# MOLECULAR MOTORS

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*Plenary Sessions* *Page*



*Poster Sessions*

*Molecular Diversity (Joint)*

J3-001 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.

*Motor Paradigms*

J3-002 KINETIC MECHANISM OF MICROTUBULE KINESIN ATPASE, Y. Z. Ma, and E. W. Taylor, The University of Chicago. Chicago, Il.

The mechanism of human kinesin motor domain, K379 was investigated by transient and steady state kinetics. A smaller (K349) and a larger construct containing part of the coiled-coil tail (K560) were also studied for comparison. All three proteins are dimers based on measurements of molecular weight by equilibrium ultracentrifugation and by sedimentation coefficient and elution from S-300 column. Maximum microtubule activated ATPase is 10 to15 $s^{-1}$ for K560 and 20 to 25 $s^{-1}$ for the smaller constructs. Kinesin 379 alone showed an isomerization rate of 200 $s^{-1}$ for binding of substrate analogs, methylanthranyloyl ATP and ADP (mant ATP, mant ADP), a hydrolysis step of 10 $s^{-1}$ (phosphate burst) and very slow dissociation of ADP. Binding to microtubules did not effect the rate of the nucleotide induced isomerization step, the phosphate burst increased to greater than 100 $s^{-1}$ and the rate constant of ADP dissociation increased to 30 $s^{-1}$. The smaller ATPase rate of K560 correlated with a smaller rate of ADP dissociation. The rate of dissociation of ADP makes a major contribution to determining the maximum rate of turnover of ATP but it is proposed that phosphate dissociation also contributes. The binding constants of kinesin, kinesin-ADP and kinesin intermediates in the presence of ATP are strongly dependent on ionic strength which indicates a contribution of electrostatic interactions to the binding similar to the results for actomyosin. The rate of dissociation of the second mant ADP from microtubule-kinesin-mant ADP dimer complex is slow as measured by decrease in fluorescence. The rate of dissociation is increased by binding of nucleotides (ATP, GTP, AMPPNP) to the available nucleotide site. The microtubule-kinesin and actomyosin schemes are compared in terms of the differences in motile properties of the two systems.

*Organellar Movement and Motors*

J3-003 GOLGI ASSOCIATED MOLECULAR MOTORS, David R. Burgess, Gina Trimbur, and Karl Fath, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

Microtubules (MT) are required for the efficient transport of membranes from the *trans*-Golgi and for transcytosis of vesicles from the basolateral membrane to the apical cytoplasm in polarized epithelia. MTs in these cells are primarily oriented with their plus ends basally near the Golgi and their minus ends in the apical cytoplasm. While the region of the cell containing the Golgi is MT-rich, MTS rarely extend to the cytoplasm immediately subjacent to the plasma membrane. This cell cortex region is very rich in actin filaments. The cytoskeletal organization of the cell suggests that Golgi membranes require translocation along both MTs and actin filaments by the sequential use of MT- and actin-based molecular motors. We have recently shown that Golgi membranes isolated from intestinal epithelial cells possess the actin-based motor myosin-I, the MT minus-end directed motor cytoplasmic dynein and its *in vitro* motility activator dynactin (p150/*Glued*)(*J. Cell* Biol. **126**:661-675, 1994). These membranes can translocate on MTs in *in vitro* motility assays. Golgi stacks, which lack dynein but contain myosin-I, can bind soluble dynein supplied from added cytosol. In cell-free Golgi stack budding assays, stacks can be induced to bud or release small membranes when incubated with cytosol and ATP. Dynein appears to bind to Golgi membranes destined to bud because dynein is present in budded membranes, but absent from the stacks after budding. Myosin-I is also associated with budded membranes. The presence of dynein on budded membranes, after incubation with GTPgS, which has been shown by others to cause an accumulation of coated vesicles, was similar to levels found in complete budding reactions. In contrast, about 10-fold more dynein was found on budded membranes incubated with NEM, which has been shown to cause an accumulation of smooth, non-coated vesicles. We propose that cytoplasmic dynein binding to Golgi membranes occurs during the late stages of membrane-protein translocation through the Golgi. We also propose that dynein transports budded Golgi membranes along MTs to the cell cortex where myosin-I provides local delivery through the actin-rich cytoskeleton to the apical membrane. Supported by NIH DK 31643.

J3-004 REGULATION AND POSSIBLE FUNCTIONS OF AMOEBA MYOSINS, Edward D. Korn, Ivan Baines, Hanna Brzeska, John Hoey, M. Jolanta Redowicz, and Zhen Yuan Wang. Laboratory of Cell Biology, National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892.

The free-living amoeba *Acanthamoeba castellanii* contains at least 3 myosin families: the heavy chains of 1 myosin II[1], 3 myosins I[2] and 1 myosin IV[3] have been characterized and, except for myosin IA, sequenced. Relatively little is known about the regulation or function of *Acanthamoeba* myosin IV. The actin-activated MgATPase activity in the N-terminal globular head of myosin II is inactivated by phosphorylation of up to 3 Ser residues in the 29-residue non-helical C-terminal end of each heavy chain. Regulation occurs only at the level of filaments and inactivation correlates with a dramatic increase in flexibility of the hinge region. As judged by immunolocalization, myosin II functions in the tail of locomoting amoeba and as a component of the contractile ring of dividing cells. The 3 myosins I are activated by phosphorylation of a single Ser or Thr located between the ATP-sensitive actin-binding site and the ATPase site in the globular head of the single heavy chain. The 97-kDa myosin I heavy chain kinase (MIHCK) is activated by autophosphorylation and by binding to acidic phospholipids or isolated plasma membranes independent of autophosphorylation. Phospholipids and membranes also accelerate autophosphorylation of MIHCK. The C-terminal 35-kDa, catalytic domain of MIHCK has been cloned and sequenced and found to have the consensus sequences of other protein kinases. As determined by antibodies specific for the 3 unphosphorylated and phosphorylated isozymes, about 80% of myosin IA (MIA) is phosphorylated and about 20% of MIB and MIC. PMIA is highly concentrated in the actin-rich, sub-membranous cortex (and particularly at regions of pseudopod formation) suggesting a role in maintaining cortical tension. PMIB also occurs in the cortex but mostly in the plasma membrane, particularly at regions of microspike and pseudopod formation and phagocytosis, suggesting a role in membrane-associated motile activity. PMIC has a similar distribution as PMIB but is the only MI associated with the contractile vacuole (CV) membrane. The concentration of PMIC is about 20-times greater in actively contracting CVs than in filling CVs and the concentration of CV-associated F-actin parallels the PMIC concentration. Anti-MIC antibodies specifically inhibit CV function *in vivo*[4]. These data provide the first direct evidence for a membrane-associated motile role for a myosin I.

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*Cell Movements*

J3-005 ACTIN FILAMENT ARRANGEMENTS AND REARRANGEMENTS IN MOTILE CELLS, J. Victor Small [1], [1] Institute of Molecular Biology, Austrian Academy of Sciences, A-5020 Salzburg, Billrothstrasse 11, Austria

The results of structural investigations of the actin cytoskeleton taken together with microinjection studies indicate that crawling of cells over a substrate involves the vectorial polymerization and depolymerization of actin filaments by actin treadmilling and not a nucleation release mechanism. In some cells, such as fish keratocytes, the rate of actin polymerization matches the rate of movement, whereas in other cells, such as fibroblasts, the relationship is more complex. Considerations of the geometric organization of actin filaments in the lamellipodia of keratocytes and fibroblasts, together with other data, suggest that a lateral flow of actin filaments may play an important role in lamellipodia motility. Apart from explaining discrepancies in measured rates of cytoskeleton and cortical flow, lateral filament flow can be invoked as a mechanism for supplying actin filaments for movement without the need for continuous necleation of new filaments at the cell front.

In view of the structural data supporting treadmilling in keratocyts we are now reinvestigating the organization of the actin comet tails that propal Listeria monocytogenes through the cytoplasm of infected cells.

J3-006 MODULATION OF MICROTUBULE DYNAMIC INSTABILITY BEHAVIOR IN LIVING CELLS, Patricia Wadsworth[1], Rama Dhamodharan[1], Doug Thrower[2], Les Wilson[2], and Mary Ann Jordan[2], [1]Department of Biology, University of Massachusetts, Amherst, Massachusetts and [2]Department of Biological Sciences, University of California, Santa Barbara, California.

Microtubules in living cells are highly dynamic, alternating between periods of growth, shortening and pause. Previous work has demonstrated that microtubule dynamic behavior is cell type-specific: microtubules in fibroblasts grow and shorten at rapid rates and MTs frequently disassemble out of the field of view. In contrast, microtubules in epithelial cells are characterized by slower rates of growth and shortening and a high frequency of rescue. MTs in epithelial cells make numerous short excursions with little change in the network over time. To explore the mechanisms that may contribute to the regulation of MT dynamics in diverse cells, we have examined the effect of brain microtubule associated proteins (MAPs), which bind along the wall of the MT, and the MT binding drug vinblastine, which interacts with MT ends, on microtubule dynamics in living cells. BSC-1 cells were injected with rhodamine labeled tubulin and then injected with brain MAPs or incubated with low concentrations of vinblastine. Images of fluorescent MTs in the cells were obtained using low-light-level video microscopy; the parameters of MT dynamic instability behavior were quantified using MT tracking methods.
Purified brain MAP-2 and heat stable brain MAPs suppressed microtubule dynamics by reducing the average rate and extent of both growing and shortening events. Both MAP preparations increased the percentage of time spent in a state of pause or attenuation, where no change in MT length could be measured by video microscopy. MAPs increased the frequency of rescue events about two-fold, but did not alter the frequency of catastrophe (measured as events/unit distance). Like neuronal MAPs, low concentrations of vinblastine (8-64 nM) also reduced the average distance and rate of growth and shortening events. At these concentrations of vinblastine, no loss of MT polymer was detected when assayed in isolated cytoskeletons. Vinblastine increased both the average pause duration and the percentage of time spent paused. In contrast to MAPs, vinblastine markedly reduced the frequency of catastrophe, to 30% of control values at 32 nM vinblastine. The frequency of rescue was increased about two-fold. Vinblastine increased the probability that a paused MT would convert to growth, rather than shortening. These experiments reveal that MAPs, which bind along the MT length, modulate the rate and extent of growth and shortening excursions with only a modest effect on transition frequencies. Vinblastine also suppresses MT dynamics, increasing pause duration and inducing a dramatic reduction in the frequency of catastrophe, demonstrating that vinblastine may kinetically stabilize MT ends. Regulation of the expression of MAPs and vinblastine-like molecules are likely to contribute to the cell type-specific behavior of MTs in living cells.

*Macromolecular Structure*

**J3-007** THE DESIGNS OF ACTIN BUNDLES, David J. DeRosier[1], Amy McGough[2] and Michael Way[3], [1]Department of Biology, Brandeis University, Waltham, MA, [2]Department of Biochemistry, Baylor College of Medicine, Houston, TX, [3]Whitehead Institute for Biomedical Research, Cambridge, MA.

Fimbrin, fascin, villin, scruin, and EF1a are examples of actin-bundling proteins that have unrelated actin-binding sequences. Do they produce bundles having different actin filament organization and does the filament organization alter the properties of the bundle? To address these questions, we are studying the organization or packing of filaments in bundles containing scruin, fimbrin, fascin, and EF1a.

A description of the packing can be divided into two parts: the spatial relationship between any pair of crosslinked filaments and the array of points that describes the positions of the helical axes of the filaments. The above bundling proteins divide into two classes regarding the relationship between neighboring filaments. All produce parallel rather than anti-parallel arrangements of filaments but EF1a produces bundles in which neighboring filaments are rotated by +/-90 degrees. The rest produce bundles in which the filaments are unrotated. The relationship between neighboring, crosslinked filaments is likely to be a direct result of the bonding properties of the bundling protein. These two different orientations can lead to differences in the extent of crosslinking bundles with the latter having the potential for greater crossbridging than the former.

In contrast to the relationships between pairs of cross-bridged filaments, the array or "lattice" of points describing the positions of filaments within a bundle is not completely specified by the bonding properties of the bundling protein, at least not of the ones examined so far. Rather the "lattice" appears to be linked to the conditions at the time of assembly. The reason for this is that there are many ways to crosslink filaments into a bundle because of the high symmetry of the actin filament. It is possible to build bundles having square, hexagonal or liquid packing with the degree of order depending on the assembly process. Thus, the organization of filaments within bundles can be controlled by the assembly process as well as the design of the bundling protein.

To understand how different crosslinking proteins might interact within a bundle we need not only the geometrical relationships between filaments but also the locations of the binding sites on the actin filaments. Three-dimensional reconstructions of filaments decorated with the actin-binding domain of alpha-actinin, which is a homolog of the actin-binding domain of fimbrin, have shown the shape of the domain and how it binds actin. We have used this information to attempt a model for the actin-fimbrin bundle.

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**J3-008** THE ACTOMYOSIN INTERACTION, Kenneth C. Holmes, Max Planck Institute for Medical Research, 69120 Heidelberg, Germany

The cross-bridge theory of muscle contraction proposes that the sliding of actin filaments passed myosin is brought about by the myosin cross-bridges which extend from the myosin filament and cyclically interact with the actin filament [1, 2]. It is proposed that cross-bridge first binds to actin in an unstrained conformation (*weak*) and then undergoes an isomerisation to the strained (*strong*) form. Release of the strain energy affects mutual movement [3, 4]. The process is modulated by the status of the nucleotide binding site. A key question is the strucutural basis for the weak to strong isomerisation.

An atomic model for the rigor complex of F-actin and the myosin head has been obtained by Rayment *et al* by combining the molecular structures of the individual proteins with the low resolution electron density maps of the actomyosin complex derived by cryo-electron microscopy [5, 6]. The actin binding sites and nucleotide binding sites of S1 are functionally linked by a cleft in the 50K domain of S1 which appears to close on binding to F-actin. The closing of the cleft is likely to be an essential part of the weak/strong sequence of the actomyosin interaction. The initial (*weak*) actomyosin interaction involves only part of the surface but is stereospecific, the full rigor complex develops on closure of the cleft. One can equate these states to the A and R states described by Geeves [7].

The stereospecific weak state (or A state) is essentially different from the unspecific electrostatic weak interactions found at low ionic strength [8]. However subsequent electrostatic interactions may well play a role in controlling the A to R transition.

X-ray analysis and electron microscopy [9, 10] show that with tropomyosin in the "off" state (no $Ca^{++}$) the myosin would block both weak (A) and strong (R) interactions In the presence of $Ca^{++}$ a weak interaction would be permitted but the development of the full rigor interface would be inhibited. A further small movement of the tropomyosin would be necessary to allow the cleft to shut and permit the full rigor interaction. These three states of the thin filament can be tentatively equated with the states "blocked", "closed", and "open" identified by McKillop *et al.* [11].

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**J3-009** FORCE MEASUREMENTS OF A SINGLE KINESIN MOLECULE, Ronald D. Vale[1], Chris Coppin[1], Jeff Finer[2], and James A. Spudich[2], [1]Departments of Pharmacology and Biochemistry, University of California, San Francisco, CA 94143, [2]Department of Biochemistry, Stanford University, Stanford, CA 94305.

Kinesin is a microtubule-based force generating protein that is involved in organelle transport. To investigate the mechanism by which kinesin converts energy from ATP hydrolysis into mechanical work, the displacements and isometric forces produced by a single kinesin molecule moving along a fixed axoneme were measured using an optical trap feedback apparatus. As a kinesin-bound bead moved up the force gradient of the optical trap, it usually exhibited discrete displacements of approximately 8 nm, which corresponds to the distance between tubulin subunits in the microtubule lattice. This suggests that kinesin moves in a methodical fashion from subunit to subunit. Analysis of the mean-square displacement of the bead in between kinesin-induced steps suggests that the spring constant of the kinesin-bead linkage is similar to values obtained previously for myosin. When the position of the bead was fixed by feedback control of the optical trap's position, kinesin achieved maximal forces of between 3 to 10 pN. Unlike myosin, which exhibits single force transients, kinesin reached its maximal force in a discontinuous fashion; each of the step wise increases in force seems to correspond to the binding of the motor to a new tubulin subunit. The relevance of these findings to understanding the mechanism of kinesin movement will be discussed.

*Mechanisms of Force Production*

J3-010 **HIGH RESOLUTION ANALYSIS OF KINESIN MOTOR MOVEMENT,** Steven M. Block†, Karel Svoboda‡, Partha Mitra‡, †Department of Molecular Biology, Princeton University, Princeton, NJ 08544, ‡AT&T Bell Laboratories, 600 Mountain Ave., Murray Hill, NJ 07074.

By marrying optical tweezers (an infrared optical trap) with a sensitive laser-based interferometer, capable of measuring position down to the ångström level, we produced a hybrid device with the required spatial and temporal sensitivity to record the movements of motor proteins on a molecular scale[1]. Using this device, in conjunction with an *in vitro* motility assay[2], it has proved possible to monitor the displacements of single molecules of kinesin moving along microtubules. The reduction in thermal noise afforded by the optical trapping geometry permits us to resolve the individual steps that underly the motion of the mechanoenzyme, under conditions of either high mechanical load or low-to-moderate ATP[3]: steps average ~8 nm in length and are completed in under 1 ms. By characterizing the potential well produced by the optical trap, we are able to subject single motors to calibrated forces up to many piconewtons. In this fashion, we measured the forces generated by single kinesin molecules as a functions of both speed and ATP level[4]. The kinesin force-velocity relationship is roughly linear at both high and low ATP concentrations, producing a peak force of 5-6 pN that is nearly independent of ATP concentration. This finding suggests that kinetically-important rates in the mechanochemical cycle (e.g., the apparent $K_m$ for movement) may be load-independent, and that the coupling of motion to ATP hydrolysis may be variable. At low mechanical loads and high ATP levels, kinesin stepping is impressively rapid (~1000 nm/s) and individual steps cannot be discerned over thermal noise. In this regime, high-precision records of displacement were subjected to a form of variance analysis[5]. Surprisingly, the variance in the motion is smaller by a factor of ~2 than that anticipated for an exponential stepper moving unidirectionally on a regular lattice of 8 nm intervals. This result may indicate that the stepping motion involves two or more sequential, rate-limiting processes.

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J3-011 FORCES GENERATED BY SINGLE MOLECULES OF THE MOTOR PROTEIN KINESIN, Jonathon Howard, Department of Physiology and Biophysics, SJ-40, University of Washington, Seattle, WA 98195.

We are interested in understanding the physical mechanism by which motor proteins convert the free energy derived from the hydrolysis of ATP into mechanical work used to power intracellular transport. We are using the microtubule-based motor protein kinesin as a model because it has the smallest motor domain, it has a simple subunit structure, and functional motors can be expressed in bacteria.

Our approach has been to develop cell-free assays in which the movement of individual microtubules across a kinesin-coated glass surface is directly observed under the microscope. Using this assay we have answered a number of questions concerning the mechanism of energy transduction. By decreasing the density of kinesin on the surface we have shown that a single kinesin molecule suffices to move a microtubule[1] and that kinesin has a highly flexible domain that permits it to move a microtubule equally well in any direction[2]. By demonstrating that kinesin's path on the microtubule's surface is parallel to the protofilaments[3], we infer that kinesin's step size, the distance between consecutive microtubule-binding sites, is a multiple of 8 nm, the spacing of tubulin dimers along the protofilament. To probe the mechanism by which kinesin generates force we have placed single motors under loads using two different techniques. In one method[4], the viscosity of the solution was raised approximately 100-fold - from the reduction in speed we estimated that one motor generates a force of 4 to 5 pN. In the second method, a motor was attached to a flexible glass fiber and a photodiode sensor was used to detect displacements as little as 1 nm and forces as small as 1 pN. Kinesin's speed decreased linearly as the load was increased. The maximum force of 5.4 ± 1.0 pN/nm (SD, n=16) was independent of the fiber's stiffness, the hydrodynamic damping, and the ATP concentration.

These results allow us to rule out a number of models of the force-generating reaction including those in which the motor is postulated to rectify the diffusion of the filament, and those that posit that the reduction in speed at high load is due primarily to increased slippage. Our results are consistent with power-stroke models in which high loads decrease the overall rate of the ATP hydrolysis cycle.

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*Regulation I*

J3-012 REGULATION OF ORGANELLE TRANSPORT IN MELANOPHORES, Leah T. Haimo[1], Fatima Gyoeva[2], Bruce Telzer [3], Kevin Pfister[4], and Mark Lazzaro[1]. [1]Department of Biology, University of California, Riverside, [2]Institute for Protein Research, Moscow, [3]Department of Biology, Pomona College, Claremont, CA, and [4]Department of Cell Biology, University of Virginia, Charlottsville, VA.

Bidirectional transport of pigment granules in melanophores is regulated by protein phosphorylation and dephosphorylation. We have undertaken studies to determine how this regulatory system controls the direction of transport. We used a series to nucleotide analogs to determine that dispersion of pigment granules towards the plus ends of microtubules is mediated by a kinesin-like protein while aggregation of pigment granules towards the minus ends of microtubules is mediated by a dynein-like protein. Using antibodies directed against the heavy chain of kinesin and against the intermediate chain of dynein, we find that both of these motors are localized to isolated pigment granules. When these organelles are isolated from aggregated versus dispersed cells, we find that the amount of each motor remains the same for the two directions of transport. Accordingly, in this system, regulation of the direction of transport is not mediated by a dissociation of one or the other motor from the organelles during one direction of transport. Pigment granules saltate during dispersion but not aggregation. These transient retrograde excursions during dispersion suggest that the retrograde motor, cytoplasmic dynein, must be active during dispersion. To determine if this motor is transiently activated by protein dephosphorylation during dispersion, we tested protein phosphatase inhibitors to determine if the saltatory behavior would be inhibited. It is not, and these results fit a model in which cytoplasmic dynein is continuously active during both directions of transport and, therefore, not regulated. We have immunoprecipitated kinesin from $^{32}$P-labeled cells and find that a protein of 70 kD immunoprecipitates with kinesin and is highly phosphorylated only during dispersion. No difference in the phosphorylation state of dynein is observed in aggregated versus dispersed cells. Accordingly, these studies suggest that the direction of organelle transport in melanophores may be regulated by controlling the activity of kinesin via its interaction with a protein that is cyclically phosphorylated. Supported by a grant from the NSF.

J3-013 REGULATION OF *CHLAMYDOMONAS* FLAGELLAR DYNEIN BY AN AXONEMAL PROTEIN KINASE, Winfield S. Sale, Geoff Habermacher, and David Howard, Dept of Anat/Cell Biology, Emory Univ. Sch. of Med., Atlanta, GA30322

Diverse experimental data support a model that the central pair and radial spoke apparatus regulate dynein driven microtubule sliding in *Chlamydomonas* flagella. However, the molecular mechanisms are not known. We used *in vitro* functional assays to test the hypothesis that an axonemal cAMP dependent protein kinase inhibits dynein in spoke deficient and central pair deficient axonemes. First, the velocity of dynein-driven microtubule sliding in spoke-deficient mutants (*pf*14, *pf*17) and a central pair mutant (*pf*18) was increased to wild type levels following treatment with the specific peptide inhibitor of cAPK PKI(6-22)amide. Half maximal activation occurred at 12-15 nM PKI, and kinase inhibitors did not effect microtubule sliding in wild type axonemes. PKI treatment of axonemes from a double mutant missing both the radial spokes and the outer row of dynein arms (*pf*14*pf*28) also increased microtubule sliding to control (*pf*28, *oda*2) velocity. Second, addition of the regulatory subunit of cAPK (RII) to spoke-deficient axonemes, increased microtubule sliding to wild type rates. Addition of 10 mM cAMP to spokeless axonemes, reconstituted with RII, reversed the effect of RII. Third, inner arm dyneins isolated from PKI-treated axonemes (strain *pf*14*pf*28) generated fast microtubule sliding velocities when reconstituted onto both PKI-treated or control extracted axonemes. In contrast, dynein from control axonemes generated slow microtubule sliding velocities when reconstituted onto either PKI-treated or control axonemes. The data indicate an axonemal cAPK inhibits dynein-driven microtubule sliding in spoke-deficient and central pair-deficient axonemes. The kinase is likely to reside in close association with its substrate(s), and the substrate targets are not exclusively localized to the central pair, radial spokes or outer dynein arms. The results are consistent with a model in which the radial spokes and central pair regulate dynein activity through suppression of a cAMP-mediated mechanism.

We also discovered that in the presence of PKI the velocity of microtubule sliding in spoke and central pair defective axonemes was dependent upon $[Ca^{2+}]$. Microtubule sliding velocity in spoke defective mutants, in the presence of PKI, was maximally stimulated to ~ 20μm/sec at low calcium ($\leq 10^{-7}$M), unstimulated at $10^{-6}$M $Ca^{2+}$, and partially stimulated to~16μm/sec at high calcium ($\geq 10^{-5}$M). Preliminary study of microtubule sliding velocity, using axonemes from outer arm mutant *pf*28 or outer arm and radial spoke mutant *pf*28*pf*14, revealed a similar, albeit attenuated, response to calcium. Thus, calcium responsive mechanisms are not exclusively localized to the spoke-central apparatus or outer dynein arms. These data indicate calcium may modulate flagellar movement and waveform through regulation of inner arm dynein activity, with a switch at $10^{-6}$ M $Ca^{2+}$.

J3-014 THE DYNACTIN COMPLEX: ACTIVATOR OF CYTOPLASMIC DYNEIN-BASED VESICLE MOTILITY, Trina A. Schroer[1], Janis K. Burkhardt[2], John A. Cooper[3], Steven R. Gill[1], Gareth W. Griffiths[2], John E. Heuser[3], Dorothy A. Schafer[3], [1]Department of Biology, Johns Hopkins University, Baltimore, MD 21218, [2]European Molecular Biology Laboratory, Heidelberg, Germany, [3]Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110.

The dynactin complex was first identified as a cytosolic factor required by cytoplasmic dynein to drive vesicle transport on microtubules *in vitro*. Recent studies in *S. cerevisiae* and *Neurospora crassa* support the hypothesis that dynactin complex participates in a variety of cytoplasmic dynein-driven functions *in vivo*. Dynactin complex is a 20S, 1.2 mD complex containing at least nine distinct polypeptides. EM analysis of individual platinum-shadowed molecules has provided an overview of dynactin complex ultrastructure, and antibody decoration experiments have allowed several subunits to be localized within the molecule. The largest structural domain, a 37 nm actin-like filament, is composed of the actin-related protein Arp1. One end of the filament (most likely the barbed end) contains actin-capping protein while the opposite end can be decorated with an antibody to a novel 62kD protein (p62). Projecting laterally from the actin-like filament is a poorly defined shoulder with a 22 nm sidearm terminating in a pair of small globular heads. The heads can be decorated with an antibody that recognizes the C-terminus of the $p160/p150^{Glued}$ subunit.

Dynactin complex and cytoplasmic dynein have been localized within cultured macrophages by EM immunogold labelling. Similar results were obtained with antibodies to dynactin complex subunits $p160/p150^{Glued}$, p62 and Arp1, as well as antibodies to whole cytoplasmic dynein and cytoplasmic dynein heavy chain. Double labelling studies indicate that dynein and dynactin complex are associated with the same membranes, which include endocytic and Golgi membranes and membrane tubules and small vesicles that are distributed throughout cytoplasm. Clusters of antibodies that do not appear to be membrane-associated are also observed, suggesting that dynein and dynactin complex may be arranged in higher order complexes in cytoplasm.

J3-015 MOLECULAR AND GENETIC ANALYSIS OF *CHLAMYDOMONAS* OUTER ARM DYNEIN, George B. Witman[1], Stephen M. King[2], Anthony Koutoulis[1], Gregory J. Pazour[1], and Curtis G. Wilkerson[1], [1]Worcester Foundation for Experimental Biology, Shrewsbury, MA and [2]University of Connecticut Health Center, Farmington, CT.

*Chlamydomonas* outer arm dynein consists of three dynein heavy chains (α, β and γ), two intermediate chains (IC69 and IC78), and 8 light chains. Previous studies showed that IC78 is located at the base of the dynein, is tightly bound to IC69, and is in direct contact with α tubulin in the axoneme. To learn more about the role of IC78, we sequenced a full-length cDNA clone encoding it. The sequence reveals that IC78 is homologous with IC69 and with the 74 kDa intermediate chain of cytoplasmic dynein. The C-terminal half of the chain contains 6 imperfect repeats, termed WD repeats, which are thought to be involved in protein-protein interactions; these repeats also are present in the other intermediate chains but were not reported previously. Using the IC78 cDNA as a probe, we screened a group of slow-swimming insertional mutants and identified eight in which the IC78 gene is either disrupted or deleted. Electron microscopy of three of these mutants revealed that each is missing the outer arm, indicating that IC78 is essential for arm assembly or attachment to the outer doublet microtubule. IC78 translated *in vitro* bound to and cosedimented with brain microtubules or axonemes, demonstrating that it is a *bona fide* microtubule-binding protein. In the same assay, IC69, which has an isoelectric point similar to that of IC78, did not bind microtubules. Analysis of the binding activities of truncated versions of IC78 allowed us to delimit two microtubule-binding domains and one IC69-binding domain within the chain. The fact that IC78 is a microtubule-binding protein essential for arm assembly strongly suggests that it provides at least part of the adhesive force for attaching the outer arm to the doublet microtubule. However, microtubule-binding activity alone is not adequate to explain binding of the outer arm to a *specific* site on the doublet microtubule. To learn more about other proteins that are necessary for outer arm assembly and attachment, we are using insertional mutagenesis to generate outer-armless mutants in which the defective gene is tagged so that it can be readily cloned. From 2978 colonies screened, we obtained 24 independently isolated cell lines that swam with reduced speed, which is characteristic of mutants lacking the outer arms. In 14 of these strains, Southern blot analysis indicated that the genes encoding the 5 cloned outer arm polypeptides (α, β and γ heavy chains, IC78 and IC69) are unaffected, suggesting that the defects are in genes not yet cloned. Of the new mutants that completely lack outer arms, two are tagged alleles of the outer-armless mutants *oda*3 and *oda*10. As the gene products of *ODA*3 and *ODA*10 are unknown, we are using the inserted DNA tag to clone the genes.

*Regulation II*

**J3-016** DYNEIN REGULATORY MUTATIONS IN *CHLAMYDOMONAS*, M E. Porter, J.A. Knott, S. Myster, & C.A. Perrone, Dept. of Cell Biology & Neuroanatomy, 4-135 Jackson Hall, 321 Church St. SE, University of Minnesota Medical School, Minneapolis, MN, 55455

Flagellar motility requires the coordinated activity of multiple dynein isoforms. Genetic analyses in *Chlamydomonas* have identified several mutations that disrupt either the assembly or the activity of the different dyneins, and the study of these mutations has led to three important conclusions. First, the primary function of the outer dynein arms is to increase the flagellar beat frequency (1-2). Second, the inner dynein arms are both necessary and sufficient to generate the complex flagellar waveforms (3). Third, microtubule sliding by the dynein arms is activated by signals from the radial spoke/central pair (RS/CP) complex (4). To gain a better understanding of how the activity of the multiple dynein motors are regulated, we have studied two different but related groups of dynein mutations. The first are extragenic suppressor mutations that were identified because they could restore motility to paralyzed RS/CP defective strains (5), and the second are flagellar mutations that have been identified by linkage to different dynein heavy chain (DHC) genes. The suppressor mutations fall into four major classes: mutations that alter a regulatory domain within the structural gene for the beta DHC of the outer arm (5-6), mutations that modify dynein arm assembly on specific axonemal microtubules (Porter, O'Toole, and Gardner, work in progress), mutations that affect specific inner arm isoforms (7-10), and mutations that disrupt a structure in the inner arm region known as the dynein regulatory complex (5, 8-10). These studies have demonstrated both the complexity and importance of the inner arm isoforms as regulatory targets. We have therefore used a PCR based strategy to isolate eleven members of the DHC gene family in *Chlamydomonas*. Genomic Southern blot analysis indicates that each gene is single copy, and that there are a total of at least 12-13 genes in this family. Comparisons to DHC sequences in the databases indicate that two sequences correspond to the alpha and beta DHC genes of the outer arm (11) but that none of the remaining nine sequences resembles cytoplasmic DHCs identified in other organisms. We predict that these nine sequences correspond to structural genes for the inner arm DHCs. Comparisons among the nine sequences indicate that they can be further subdivided into at least two or three subgroups. RT-PCR experiments have confirmed that each sequence is present as a mature transcript in RNA isolated from deflagellated cells. To correlate the DHC sequences with specific DHC isoforms, we have placed each gene on the genetic map of *Chlamydomonas* using RFLP mapping procedures. Four genes are linked to flagellar loci not previously identified as potential DHC mutations, but a fifth sequence (DHC1) is tightly linked to the inner arm locus *PF9/IDA1*. We are now using insertional mutagenesis strategies (12) to confirm the identity of the DHC loci. Thus far, we have established that the DHC1 gene encodes an I1 DHC isoform by the characterization of a mutant allele lacking ~13kb of the DHC1 gene. This is the first inner arm DHC locus to be identified in any organism.

(1) Mitchell & Rosenbaum, 1985, J. Cell Biol. 100:1228 (2) Kamiya, 1988, J. Cell Biol. 107:2253 (3) Brokaw & Kamiya, 1987, Cell Motil. Cytoskel. 8:68 (4) Smith & Sale, 1992, Science 257:1557 (5) Huang et al., 1982, Cell 28:115 (6) Porter et al., 1994, J. Cell Biol. 126:1495 (7) Porter et al., 1992, J. Cell Biol. 118:1163 (8) Mastronarde et al., 1992, J. Cell Biol. 118:1145 (9) Piperno et al., 1992, J. Cell Biol. 118:1455 (10) Gardner et al., 1994, J.Cell Biol., in press; (11) Mitchell & Brown, 1994, J. Cell Sci. 107:635 (12) Tam & Lefebvre, 1993, Genetics 135:375

**J3-017** DYNEIN REGULATION BY cAMP-DEPENDENT LIGHT CHAIN PHOSPHORYLATION, Peter Satir[1], M.E.J. Holwill[2], Kurt Barkalow[1], Hali Wang[1], and Toshikazu Hamasaki[1], [1]Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY 10461 and [2]Department of Physics, Kings College London, University of London, Strand.

Hamasaki *et al.* (1991; PNAS **88:**7918) demonstrated that a 29kD axonemal polypeptide (p29) that copurifies with 22S outer arm dynein in *Paramecium* becomes phosphorylated in a cAMP-dependent manner. p29 appears to be a regulatory dynein light chain in that it can be separated from 22S dynein by high salt extraction, but will specifically rebind to the a H chain of 22S dynein in a functional way across species boundaries (Barkalow *et al.*, 1994; J. Cell Biol. **126:**727). The recombined p29-22S dynein complex can be immunoprecipitated by antibodies against dynein H or I chains. When phosphorylated, p29 increases the velocity of microtubule translocation over 22S dynein in *in vitro* motility assays. This can account for the increase of swimming speed of permeabilized ciliated cells, which reflects an increase in ciliary beat frequency, by cAMP.
The mechanism by which microtubule translocation velocity is increased by phosphorylation is not well understood; however, our results suggest that the translocation velocity changes seen upon p29 phosphorylation depend on changes in the mechanochemical properties of the 22S dynein itself.

**J3-018** EXPRESSION, PURIFICATION AND ATPase MEASUREMENTS OF THE ncd MOTOR DOMAIN, Takashi Shimizu[1,2], Elena Sablin[3], Robert Fletterick[3], Ronald D. Vale[1,4], Elen Pechatnikova[5], and Edwin W. Taylor[1,5], [1]Marine Biological Laboratory, Woods Hole, MA 02543, [2]National Institute of Bioscience and Human-Technology, and National Institute for Advanced Interdisciplinary Research, Tsukuba, 305 Japan, [3]Department of Biochemistry and Biophysics, and [4]Department of Pharmacology, University of California, San Francisco, San Francisco, CA 94143, and [5]Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637.

ncd is a kinesin-related motor protein from *Drosophila* that moves in the opposite direction along microtubules to kinesin. To learn about the ncd mechanism, ncd motor domain (R335-K700) was expressed in *E. coli* and its enzymatic characteristics were studied. The ncd motor domain was purified from the cell lysate by S-Sepharose chromatography and trace amounts of contaminants were mostly removed by passing through a Q-Sepharose column. The yield was 20 mg out of 0.5 l culture of *E. coli*. The purified ncd motor domain exhibited unusual UV spectrum with a broad peak around 272-275 nm. Upon incubation with radioactive ATP, $^{3}H$ at adenine but not $^{32}P$ at $\gamma$-phosphate was incorporated into the protein, indicating it bound ADP but not ATP. The unusual UV spectrum as above was at least partly due to the bound ADP. Thus, like kinesin, nucleotide binding to the ncd motor domain is tight, although there is an equilibrium between the protein and free nucleotide. We also used a fluorescent ATP analogue, mantATP, for the kinetic study of ncd motor domain. MantATP was turned over by ncd motor domain slowly in the absence of microtubules but microtubules activated the turnover to a large extent, and the overall profile of microtubule-activation was similar to that of ATP turnover. Similar to ATP, mantATP was incorporated in the protein upon incubation, and the bound nucleotide was probably mantADP. Upon mixing of mantATP with ncd motor domain, the fluorescence intensity increased at 0.005 $s^{-1}$, which is likely to reflect the release of ADP from the protein. This rate was nearly equal to the ATPase rate. The fluorescence intensity of the ncd motor domain having bound mantADP, likewise, decreased upon mixing with ATP, representing the mantADP release. The rate was accelerated more than 1000 fold to 3.3 $s^{-1}$ by the presence of saturating microtubules. The profile of the rate vs microtubule concentration was nearly superimposable with that of mantATPase vs microtubule concentration. This strongly suggests that the ADP (and mantADP) release is the rate-limiting in the substrate turnover cycle by ncd motor domain. These characteristics are very similar to those of kinesin.

*Mitosis (Joint)*

**J3-019** 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.

**J3-020** 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.

*Cytokinesis*

**J3-021** DIVISION MECHANISM ESTABLISHMENT BY THE MOVING MITOTIC APPARATUS, R. Rappaport, The Mount Desert Island Biological Laboratory, Salsbury Cove, ME 04672.

The division mechanism is organized in the animal cell equatorial cortex by the mitotic apparatus (MA). Before, during, and after the interaction that establishes the mechanism the midpoint of the MA is fixed in the future division plane and that relation has been often incorporated as an essential feature in theories of both cell division function and establishment. The purpose of this investigation was to determine whether cytokinesis can occur when the normal fixed relation between the MA and the equatorial cortex is prevented. Eggs of the sand dollar (Echinarachnius parma) were reshaped into cylinders by confining them in a thin-roofed, transparent silicone rubber capillary with a tapered lumen 85-120 μm in diameter. In this circumstance the orientation of the MA and the distance from its axis to the cortex is constant from cell to cell and the division mechanism is established in the equatorial surface 4 min before the furrow appears. The MA was kept in constant reciprocating motion by alternately compressing the capillary lumen at the cell poles beginning on average 22 min before furrowing. Initially the asters were relatively small and the intact MA slid back and forth with the surrounding endoplasm. The cortex did not move and its visible motionless inclusions extended 3-5 μm below the cell surface. As the aster rays elongated they remained straight and radial and they appeared to entrap vesicular elements of the cytoplasm. When they attained maximum length their ends slid along the inner boundary of the cortex like the bristles of a stiff brush. The midpoint between the asters was moved 25, 50 and 75 μm in three experimental groups. One or two minutes before the furrow indentation appeared, the surface shrank away from the capillary wall in the region underlain by the moving MA. The indentation appeared near the midpoint of the shrunken region and its acuteness was greater in the 25 μm group than in the 75 μm group. As the furrow deepened the interastral cytoplasm flowed freely through the constriction but the asters mechanically distended the region when they were pushed against it or through it. These results imply that neither the establishment of the division mechanism nor its subsequent function require attachment or a stable relationship between the MA and the cortex. The behavior of the cortex suggests that the MA effect is spread more thinly over a wider area when the MA is moved. Its effect may be based upon regional differences in the concentration of contraction promoter that arise on its periphery by transport along microtubules from the vicinity of the mitotic axis. The cortex can apparently resolve a narrow contractile zone from an abnormally wide affected area.

J3-022 REGULATION OF CORTICAL DYNAMICS BY THE MITOTIC SPINDLE DURING CELL DIVISION, Yu-li Wang, Long-guang Cao, Douglas J. Fishkind, and John D. Silverman, Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545.

We have used a combination of single particle tracking, micromanipulation, and fluorescence imaging techniques to analyze the mechanism and regulation of cortical reorganization in dividing cultured animal cells. Surface receptors, which associate and comigrate with the cortical cytoskeleton, were labeled with fluorescent latex beads during metaphase. The beads start to move toward the equator ~1 min. after the onset of anaphase, when the spindle interzone becomes well defined. The movement is most active in the central region of the cell near separating chromosomes and appears to initiate or accelerate upon the passage of chromosomes, suggesting that cortical reorganization may be regulated by signals originating from separating chromosomes or the spindle interzone. To investigate the role of microtubules in regulating cortical activities, we have applied microtubule drugs nocodazole and taxol to anaphase cells. Nocodazole, when applied during the first 2 min. of anaphase, induces a simultaneous inhibition of chromosomal separation and cortical movement. Taxol on the other hand induces a striking enhancement in the converging movement of beads toward the center of the cell and an increase in the organization of actin filaments on the dorsal cortex. These observations suggest that microtubules may play a direct or indirect role in modulating cortical activities. To further characterize the relationship between the mitotic spindle and cleavage, we have used a microneedle to create physical barriers between the spindle interzone and the equatorial cortex. Barriers created before or during early anaphase cause inhibition of cleavage on the side away from the mitotic spindle, while the side containing the mitotic spindle undergoes active cleavage. The inhibited side contains a random network of microtubules and shows no directional movement of surface-bound beads. On the other hand, the side undergoing cleavage shows directional movement of beads and contains organized microtubules with their ends terminating at the equator. Our results point to the possibility that signals from the anaphase spindle may cause the organization of a specific set of microtubules near the interzone, which then mediate the regulation of cortical contractile activities in the cleavage furrow (Supported by NIH GM32476 and Human Frontier Science Program).

*Late Abstract*

MECHANISTIC STUDIES OF THE MICROTUBULE•KINESIN ATPASE, Susan P. Gilbert[1], Martin R. Webb[2], Martin Brune[2], and Kenneth A. Johnson[3], [1]Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, [2]National Institute for Medical Research, Mill Hill, London, UK NW71AA, [3]Department of Biochemistry & Molecular Biology, The Pennsylvania State University, University Park, PA 16802.

Rapid kinetic techniques were used to define a mechanism for the microtubule•kinesin (M•K) ATPase using the *Drosophila* kinesin motor domain (K401) expressed in *Escherichia coli* (Gilbert & Johnson, 1993, *Biochemistry* **32**, 4677; *Biochemistry*, 1994, **33**, 1951). The chemical quench flow results indicate that ATP binds the M•K complex rapidly ($k_{on,ATP}$ = 2 $\mu M^{-1}s^{-1}$, $k_{off,ATP}$ = 200 $s^{-1}$) but weakly with an apparent $K_{d,ATP}$ = 100 $\mu M$. ATP hydrolysis occurs at the active site at 100 $s^{-1}$ which is significantly faster than steady state turnover at 20 $s^{-1}$, showing that the rate limiting step occurs after ATP hydrolysis. The kinetics of K401 dissociation from the M•K complex, Pi release, K•ADP rebinding to the microtubule, and ADP product release were measured directly using the stopped-flow. These studies indicate that the rate limiting step in the pathway is the release of the K•ADP•Pi complex from the microtubule after ATP hydrolysis. After Pi release, the rebinding of the K•ADP intermediate to the microtubule is fast (20 $\mu M^{-1}s^{-1}$), thus accounting for the dramatic activation of the ATPase at low microtubule concentrations. The cycle is then completed by fast ADP product release (300 $s^{-1}$). These results lead to a model for motility in which the apparent processivity of the kinesin dimer is due to sequential release of each motor domain from the microtubule followed by fast rebinding to the microtubule once dissociated. Supported by grants from NIH (GM 26726) to KAJ, University of Pittsburgh to SPG, and European Community Twinning Grant, the Human Frontiers Science Program & the Medical Research Council, UK to MRW.

*Molecular Diversity; Motor Paradigms*

J3-100 TWO-POINT MUTATIONS ALLOW THE OVER-EXPRESSION OF HUMAN CARDIAC TROPONIN-I IN *ESCHERICHIA COLI*, Eman Al-Hillawi, Stephen D. Minchin, Hylary R. Trayer and Ian P. Trayer, School of Biochemistry, University of Birmingham, Birmingham B15 2TT, U.K.

We have subcloned the cDNA encoding the human cardiac Tn-I gene into the bacterial expression vector, pET11c. Initial attempts at overexpression in *E. coli* were not successful indicating very low levels of protein yields in the prokaryotic system. Since the overexpression of the skeletal isoform of Tn-I has been achieved previously, we decided to overexpress a truncated cardiac protein from which the cardiac-specific N-terminal extension has been eliminated. The overexpression of this mutant was successful and it led us to a series of deletion experiments whereby the first 20, 10, 5 and 3 amino acid residues were eliminated from the wild-type sequence. All mutants were overexpressed to varying levels in *E. coli*. These experiments helped us to narrow the problem down to the first four codons. Random mutagenesis of these codons to all possible permutations allowed us to isolate a mutant which was able to express the whole cardiac protein to high levels. Closer inspection of both the protein and DNA sequences revealed no change in the amino acid residues. The the second and fourth codons, however, were different: Ala2, GCG→GCC and Gly4, GGG→GGT. These two point mutations allowed the overexpression of the human cardiac Tn-I to high levels; over 60mgs of pure protein was obtained from 1L culture. We have also overexpressed the human cardiac Tn-C to high levels in the same system. Both proteins were proved to be biologically active by the following criteria: (a) Tn-I was able to bind to a Tn-C-affinity column in 9M urea in a calcium-dependent manner; (b) the highly-specific affinity between Tn-I and Tn-C in the presence of calcium was shown by the use of alkaline-urea polyacrylamide gel electrophoresis, and (c) Tn-I inhibited the acto-S1 Mg-ATPase activity and this inhibition was potentiated by the presence of tropomyosin and relieved by the addition of Tn-C to the system.

J3-101 MICROTUBULE SLIDING IN FLAGELLA DURING CELLULAR AVOIDANCE RESPONSES,

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Many cells undergo avoidance responses as a direct result of changed patterns of beating in the propulsive cilia or flagella. The changes can involve a reorientation of the organelle, alterations to the bend shape or symmetry, reversal of the direction of bend propagation, transient cessation of motion or a combination of these. The responses demonstrate that the beat patterns of the propulsive organelles are under the control of the cell. The patterns of microtubule sliding within a cilium can be determined from a critical analysis of the bend shapes. In cells such as *Crithidia oncopelti*, which reverse the direction of propagation of essentially planar waves, the relative directions of microtubule sliding in the axoneme must reverse during the avoidance response. Since *active* microtubule sliding is probably unidirectional, wave reversal could be achieved by switching activity from the set of doublets on one half of the axoneme to the diametrically-opposed set. Such a mechanism represents a development of the switch-point hypothesis proposed by Satir (1985, Mod. Cell Biol. 4:1) for normal ciliary movement. For organisms such as *Phytophthora* zoospores, where one flagellum stops beating during cell reorientation, sliding in both halves of the axoneme is switched off simultaneously. Other patterns of avoidance response can be explained by variants of a mechanism which switches active sliding from one microtubule group to another, with appropriate phasing.

J3-102 A FAMILY OF DESMOSOMAL MYOSIN-LIKE MOLECULES IN EPITHELIAL TISSUES.

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A family of monoclonal antibodies that react with skeletal muscle myosin heavy chains II (MyHC) were found also to recognise epitopes associated with the plasma membrane of a variety of epithelial tissues. In rodent epidermis each antibody distinguished a unique set of cells. In some cells more than one epitope could be found in partially overlapping cell subsets. For example, antibody A4.840 that recognises slow skeletal MyHC reacts with all epidermal cells, while N3.36 against neonatal/fast MyHC detects cells of only the basal layer and part of the hair bulb, and a third antibody A4.74 (against fast type IIa MyHC) detects only cells of an outer layer of the hair bulb. Antibodies were found to give a punctate pattern of staining on the cell surface in a variety of tissues and co-localise with desmoplakin at the light microscopic level in rodent and human epidermis. The distinct MyHC-like immunoreactivities arise sequentially during embryonic and postnatal development. The conservation of these epitopes both on skeletal muscle MyHC across species and on the new family of molecules in epithelial tissues indicates functional significance for these epitopes. The MyHC-like molecules in skin may have a motor function and the possession of distinct epitopes may confer distinct mechanical properties on these molecules. The possibility that this family MyHC-like molecules localised at the desmosomal junction could be involved in movement of cells or their components is being investigated.

J3-103 ANTITUBULIN ANTIBODIES SHOW A NON UNIFORM DISTRIBUTION ALONG THE AXONEME IN FLAGELLA

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Newly obtained monoclonal antibodies (mAb) (B3, C9, D66) block flagellar motility of sea urchin axonemes ATP reactivated *in vitro* (see accompanying abstract). We have used these MoAb for immunolocalizations on sea urchin spermatozoa and compared them to well known anti tubulin mAb and an anti dynein (D1) that also blocks flagellar motility (Mol.Biol.Cell, 1994, in press). Surprisingly, D1 shows a decreasing (base to tip) gradient of staining on the axoneme, even though it specifically recognizes IC1, one of the regulatory chain of outer dynein arms, supposed up to now to be evenly distributed. YL1/2, DM1A and DM1B do not interfere with motility (100µg/ml) and show an homogeneous staining throughout the whole length of the axoneme, a property shared with C9, a strong inhibitor of motility (0.1µg/ml). B3, another strong inhibitor, shows an homogeneous staining, but does not stain the distal tip of the axoneme. GT335, also strong inhibitor of motility, shows a gradient decreasing from base to tip and does not stain the distal tip. Finally, D66, a good inhibitor, shows a gradient increasing from base to tip, and labels the distal tip. Those various gradients have been quantified by digitized image treatments and segmentation. All these results suggest that outer dynein arm composition (as for inner arms) or accessibility may vary along the axoneme and that various isoforms of tubulin coexist along the axoneme, with different localization or accessibility that may have a functionnal significance.

**J3-104** A *CHLAMYDOMONAS* PHOTOTACTIC MUTANT STRAIN HAS A NOVEL INNER DYNEIN ARM DEFECT.

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We have characterized several *Chlamydomonas reinhardtii* strains that are unable to orient to a light source; they are phototactic defective. One of these strains, *ida7–1*, has an inner dynein arm defect. *ida7–1* cells exhibit a motility phenotype that is distinct from the phenotypes of strains that have affected outer dynein arms or inner dynein arms. The phototactic and motility phenotypes cosegregate in 36 tetrads, indicating that the phenotypes are linked and likely to be caused by the same mutational event. The *ida7–1* mutation is recessive to the wild–type allele in both heterozygous dikaryon and diploid strains and has been mapped to a new locus. Double mutant strains have been constructed with *ida7–1* and other mutations that affect the outer (*oda2*) and inner (*ida4*, *pf9*, *bop2–1*) dynein arms. Double mutant strains with *oda2* are aflagellate, with *ida4* have predominantly paralyzed flagella, and double mutant strains with *bop2–1* or *pf9* are slower than either parental strain. These data suggest that the *ida7–1* mutation affects a structure not affected by the *oda2*, *ida4*, *pf9* or *bop2–1* strains. The high molecular weight (HMW) dyneins of *ida7–1* axonemes were examined by FPLC fractionation. FPLC fractionation of axonemal high salt extracts has previously identified seven inner dynein arm subspecies that contain eight HMW polypeptides; so far five of these subspecies are affected in mutant strains (Kagami and Kamiya, *J. Cell Sci.*, 103: 653–664, 1992; Kato et al., *Cell Struct. Funct.*, 18: 371–377, 1993). *ida7–1* axonemes are missing the *g* subspecies dynein, which is not affected in previously characterized inner dynein arm mutant strains. We are currently examining *ida7–1* axonemes by electron microscopy to determine what axonemal structures are affected.

**J3-105** MULTIPLE NUCLEOTIDE BINDING SITES ON DYNEIN, ROLE IN REGULATION?, Charlotte K. Omoto, Department of Genetics and Cell Biology, Washington State University, Pullman WA 99164-4234.

Over the years, there has been increasing evidence for multiple nucleotide binding sites on dynein. The large size and complexity of dynein heavy chains may be related to the presence of multiple and functionally distinct nucleotide binding sites. Sequence analysis of dynein heavy chain also suggests multiple nucleotide binding site. Recently, experiments using a wide range of organisms from Tetrahymena, Chlamydomonas and trout sperm have provided evidence for functionally distinct nucleotide binding sites. The evidence suggests a common feature among axonemal dyneins, the existence of multiple nucleotide binding sites, and more importantly, the existence of a regulatory nucleotide binding site. The evidence includes: 1) sliding disintegration of Tetrahymena and Chlamydomonas axonemes are inhibited at high [ATP]. Addition of ADP overcomes this ATP-inhibition. 2) High [ATP] inhibition of movement of Chlamydomonas pf mutants is similarly overcome by the addition of ADP. 3) High [ATP] also inhibits trout sperm movement. The circumferentially symmetric axonemal structure may require a type of regulation dependent on inhibition at high [ATP]. A model for such regulation will be presented. The evidence presented for multiple and functionally distinct nucleotide binding sites invites reinterpretation of earlier experiments which assumed the presence of a single nucleotide binding site/dynein heavy chain.

**J3-106** GENETIC PERTURBATION OF A MODEL ENERGY-TRANSDUCING SYSTEM: THE DNA PACKAGING APPARATUS OF BACTERIOPHAGE T7, Philip Serwer, Shirley J. Hayes, Robert H. Watson, and Saeed A. Khan, Department of Biochemistry, The University of Texas Health Science Center, San Antonio, TX 78284-7760.

The packaging of double-stranded DNA in the pre-formed DNA-free capsids of bacteriophages is an example of ATP-driven translocation that is unusually susceptible to analysis by use of both genetics and biochemistry. For bacteriophage T7, particles in the DNA packaging pathway (intermediates) have been detected during both in vivo and high efficiency (20-35%) in vitro DNA packaging. These intermediates include capsids with incompletely packaged DNA (ipDNA-capsids). The ipDNA-capsids are an archive of the intermediate states of DNA packaging, a process that includes ATP-driven entry of DNA through a six-fold symmetric ring at the site of attachment of the bacteriophage tail. To increase the number of intermediate states represented among the ipDNA-capsids, we have developed high-efficiency procedures of (a) region-specific mutagenesis, performed by use of the polymerase chain reaction, followed by in vitro recombination-packaging, and (b) screening for DNA packaging intermediates, performed by nondenaturing gel electrophoresis. A mutant that accumulates ipDNA-capsids has been isolated. Our current collection of intermediates includes capsids that have altered permeability. The use of gating to transduce energy during DNA packaging (i.e., use of an ATP-dependent Maxwell's demon) is a possible explanation for the existence of more than one permeability state. Analysis of mutant phenotypes will assist in testing both this and other hypotheses that describe energy transduction during DNA packaging.

Supported by NSF and NIH.

*Organellar Movement and Motors; Cell Movements*

**J3-107** ARE MYOSINS I INVOLVED IN MEMBRANE TRAFFICKING? Antoine Durrbach, Katleen Collins, Paul Matsudaira, Daniel Louvard, and Evelyne Coudrier.Institut Pasteur 75015 Paris France.

Myosin Is, an ubiquitous monomeric class of myosins which exhibit actin based motor properties are associated with plasma membrane and/or vesicular membranes, and have been suggested as key players for trafficking events between surface and intracellular membranous structures. In order to investigate the functions of myosin Is, we have transfected a mouse hepatoma cell line (BWTG3), with cDNAs encoding the chicken brush border myosin I (BBMI) and 2 variants truncated in the motor domain. One variant lacks the first 40 amino-acids (BBMID40) while the other, the entire motor domain (BBMI tail). Production of each of the two variants impaired the organization of: 1) the juxtanuclear recycling compartment of internalized transferrin (uptake for 30min), 2) the late endocytic compartment characterized by the distribution of internalized a2-macroglobulin (uptake for 30min), 3) lysosomes characterized by the distribution of cathepsin D, and 4) the acidic compartment labelled with Acridine Orange. Furthermore these two variants codistributed with the late endocytic compartment characterized by the distribution of internalized a2-macroglobulin (uptake for 30min). In contrast, the full length BBMI which is recruted in lamellipodia did not modify the distribution of these compartments. The number of receptors and the kinetics of internalization and recycling of transferrin and a2-macroglobulin were similar in control cells and cells producing BBMI or its variants. However, the rate of a2-macroglobulin degradation was increased by 80% and 45% over control cells, in cells producing BBMID40 and BBMI tail, respectively. In contrast, the rate of a2-macroglobulin degradation was decreased by 25% in cells producing BBMI. We have also produced chicken BBMI and the two variants in Caco-2 cells which are derived from a human colonic adenocarcinoma. In contrast to the entire protein the two variants did not reach the microvilli of fully differentiated Caco-2 cells but are distributed along the lateral membrane domain. We suggest that the BBMI variants, truncated in the motor domain, are non functional in both cell types. These observations might suggest that, chicken BBMI or an endogenous myosin I which could be competitively inhibited by the truncated variants, is involved in membrane trafficking in the unpolarized hepatoma cell line. We are currently studing whether the truncated variants have a dominant negativ effect on membrane trafficking in polarized cells.

**J3-108** AN ESSENTIAL MYOSIN I ISOFORM IN *ASPERGILLUS NIDULANS* IS REQUIRED FOR SECRETION AND POLARIZED GROWTH, Gregory S. May and Carol A. McGoldrick, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

Myosins are actin activated ATPases capable of supporting contractile and motile activities. Myosin isotypes are classified at either conventional and unconventional myosins based on their structural organization (ref). The conventional myosins are those that are structurally similar to muscle myosin and are able to form bipolar thick filaments. The unconventional mysosins are structurally diverse but all contain a motor domain characteristic of myosins. The first unconventional myosins described were the ameoboid myosins I. Myosins I have been shown to be membrane and/or vesicle associated and are thought to have roles both in cell motility and organelle transport. However a precise role for the function of myosins I has been hard to determine because in most systems there is functional redundancy amongst the isoforms. We have identified an essential myosin I, *myoA*, from the filamentous fungus *Aspergillus nidulans*. We have used the *alcA* alcohol dehydrogenase promoter to develop conditionally lethal *myoA* strains. When conidiaspores of the conditionally lethal strain are germinated on repressing medium they swell and eventually lyse. In contrast, the conditional lethal strains when grown on inducing media grow like wild type. The mutant phenotype indicates an essential role for *myoA* in secretion and polarized hyphael growth. Using affinity purified antibodies against the myosin I carboxyl terminus we have determined that MYOA is enriched at growing hyphael tips. Thus it seems likely that MYOA transports components essential for polarized growth or that myosin I is required for organization of the actin cytoskeleton at the growing tip.

**J3-109** IDENTIFICATION AND LOCALIZATION OF THREE CLASSES OF MYOSINS IN POLLEN TUBES OF *LILIUM LONGIFLORUM* AND *NICOTIANA ALATA*, Deborah. D. Miller[a], Stylianos. P. Scordilis[b] and Peter. K. Hepler[a,c], [a]Molecular and Cellular Biology Program, University of Massachusetts, Amherst MA 01003, [b]Department. of Biological Sciences, Smith College, Northampton, MA 01063, [c]Department. of Biology, University of Massachusetts, Amherst MA 01003.

Pollen tubes, the fastest growing plant cells known, have active cytoplasmic streaming and growth processes that deliver the male gametes to the egg apparatus. Vesicular movement, brought about by cytoplasmic streaming, is supported by an actomyosin system. In this study, identification and localization of actin and myosin in pollen tubes has been accomplished. Immunoblot analysis of pollen tube extracts with antibodies to actin, *Acanthamoeba* myosins IA and IB and chicken brain myosin V revealed the presence of these contractile proteins. Immunofluorescence microscopy indicated that actin is localized longitudinally in the active streaming lanes and near the cortical surface of the tube. Three dimensional reconstruction of optical sections with confocal laser scanning microscopy through a pollen tube near a vegetative nucleus indicated that actin was located very near the surface of the nucleus and free in the cytoplasm. Myosin I was localized to the plasma membrane, vesicles and/or larger organelles, surfaces of the generative cell and vegetative nucleus, and within the cytoplasm of the generative cell, whereas, myosin V was found in the vegetative cytoplasm in a punctate fashion representing secretory vesicles and/or organelles. Conventional myosin subfragment 1 and light meromyosin antibodies were localized in a punctate fashion throughout the vegetative cytoplasm. Isolated generative cells and vegetative nuclei were only fluorescently labeled with myosin I antibody. Chemical fixation, rapid freeze fixation and freeze-substitution followed by rehydration or embeddment in a methacrylate mixture were performed in order to optimize preservation of pollen tubes for immunofluorescence microscopy. These results lead to the following hypothesis: Myosin I moves the generative cell and vegetative nucleus unidirectionally through the tube to the pollen tube tip while myosin V moves the organelles and the vesicles involved in growth. (Supported by grants to P.K.H. DCB 93-04953, and to S.P.S. NIH GM 47569, Howard Hughes Med. Inst.)

**J3-110** IDENTIFICATION OF ACTIN-DEPENDENT MOTOR ACTIVITY ON ISOLATED YEAST MITOCHONDRIA Liza A. Pon and Viviana R. Simon, Department of Anatomy and Cell Biology, Columbia University, 630 W. 168th Street, New York, NY 10032

Phenotypic analysis of yeast actin mutants has implicated the actin cytoskeleton in control of mitochondrial postion and inheritance. Consistent with this, we find that isolated yeast mitochondria bind to actin filaments. These actin-mitochondrial interactions are ATP-sensitive, reversible, saturable and mediated by a mitochondrial outer membrane protein. Here, we used the microfilament sliding assay developed by Kron and Spudich to study motor activity in isolated yeast mitochondria. Mitochondria are immobilized in a microscope flow cell and incubated with rhodamine-phalloidin yeast F-actin. In the absence of ATP, microfilaments bind to mitochondria. Upon addition of high levels of ATP (2mM), all bound filaments are released. However, in the presence of low levels of ATP (10μM), we observe microfilament sliding. Microfilament sliding co-localizes with immobilized mitochondria, and occurs in the absence of cytosolic extracts. Thus, all of the determinants required for microfilament sliding are present on the mitochondrial surface. Preliminary characterization indicates that this motor activity shares properties with members of the myosin superfamily. Previous studies indicate that myosin activity requires critical $Mg^{2+}$-ATP concentrations ($pMg^{2+}$-ATP = 5.5). Therefore, we examined the ATP concentration dependence of mitochondrial motor activity. The minimum concentration of ATP required for microfilament sliding is 1 μM. At 1 - 10 μM ATP, the velocity F-actin sliding is directly proportional to ATP concentration. Thus, the ATP concentration dependence of the mitochondrial motor is similar to that of other myosins tested. In addition, microfilament sliding requires ATP hydrolysis: 10 and 100 μM AMP-PNP will not support microfilament sliding. Finally, the maximum velocity of microfilament sliding is $3.6 \times 10^{-2}$ μm/sec. Although this velocity is lower than the rates of some myosin-driven movements, it is similar to the velocity of mitochondrial movement in living yeast ($5.4 - 5.8 \times 10^{-2}$ μm/sec).

J3-111 UNDERSTANDING THE ROLE OF CALMODULIN AND THE UNCONVENTIONAL MYOSIN, MYO2P, IN BUD GROWTH, Richard C. Stevens and Trisha N. Davis, Dept. of Biochemistry, SJ-70, University of Washington, Seattle, WA 98195

In *S. cerevisiae*, the intracellular $Ca^{2+}$-binding protein calmodulin plays essential roles in cytokinesis, chromosome segregation and budding. We have previously shown that $Ca^{2+}$ binding by calmodulin is not required for growth and that the essential target for calmodulin in budding is the unconventional myosin, Myo2p (Geiser *et al.* (1991) Cell 65:949-959; Brockerhoff *et al.* (1994) J. Cell Biol. 124:315-323). The interaction between calmodulin and Myo2p does not require $Ca^{2+}$. *MYO2* was first identified as a gene required for polarized growth and is now known to be a member of the Myosin V class of unconventional myosins (Johnston *et al.* (1991) J. Cell Biol. 113:539-551). Myosin V proteins have a large myosin-like head domain with six $Ca^{2+}$-independent calmodulin binding sites or "IQ" sites. In the tail is a short region of heptad repeats thought to be involved in dimerization. The chicken homolog, p190, has been shown to form dimers (Cheney *et al.* (1993) Cell 75:13-23). Most of the tail is poorly characterized but believed to play a role in vesicle transport (Cheney and Mooseker (1992) Curr. Opin. Cell Biol. 4:27-35).

To better understand the essential interaction between calmodulin and Myo2p, I created internal deletions in the *MYO2* gene. I deleted "IQ" sites in order to further define the region required for calmodulin function. I used oligo-directed mutagenesis to remove the C-terminal four of the six "IQ" sites. This mutant is nonfunctional most likely due to problems with protein stability. I also made nested deletions in the "IQ" site domain using exonuclease III digestion of plasmids carrying the *MYO2* gene to further define the required elements. Removing the first three or the last three "IQ" sites does not impair function. Additional internal deletions in *MYO2* were made in order to understand the role of the tail of Myo2p in polarized growth. Removal of a small region near the C-terminus of Myo2p results in a very poorly growing yeast strain. Larger deletions near the C-terminus of Myo2p are lethal. Further mutations in the tail domain may help determine the role of Myo2p in budding. Interestingly, removal of the heptad repeat region in the tail of Myo2p does not hinder function. The heptad repeats are believed to form a coiled-coil and allow Myo2p to dimerize. (RS supported by PHS NRSA T32 GM07270, NIGMS. Work supported by NIGMS RO1 GM40506)

J3-112 IDENTIFICATION OF A CYTOSOLIC FACTOR REQUIRED FOR GOLGI LOCALIZATION AT THE CENTROSOME, Nirit Ulitzur, Martine Humbert & Suzanne Pfeffer, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305-5307

The localization of the Golgi complex depends upon the integrity of the microtubule apparatus. At interphase, the Golgi has a restricted pericentriolar localization. During mitosis, it fragments into small vesicles that are dispersed throughout the cytoplasm until telo-phase, when they again coalesce near the centrosome. These observations have suggested that the Golgi complex utilizes a dynein-like motor to mediate its transport from the cell periphery towards the minus ends of microtubules, located at the centrosome. We have shown that isolated Golgi complexes can enter the cyto-plasm of gently broken, semi-intact CHO cells, and accumulate in the vicinity of the centrosome. This process, which we term "Golgi capture," requires ATP hydrolysis, intact microtubules, and occurs maximally at physiological temperature in the presence of added cytosolic proteins. In addition, the microtubule-based motor, cytoplasmic dynein, is absolutely required[1]. Since little is known about the molecular interactions responsible for the cell cycle-dependent association of dynein with membrane-bound organelles, we are trying to identify cytosolic proteins that are required for Golgi capture. Golgi complexes, stripped of peripheral proteins by treatment with 1M KCl, fail to be captured. We are fractionating cytosolic proteins that restore the ability of KCl-stripped Golgi complexes to undergo capture. We have identified and highly purified a potentially novel factor that appears to be essential for Golgi accumulation at the centrosome in vitro. The factor appears to be distinct from previously identified subunits of cytoplasmic dynein and the dynactin complex. Experiments are in progress to determine the role of this factor in Golgi complex motility and/or anchoring at the centrosome.

1. Corthésy-Theulaz, I., Pauloin, A. and Pfeffer, S.R. (1992). Cytoplasmic dynein participates in the centrosomal localization of the Golgi complex. J. Cell Biol. 118, 1333-1345.

*Macromolecular Structure; Mechanisms of Force Production*

J3-113 Analysis of *Drosophila* Muscle Myosin Isoform Function by Chimeric Exon Substitution. Rodney A. Brundage, *Joseph E. Smith, *H. Lee Sweeney, and Charles P. Emerson, Jr. Department of Cell and Developmental Biology, and *The Pennsylvania Muscle Institute, University of Pennsylvania, Philadelphia, PA 19104

The *Drosophila* muscle myosin heavy chain (MYHC) gene provides a unique opportunity to investigate the structural aspects of myosin function. A single 19 exon MYHC gene provides for the expression of as many as 480 distinct protein isoforms via regulated alternative RNA splicing. These alternative exons encode biochemically defined domains of the myosin protein and are expressed in specific muscle types during the development of the organism. Analysis of the recently solved chicken S1 subfragment crystal structure indicates that the alternatively expressed exons contribute to important structural domains of the protein. As a first step towards understanding how alternative exon usage influences MYHC function, we have constructed three synthetic *Drosophila* MYHC cDNA constructs. One construct contains the wild type exons which are expressed in the indirect flight muscle (IFM) of the adult fly. The other two constructs are synthetic chimeras which contain substitutions of specific alternative exons, derived from (2) partial, late pupal cDNAs, into the wild type, IFM specific MYHC. We are currently exploiting two methods to express these synthetic MYHCs and to characterize their biochemical properties. Employing a *Baculovirus* system, we have expressed full length *Drosophila* MYHC in insect cells. When Drosophila MYHC is co-expressed with smooth muscle myosin light chains, soluble complexes form. We will biochemically characterize these hybrid complexes and determine their relative ATPase activities, actin affinities, and their *in vitro* motility properties. Also, we have generated chimeras based on vertebrate smooth muscle MYHC which contain *Drosophila* alternative exons. For *in vivo* expression and characterization, constructs have been placed under the control of an IFM specific promoter for the generation of transgenic *Drosophila*. Extant mutants in chromosomal MYHC will allow the expression of these transgene constructs in a null MYHC background. Measurement of the mechanical properties and microscopic structural analyses of transgenic and wild type IFM fibers will be undertaken to define the role of specific alternative exon domains in MYHC isoform function and structure in intact muscle.

J3-114 STRUCTURAL AND FUNCTIONAL STUDIES OF THE NECK DOMAIN OF CHICKEN BRAIN MYOSIN-V. E.M.Espreafico*, A.A.C.Nascimento, F.Mani, M.C.R.Costa, S.L.Silva, S.B.F.Tauhata, L.C.Alcântara and R.E.Larson. Departments of Morphology* and Biochemistry, University of São Paulo, Ribeirão Preto, 14049-900, Brazil.

Myosin-V (M-V) is an unconventional, dimeric, molecular motor consisting of three major structural domains: the motor head domain, a neck region containing multiple calmodulin-binding sites (6 IQ motifs), and a distinct tail with coiled-coil and globular regions. Biochemical studies with highly purified brain M-V suggest that $Ca^{2+}$ tightly regulates the actin-activated ATPase activity via its binding to the calmodulin-IQ sequences in the neck domain. Calpain, a $Ca^{2+}$-dependent protease, cleaves M-V between the head and neck domains and, thus, the actin-activated ATPase activity loses the $Ca^{2+}$ control. The whole neck *domain*, expressed in bacteria, shows $Ca^{2+}$-independent calmodulin-binding as does the native protein. However, a synthesized peptide, corresponding to the fifth IQ motif alone, showed $Ca^{2+}$-dependent calmodulin-binding, suggesting either that not all IQ motifs bind light chains in the absence of $Ca^{2+}$ or that more complex interactions between light chains and multiple IQ motifs are necessary for the $Ca^{2+}$-independent binding. Molecular modeling studies suggest that the neck domain forms a long α-helix with clusters of basic and hydrophobic residues oriented on opposite sides of the helix. Along the longitudinal axis, the even-numbered IQ motifs are rotated by 120° in a clock-wise direction in relation to the immediately preceding odd-numbered motif. These odd-even pairs of IQ motifs, however, are rotated clock-wise by 60° in relation to the preceding pair. This suggests that there is structural and, maybe, functional significance for a pair of IQ motifs. M-V has been localized to an unidentified particulate fraction in synaptosomes, as well as being present in a purified synaptic vesicle preparation, suggesting a role for this unconventional myosin in $Ca^{2+}$-regulated synaptic processes. **Financial support: FAPESP, CNPq and CAPES.**

J3-115 NEW ANTITUBULIN ANTIBODIES PREVENT AXONEMAL MOTILITY IN FLAGELLA

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Among monoclonal antibodies (mAb) erected against sea urchin axonemal proteins and selected for their capacity to inhibit at low concentrations motility of ATP reactivated flagella, we have obtained 3 mAb against tubulin (B3,C9,D66). The corresponding epitopes have been conserved across long term evolution since these mAb inhibit axonemal motility in 3 species of sea urchins, in human spermatozoa and in a dinoflagellate. The inhibition patterns induced by these 3 mAb, observed on the various models, differ from each other suggesting that they are targetted to different epitopes: B3 (0.1μg/ml) first reduces the beat amplitude, whereas C9 (0.1μg/ml) first reduces the beat frequency (BF) not the wave form. D66 (5μg/ml) mainly blocks the distal part in a curled shape, with a slight effect on BF. Other well characterized mAb, DM1A,YL1/2,Tub 1A2,DM1B,Tub 2.1 (up to 200μg/ml) saturate the axoneme without effect on motility. GT 335 (anti poly-Glu Tub) blocks motility at 0.1μg/ml. Affinity purified polyclonal Ab L141 and L142 (anti αTyr Tub) bind but have no effect (up to 300μg/ml), while L3 (anti αGlu Tub) is a good inhibitor (5μg/ml), and L7 (anti αΔ2 Tub) is a strong inhibitor (1μg/ml). These results indicate an important involvement in axonemal movement of a small region of α-tubulin c-terminus which lies close to Glu 445 as indicated by comparing the activities of anti poly-Glu, anti Δ2, anti Glu and anti Tyr mAb.

J3-116 ACTIVATION OF PARAMECIUM 22S AXONEMAL DYNEIN VIA PHOSPHORYLATION OF A 29KD LIGHT CHAIN, Toshikazu Hamasaki, Kurt Barkalow and Peter Satir. Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY 10461

We have demonstrated that a 29kD polypeptide (p29) which copurifies with *Paramecium* 22S axonemal dynein (outer arm dynein) activates *in vitro* microtubule translocation activity via its cAMP-dependent, $Ca^{2+}$-sensitive phosphorylation (PNAS 1991, 88:7918). Dynein with a phosphorylated p29 (pp29) caused 40-50% faster gliding of microtubules compared to its matched controls. Partially purified p29 is able to rebind to *Paramecium* or *Tetrahymena* 22S dynein (J. Cell Biol. 1994, 126:727). *Paramecium* 22S dynein reconstituted with pp29 increases the microtubule velocity by 31% compared to reconstitution with unphosphorylated p29. Further, isolated cAMP-dependent protein kinase (PKA) from *Paramecium* (from Walczak and Nelson, U. Wis.) phosphorylates p29 as well as dynein H-chain(s) *in vitro* and increases the gliding velocity (by 50%) when 22S dynein is incubated with PKA. Calculations suggest that only a few percent of p29 is phosphorylated even after *in vitro* phosphorylation by PKA; in the reconstituted dynein, about 1% of molecules contain pp29.

How could such a small number of phosphorylated, activated dyneins increase microtubule translocation velocity? p29 might act catalytically on some other dynein component or the small number of activated dyneins would have to change their mechanochemical properties; an individual molecule might cycle faster or produce a bigger translocation step, so that the few activated dyneins pushing the microtubule at any short time determine the overall gliding velocity. To test the latter hypothesis, we are monitoring local changes in sliding velocity in *in vitro* assays. We have observed that indeed some gliding microtubules are faster in a particular spot in a field. Such an activated dynein might work as a pacemaker to set gliding velocity of a microtubule *in vitro*.

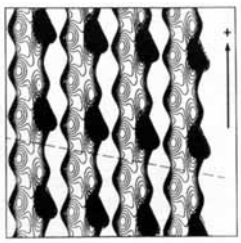
J3-117 INTERDOUBLET SLIDING IN BULL SPERM: AN ASSESSMENT OF SLIDING RATES AND THE EFFECTS OF INHIBITORS. Charles B. Lindemann and Zachary C. Bird, Dept. of Biol. Sci., Oakland Univeristy, Rochester, MI 48309. Bull sperm, suspended in demembranating medium containing 0.1% Triton X-100, 1 mM DTT, 1 mM $MgCl_2$, 0.5 mM EGTA, 0.13 M sucrose, 0.024 M $K^+$ glutamate and 0.02 M Tris-HCl (pH 7.8) would reactivate in response to 1 mM Mg-ATP. If the suspension is frozen at -20° for 48-72 hours without Mg-ATP, then thawed, the sperm disintegrate by microtubule sliding when Mg-ATP is added. Upon analysis of axonemal element extrusion in these disintegrating axonemes, we found an average interdoublet sliding rate of 7.32 ± 2.0 μm/s upon rapid perfusion of the cells with 1 mM ATP in a slide chamber. Using strobed video images of live cells, and Triton X-100 reactivated cells, we have determined the interdoublet sliding velocities in bull sperm axonemes during normal motility. The maximal rates were 6.0 μm/s for intact cells and 5.8 μm/sec for reactivated models. We examined the impact of agents that effect motility on the rate of interdoublet sliding in disintegrating axonemes. The state of activation and deactivation, as controlled by cAMP-kinase A , did not greatly impact sliding rate. Even sperm samples showing little motility in the presence of 1 mM MgATP none-the-less exhibited sliding rates near the control value (6.24 ± 2.03 μm/sec). High concentrations (8 μM) of $NaVO_3$ did noticeably slow sliding (4.0 ± 9 μm/sec), but 2 μM $VO_3^-$ blocked all motility, with only a small effect on the free sliding rate (5.76 ± 1.3 μm/s). 0.66 mM $NiSO_4$ blocked free swimming motility in sperm models, and reduced interdoublet sliding to 5.37 ± 1.1 μm/sec. Lithium abolished motility at <5 mM, yet up to 60 mM lithium allowed normal sliding rates (7.6 ± 2.0 μm/s). These results indicate that there is no simple relation between interdoublet sliding rates and the capacity for motility. These observations may be reconciled if near maximal sliding rates are produced by few functional dynein arms, while coordinated beating may depend on the summation of motive force. Supported by N.S.F. grant MCB-9220910.

J3-118 ROLES FOR MICROTUBULES AND KINESIN IN MEMBRANE TRAFFIC BETWEEN THE ER AND GOLGI COMPLEX, Jennifer Lippincott-Schwartz, Nelson Cole, Alex Marotta, Patricia Conrad* and George Bloom*, Cell Biology and Metabolism Branch, NICHD, NIH, Bethesda, MD and Dept Cell Biology and Neuroscience*, Univ. Texas SW Med Ctr, Dallas, TX

The roles of microtubules and the microtubule plus end-directed motor kinesin in membrane transport between the ER and Golgi were investigated using a monoclonal antibody to kinesin, and membrane markers for the early secretory pathway. Kinesin was localized at the light and electron microscope levels predominantly to COP-coated transport intermediates moving from the ER to the Golgi. These kinesin-enriched intermediates required intact microtubues to move from multiple peripheral sites near the ER to the centrally located Golgi, and accumulated newly synthesized plasma membrane proteins when cells were treated with the microtubule disrupting agent, nocodazole. Kinesin redistributed onto Golgi, Golgi-to-ER intermediates or ER when membrane traffic between the ER and Golgi was perturbed by microtubule disruption, temperature reduction or brefeldin A-treatment. This suggested that kinesin associates with membranes that constitutively cycle between the ER and Golgi complex. Microinjected kinesin antibody inhibited Golgi-to-ER but not ER-to-Golgi membrane transport, suggesting kinesin powers the microtubule plus end-directed recycling of membrane to the ER, and remains inactive on pre-Golgi intermediates that move along microtubules in the opposite direction toward the Golgi complex.

J3-119 STRUCTURE OF THE KINESIN-MICROTUBULE COMPLEX E. Mandelkow, Y.-H. Song, A. Marx, M. Thormaehlen, E.-M. Mandelkow, Max-Planck-Unit for Struct. Mol. Biol., Hamburg, Germany.

The structure and interactions between the motor protein kinesin and microtubules has been studied by structural and biochemical methods. Starting from the clone of squid kinesin (Kosik et al., JCB 254:3278, 1992) we made several constructs of the head domain and expressed them in E. coli. This protein was then incubated with microtubules repolymerized from porcine brain tubulin and analyzed by electron microscopy, optical diffraction, and computer image processing (Song & Mandelkow, PNAS 90:1671, 1993). The kinesin binds tightly to microtubules in the absence of ATP or in the presence of AMP-PNP. Chemical cross-linking with several cross-linkers shows that the kinesin head preferentially interacts with beta-tubulin but not alpha-tubulin. Thus the binding stoichioimetry is one kinesin head per tubulin dimer. The spacing of kinesin along microtubules is periodic with a spacing of 8 nm, consistent with one kinesin head for every tubulin dimer. Kinesin head molecules are aligned along the helical lines of the 3-start helix of microtubules, consistent with the B surface lattice. This lattice is found in flagellar A, B, or C microtubules, and in brain microtubules. The kinesin decoration reveals the dimer polarity (the plus end of MT has a crown of α tubulin). Finally, the reconstructions of decorated tubulin sheets show the shape of the kinesin head and its position relative to the tubulin subunits (see Figure).



J3-120 SITE DIRECTED MUTAGENESIS OF A HYDROPHOBIC PLUG IN YEAST ACTIN THAT MAY BE IMPORTANT IN ACTIN HELIX STABILIZATION, Peter A. Rubenstein and Bing Kuang, Department of Biochemistry, University of Iowa College of Medicine, Iowa City, IA 52242

The Holmes model of the F-actin helix proposes that a hydrophobic plug (residues 265-268) at the end of a loop between subdomains 3 and 4 inserts into a hydrophobic pocket on the opposing strand resulting in cross strand stabilization of the actin helix. We have mutated residues in this plug in yeast actin to decrease the extent of its hydrophobicity and have assessed the effects of the mutations in a yeast expression system. $V_{265}D$ and conversion of $V_{265}$ and $L_{266}$ to glycines (GG) produce actins which by themselves are not compatible with yeast viability. In the presence of wild-type actins, these mutations allow near normal growth at 30°, but the cells exhibit a severly decreased ability to grow in hyperosmolar medium Growth of the GG mutant is also severely retarded at cold temperatures or on glycerol as a sole carbon source. The single mutations, $V_{265}G$ and $L_{266}G$, support yeast life by themselves with little or no difference in growth rates at temperatures between 10° and 37° although $V_{265}G$ show altered budding patterns. The $V_{265}G$ actin is more susceptible to subtilisin digestion in subdomain 2 where the actin monomer on one strand is believed to interact with the plug of a monomer on the opposing strand suggesting increased accessibility of the protease to subdomain as a result of a plug mutation. However, polymerization of the $V_{265}G$ actin is not cold-sensitive in vitro contrary to what we previously observed with the $L_{266}D$ mutation. These results suggest that if the Holmes model is correct, a cumulative energy threshold must be crossed before weakening of the plug-pocket interaction is sufficient to cause substantial helix disruption.

J3-121 MOTILITY ASSAYS USING MYOSIN ATTACHED TO SURFACES VIA MONOCLONAL ANTIBODIES, Donald A. Winkelmann[1], Laurent Bourdieu[2], Fumi Kinose[1] and Albert Libchaber[2,3]. [1]Department of Pathology, Robert Wood Johnson Medical School, Piscataway, NJ, [2]Department of Physics, Rockefeller University, New York, NY and [3]NEC Research Institute, Princeton, NJ.

The development of in vitro motility assays has contributed enormously to the analysis of the minimal requirements for myosin-catalyzed movement of actin filaments. A wide range of actin filament velocities have been reported that are dependent on not only the myosin isoform but also the details of the experimental protocol. We have analyzed the dependence of actin filament movement on the mode of myosin attachment to surfaces. Monoclonal antibodies (mAbs) that bind to three distinct sites were used to tether myosin to nitrocellulose coated glass. One antibody reacts between residues 17-51 of the regulatory light chains (LC2) which are located at the myosin head-rod junction (anti-$LC2_{17\text{-}51}$), and tethers the myosin heads close to the surface. The other two mAbs react with sites in the rod domain, one in the S2 region near the S2-LMM hinge (anti-$S2_{10F12}$), and the other at the C-terminus of the myosin rod (anti-$LMM_{5C3}$). These mAbs provided increasing flexibility in the mode of myosin monomer attachment. The sliding movement of fluorescently labeled actin filaments was analyzed by video microscopy. Each mAb produced stable myosin-coated surfaces that supported uniform movement of actin over the course of several hours. Attachment of myosin through the anti-$S2_{10F12}$ and anti-$LMM_{5C3}$ mAbs yielded maximum velocities of 9-10 μm/sec at 30°C, while attachment through anti-$LC2_{17\text{-}51}$ produced velocities of 4-5 μm/sec. Thus, the specific mode of attachment can influence the characteristic velocity of actin filaments. Maximum sliding velocity was achieved over a broad range of myosin concentrations (5-500 μg/ml). Each mAb showed a characteristic minimum myosin concentration (0.25-1.5 μg/ml) below which sliding movement was no longer supported and an exponential dependence of actin filament velocity on myosin concentration below $V_{max}$. This method provides a means of controlling the mode of attachment and density of myosin on the surface. The high affinity of the antibody-antigen interaction results in a significant decrease in the total myosin needed to produce surfaces promoting rapid sliding movement of actin filaments. Furthermore, the specificity of the antigen-antibody interaction facilitates the selective isolation of myosin isoforms from complex mixtures. These features are being exploited for rapid isolation and analysis of recombinant myosin fragments expressed in vitro.

*Mitosis; Cytokinesis*

J3-122 CONCENTRATION OF ACIDIC CALPONIN IN THE CLEAVAGE FURROW DURING CYTOKINESIS,
Dianne Applegate, *Sven Beushausen, and Yufeng Tang, Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029, and *Laboratory of Neurobiology, NINDS, NIH, Bethesda, MD 20892

We have recently identified a novel acidic isoform of calponin (Mr 36-kDa) that is expressed in a wide range of nonmuscle tissues and cell types (Applegate et al.(1994) J. Biol. Chem. 269: 10683-10690). We have shown that like smooth muscle calponin, acidic calponin (AcCaP) synthesized in a bacterial expression system binds F-actin and inhibits actomyosin MgATPase in vitro. In the present work we have analyzed the distribution of AcCaP in dividing 3T3 fibroblasts. For this purpose, we obtained rabbit polyclonal antibodies directed against a synthetic peptide that is specific to the unique carboxyl terminal domain of acidic calponin. Immunoblot analysis of lysates from diverse cultured cell types, including NIH 3T3 fibroblasts, using the affinity purified antibody, designated pAb-AcCaP-C, revealed a single band migrating at 36-kDa. To examine the intracellular distribution of AcCaP at various stages of the cell cycle, 3T3 fibroblasts at various times following their release from metaphase arrest were stained with pAb-AcCaP-C. In some cases cells were double labeled with either rhodamine phalloidin or with a mouse monoclonal antibody specific for acetylated alpha tubulin. Examination of indirect immunofluorescence by confocal laser scanning fluorescence microscopy revealed that during metaphase, AcCaP appeared to co-localize with F-actin in the cortical meshwork. At the onset of furrowing, AcCaP appeared to accumulate at the cleavage furrow and continued to be concentrated in the furrow throughout telophase. At late stages of cytokinesis, AcCaP remained enriched at the midbody. When daughter cells began to spread, AcCaP appeared to co-localize with F-actin-containing structures. In interphase cells, AcCaP co-localized with F-actin in both membrane ruffles and along stress fibers. These results suggest that AcCaP plays a specific role in cytokinesis. By analogy with the role attributed to smooth muscle calponin in modulating contraction, we hypothesize that AcCaP participates in regulating actomyosin interactions.

J3-123 A PUTATIVE CENTRACTIN HOMOLOGUE, *ACT3*, IS ESSENTIAL FOR PROPER ORIENTATION OF THE MITOTIC SPINDLE IN *S. CEREVISIAE*,
Sean W. Clark and David I. Meyer, Department of Biological Chemistry and the Molecular Biology Institute, University of California, Los Angeles, California 90024. Using a PCR-based approach, we have sought out a vertebrate centractin homologue in *S. cerevisiae*. We refer to this gene as *ACT3*. *ACT3* lies on chromosome VIII and is convergently transcribed with respect to *FUR1*. By comparison with all known actin-related proteins, *ACT3* is most related to human and canine α-centractin. We sought additional data to substantiate *ACT3* as a functional centractin homologue. As vertebrate centractins are the major component of a regulator of cytoplasmic dynein, we compared the phenotype of yeast harboring mutations in *DYN1* and *ACT3*. Monitoring both nuclear migration and spindle orientation, we found an equivalent defect, which is exacerbated by growth at low temperatures. However, the growth rate of strains lacking Act3p is not significantly different from wildtype, suggesting that the *ACT3* defect can be compensated for within the normal time required for cell division. Double mutants lacking both Act3p and Dyn1p or Act3p and Jnm1p are viable and have no gross growth defects. This further suggests *ACT3* acts in the same pathway as *DYN1* and *JNM1* and is thus a candidate for a functional homologue of vertebrate centractins.

J3-124 *IN VIVO* FORMATION OF SINGLE-HEADED MYOSIN II ACTS AS A DOMINANT-NEGATIVE MUTATION IN *DICTYOSTELIUM*.
Arturo De Lozanne, C. Geoffrey Burns, Denis A. Larochelle, Harold Erickson and Mary Reedy, Department of Cell Biology, Duke University Medical Center, Durham, NC 27710.

Conventional myosin II is an essential protein for cytokinesis, capping of cell surface receptors and development of *Dictyostelium* cells. Myosin II also plays an important role in the polarization and movement of cells. All conventional myosins are two-headed molecules but the significance of this structure is not understood since single-headed myosin II can produce movement and force *in vitro*. We found that expression of the tail portion of myosin II in *Dictyostelium* led to the formation of single-headed myosin II *in vivo*. The resultant cells contain an approximately equal ratio of double- and single-headed myosin II molecules. Surprisingly, these cells were completely blocked in cytokinesis and capping of concanavalin A receptors although development into fruiting bodies was not impaired. Despite these physiological impairments the biochemical properties of myosin II in these cells were similar to wild-type. We found that phosphorylation of the regulatory myosin light chain in response to concanavalin A capping was normal in these mutants. In addition, filament formation by the single-headed molecules was normal. Therefore, our results suggest that double-headed myosin II may be required for proper force production *in vivo*. Clearly, single-headed myosin II cannot function properly *in vivo* and, therefore, it acts as a dominant-negative mutation for myosin II function.

J3-125 *CHLAMYDOMNAS REINHARDTII* STRAINS DEFECTIVE IN CYTOKINESIS.
Linda L. Ehler and Susan K. Dutcher. Dept. of MCD Biology, University of Colorado, Boulder CO 80309-0347. During mitosis in wild-type *Chlamydomonas* cells the position of the cleavage furrow is correlated both with the center of the mitotic spindle and with distal regions of the four-membered microtubule rootlets. We have proposed that the position of the microtubule rootlets is important in determining the position of the cleavage furrow and we are interested in identifying components involved in both the proper orientation and assembly of cleavage furrows. We have found mutant strains that position cleavage furrows aberrantly and show incomplete or no cytokinesis ($Cyt^-$). Two $Cyt^-$ strains, AT2-17 and *cyt2*, were generated by nonhomologous integration of a plasmid containing a wild-type copy of *ARG7*. AT2-17 maps to the mating-type locus (108:0:0) and is likely to be a new allele at the *CYT1* locus, originally isolated by Warr (*J. Gen. Microbiol.*, 52: 243-251, 1968). The *cyt2* locus is unlinked to the *CYT1* locus. The primary defect of strains AT2-17 and *cyt2* appears to be in the initiation and assembly of the cleavage furrow. Both strains are being characterized by immunocytochemistry to determine both the relationship between cleavage furrows and microtubule rootlets and the location of cleavage furrow components such as actin. Actin localizes to the parental four-membered microtubule rootlets during cytokinesis in wild-type cells. Preliminary characterization of the *cyt2* strain indicates that actin may be localized to additional microtubule rootlets during cytokinesis. This aberrant localization may lead to the abnormal initiation and completion of cytokinesis. The $Arg^+$ phenotype of AT2-17 and *cyt2* and vector sequence cosegregates with the $Cyt^-$ phenotype by genetic analysis (108:0:0 and 61:0:0, respectively) and by Southern analysis (5:0:0 and 3:0:0, respectively), indicating that the mutant alleles are tagged by the inserted DNA. Anchored RAPD PCR using a 26 bp primer in the vector sequence and random 10 bp oligonucleotides are being used to identify RFLPs that cosegregate with the $Cyt^-$ phenotype. RFLP bands will be used to probe a genomic λ library.

J3-126 A XENOPUS NONMUSCLE MYOSIN HEAVY CHAIN ISOFORM IS PHOSPHORYLATED BY CYCLIN-p34$^{cdc2}$ KINASE DURING MEIOSIS, Christine A. Kelley, Fromer Oberman*, Joel K. Yisraeli* and Robert S. Adelstein, Laboratory of Molecular Cardiology, National Heart, Lung and Blood Institute, Bethesda, MD 20892 and *Department of Anatomy and Embryology, The Hebrew University-Hadassah Medical School, Jerusalem 91010, Israel

There are two vertebrate nonmuscle myosin heavy chain (MHC) genes that encode two separate isoforms of the heavy chain, MHC-A and MHC-B. Recent work has identified additional, alternatively spliced, isoforms of MHC-B cDNA with inserted sequences of 30 nucleotides (chicken and human) or 48 nucleotides (Xenopus) at a site corresponding to the ATP binding region in the MHC protein (Takahashi et al., J. Biol. Chem. 267: 17864, 1992 and Bhatia-Dey et al., Proc. Natl. Acad. Sci. USA 90: 2856, 1993). The deduced amino acid sequence of these inserts contains a consensus sequence for phosphorylation by cyclin-p34$^{cdc2}$ kinase. In cultured Xenopus XTC cells, we have identified two inserted MHC-B isoforms and a non-inserted MHC-A isoform by immunoblotting of cell extracts. When myosin was immunoprecipitated from XTC cells and phosphorylated *in vitro* with cdc2 kinase, the kinase catalyzed the phosphorylation of both inserted MHC-B isoforms, but not MHC-A. Isoelectric focusing of tryptic peptides generated from MHC-B phosphorylated with cdc2 kinase revealed one major phosphopeptide which was purified by reverse-phase HPLC and sequenced. The phosphorylated residue was Ser-214, the cdc2 kinase consensus site within the insert near the ATP binding region. The same site was phosphorylated in intact XTC cells during log phase of growth and in cell-free lysates of Xenopus eggs stabilized in second meiotic metaphase, but not interphase. Moreover, Ser-214 phosphorylation was detected during maturation of Xenopus oocytes when the cdc2 kinase-containing maturation-promoting factor was activated, but not in G2 interphase-arrested oocytes. These results demonstrate that MHC-B phosphorylation is tightly regulated by cdc2 kinase during meiotic cell cycles. Furthermore, MHC-A and MHC-B isoforms are differentially phosphorylated at these stages suggesting that they may serve different functions in these cells.

J3-127 Jnm1p MAY BE PART OF A DYNACTIN-LIKE COMPLEX IN YEAST REQUIRED FOR NUCLEAR MIGRATION AND SPINDLE ORIENTATION DURING THE MITOTIC CELL CYCLE, John N. McMillan, Guglielmo Venturi and Kelly Tatchell, Dept. of Microbiology, Box 7615, North Carolina State University, Raleigh, N.C. 27895

*jnm1* null mutants have a temperature-dependent defect in nuclear migration that is strikingly similar to that for mutants in the dynein heavy chain gene (*DHC1/DYN1)* or in *ACT5* which encodes an actin-related protein similar to that found in the dynactin/glued complex. In these mutants at 30°C, a significant proportion of the mitotic spindles is not properly located at the neck between the mother cell and bud. This defect is more severe at low temperature. At 11°C, cells accumulate with large buds, most with two DAPI staining bodies in the mother cell, however, the cells continue to grow exponentially, albeit at a slower rate. At low temperature the large budded cells of *jnm1* also exhibit extremely long astral microtubules that often wind around the cell periphery. No significant defect has been found in two other microtubule dependent processes, chromosome segregation and nuclear migration during conjugation. No synthetic phenotype is observed in a *jnm1/dhc1* double mutant, indicating that Jnm1p and Dhc1p may be involved in the same cellular process. A *JNM1:lacZ* gene fusion is able to complement the cold sensitivity and microtubule phenotype of a *jnm1* deletion strain. This hybrid protein localizes to a single intensely staining spot, often at a spindle pole body (SPB), and is visible during all stages of the mitotic cell cycle. In cells with two SPBs the Jnm1p fusion protein most often localizes to the SPB in or closest to the bud. The Jnm1p:ß-gal spot does not localize to the SPB in a *dhc1* mutant and completely disappears in an *act5* null mutant. Together, these results suggest that Jnm1p is part of a complex which requires Act5p for its integrity. Both proteins could be components of a dynactin-related complex in yeast that is required for dynein motor activity.

J3-128 PKL 1 : A KINESIN-LIKE PROTEIN FROM *SCHIZOSACCHAROMYCES POMBE.*, Alison L. Pidoux and W.Zacheus Cande, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

Kinesin-like microtubule motor proteins are thought to contribute to motility in the spindle and in the cytoplasm. PKL1 is a 96kD kinesin-related protein which was isolated from an *S.pombe* cDNA library using peptide antibodies (anti-LAGSE and anti-HIPYR) specific for the motor domains of kinesin-related proteins. The putative motor domain of PKL1 is at the C-terminus and the greatest degree of homology is with the KAR3/NCD subfamily. PKL1 is a non-essential gene, but disruption causes an increased sensitivity to microtubule-depolymerising drugs. PKL1-disrupted strains are able to mate and sporulate, so unlike KAR3, PKL1 is not required for karyogamy. Disruption of PKL1 partially suppresses the temperature sensitivity associated with a mutation in CUT7, another kinesin-related protein. This observation is similar to those made in *S. cerevisae* and *Aspergillus* and may support the idea that maintenance of spindle structure requires balancing forces. In permeabilised, fixed cells the PKL1 protein localises to the mitotic spindle and when overexpressed it is readily detectable on cytoplasmic microtubules as well. PKL1 behaves as a motor protein in that bacterially expressed fusion proteins bind microtubules, show microtubule-stimulated ATPase activity and bundle microtubules *in vitro* in an ATP-dependent manner. Genetic screens are currently underway to identify genes which are synthetically lethal with PKL1. We believe that PKL1 may function as a motor in the mitotic spindle (eg anaphase A, spindle integrity) and/or cytoplasm (eg nuclear movement) though its precise role is not yet clear.

J3-129 IN VIVO IMAGING OF SPINDLE POLE BODIES IN SACCHAROMYCES CEREVISIAE, Bruce J. Schnapp*, Pamela A. Silver#, and Jason A. Kahana#, *Dept. Cell Biology, #Dept. of Biological Chemistry & Mol. Pharmacology, Harvard Medical School, and #Dana-Farber Cancer Institute, Boston, MA 02115.

The spindle pole body (SPB) of Saccharomyces cerevisiae has functional similarities to the mammalian centrosome. Like the centrosome of higher eukaryotic cells, the SPB serves as a microtubule-organizing center (MTOC). During the cell cycle, the SPBs undergo a characteristic sequence of behaviors including duplication, spindle formation, and migration. These activities of the SPB can be directly correlated to the progress of the cell cycle. Investigations of mitosis and SPB behavior in yeast have been limited by the lack of a method to visualize SPBs in living cells. Herein, we report fusing green-fluorescent protein to a yeast SPB-associated protein, NUF2p (Osborne et al. 1994, J.Cell Biol. 125:853), enabling *in vivo* observations by time-lapse digital microscopy of the rate, direction, and pattern of SPB movement during the cell cycle. During the period between cytokinesis and bud emergence, the SPB migrates to the bud site and duplication of the SPB occurs. During bud growth, the pair of SPBs separate by ~1 µm defining a short spindle. When the bud grows to 60-70% the size of the mother, the short spindle becomes oriented along the mother-bud axis. The spindle pole bodies then separate as the spindle elongates at a rate of 0.1 - 0.3 µm/second, although the position of the daughter's SPB remains relatively constant. Once the spindle has elongated to approximately 60% the length of the mother, it is translocated **as a unit** through the bud neck. Elongation then continues for a period preceeding cytokinesis (and nuclear division). The ability to track the dynamics of SPB's in living cells creates unique experimental opportunities for investigations of microtubule organizing centers and their roles in mitosis.

*Late Abstracts*

**Actin-based motility of squid axoplasmic organelles and the identification of a putative motor.** E. L. Bearer, J. DeGiorgis, and T.S. Reese. Brown University, Providence R.I., NINDS, Bethesda, MD, and Marine Biological Labs, Woods Hole, MA, 02543

Using a novel video assay which simultaneously images both the organelles and bundles of actin by video microscopy, we showed that axoplasmic organelles move on exogenous actin filaments. A pan-myosin antibody recognized a single 235 kDa band in Western blots of isolated axoplasmic organelles. By immunogold cytochemistry, this myosin was found to be present on the surface of a subset of organelles (Bearer et al, 1993). We now show that KI-washed, sucrose-density gradient purified organelles bind actin filaments in the absence of ATP, have a high actin-dependent ATPase activity, and move on exogenous actin filament bundles at rates intermediate to that of kinesin, with an instantaneous velocity of ≈1.6 μm/sec.

Purification of the 235 kDa putative organelle myosin from axoplasm reveals that it is a myosin: it cross-reacts with a pan-myosin antibody in Western blots; it has a 40-fold higher specific activity for actin-dependent Mg-ATPase than axoplasm or KI supernatant; and it causes actin to hypersediment, a characteristic of two-headed myosins. However, it appears to be an unusual myosin since by electronmicroscopy of rotary-shadowed, glycerol-sprayed purified protein, the globular head is larger than that of myosin II, and it has a shorter, thicker tail. It fails to form bipolar filaments under the conditions which induce myosin II to form filaments. In Western blots, antibodies raised against a peptide sequence obtained by Edman degradation of the purified 235 kDa band recognize the original protein as well as the 235 kDa band which co-purifies with KI-washed organelles. By immunocytochemistry, this protein is present on KI-washed organelles. Two low molecular weight species co-purify with the 235 kDa protein. Amino acid sequence analysis reveals that one of these low molecular weight bands consists of a calmodulin-like protein, suggesting it is a light chain.

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MULTIPLE KINESIN-LIKE TRANSCRIPTS IN THE FISH RETINAL PIGMENTED EPITHELIUM, B. Burnside and L.M. Bost-Usinger, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

We have used a degenerate primer reverse transcriptase polymerase chain reaction (PCR) cloning strategy to identify and isolate putative kinesin family members expressed in the retinal pigmented epithelium (RPE), freshly isolated from the striped bass, *Morone saxitilus*. Since adult fish RPE contains a single postmitotic cell type, this approach should avoid mitotic or meiotic kinesins and identify only those associated with differentiated epithelial cell functions. Six classes of kinesin family members (FKLP1-6) were identified, and the regions spanning the motor domains were cloned and sequenced. A homology search at the amino acid level suggests that FKLP1 is the fish counterpart of the kinesin heavy chain (KHC) and FKLP5 is closely related KHC homolog. FKLP3 and FKLP4 are most closely related to unc104 and KIF2, respectively, whereas FKLP2 and FKLP6 appear to be novel species phylogenetically associated with the KAR3 family. Northern blot analysis revealed that the FKLPs are abundantly expressed in the neural retina and intact retina-RPE, but expression levels were detectable in the isolated RPE only for FKLP2 and only after long term exposure, suggesting a low abundance of FKLP messages in RPE cells. The FKLPs showed different patterns of expression in a variety of tissues examined, with the KHC homolog FKLP-1 being most widely expressed, while the most abundant member, FKLP-2, showed strong expression in the retina and less in brain. Thus, we have shown that six different KLPs can be PCR amplified from the postmitotic cells of the fish RPE. These FKLPs may participate in known RPE transport processes such as vectorial protein secretion, phagosome and lysosome translocation, pigment granuale positioning, and chromophore recycling. (Supported by NIH grants EY03575 and EY06399-02).

CELL MOVEMENT IN THE ADULT BRAIN. Lois A. Lampson and Anthony Chen. Dept. of Neurology, Harvard Medical School, Boston MA 02115

Cells that move in the adult brain include inflammatory cells and tumor. While some aspects of the movement are well-studied, little is known about the internal mechanisms or their control. Features that permit differential control are of special interest. For example, it is desirable to selectively enhance surveillance by inflammatory cells, without increasing tumor spread. Given the regulatory and anatomic complexity of the brain, an in vivo model is needed, to complement in vitro work. We have developed a model that permits quantitative analysis of the migration of tumor and inflammatory cells in situ.* *Model. (1) Identified cells.* Injected tumor and responding leukocytes are studied in the rat brain. Tumor cells expressing the *lacZ* reporter gene are identified by histochemical stain for the reporter gene product. T cells and other leukocytes are identified by monoclonal antibody immunocytochemistry on the same slides. *(2) Quantitative analysis.* Images are digitized directly from the microscope slide. The numbers of tumor cells or leukocytes, and the areas covered, are computed. Simple reconstructions demonstrate the distributions in 3 dimensions. *Results and discussion. (1)* Tumor and leukocytes are found at the same sites. This underlies the challenge of achieving differential control. *(2)* External manipulations, such as cytokine injection, can alter the cells' movement, but may not be sufficiently selective. *(3)* We suggest that internal changes to the T cells' migratory mechanisms may be the best way of selectively enhancing T cell surveillance. The model system will help define the effects of such changes on cell movement in situ. **Cancer Res.* 53: 176-182, 1993; *Brain Pathol.* 4: 125-134, 1994.