189 Molecular analysis of a differentiated microtubule organelle: components of the marginal band. E. Birgbauer and F. Solomon, Department of Biology and Center for Cancer Research, M.I.T., Cambridge, MA.

The marginal band (MB) of the chicken erythrocyte is a model for a stable, differentiated microtubule organelle. Unlike the microtubule organelles of cultured cells, the MB is stable to depolymerizing drugs and is apparently independent of a microtubule organizing center. In mature cells, the MB is rigidly specified with respect to the shape of the microtubules, their number, and their position in the cell. Polymerized actin co-localizes with the MB. Several previous experiments on the formation of the MB during development, and its reformation in vivo and in vitro after disassembly, suggest that the organization of the adult MB is specified by lateral interactions between microtubules and other elements of the cell. Other results imply that the determinants of microtubule organization in the MB are stably associated with the cytoskeleton even after all of the microtubules have been removed from the cell. We describe here the results of one approach to identifying the proteins that might mediate such interactions. We raised monoclonal antibodies against the proteins that assemble in vitro with chicken brain tubulin, then screened them against preparations of chicken red blood cell detergent extracted cytoskeletons. One antibody binds to a single polypeptide of 78kd in chicken erythrocytes. That protein remains associated with extracted cytoskeletons of chicken erythrocytes even after treatments which release all cellular tubulin. The antibody stains the position of the marginal band in the presence and the absence of microtubules. The antibody also stains chicken embryo fibroblasts, especially filopodia and ruffling edges. These results raise the possibility that a 78kd protein is, like tubulin and actin, a component of the marginal band.

190 The Immunosuppressive Agent Cyclosporine A Modulates Microtubule Organization and Directed Cell Migration in Cell Cultures. B.N. Mascardo and E. Barton, Danbury Hospital and Boehringer-Ingelheim Research Lab., Danbury, CT.

Cyclosporine A(CsA), a lipophilic, cyclic undecapeptide, is an immunosuppressive agent used in organ transplantation. In addition to its immunosuppressive action, CsA has cytotoxic effects on renal, hepatic and neural tissues. Although the immunosuppressive action of CsA has been attributed to CsA-calmodulin binding and consequent inhibition of calmodulin-dependent protein kinases, the mechanism/s of the cytotoxic actions of CsA remain unclear. We describe here a possible mechanism of its cytotoxic actions. Human umbilical vein endothelial(HUVE), epithelial(A549), adenocarcinoma(SKHEP1), fibroblast(SKPS), and bovine pulmonary artery endothelial(BPAE) cells were cultured in complete media, serum-deprived for 24-36h, and exposed to CsA(10^{-9}-10^{-4}M). Cells were fixed and processed for immunofluorescence localization of microtubules(MTs) with a monoclonal antibody to α-tubulin. BPAE and SKPS cell monolayers were also subjected to experimental wounding and monitored for centrosomal orientation and cell migration responses. We found that CsA induced a rapid(@ 5min) disassembly of MTs in situ. The effect was concentration-dependent with the maximal effect being observed at 10uM. MT disassembly persisted for 4-5h after removal of CsA. CsA also partially blocked serum-induced centrosomal orientation and cell migration responses to wounding. CsA-induced MT disassembly was blunted by interleukin 1 and γ-interferon but not by 10% calf serum, insulin-like growth factor I, or the phorbol ester TPA. This novel action of CsA could be an important mechanism of CsA's clinical toxicity.

191 Capping of Con A-coated gold is driven by the cytoskeleton. M.P. Sheetz, S. Turney, H. Qulan, E. Elson, E. Brown. Depts. of Cell Biology and Biochemistry, Wash. Univ. Med. School, St. Louis, MO. 63110.

Concanavalin A-induced capping and endocytosis of membrane glycoproteins has been explained as either the consequence of lipid flow or active movement of glycoproteins along cytoskeletal elements. In mouse peritoneal macrophages, our analyses of bound Con A-coated gold particles (40 nm in diameter) reveals two major classes of behavior; either particles are diffusing or they appear trapped. The trapped particles move in a straight line (cap) and are endocytosed whereas the diffusing particles always become trapped before they are endocytosed. The trapping is reversible. When particles are followed on lamellipodial regions, they move toward the cell center and are endocytosed at the boundary between the lamellipodium and the endoplasm. During capping, the random movements of the particles are less than 50 nm. After endocytosis, the particles often move rapidly (1-2 microns/sec) along straight tracks. In contrast the diffusing particles move randomly across the cell over greater distances at a much faster rate than capping. Since the small size of the gold particles limits the size of the protein aggregates in the membrane and those aggregates are only slightly larger than individual membrane glycoproteins, the 100-1000-fold decrease in the diffusion coefficient of the particles during capping is inconsistent with the lipid flow model. Further, nanometer level measurements of the particle movements (Gelles, et al., (1988) Nature 33; 450-453) have revealed order in the movements, which indicates that an ordered motile process drives capping.

192 Endoplasmic Reticulum and Motility of Intact and Extruded Plant Cells. N.S. Allen and J.C. Schumm, Wake Forest University, Winston-Salem.

Recent fluorescense, AVEC-DIC and confocal laser scanning microscopic studies have revealed the dynamic nature and structural extent of a calcium-sequestering endoplasmic reticulum (ER) in plant cells. The ER has been postulated to play a direct role in cell movement generation as well as an indirect regulatory role. Video microscopy of intact Acetabularia revealed multistriate streaming in thin, anastomosing, membrane-bound networks within which the ER can be seen as interconnecting membrane tubules. Particle movements can be transiently stopped by IP_2, IP_3 and IP_4. Acetabularia cells were cut and the entire cytoplasmic content extruded into Buffer A solution (Sheetz and Spudich, 1983, Nature 303:31) containing the fluorescent dye $DiOC_6(3)$, which stains cytoplasmic membranes and particularly mitochondria and ER (Terasaki et. al., 1984, Cell 38: 101). The brightly stained ER network is seen on extrusion to 1) break into specific lengths and consists of tubules of varying diameter which 2) often intersect in characteristic Y-shaped junctions forming very specific angles to which 3) other tubules and organelles appear to attach. Small, brightly fluorescent vesicles (calciosomes?) move along the ER. This system will provide insights into relationship(s) between the ER and microfilament generated intercellular movements. This work was supported in part by a grant from the North Carolina Biotechnology Center (Allen and Jaffe).

193 Cytoskeleton and Membrane Assembly in Vertebrate Photoreceptors. B. Matsumoto, D.K. Vaughan, S.A. Bernstein, J.L. Hale, S.K. Fisher. Neuroscience Research Program, University of California, Santa Barbara, 93106 (Spon. by S.K. Fisher)

The organization of the rod photoreceptor's cytoskeleton suggest that microtubules (MT) and actin are important in outer segment (OS) membrane renewal. We studied the role of the cytoskeleton in this process by first quantifying membrane assembly with a video assay for disc morphogenesis and then determining if it was reduced following drug disassembly of either MTs or actin. Membrane assembly was quantified by continuously labelling newly forming OS membranes with Lucifer yellow VS and following the tagged membranes' distal displacement along the OS. We observed that rods from explanted eyecups displayed a linear increase in LY band displacement with time for at least 11 hours in culture. These cells possessed a longitudinally oriented network of ellipsoid MTs linking the sites of protein synthesis and membrane assembly. Incubation of eyecups in 16.5 uM nocodazole disassembled the ellipsoid MTs. In spite of their absence, photoreceptors maintained a normal rate of OS assembly. Rods from eyecups treated with either vinblastine, podophyllotoxin or colchicine also retained normal rates of assembly. These results indicate that the vectorial transport of OS membrane constituents through the ellipsoid and their assembly into disc membranes is not dependent on MT integrity. In contrast, eyecups treated with cytochalasin D displayed a reduced distal displacement of labelled membranes. Williams *et al.* (J. Comp. Neurol. 1988) showed that this drug induced abnormal lateral growth of disc membranes and we conclude that it was this lateral movement which reduced their apical displacement.

194 Microtubule Motility Driven Construction of the Endoplasmic Reticulum in Living Cells. C. Lee and L.B. Chen, Dana-Farber Cancer Inst. Boston MA 02115.

To study the construction of the endoplasmic reticulum (ER), we used the anti-microtubule drug nocodazole to induce the complete breakdown of ER structure in living cells (Terasaki et al., *J. Cell Biol.* 103, 1557 (1986)), followed by recovery in drug-free medium, which regenerates the ER network within 15 minutes. Using the fluorescent dye 3,3'-dihexyloxacarbocyanine iodide ($DiOC_6(3)$) to visualize the ER, we have directly observed the network construction process both in cells fixed with glutaraldehyde at different times during recovery, and, through video recording techniques, in living cells recovering from nocodazole. We conclude that the ER network is constructed through an iterative process of extension, branching, and intersection of new ER tubules, driven by the ER motility previously described as tubule branching (Lee and Chen, *Cell* 54, 37 (1988)). We have also examined correlations between ER network construction and the cytoskeleton through immunofluorescent staining, and investigated the cytoskeletal requirements of this process. We find that newly formed ER tubules are aligned with single microtubules, and advance outwards apparently along the line of newly polymerized microtubules. ER tubule branching followed and did not precede microtubule polymerization. To characterize the role of other elements of the cytoskeleton, we have done similar comparisons of ER network construction with actin and vimentin structure, and have sought to evaluate the effect of perturbations of these structures on ER network formation. Cytochalasin B had no apparent effect on construction of the ER network during recovery, despite drastic reduction in microfilaments (confirmed by both phalloidin staining and electron microscopy). We have also examined the effects of perturbations in the pattern of microtubule recovery (as produced by taxol, for example) on the pattern of ER regrowth, and tested the metabolic requirements of this process through microinjection of inhibitors.