhaps through the binding of poly(L-proline) or a proline-enriched peptide.

J4-112 IMPLICATIONS OF MICROFILAMENT DIVERSITY FOR NEURONAL DEVELOPMENT. Ron Weinberger, Galina Schevzov, Tony Hannan, Peter Jeffrey, and Peter Gunning, Children's Medical Research Institute, Locked Bag 23, WENTWORTHVILLE NSW 2145, Australia

Differentiation and maturation of neurons involves a series of profound changes in morphology which are intrinsic to the ultimate function of a neuron. Morphogenesis is most often associated with alterations in the actin based microfilament system of non-muscle cells. We have evaluated whether changes in the composition of microfilaments accompany neuronal morphogenesis. Antisera were raised against the tropomyosin (Tm) isoforms Tm-5 and TmBr-1/-3. In combination with actin isoform specific antibodies, we find that during early morphogenesis *in vivo*, immature growing axons contain β- and γ-actin and Tm-5. In particular, β-actin is enriched in, and Tm-5 exclusively located in, the growing axonal processes relative to the neuronal cell body. β-actin and Tm-5 are lost from mature quiescent axons suggesting that these isoforms are associated with membrane remodelling. The localization of Tm-5 protein to growing axons appears to be achieved by the segregation of its mRNA almost exclusively to the axon hillock and at least the proximal portion of the axon. Cortical neuronal cultures show that the Tm-5 protein is highly concentrated at the base of one process well before a clearly defined axon is observed. The localization of mRNA and protein is not observed for other Tms and suggests a specific role of Tm-5 in the early decisions associated with neuronal polarity.

The loss of β-actin and Tm-5 from axons *in vivo* is accompanied by a progressive appearance of TmBr-1/-3 in axons. The loss of β-actin and Tm-5 from axons involves a redistribution of these molecules to other intracellular sites. Tm-5 becomes associated with the cell body and dendrites whereas β-actin is most prominently localized in synapse-rich structures. At all times, γ-actin is uniformly distributed over all cellular structures. We conclude that both Tm isoforms and β-actin are subject to specific patterns of segregation associated with axonal growth and synaptic maturation. This provides a potential molecular basis for the temporal and spatial specificity of microfilament function during neuronal differentiation. Based on our data we propose a model which suggests associations of specific Tms with specific actin isoforms and a "subunit exchange" scheme of axonal microfilament components during neuronal development that incorporates mRNA localization

Intermediate Filaments in Differentiation and Cell Adhesion; Tau Protein Function and Its Relation to Alzheimers' Disease

J4-200 **T3 EFFECTS ON CEREBELLAR ASTROCYTE DIFFERENTIATION OF HYPOTHYROID RATS**

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Instituto de Biofísica Carlos Chagas Filho - UFRJ - Rio de Janeiro - Brasil - FAX: 5 21 2808193.

The thyroidian hormone T3 plays a crucial role in normal development of Nervous System. It's diminished expression on hypothyroidism induces deficiency in neural myelination, arborization and migration, so as in synapses formation. The effects of thyroidian hormone T3 on proliferation and differentiation of rat astrocytes have been investigated. T3 stimulates differentiation in cultured cortical astrocytes. In this work we analyze the effect of T3 on cerebellar astrocytes morphogenesis and its effect on cytoskeletal proteins in hypothyroidian rats. T3 was added for 3 days with daily medium change letting cells incubate for four days. The proteic extracts of cells analyzed by SDS-PAGE and western blotting showed that in treated cells vimentin expression raised whereas GFAP diminished. This altered balance of cytoskeletal proteins could indicate that T3 favors proliferation to the detriment of differentiation. Labeling the cultures with [^{35}S] metionine we were able to see that the conditioned medium was enriched in proteins if compared to the control. Neurons were plated onto T3 treated astrocytes. Although no effect on neuronal number or length of processes was seen it seems that T3 treated astrocytes cultures showed a higher number of neurons than the control, suggesting an increased neuronal survival.

J4-201 CROWDING-INDUCED ORGANIZATION OF CYTOSKELETAL FILAMENTS: SPONTANEOUS BUNDLING AND SORTING, MODULATED BY CAPPING AND CROSS-LINKING,

Judith Herzfeld, Daniel T. Kulp, Thomas L. Madden and Boris Itin, Department of Chemistry, Brandeis University, Waltham, MA, 02254-9110

Naive expectations, based on the behavior of dilute solutions, are that cytoskeletal filaments will be randomly dispersed unless they are cross-linked into bundles by accessory proteins. However, the cytoplasm is a very crowded, and therefore highly non-ideal, solution. The effects of packing constraints have been calculated using statistical thermodynamics approaches validated on simpler systems. The theory combines a phenomenological description of reversible filament self-assembly and a scaled particle treatment of excluded volume. Filament flexibility is treated according to Khokhlov and Semenov. We find that, at the concentrations found in cells, filaments will spontaneously separate from globular proteins, the loss of mixing entropy being compensated by a greater gain in translational entropy. The theory explains the bundling that has been reported when ovalbumin has been added to F-actin in cell-free solutions. In further calculations, we find that this spontaneous filament bundling occurs for filaments as flexible as microfilaments, as well as for more rigid filaments such as microtubules. When both types of filaments are present, further demixing occurs such that segregated bundles are formed. In this case the entropy of mixing is compensated by the translational entropy gained by the stiffer filaments and the bending entropy gained by the more flexible filaments. Since bundling is spontaneous, the cell needs mechanisms to suppress it when it is not desirable. Model calculations show that capping proteins can prevent bundling by shortening the filaments and cross-linking proteins can prevent bundling by preventing alignment.

J4-202 STRUCTURAL AND FUNCTIONAL ANALYSES OF THE ß-ACTIN MESSENGER RNA ZIPCODE.

E. H. Kislauskis, X. Zhu, and R. H. Singer. Department of Cell Biology, University of Massachusetts, Worcester, MA 01655.

In motile chicken embryonic fibroblasts (CEFs), ß-actin mRNA is highly localized to the leading lamellae. The mechanism is highly responsive to the chemotactic factor (PDGF) and serum, is sensitive to chemical modulators of signal transduction, and requires the integrity of the actin cytoskeleton. To characterize the cis-acting RNA for peripheral RNA localization, chimeras between ß-actin sequences and a LacZ reporter gene were evaluated in transiently transfected CEFs. Intracellular ß-galactosidase activity (blue staining) correlated with the distribution of the chimeric mRNA. The ß-actin 3'-untranslated sequences (3'UTR) necessary and sufficient to target ß-galactosidase activity appropriately to the cell periphery could be determined in this way. Detailed mutational analysis indicated that the RNA localization sequences ("zipcode") occured within a 54 nt segment and a homologous but weaker, 43 nt segment. These regions contain motifs of 5-7 nt that are conserved betweeen the chicken and human clones. Complementary (antisense) phosphorothioate oligonucleotides against the zipcode were found to delocalize the endogenous actin mRNA, whereas control oligonucleotides did not. Actin mRNA and protein levels were not affected by oligonucleotide treatment. However, dramatic changes in lamellipodia structure and stress fiber organization occurred using antizipcode oligonucleotides that delocalized actin mRNA. These same oligonucleotides also inhibited cell motility and RNA localization in response to chemotactic stimuli in serum. Hence, discrete 3'UTR sequences that direct ß-actin synthesis to the leading lamellae also affect cell morphology and motility, presumably through the actin cytoskeleton.

J4-203 **MORPHOLOGICAL CHANGES INDUCED BY THE THYROID HORMONE (T_3) IN HUMAN EPENDYMOMA AND RAT GLIOMA CELLS (C_6).** ***C. Lins#, C. M. Takiya§, F. Rodrigues§, F. Duarte§, C. Chagas#, V. Moura Neto#.***

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A great variety of tumors arise in the Nervous System due to the transformation of normal resident cell types.The most common tumors are: gliomas, oligodendrogliomas, ependymomas and neuroblastomas. We have analyzed a human ependymal tumor, established by us in culture, using either explants or dissociated tumor cells. More than 90% of plated dissociated cells showed a flat or ovoid morphology and were positively labeled for the cytoskeletal protein vimentin and negatively labeled for GFAP. Cells cultivated in the presence of the Thyroid hormone (T_3) for three days, exhibited progressive morphological transformation from flat shaped to process-bearing cells. The rat glioma cell line C_6 was also responsive to the hormonal treatment, changing from triangular shaped cells to fine long process bearing cells. The same transformation was previously obtained in normal rat glial cells and related to an indirect effect of T_3, most likely going through the release of cytokines, themselves responsible for the morphological change. These results suggest that like normal astroglia, tumoral ependymocytes and astrocytes are able to release T_3-induced factors. Cytokine-like molecules induced and released by the hormonal treatment can play an important role in the rearrangement of the cytoskeleton, proliferation and differentiation of these tumoral cells.

J4-204 CYTOSKELETAL BASED TRANSLOCATION OF SPECIFIC MATERNAL RNA TRANSCRIPTS AND ORGANELLES DURING THE FIRST CLEAVAGE CYCLE OF XENOPUS EMBRYOS. Carey R. Phillips, Bethany Whalon and Michael Danilchik*. Department of Biology, Bowdoin College, Brunswick ME. 04011 and *Oregon State Health Sciences University, Portland OR.

A subset of the maternally derived RNA transcripts become localize to specific regions of the single celled Xenopus laevis embryo during the first cell cycle. It is believed that some transcripts are involved in providing spatial information for subsequent differentiation of dorsal axial structures. We are using the PCR display method to quantify the number of localized maternal RNAs within the newly fertilized amphibian egg. The PCR method is also used to generate the appropriate DNA clones in order to prepare in situ hybridization probes to map the spatial locations and to study the mechanisms involved in the translocation process. We have found a small number of maternally derived RNAs which are translocated to specific regions or compartments of either the presumptive dorsal or presumptive ventral side of the developing embryo. We have isolated several clones for translocated RNAs and we are in the process of examining the mechanisms involved in the translocation process. The movements of these RNAs is dependent upon the integrety of the microtubule system and has been found to be gravitationally sensitive. Sequence analysis of one dorsal enriched RNA indicated that it is the large mitochondrial ribosomal subunit. We have shown that mitochondria become enriched on the presumptive dorsal side of the embryo at the one cell stage.

J4-205 ISOLATION AND CHARACTERIZATION OF PROTEINS BINDING TO THE LOCALIZATION "ZIP CODE" OF β-ACTIN mRNA IN CHICK EMBRYO FIBROBLASTS. A.F. Ross, E.H. Kislauskis, K.L. Taneja, and R.H. Singer, Department of Cell Biology, University of Massachusetts Medical School, Worcester, MA 01655.

It is now well established that the mRNA for β-actin in chick embryo fibroblasts is localized to the leading edge of the cell in a region referred to as the lamella. This peripheral localization may play a role in the establishment of a polarized actin cytoskeleton, a requirement for cellular motility. Although the mechanism of β-actin mRNA sorting is not understood, evidence suggests that the mRNA is physically associated with actin filaments at the cell periphery. A short (54 base) sequence has been identified in the proximal 3' untranslated region of β-actin mRNA which localizes a reporter gene (β-galactosidase) in a heterologous expression system (Kislauskis et al., 1994, J. Cell Biol. 127:441-451). It is possible that a protein or complex of proteins interacts with this localization "zip code" to transport and/or link β-actin mRNA to peripheral actin filaments. To isolate such proteins we have constructed affinity resins consisting of oligoribonucleotide probes modified with biotinylated bases at the 3' end and linked to streptavidin-agarose. Using these resins we have isolated a 70kDa protein from extracts of chick embryo fibroblasts which binds specifically to the proximal 27 bases of this localization zip code. Microsequence analysis and GenBank comparison to known proteins indicates that this protein has not been identified. In addition, several other proteins of molecular mass 90kDa, 53kDa, 35kDa, and 30kDa bind to this sequence in a variable manner. These data suggest that a complex of proteins binds specifically to a segment of the 3'UTR of β-actin mRNA which has been shown to be responsible peripheral localization.

J4-206 ANALYSIS OF THE INTERMEDIATE FILAMENT NESTIN IN MUSCLE UNDER NORMAL AND PATHOLOGICAL CONDITIONS, Gunnar Sjöberg, Lars Edström*, Urban Lendahl and Thomas Sejersen, Department of Cell and Molecular Biology, Karolinska Institute, *Department of Neurology, Karolinska hospital

The intermediate filament nestin, a class VI intermediate filament, has been shown to be expressed in neuroepithelial stem cells, somites and in developing muscle. In addition to this we have found nestin to be expressed in developing heart muscle and also in Purkinje fibers of the adult heart. Though quite distantly related to class III intermediate filaments, we present results from 3-D fluorescence digital imaging microscopy showing that the filamentous network of nestin colocalizes with that of desmin and vimentin in myoblasts and in multinuclear myotubes of the human fetal myogenic cell line G6. Comparisons in amino acid sequence of the rod domains and regulatory regions of the genes are discussed.

Since regenerating muscle are assumed to pass through the same sequential events as developing muscle we analyzed the expression patterns of the above mentioned intermediate filaments in biopsies from patients with Duchenne/Becker muscular dystrophy and polymyositis. In both types of diseases areas of muscle regeneration stained positive for nestin and vimentin, and also stained more intensely for desmin than surrounding fibers. Nestin was, however, the only intermediate filament to be found specifically in regenerating muscle cells.

Studies of nestin expression in rhabdomyosarcomas in comparison to myogenic regulatory factors and desmin show that nestin can potentially be an interesting marker for undifferentiated muscle tumors. All 35 desmin positive rhabdomyosarcomas also stained positive for nestin and Myf3. Additionally, one tumor expressed nestin and Myf3 but did not express desmin. An interesting finding was that non-tumor muscle cells surrounding tumors quite often also stained positive for nestin. We believe this to represent muscle influenced by tumor growth to undergo regeneration.

J4-207 **INTERACTION BETWEEN VIMENTIN AND THE HIV-1 VIF PROTEIN RESULTS IN THE STRUCTURAL REORGANIZATION OF BOTH PROTEINS,** Klaus Strebel and Mary K. Karczewski, Laboratory of Molecular Microbiology, NIAID, NIH, Bethesda, MD 20892.

The Human Immunodeficiency Virus Type-1 (HIV-1) Vif protein is a 23 kDa basic protein which is known to regulate infectivity of newly synthesized virions in a cell-type specific manner. In restrictive cells, virions produced in the absence of Vif are completely non-infectious. Although easily detectable in infected cells, Vif has thus far not been found in virion preparations suggesting that its activity is required at the time of virus assembly and/or virion maturation. Cell fractionation studies reveal that a significant portion of Vif in virus-producing cells is highly insoluble and cannot be extracted with non-ionic or ionic detergents. Immunocytochemical analyses reveal a filamentous pattern for Vif in HeLa cells and subsequent studies involving confocal microscopy demonstrated a clear co-localization of Vif with the intermediate filament vimentin. Surprisingly, treatment of Vif-producing HeLa cells with brefeldin A (BFA) resulted in a dramatic re-organization of both vimentin and Vif from a filamentous form to a 5 um perinuclear cap. This effect of brefeldin A on vimentin is dependent on the presence of Vif and requires intact microtubules. Perinuclear capping of Vif can be blocked or reversed when cells are treated with drugs causing depolymerization of microtubules. Hyper-phosphorylation of vimentin induced by the treatment of cells with a phosphatase inhibitor also resulted in perinuclear capping of Vif and vimentin but in a Vif-independent fashion. One of the possible interpretations of these findings is that Vif, in the presence of BFA, causes hyperphosphorylation of vimentin, resulting in the observed structural changes of vimentin as well as Vif. In the colon carcinoma cell line SW480, both vimentin and Vif form a nuclear cage rather than a filamentous network. Interestingly, BFA treatment of SW480 cells does not result in perinuclear capping of vimentin nor Vif. The mechanistical implications of these findings as well as the relevance of our observations with respect to the regulation of virus infectivity will be discussed.

J4-208 INFLUENCE OF AGING ON PLATELET DERIVED GROWTH FACTOR STIMULATED CYTOSKELETAL CHANGES IN FIBROBLASTS, Beatrice Wang, Jean Rischert-Buhac, Ian Penner, Wende R. Reenstra, Department of Dermatology, Boston University School of Medicine, Boston, MA 02118.

The effect of donor age on platelet derived growth factor (PDGF) modification of cytoskeletal proteins was investigated. Interactions between the actin cytoskeleton and cell membrane receptors are mediated by a population of proteins that play both a structural and regulatory role in cell proliferation. Modifications in these interactions occur when cells change shape or proliferate in response to PDGF. One of the modifications is hypothesized to be phosphorylation of talin and focal adhesion kinase (FAK). It is also well documented that there is an age associated loss of proliferative response to serum mitogens, one of which is PDGF. To initiate fibroblast proliferation, PDGF binds the transmembrane PDGF-receptor (PDGFR), which becomes phosphorylated. Further protein phosphorylation is known to modulate cytoskeleton organization via protein kinases. Since fibroblast proliferative response to PDGF is known to be decreased with age, we determined the influence of donor age on PDGF cytoskeletal changes by confocal microscopy. Fibroblasts from newborn, young and old adult donors were stimulated with PDGF and incubated with monoclonal antibodies to PDGFR, talin, FAK and phosphotyrosine, as well as stained with phalloidin. PDGF stimulation was found to induce changes in actin filament distribution that were age-specific. Newborn cells responded within 5 minutes whereas young and old adult did not undergo actin reorganization until 20 and 60 minutes after PDGF stimulation. Determination of phosphorylation on tyrosine residues revealed a punctate staining pattern on old adult fibroblasts that did not change with PDGF stimulation. In contrast, newborn and young adult fibroblasts displayed a diffuse upregulation of tyrosine phosphorylation. By morphologic analysis, the phosphorylation appeared to be actin-associated. The inablity of old adult cells to reorganize the actin cytoskeleton upon PDGF stimulation may play an important role in the observed decreased proliferative response with age.

Transgenic Mice-Models for the Study of Differentiation and Disease; Cell Motility: Mechanisms and Extracellular Signaling

J4-300 STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF BOVINE PROFILIN II,

Christophe Ampe, Anja Lambrechts, Jozef Van Damme, Marc Goethals and Joël Vandekerckhove, Laboratory of Physiological Chemistry, University Ghent, Belgium.

Profilins are small ubiquitous proteins that are capable of regulating actin dynamics through a dual activity[1]. They may act as sequestering proteins or may facilitate actin polymerization depending on the conditions used. In a number of lower eukaryotes two profilin isoforms are present. However, only recently a gene encoding a second profilin isoform in human was identified[2]. To investigate the functional properties of this isoform and to compare them with those of profilin I, we purified the bovine homologue of this isoform. We determined its entire sequence. This isoform is NH_2-terminally acetylated and is more basic than profilin I. It shows 93.7% similarity with profilin II from human and 74.7% similarity with either profilin I. The similarity with profilins from lower eukaryotes ranges from 35% to 42% and is not significantly higher than the similarity of profilin I with profilins of lower eukaryotes. Profilin II binds more tightly to poly-L-proline sepharose columns than profilin I. Also, the affinity for actin is higher because less profilin II is needed in a falling ball viscosimetry experiment to achieve the same reduction in viscosity. Like profilin I, this second isoform binds the second messenger molecule phosphatidyl inositol 4,5 bisphosphate.

1) Pantaloni D. and Carlier M.-F. (1993) - Cell. 75, 1007-1014.
2) Honoré B. et al. (1993) - FEBS Lett. 330, 151-155.

J4-301 HIGH-LEVEL EXPRESSION OF THE HYPERPROLIFERATION-ASSOCIATED KERATINS IS ASSOCIATED WITH MAJOR CHANGES IN KERATINOCYTE CYTOARCHITECTURE.

Pierre A. Coulombe, Kenzo Takahashi, Rudy Paladini, and Janet Folmer, Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Injury to epidermis and other stratified epithelia triggers profound changes in the pattern of keratin gene expression. In post-mitotic cells located at the wound edge, a strong induction of keratins K6 (56 kD), K16 (48 kD) and K17 (46 kD) occurs at the expense of the keratins produced under the normal situation.This induction correlates spatially and temporally with significant changes in the shape and cytoarchitecture of epidermal keratinocytes. Such changes take place early in the regeneration response and precede the onset of hyperproliferation per se. We have initiated a series of studies aimed at elucidating whether K6, K16 and K17 play a direct role in the process of keratinocyte "activation" at the wound edge. We recently showed that increased expression of human K16 in a tissue-specific fashion in transgenic mice results in skin lesions caused by anomalies in the program of terminal differentiation in the hair follicle outer root sheath and proximal epidermis (Takahashi et al., J. Cell Biol. October 1994). Biochemical studies established that the onset and progression of skin lesions depend upon the levels at which the transgene product is expressed relative to other epidermal keratins, and that transgenic lines which do not develop skin lesions are characterized by low transgene expression. Morphologically, the epidermis of phenotypic mice features various degrees of acanthosis (increase in thickness), acantholysis (loss in keratinocyte cell-cell contacts), and hyperkeratosis. Interestingly, these changes are similar to those occuring after skin wounding in a normal mouse. We are currently investigating the cellular mechanisms underlying the production of this phenotype by conducting in vitro cell culture studies with epidermal keratinocytes derived from the human K16-expressing transgenic mice, and by examining the assembly properties of purified human K6 and K16 in vitro. These studies are supported by the American Cancer Society.

J4-302 THE MOLECULAR GENETICS OF NEURONAL GROWTH: CHARACTERIZATION OF THE Tα1 α-TUBULIN PROMOTER IN TRANSGENIC MICE,

Andrew Gloster, Wendong Wu, Audrey Speelman, Jean Toma, Edward Chang, and Freda D. Miller. Montreal Neurological Institute, McGill University, Montreal, Canada.

We have previously demonstrated that the Tα1 α-tubulin gene represents one member of the α-tubulin multigene family that is expressed at abundant levels as a function of neuronal growth. To determine the molecular mechanisms whereby neuronal gene expression can be coupled to morphological differentiation, we have analyzed the promoter for this gene in transgenic mice. More specifically, we have generated transgenic mice carrying a fusion gene comprised of 1,100 nucleotides of the upstream, putative Tα1 promoter region linked to a nuclear β-galactosidase reporter gene. Analysis of these mice demonstrated that expression of the transgene is limited to the nervous system, and that the onset within the neural tube, retina, dorsal root, sympathetic, trigeminal, and fascio-acoustic ganglia, intrinsic neurons of the heart and gut suggests that expression first occurs, as or just before, neurons undergo their final mitosis. In dispersed E15 brain cultures, BrdU labelling revealed cells which were immunoreactive for both BrdU and the transgene, consistent with the notion that cells express the transgene before their final mitosis. Marker gene expression remains high during neuronal morphogenesis, at birth, and is subsequently downregulated following neural maturation, as indicated both by X-gal staining and by Western blot analysis. However, in the adult the marker gene continues to be expressed at high levels in the olfactory epithelium where neurogenesis is ongoing, and is dramatically upregulated in facial motoneurons during axonal regeneration. These results suggest that the Tα1 α-tubulin gene is activated coincident with the cells commitment to being a neuron, and that sequences exist within this promoter region which are responsible for coupling gene expression to neuronal growth during both development and regeneration.

J4-303 INHIBITION OF CYTOSKELETAL REORGANIZATION STIMULATES ACTIN AND TUBULIN SYNTHESIS DURING INJURY-INDUCED CELL MIGRATION,

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A single layer of squamous epithelial cells termed the "endothelium" lines the posterior surface of the vertebrate cornea. A well-defined circular freeze injury to the center of the tissue results in the directed migration of surrounding cells into the wound center, and the reorganization of actin and tubulin. In these cells, circumferential microfilament bundles (CMBs) are replaced by prominent stress fibers while microtubules (MTs), observed as a delicate lattice in non-injured cells, are reorganized into distinct web-like patterns. To determine whether cytoskeletal reorganization requires actin and tubulin synthesis, injured endothelia were organ cultured for various times and metabolically labeled with Trans ^{35}S-label (ICN, 250 μCi/ml) for the final 6 h of each experiment. Actin and tubulin immunoprecipitates were analyzed by SDS PAGE, fluorography and scintillation counting. No significant increase in ^{35}S incorporation was detected when compared to noninjured controls. If cytoskeletal reorganization is prevented, either by pre-treating tissues with phalloidin (7 μM) to stabilize CMBs, or culturing in 10^{-8}M colchicine to dissociate MTs, ^{35}S incorporation into actin and tubulin increases significantly. This result indicates that pre-existing actin and tubulin are used for cytoskeletal reorganization during injury-induced cell movement in the endothelium. If this process is inhibited, then actin and tubulin synthesis is initiated. [Supported by the Oakland University Program in Biochemistry and Biotechnology].

J4-304 MECHANISMS OF THIN FILAMENT ASSEMBLY: TROPOMODULIN REQUIRES TROPOMYOSIN FOR ASSEMBLY, Carol C. Gregorio and Velia M. Fowler, Department of Cell Biology, The Scripps Research Institute, La Jolla, CA 92037.

Actin filament lengths are precisely maintained in striated muscle sarcomeres. Tropomodulin is an actin and tropomyosin-binding protein that is associated with the pointed ends of thin filaments (Fowler *et al.*, 1993, JCB 120:411). Recent in vitro studies demonstrate that tropomodulin is a pointed end capping protein; it completely blocks elongation and depolymerization at the pointed ends of tropomyosin-containing actin filaments (Weber *et al.*, 1994. JCB, in press). We are presently studying the subcellular distribution and biosynthesis of tropomodulin during striated myofibril assembly in primary embryonic chick cardiomyocyte cultures to give us further insight into mechanisms of thin filament assembly. Results from these experiments indicate that tropomodulin is not assembled coordinately with other thin filament proteins. Specifically, a significant proportion of tropomodulin is present as a stable pool of soluble tropomodulin throughout myofibril assembly. Kinetic data obtained from pulse-chase experiments and results obtained utilizing a permeabilized cell model for thin filament assembly indicate that 1) tropomodulin is synthesized as a soluble protein and 2) tropomodulin requires tropomyosin to be assembled on actin filaments prior to its own assembly into myofibrils. In fact, tropomodulin appears to assemble later than all other myofibrillar proteins studied; it is incorporated in its characteristic sarcomeric location at the pointed ends of the thin filaments after the appearance of I and A bands periodicities. Our results suggest that the absence of tropomodulin in some striated myofibrils is not due to limiting quantities of the protein, but is due to its inability to incorporate into thin filaments. We hypothesize that tropomodulin may be involved in maintaining the final length of thin filaments in mature striated myofibrils after assembly.

J4-305 **THE KELOID FIBROBLAST PHENOTYPE -- ABNORMAL ORGANIZATION OF THE CYTOSKELETON MAY ALTER MOTILITY AND OTHER FUNCTIONS**, Patricia A. Hebda, Robert G. Rodgers and Sandra A. Murray, Departments of Dermatology and Cell Biology & Physiology, University of Pittsburgh School of Medicine, Pittsburgh PA 15261

Keloid fibroblasts in culture have been shown to be metabolically distinct from normal skin fibroblasts, with respect to proliferation, collagen and other protein synthesis and response to various cytokines and growth factors; these metabolic differences persist for at least 8 passages. *In vivo* keloid fibroblasts appear to have the ability to invade and involve surrounding normal connective tissue in a process of aberrant dermal repair culminating in excessive fibrosis. This report describes the distinctive features of morphology and motility exhibited by keloid fibroblasts in culture. They are smaller than normal fibroblasts, as measured by flow cytometry, and they have an altered cell shape. By light microscopy they lack the classic thin, bipolar fibroblast conformation and appear more flattened and somewhat stellate. By scanning electron microscopy they display abnormal, lateral cell processes that contrast with the bipolar alignment of normal fibroblasts. They also exhibit alterations in cell motility as measured in chemotaxis assays, with keloid fibroblasts requiring higher concentrations of chemoattractant. Altered cell shape and cell motility of keloid fibroblasts suggest abnormalities of microfilament or microtubule organization and interaction with cell surface components such as integrins; therefore, immunohistology was used to compare cytoskeletal features of keloid and normal fibroblasts. These results indicate a significant alteration in the pattern of stress fibers and the location of attachment plaques. Taken together, these observations demonstrate an abnormal arrangement of the cytoskeleton of keloid fibroblasts that is reflected in its altered phenotype.

J4-306 SKELETAL MUSCLE GENE DISRUPTION, Brian Lu[†], Leslie Acakpo-Satchivi[‡], Winfried Edelmann[‡], Leslie Leinwand[†], and Raju Kucherlapati[‡], [†]Department of Microbiology and Immunology, [‡]Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY 10461

Skeletal muscle is built of a complex array of myosin and actin isoforms whose genes exhibit temporally and spatially distinct patterns of expression. While quantitatively different ATPase activities have been demonstrated for some myosins, the precise function served by most myosin isoforms remains obscure. The skeletal muscle myosin gene family consists of at least 7 members, 6 of which are located on a single chromosomal cluster. The genes are not organized in their developmental pattern. During muscle development the embryonic skeletal myosin heavy chain gene is activated first and decreases to barely detectable levels at birth. The perinatal skeletal myosin heavy chain gene is activated shortly after the embryonic gene and decreases after birth. The fast and slow genes activate during development and remain active throughout adult life. Their relative abundance is dependent upon exercise state, hormonal status and the fiber-type composition of the individual muscle group. In order to determine the role of individual members of the myosin gene family, we have begun experiments to inactivate three mouse myosin heavy chain genes by homologous recombination. The genes we have chosen are embryonic skeletal, perinatal skeletal and adult fast IIb. We have isolated genomic clones from the 129/J mouse strain encoding these genes. Constructs were made in which the PGK-neo gene was inserted into the third exon (first coding exon) of each gene. The neo gene was used for positive selection. These constructs were transfected into ES cells and neomycin resistant clones were screened by PCR and by Southern blot for the homologous recombination events. We obtained multiple clones for each construct in which a homologous recombination event had occurred. Blastocyst injections have been carried out for two of the genes and the third is in progress. These experiments should provide insight into both muscle development and function.

J4-307 ***Merlin*, a tumor suppressor gene which is a member of the ERM family of cytoskeletal-associated proteins, is required for early development of the mouse embryo.**
Andrea I. McClatchey* and Tyler E. Jacks†, *†Center for Cancer Research and Department of Biology, †Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

Neurofibromatosis type 2 (NF2) is a hereditary disorder featuring the development of nervous system tumors, including schwannomas, meningiomas and ependymomas. The hallmark of NF2 is the development of schwannomas on or around the 8th cranial nerve. The tumor suppressor gene responsible for NF2 has recently been identified as a member of a class of cytoskeletal-associated proteins, and therefore represents a new type of tumor suppressor. In an effort both to develop an animal model for NF2, and to launch an investigation of the NF2 protein *merlin*, we have employed the method of gene targeting to create a mouse which is mutant at the NF2 locus. We have generated mice which are heterozygous for a mutation at the NF2 locus, and therefore genetically represent mouse models for NF2; these mice are being monitored for symptoms of NF2 or other etiology. We have intercrossed these mice in order to determine what role if any, *merlin* plays during development. We found that NF2 homozygotes die very early in gestation during a critical time in mouse embryogenesis, indicating that *merlin* plays a crucial role in the development of the early embryo in addition to its growth suppressing activity in some differentiated cell types.

We have also undertaken an extensive analysis of the pattern of NF2 expression in the mouse, as a basis for evaluating any phenotype seen in our heterozygous and homozygous mutant animals and as an important preliminary investigation into the normal role of *merlin* in the mouse. This includes a comparison of the tissue distribution of NF2 mRNA with that of the three other closely related family members, and the investigation of the expression patterns of various alternatively spliced forms of the NF2 message.

J4-308 **FUNCTIONAL ANALYSIS OF TROPOMYOSIN IN MURINE HEART**, Mariappan Muthuchamy, Ingrid Grupp, Gunter Grupp, Barbara O'Toole, Ann Kier, Gregory Boivin, Jon Neumann, and David Wieczorek, Department of Molecular genetics, University of Cincinnati, Cincinnati, OH 45267.
Tropomyosins (TMs) comprise a family of actin-binding proteins which are central to the control of calcium-regulated, striated-muscle contraction. In the myocardium of adult small mammals, the principle striated muscle isoform of TM is α, while fetal and hypertrophic myocardium express both α- and β-TM isoforms. Our previous studies have shown that the ratio of striated α- to β-TM mRNA in murine cardiac tissue dramatically changes during the embryonic to adult transition. To comprehend the functional role of TM isoform differences in the murine heart, we have generated transgenic mice which overexpress striated muscle-specific β-TM in the heart by using a cardiac-specific promoter. Nine independent transgenic lines were generated and show an increase in β-TMstr mRNA expression ranging from 50 - 200 fold, and an associated protein increase of 50 fold. This expression is restricted to cardiac muscle. Interestingly, the increase in β-TMstr message and protein in the transgenic mouse heart causes a concomitant decrease (20 - 30%) in the level of α-TMstr transcripts and its associated protein. Two-dimensional protein gel analysis reveals that there is a preferential formation of the αβ heterodimer in the transgenic heart myofibrils. Further, when the expression from the β-TMstr transgene is terminated, the expression of α-TMstr isoform returns to normal levels. Very interestingly, physiological analyses of these hearts using the work-performing model reveal that functional parameters associated with myocardial contractility appear normal, however, there is a significant delay in diastolic function. As such, this study demonstrates a coordinate regulatory mechanism exists between α- and β-TM gene expression in the murine heart, and there is a significant functional correlation between α- and β-TM content and cardiac performance.

J4-309 ASSOCIATION OF MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) AND KINASE KINASE (MAPKK) WITH THE CYTOSKELETON. Alfred A. Reszka[1], Rony Seger[2], Curtis D. Diltz[1], Edwin G. Krebs[2,3], and Edmond H. Fischer[1]. From the Departments of Biochemistry[1] and Pharmacology[2] and Howard Hughes Medical Institute[3], University of Washington, Seattle, WA 98195.
We have determined the subcellular distribution of mitogen-activated protein kinase (MAPK) and kinase-kinase (MAPKK) in NIH/3T3 cells using both microscopy and biochemical approaches. In addition to being diffuse in the cytoplasm, MAPK and MAPKK associate with cytoplasmic, mitotic, and cytokinetic microtubules. In cycling cells, the cytoskeletal pools comprise one-half of the MAPK and one-third of the MAPKK, respectively. A correlation exists between cytoskeletal dynamics and the association of MAPK and MAPKK with the cytoskeleton. In G_0 cells, cytoskeleton-associated MAPK and MAPKK levels are one third and one quarter of the total, respectively. Mitogen-stimulation of these cells generates a specific activity of cytoskeletal MAPK that is two-fold higher than that of soluble MAPK without immediately increasing the size of the cytoskeleton-bound pool. This coincides with dramatic cytoskeletal reorganizations that occur within the cell. After v-src transformation, MAPKK-1-cytoskeleton association increases nine-fold over that of G_0 cells. This is due in part to a four-fold increase in total expression. These findings suggest that the cytoskeleton participates in the activation of MAPK and, in turn, MAPK participates in the regulation of cytoskeletal dynamics.

J4-310 TWO ACTIN ISOFORMS FROM THE FISSION YEAST *SCHIZOSACCHAROMYCES POMBE*. Stylianos P. Scordilis, Deborah M. Anable and Masha Huseinovic, Department of Biological Sciences, Smith College, Northampton, MA 01063
Actin has been isolated and purified from *Schizosaccharomyces pombe* strain 975 (*wt*). Either spheroplasts or an acetone powder is extracted in an actin depolymerizing buffer (buffer G), centrifuged and the supernate chromatographed on an ATP-saturated DEAE-Sepharose column. Elution is with a linear KCl gradient. The pooled DEAE actin-containing fractions are concentrated and chromatographed on a Sephacryl S-100 HR column equilibrated with in buffer G with or without 1.0 M KCl. Actin typically elutes as a single peak. These S-100 actin-containing fractions are concentrated and polymerized by dialysis overnight in G-buffer with 2 mM $MgCl_2$ and 100 mM KCl. The polymerized actin is centrifuged and the pellet is resuspended in buffer G. Far more actin is left in the supernate, ≈8 mg/mL, than can be accounted for due to its critical concentration. Hence, we have termed the two actins polymerizable, P, and non-polymerizable, NP. The apparent molecular weight of the *S. pombe* actins on SDS gels are Mr = 46,800 (P) and 47,800 (NP) Da. The actins resolved as two protein bands at pI 5.56 ± 0.01 (P) and 5.18 ± 0.03 (NP) on isoelectric focusing gels. Immunoblots demonstrate a strong binding affinity of the monoclonal antibody to chicken gizzard actin (JLA-20) to the (P) *S. pombe* actin isoform, but not for the (NP) one. The actins were cleaved with *Staphylococcus aureus* V8 protease and electrophoresed on SDS gels. This mapping resulted in identical peptide fragment banding patterns. Preliminary results indicate that the difference between these two isoactins is that the non-polymerizable form is ADP-ribosylated. This post-translational modification could account for the isoelectric point difference and for the change is polymerization properties of the two actin isoforms.
This work was supported by grants from the NIH GM-47569 and the Blakeslee Fund for Genetics Research at Smith College to SPS.

Mitosis; Interaction of the Cytoskeleton with Cell Surface Adhesive Junctions

J4-400 ANALYSIS OF CENTROSOME REPLICATION EVENTS IN CULTURED CHO CELLS, Ron Balczon, Liming, Bao, and Warren E. Zimmer, Department of Structural and Cellular Biology, The University of South Alabama, Mobile, AL 36688.

The fidelity of chromosome segregation is dependent upon the regulated replication of the centrosome complex. Relatively little is known about the strategies used by somatic cells to insure that centrosome doubling occurs once, and only once, during each cell cycle. Studies were performed to investigate the cellular regulation of centrosome replication. For these studies, CHO cells were arrested at the G_1/S boundary of the cell cycle and then maintained in the blocked state for a period that approximated 4-5 cell cycles. When the arrested cells were examined by either electron microscopy or anticentrosome immunofluorescence it was determined that the experimentally treated cells had undergone multiple rounds of centrosome replication in the absence of cycles of either DNA synthesis or mitosis. Northern blot analysis using the cDNA encoding portion of the centrosomal autoantigen PCM-1 demonstrated that cells arrested at the G_1/S boundary of the cell cycle maintained centrosomal mRNA at artificially elevated levels when compared to control cells, providing a possible molecular explanation for how the arrested cells were able to perform multiple rounds of centrosome replication in the absence of either S phase or mitosis. In addition, the timing of the cell cycle arrest was important as cells that were arrested for prolonged periods using etoposide, a drug that arrests cells in G_2, were unable to perform multiple rounds of centrosome replication. Finally, the capacity to replicate centrosomes could be abolished in arrested cells by culturing the cells in dialyzed serum. The ability to replicate centrosomes and to synthesize PCM-1 mRNA could be re-initiated by adding EGF to the dialyzed serum. These results argue against the presence of an endogenous cellular centrosome counting mechanism and suggest that the somatic cell nucleus, probably under the direction of cell cycle regulatory molecules, directs cycles of centrosome replication by controlling the activation of centrosome genes at appropriate times during each cell cycle.

J4-401 SRP1p, A YEAST NUCLEAR PORE COMPLEX PROTEIN, PHYSICALLY INTERACTS WITH NUCLEOPORINS NUP1p AND NUP2p. Kenneth D. Belanger[1] and Laura I. Davis.[2] 1) Dept. of Cell Biology, Duke University, Box 3646 DUMC, Durham, NC 27710, 2) Howard Hughes Medical Institute, Duke University, Durham, NC.

The nuclear pore complex is a highly structured unit that is essential for transport of macromolecules between the nucleus and the cytoplasm of the cell. Recent evidence suggests that the nuclear pore complex is also required for proper formation and maintenance of nuclear envelope structure. We initiated a synthetic lethal screen in *Saccharomyces cerevisiae* to identify factors that interact with yeast nuclear pore complex protein, Nup1p. Seventeen *nle* (*n*up1 *l*ethal) mutants were recovered. Mutant *nle1* was shown to interact in an allele-specific manner with *nup1* mutants. *NLE1* was cloned by complementation of a cold sensitive phenotype and found to encode Srp1p, an essential protein that has been shown to colocalize with nuclear pore proteins by indirect immunofluorescence (Yano et al. 1992. MCB 12, 5640). Using biochemical methods, we have shown that Srp1p and Nup1p directly interact. Srp1p also forms a complex with a related nucleoporin, Nup2p. Because Nup1p does not physically interact with Nup2p, we think that Srp1p is a common component of distinct nucleoporin complexes (Belanger et al. 1994. JCB 126, 619).

Mutations in Nup1p lead to morphological changes in the yeast nuclear envelope, suggesting an interaction with structural components of the cell involved in maintenance of nuclear structure. The central region of Srp1p contains a repeat sequence with homology to the repeat domain of the β-catenin/armadillo/plakoglobin protein family. Srp1p fragments fused to *E. coli* β-galactosidase have been used in an attempt to determine the function of the specific regions of Srp1p. A fusion protein with the central repeat domain is found within the nucleus by indirect immunofluorescence. The N-terminus fused to β-gal appears to be found at the nuclear envelope Interestingly, a short region of the carboxy-terminus of Srp1p localizes specifically to the bud site of mitotically active cells. These data suggest that the nuclear pore complex may have a functional or evolutionary relationship with cytoskeletal organizing structures found at the cell membrane. Further biochemical and genetic studies are being carried out to elucidate the role of each of these domains in the overall function of Srp1p.

J4-402 MATERNAL CONTROL OF CYTOKINESIS IN THE EARLY *C. ELEGANS* EMBRYO. Bruce Bowerman, Chris Thorpe, and Ann Schlesinger, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

Large (50μm x 20 μm) and accessible, the 1-cell stage *C. elegans* embryo is well suited for a genetic and molecular analysis of cytokinesis. We recently isolated a mutant allele of a *C. elegans* gene we call *odd-1* (for *oddball*) that appears to be required specifically for the completion of embryonic cytokinesis. Although the first cleavage furrow in *odd-1* mutant embryos initiates normally and even pinches in extensively, cytokinesis is never completed. Eventually the furrow regresses, leaving the daughter nuclei in a single cell. All subsequent attempts at cytokinesis also fail, but *odd-1* embryos continue to undergo a normal or nearly normal number of mitotic nuclear divisions and even exhibit morphological signs of differentiation. The large numbers of nuclei in oddball embryos eventually develop morphologies characteristic of epidermal, neuronal, or intestinal cell nuclei, with all the nuclei in a given embryo usually, but not always, appearing to adopt the same "fate". The defect in *odd-1* mutant embryos appears to be specific for embryonic cytokinesis: events both preceding and following cytokinesis occur normally, including events that require components of the cytoskeleton. Moreover, the *odd-1* allele we identified in our genetic screens is recessive and strictly maternal-effect. These results suggest that the wild-type *odd-1* gene product is supplied by the mother during oogenesis and may be required for only embryonic, and not post-embryonic, cytokinesis. We are currently working to isolate additional mutant alleles, and are continuing our characterization of the mutant phenotype.

J4-403 THE MAJOR KIDNEY AE1 ISOFORM DOES NOT BIND ANKYRIN. An essential role for the 79 NH_2-terminal amino acid residues of band 3. Yue Ding, Joseph R. Casey and Ron R. Kopito, Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020

The AE1 (band 3) protein mediates the exchange of anions across the erythrocyte plasma membrane and, via association with the adapter molecule, ankyrin (Ank1), forms the major link between the membrane and the underlying spectrin cytoskeleton. The major kidney isoform of AE1 (kAE1), a protein that is otherwise identical to erythroid AE1, but lacks the NH_2-terminal 79 amino acids, is localized to the basolateral plasma membrane of acid-secreting (α-type) intercalated cells of distal nephron. It has been proposed that this polarized distribution of kAE1 is due, at least in part, to its association with the ankyrin-spectrin cytoskeleton. In this study we have used cell-free binding assays to investigate the interaction of anion exchangers with an Ank1 fragment, R13-H, that contains the AE1 binding site. Microsomes from cells expressing full-length erythroid AE1 bound ^{125}I-labeled R13-H, revealing the presence of both high (K_d = 12.5 nM) and low (K_d = 166 nM) affinity sites. This binding was specific, as evidenced by the failure to observe high affinity binding of ^{125}I-R13-H to microsomes from cells transfected with vector alone or with AE1m, a mutant lacking the ~400 amino acid NH_2-terminal cytoplasmic ankyrin binding domain. Using this assay, we could detect no high affinity association between kAE1 and ^{125}I-R13-H. We conclude that the NH_2-terminal 79 amino acids play an essential role in high affinity and specific binding of AE1 to Ank1.

J4-404 ANKYRIN ASSEMBLY AT SITES OF CELL ADHESION, Ronald R. Dubreuil and May Yau, Pharm./Phys. & Cell Physiology, University of Chicago, Chicago, IL 60637.

We are using *Drosophila* as a model system in which to study membrane skeleton function. Most previous research on the membrane skeleton has focused on its role in cell shape and membrane stability. Another concept, emerging from the work of several different laboratories, is that the membrane skeleton provides a scaffold that is important for the correct assembly of integral membrane proteins. Some integral membrane proteins appear to be inducers of membrane skeleton assembly, and other proteins appear to be responders whose stable assembly depends on the membrane skeleton. The spectrin membrane skeleton is attached to the plasma membrane through ankyrin, which interacts with several different integral membrane proteins. We recently described *Drosophila* ankyrin (Dubreuil & Yu (1994) *Proc. Natl. Acad. Sci. USA* 91:10285-10289). The protein sequence is closely related to mammalian ankyrins through the membrane binding and spectrin binding domains, but differs in a carboxy terminal domain of unknown function. We used an antibody against the carboxy terminal domain of fly ankyrin to localize it in larvae and tissue culture cells. Ankyrin was found to be associated with the plasma membrane in several cell types of larvae. Its assembly did not appear to be affected by α spectrin mutations, which are ultimately lethal. In contrast, ankyrin in S2 tissue culture cells appeared to be diffusely distributed in the cytoplasm. Transfected S2 cells expressing the *Drosophila* cell adhesion molecule neuroglian (generously provided by Dr. Michael Hortsch, University of Michigan) exhibited a striking redistribution of ankyrin to sites of cell-cell interaction. These results indicate ankyrin assembly (and perhaps some aspects of its function) does not depend on α spectrin, but is promoted by neuroglian-mediated cell adhesion.

J4-405 ON THE ROLE OF CALPAIN IN REGULATING SIGNALING ACROSS THE INTEGRIN α_{IIb}-β_3. Joan E.B. Fox, Susanne Zuerbig, George Santos, and Takaomi C. Saido. Children's Hospital Oakland, Oakland, CA 94609 andTokyo Metropolitan Institute of Medical Science, Tokyo 113 Japan

Interaction of integrins with extracellular matrix proteins leads to clustering of integrins and the formation of cytoskeletal structures known as "focal contacts". Formation of focal contacts in turn regulates the adhesive properties of integrins. Little is known about the molecular mechanisms regulating this two-way signaling across integrins. A variety of signaling molecules have been detected in focal contacts. One is calpain. The goal of the present study was to determine whether calpain is involved in signaling across integrins. Platelets were activated with thrombin. Ligand-occupied integrin, α_{IIb}-β_3, was detected with antibody PAC-1. Immunofluorescence studies showed that while unoccupied α_{IIb}-β_3 remained uniformly distributed, ligand-occupied α_{IIb}-β_3 selectively clustered in patches that also contained the "focal-contact" protein, talin. Activation of calpain was detected on western blots by the autolysis of calpain or the appearance of hydolytic fragments of spectrin. Occupancy of α_{IIb}-β_3 with ligand resulted in activation of calpain. Immunofluorescence experiments utilizing an antibody that selectively recognizes the proteolyzed form of spectrin, confirmed that as α_{IIb}-β_3 bound ligand, spectrin was cleaved. Moreover, the proteolyzed form of spectrin was present exclusively in the "focal-contact like" structures, along with ligand-occupied α_{IIb}-β_3. The formation of these "focal-contact like" structures was prevented by preincubation of platelets with cytochalasin. Cytochalasin prevented autolysis of calpain and cleavage of spectrin. Preincubation of platelets with the membrane permeable inhibitor of calpain, calpeptin did not prevent the formation of the "focal-contact like" structures, however, it did inhibit cleavage of spectrin and decreased the binding of ligand to α_{IIb}-β_3. We suggest that the formation of focal-contacts is involved in activation of calpain and that the calpain-induced cleavage of focal-contact components is involved in signaling across the integrin, α_{IIb}-β_3 in platelets.

J4-406 THE PROLINE RICH REGION OF THE *SACCHAROMYCES CEREVISIAE* CYCLASE-ASSOCIATED PROTEIN (CAP) AND ITS HOMOLOGS CONTAIN A CONSERVED SH3 BINDING SITE Nancy L. Freeman, Keith A. Mintzer* and Jeffrey Field, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia PA 19104. *Department of Biological Sciences, Columbia University, New York NY, 10027

CAP (cyclase-associated protein) is a 70 kDa protein which mediates RAS activation of adenylyl cyclase in the yeast *Saccharomyces cerevisiae*. Previous studies have demonstrated that CAP is a bi-functional protein: the N-terminus is associated with RAS/cAMP signaling and the C-terminus is involved with cytoskeletal integrity. Here, we identify a third functional domain for the middle region of CAP as an SH3 binding site. This region is rich in proline residues and is conserved in all CAP homologs. Using yeast and mammalian GST SH3 domains as biotinylated probes, several SH3 domains were shown to bind the *S. cerevisiae* CAP. Two yeast proteins which seem to interact with the *S. cerevisiae* CAP are CDC-25 and ABP1. Biochemical evidence suggests that SH3 domains and CAP can interact directly in their native forms. CAP is easily co-precipitated with ABP1 and can also be purified by ABP1 affinity chromatography. Co-precipitation experiments also demonstrate that CAP can concurrently bind actin and an SH3 domain. Similarly, using adenylyl cyclase assays, binding of an SH3 domain does not preclude CAP from interacting with adenylyl cyclase. Genetic experiments suggest the proline region is important in RAS/cAMP signaling. Heat shock analysis using *ira1cap* or *ira2cap* knockout strains indicate that deletion of the proline region attenuates the heat shock phenotype. Finally, the human CAP also binds SH3 domains binding strongest to the *Abl* proto-oncogene's SH3. This suggests that the SH3 binding site on CAP is conserved in evolution.

J4-407 INTERACTION OF E-CADHERIN WITH THE CYTOSKELETON IN THE MAINTENANCE OF EPITHELIAL INTEGRITY. Giroldi L, Bringuier PP, *Isaacs W, Schalken J. University Hospital Nijmegen, the Netherlands & *Brady Urological institute, Johns Hopkins hospital, Baltimore, MD

Disturbed E-cadherin function is implicated in loss of epithelial integrity (i.e. dedifferentiation and aggressiveness) in most carcinoma. E-cadherin is the cell-cell adhesion molecule involved in the formation of *adherens* junctions. It is thought to be linked to the cytoskeleton via complex formation with β and α catenin on one hand or γ and α catenin on the other hand (γ catenins is also known to take part in desmosomes). We have transfected the human prostate carcinoma cell line PC3 which lacks α catenin with an α catenin expression vector. The transfectants acquire an epithelial morphology and loose invasion capacity in collagen or matrigel. Thus, restoration of the E-cadherin/cytoskeleton interaction is probably responsible for reversion of PC3 cells to a non invasive epithelial phenotype. To get more information on the frequency of loss of E-cadherin / cytoskeleton interaction in cancer, we analysed 17 bladder carcinoma cell lines for E-cadherin and catenins expression. All but one line expressing E-cadherin display the normal set of catenins. However, γ catenin is not detected in 4 lines that have lost E-cadherin mRNA expression. This suggests that absence of E-cadherin destabilizes γ catenin and that consequently cells also lack desmosomes.

J4-408 CONSEQUENCES OF STABLE EXPRESSION OF FULL-LENGTH AND TRUNCATED FORMS OF RADIXIN IN CULTURED CELLS. Michael D. Henry, Charo Gonzalez Agosti, Nancy-Lorena Torres, and Frank Solomon. Department of Biology and Center for Cancer Research, Massachussetts Institute of Technology, Cambridge, MA 02139.

ERM (ezrin, radixin, moesin) proteins are localized to various regions of the cell in which there is a close apposition of the cytoskeleton and plasma membrane, including microvilli, filopodia, ruffling edges, growth cones, cleavage furrows, and marginal bands (Goslin et al., J. Cell Biol. 109:1621-1631; Sato et al., J. Cell Biol. 113:321-330). These subcellular localizations, along with the sequence similarities between their amino termini and that of band 4.1, suggest that ERM proteins may play a role in linking components of the cytoskeleton to the plasma membrane. In an effort to identify the domain(s) of radixin necessary for its appropriate localization, we have established lines of NIH-3T3 and embryonal carcinoma P19 cells that stably express full-length and truncated forms of radixin. Both of these cell types normally express all three ERM proteins, and at least moesin and radixin are appropriately localized. These constructs do not affect cell morphology or, in the case of P19 cells, neuronal differentiation. We used variously placed epitope tags to monitor the production and subcellular distribution of the radixin constructs. The data show that, depending on the position of the epitope tag, the localization of the full-length radixin construct can be comparable to that of endogenous radixin. A carboxy-terminal fragment, gives a similar, but not identical localization pattern; strikingly, it is absent from cleavage furrows. Expression of a full-length radixin molecule or a carboxy-terminal fragment perturbs the localization of the endogenous moesin. The results suggest the presence of finite, common sites necessary for localization of the ERM proteins. The results also suggest that the carboxy-terminus of radixin contains structural elements necessary for association with a wide variety of actin-containing structures but that the amino-terminus is necessary for the appropriately restricted localization pattern of the full-length molecule.

J4-409 REDUCED EXPRESSION OF GELSOLIN IN HUMAN GASTRIC AND URINARY BLADDER CANCERS

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The multistage model for carcinogenesis during tumor progression requires consecutive genetic abnormalities of both oncogene and tumor suppressor genes. This appears to be the case with the well-studied colorectal carcinoma and similar patterns are beginning to emerge in studies of the gastric and urinary bladder cancers. Previously, we have demonstrated that human authentic gelsolin, if expressed at increased levels, may have a suppressive potential against the tumorigenicity of murine fibroblasts transformed by human activated Ha-ras oncogene (Oncogene 8:253,1993). We have examined the expression of gelsolin in human gastric and bladder cancer cell lines and tissues compared with normal tissues, using Western blot analysis with a monoclonal anti-gelsolin antibody. The immunoblotting showed that the anti-gelsolin antibody detects a single band of 90 KD comigrating with purified gelsolin in E. coli. The production of gelsolin was notably down-regulated in 7 out of 8 gastric carcinoma (adenosquamous, well, moderately, poorly differentiated adeno, scirrhous) cell lines in comparison with that in normal gastric mucosa. All of the 6 human urinary bladder cancer cell lines and 3 out of 5 transitional carcinoma tissues also lost gelsolin expression or the expression was reduced. Control normal bladder epithelial tissues showed intense staining. The cDNAs encoding authentic gelsolin were transfected into a gastric adenocarcinoma cell line and a urinary bladder cancer cell line. The growth potential of both the transfectants which recovered gelsolin production was reduced. Our current research has shown that the reduced expression of gelsolin may be involved in the initiation of gastric and bladder cancers and plays any important role in their carcinogenesis.

J4-410 **EXPRESSION OF THE PLATELET GP Ib-IX COMPLEX IN HETEROLOGOUS CELLS INDUCES SHAPE CHANGE AND INCREASES ACTIN-BINDING PROTEIN SYNTHESIS.** **José A. López, Chester Q. Li, Stuart Weisman, Margaret Chambers, and Jing-fei Dong, Gladstone Institute of Cardiovascular Disease, University of California, San Francisco, 94110**

Many adhesive cell-membrane receptors depend upon interactions with cytoskeletal elements to function normally. An example is the platelet glycoprotein (GP) Ib-IX complex, which functions as a receptor for von Willebrand factor in attaching platelets to the blood vessel wall and which associates with actin-binding protein (ABP) in the cytoskeleton. We expressed the entire GP Ib-IX complex or its components in heterologous cell lines—CHO and L cells—and found that the receptor had a profound effect on cell morphology. Cells expressing the highest amounts of cell-surface GP Ibα (the polypeptide through which the complex associates with the cytoskeleton) went from being firmly attached and spread to having a much more rounded morphology and detaching easily from the culture plates. The cells expressing the highest levels of the complex also took much longer to reach confluence than did wild-type CHO or L cells. Because of the known association between ABP and GP Ib-IX, we examined the levels of ABP in cells expressing the complex. Metabolic labeling and western blotting studies revealed that the cells undergoing the morphological change expressed higher levels of ABP than did untransfected cells or than did transfected cells that lacked GP Ibα. Confocal microscopy of high-expressing cells that had been incubated with ABP antibodies also revealed higher levels of ABP, with a marked redistribution toward the cell periphery when compared with cells that did not express GP Ibα. Expression of the GP Ib-IX complex may have altered the level of ABP in the cells by one of two mechanisms, either by stabilizing the protein or by increasing its synthesis. We investigated these possibilities by performing pulse-chase experiments to follow the levels of ABP in the cells. Even at the earliest chase times the amount of labeled ABP was greatest in complex-expressing cells, indicating that more ABP was being synthesized in these cells. In summary, we have found that over-expression of the GP Ib-IX complex in heterologous cells leads to a change in cell morphology that is concomitant with increased expression of ABP.

J4-411 CHARACTERIZATION OF THE *OST* ONCOGENE, A POTENTIAL REGULATOR OF GROWTH FACTOR-INDUCED CYTOSKELETAL ORGANIZATION

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The Rho family of small GTP-binding proteins, including Rho, Rac and Cdc42, function as molecular switches in signal transduction pathways for cytoskeletal organization. We have isolated a novel oncogene, *ost*, from rat osteosarcoma cells. The *ost* oncogene possesses high transforming activity in NIH/3T3 cells. The *ost* gene product was activated by truncation of its N-terminal domain and was highly tumorigenic in nude mouse assays. The full-length *ost* cDNA encodes a predicted protein of 100 kilodaltons containing the DH (dbl homology) and PH (pleckstrin homology) domains.

Purified Ost protein can catalyze guanine nucleotide exchange on RhoA and Cdc42. Ost did not detectably associate with RhoA or Cdc42, but interacted specifically with the GTP-bound form of Rac1. These results implicate Ost as a critical regulatory component which links the signal transduction pathways that flow through Rac1, RhoA and Cdc42. Ost is mainly phosphorylated on serine and localized in the cytoplasm. Among the tissues examined, brain showed the highest expression of Ost especially in neurons and α-tanycytes. These cells contain actin fibers as well as microtubules in their processes. Taken together with involvement of Rho family proteins in cytoskeletal organization, a local function of Ost may be the regulation of actin remodeling in these highly specialized cells.

J4-412 α-CATENIN BINDS TO BOTH ACTIN AND β-CATENIN: POTENTIAL LINKAGE OF THE THE CADHERIN COMPLEX TO THE CYTOSKELETON, David L. Rimm, Erika R. Koslov, Partow Kebriaei, and Jon S. Morrow, Dept. of Pathology, Yale University School of Medicine, New Haven, CT 06510

The linkage of E-cadherin to the cortical cytoskeleton has been implied by co-distribution experiments and deletion experiments showing loss of adhesive function correlating to loss of the cadherin cytoplasmic domain. Two cytoplasmic proteins, α-catenin and β-catenin are involved in this interaction as shown by co-precipitation. To understand these interactions on a molecular level, we have recombinantly produced and purified the components of this complex. Actin co-sedimentation assays were used to show direct binding of α-catenin to actin, and β-catenin binding to α-catenin but not actin. Low speed sedimentation and electron microscopy of negatively stained proteins showed that α-catenin is also capable of bundling actin filaments. In a series of recombinants all of the binding activity is accounted for by a fragment that contains the N-terminal 228 amino acids, however this fragment does not bundle actin. We propose that the C-terminus mediates α-catenin dimerization, and hence allows filament bundling. Quantitation using surface plasmon resonance detected by a Pharmacia Biacore™ Biosensor suggests that the interaction between α-catenin and F-actin has a Kd of 6μM. Similarly, β-catenin binds directly to α-catenin immobilized on the biosensor chip. We are currently characterizing this interaction. It has been shown previously by others that β-catenin is tightly associated with E-cadherin in numerous extraction experiments. It appears that E-cadherin binds to β-catenin, which binds α-catenin which binds actin. These studies suggest that α- and β-catenin may form the linkage between the membrane based cadherin homotypic interaction site and the cytoskeleton.

J4-413 SITE DIRECTED MUTAGENESIS OF THE μ CALPAIN CLEAVAGE SITE IN αII SPECTRIN. Paul R. Stabach, Susan Glantz-Tuschman, and Jon S. Morrow Yale University School of Medicine, Dept. of Pathology, New Haven, CT 06510.

The cleavage of proteins by calcium activated neutral proteases such as μ-Calpain is both ubiquitous and highly specific. While the precise role of these enzymes remains enigmantic, they are believed to play an important role in mediating cytoskeletal changes during synaptic remodeling, and may be excessively activated in cells subjected to ischemic stress. One important target of μ-calpain action is the αII (non-erythroid spectrin or fodrin). *In vitro* and *in vivo*, μ-calpain cleaves this subunit of spectrin between tyr^{104} and gly^{105} of the 11th repetitive unit of αII spectrin (Harris et al., JBC 263:15754, 1988). This cleavage is highly specific, despite the occurrence of presumably susceptible X-tyr and X-arg sequences at least 35 times in αII spectrin. In order to better understand the site specificity of μ-calpain for spectrin, and as a first step to preparing transgenic mice with μ-calpain resistant spectrin, site directed mutatgenesis has been used to prepare recombinant αII spectrin with 20 different amino acid substitutions at the -2 position. Each of these were evaluated for their susceptibility to μ-calpain using *in vitro* cleavage assays. The replacement of the wild type valine at the -2 position with glycine, proline, and tryptophan abolished susceptibility. This replacement, however did not effect the peptides' ability to bind to calmodulin, indicating an overall preservation of secondary conformation. Several other amino acids yielded reduced susceptibility, although overall a surprising range of substitutions were well tolerated at this position without substantive loss of cleavabilty. These studies suggest that secondary and tertiary structural factors resident in proteins, and difficult to evaluate in synthetic peptides, may be the primary determinants of μ-calpain specificity.

J4-414 XKLP1: A KINESIN-LIKE PROTEIN ASSOCIATED TO CHROMOSOMES, I.Vernos[a], J.Raats[b], J.Heasman[b], C.Wylie[b] and E.Karsenti[a]. EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany and [b]University of Minnesota, Institute of Human Genetics,420 Delaware Street S.E., Mn 554550392, USA.

The kinesin family of microtubule motor molecules includes an increasing number of proteins involved in organelle transport and different aspects of the meiotic and mitotic spindles organization and function.

Using a PCR approach, we have identified 4 new kinesin-like transcripts in Xenopus oocytes. We report here the characterization of one of them: the Xenopus kinesin-like protein 1 (Xklp1) that belongs to a new subfamily of Klps. This protein has a motor domain at the amino-terminus, several nuclear localization sequences (NLS) in the stalk domain and a putative zinc finger-like cysteine-rich sequence in the tail as well as a consensus phosphorylation site for cdc2.

Using several specific antibodies we have shown that the protein binds to taxol stabilized microtubules in the presence of AMP-PNP and apyrase and is released to different extents from them by addition of Mg-ATP.

The protein is localized in the nucleus during interphase and on the chromosomes during mitosis. During late anaphase, a fraction of the protein relocalizes to the spindle interzone and accumulates in the midbody during telophase. The chromosomal localization of Xklp1 suggests that this motor protein may have a role in generating the polar ejection forces in pro-metaphase. The relocalization of Xklp1 to the midzone during late anaphase A suggests that it may have a function during anaphase B or in cytokinesis.

To determine the function of XKlp1, anti-sense oligonucleotides have been injected in Xenopus oocytes in order to deplete the system of endogenous XKlp1 mRNA. The depleted embryos show a specific arrest in cell cleavage. In the most severe cases no mitotic spindles can be found while in other abnormal spindles are observed. This data indicates that XKlp1 performs an essential role during mitotic spindle assembly and maybe cytokinesis..

Late Abstracts

CHARACTERIZATION OF A MURINE PROTEIN KINASE, Plk, Kyung S. Lee*, Ryoko kuriyama† and Raymond L. Erikson*, Department of Molecular and Cellular Biology, Harvard University, 16 Divinity Ave., Cambridge, MA 02138*; and Department of Cell Biology and Neuroanatomy, University of Minnesota Medical School, 321 Church St., S. E., Minneapolis, MN 55455†

PLK (STPK13) encodes a murine protein kinase closely related to those encoded by the *Drosophila melanogaster polo* and the *Saccharomyces cerevisiae CDC5*, which are required for normal mitotic and meiotic divisions.

Affinity-purified antibody generated against the C-terminal 13 amino acids of Plk specifically recognizes a single polypeptide of 66 kDa in MELC, NIH3T3, and HeLa cellular extracts. In NIH3T3 cells, poly $(A)^+$ *PLK* mRNA and its encoded protein are most abundant about 17 hours after serum stimulation, indicating that it may function at the late stage of cell cycle.

Plk immunoprecipitates preferentially phosphorylate casein rather than histone H1 *in vitro*. The kinase activity of Plk immunoprecipitates peaks at the early M phase of the cell cycle and persists longer than cyclin B-associated cdc2 kinase activity, correlating with $p66^{Plk}$ expression levels. Phosphoamino acid analysis of Plk following autophosphorylation reveals the phosphorylation of both serine and threonine residues but not tyrosine residues.

Plk is localized to punctuate spots within the nucleus in prophase, moves to the area surrounding the chromosomes in metaphase, appears condensed as discrete short lines along the spindle axis at the interzone in anaphase, and finally concentrates at the midbody during telophase and cytokinesis. In addition, co-localization of Plk and CHO1 antigen (mitotic kinesin-like protein-1), which induces microtubule bundling and anti-parallel movement *in vitro*, persists throughout mitosis.

KT5720, A MAP KINASE INHIBITOR, ALTERS MICROTUBULES AND ACTS AS A TAXOL-MIMIC IN CELLS. Mary K. Olsen, Kathleen E. Sampson, Cindy L. Wolf, Alice L. Laborde, and Irene Abraham. The Upjohn Company, Kalamazoo, MI. 49007.

Taxol has been shown to inhibit mitosis by stabilizing microtubules. We devised a screen [Abraham et al., *Proc. AACR* (1994) **35**:75] to select compounds with functional characteristics similar to that of taxol by using taxol dependent, Tax 2-4, cells (Cabral, J. *Cell Biol.*, 1993, **97**:22) that die in the absence of taxol. Compounds were screened for their ability to support cell survival in the absence of taxol using a multi-well format. We identified certain commercially available indolocarbazoles, such as KT5720, as a class of compounds that can support some growth of Tax 2-4 cells in the absence of taxol. However, other indolocarbazoles, such as the PKC inhibitor staurosporine, show no activity in supporting this cell growth. The active indolocarbazoles also potentiate the effects of taxol so that less taxol is required to support growth of Tax 2-4 cells. In wild type cells, isobologram analysis shows that these compounds synergize with taxol to induce cell death. In intact cells, the indolocarbazoles cause changes in the apparent length and distribution of microtubules and a rearrangement of cell borders. However, in an *in vitro* tubulin polymerization assay in which GTP is omitted, the indolocarbazoles, unlike taxol, are unable to support tubulin polymerization. The indolocarbazoles are known to have activity as serine-threonine kinase inhibitors. We have found that the ability of the active compounds to cause cytoskeletal changes correlates with their relative specificity for inhibition of MAP kinase, as compared to other kinases such as PKA. This points to MAP kinase as a potential target mediating the cellular effects seen with KT5720 and its analogs and supports a role for MAP kinase in the cytoskeleton.

MICE DEFICIENT FOR GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) GENERATED BY GENE TARGETING, Milos Pekny, Per Levéen, Marcela Pekna, Camilla Eliasson, Bengt Westermark and Christer Betsholtz, Department of Medical Biochemistry, Gothenburg University, Sweden.

Glial fibrillary acidic protein (GFAP) is the main component of the intermediate filaments in cells of astroglial origin. These cells include astrocytes in the brain and non-myelin forming Schwann cells and enteric glia in the peripheral nervous system. To address the question about the function of GFAP in vivo, we have disrupted the gene coding for GFAP in mice via targeted mutation in embryonic stem cells.

The mice lacking GFAP develop normally, reach adulthood and reproduce. We did not find any abnormalities in their behavior, memory, motility, nor in the histological architecture of the central nervous system. The histology of myenteric plexi and peristaltic intestinal movement appeared normal. Mutants also demonstrate an intact blood brain barrier function. Quantification of GFAP and S100 immunohistochemical staining in the hippocampus of wild type and mutant mice suggested a normal abundance of astrocytes in mutants.

These and other aspects of the phenotype of the GFAP deficient mice will be presented.