SIGNAL TRANSDUCTION IN CYTOPLASMIC ORGANIZATION AND CELL MOTILITY

Peter Satir, John Condeelis and Elias Lazarides, Organizers
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Poster Sessions

Assembly of the Cytomatrix - I. Thermokinetics and Structural Basis of Assembly

1001 THE ADHESION PLAQUE: INTERFACE BETWEEN THE EXTRACELLULAR MATRIX AND THE CYTOSKELETON, Keith Burridge and Mary Beckerle, Dept. of Anatomy, University of North Carolina, Chapel Hill, NC 27514.
The adhesion of cells to the substrate or extracellular matrix induces a transmembrane organization of microfilaments at the cytoplasmic face of the plasma membrane. We are interested in understanding this organization and how it is regulated. Several proteins are concentrated at the sites of tightest adhesion to the substrate (adhesion plaques) and amongst these we have identified interactions between vinculin and talin and between talin and the fibronectin receptor. This establishes a link across the plasma membrane from the extracellular matrix on the outside to these cytoskeletal elements on the inside. The connection to actin filaments, however, has not been established and undoubtedly other proteins remain to be identified that function in this transmembrane association between the cytoskeleton and extracellular matrix. The adhesion of cells to specific substrates affects their growth, differentiation and morphology, suggesting that these sites of adhesion are also involved in signal transduction. Consistent with this idea, a number of tyrosine protein kinases have been identified in adhesion plaques and several of the proteins concentrated in these regions are substrates for these kinases. Talin is also a particularly good substrate for the calcium-dependent protease. Recently we have demonstrated that a calcium-dependent protease isoform is concentrated in adhesion plaques, raising the possibility that this enzyme may be important in regulating the disassembly of these transmembrane linkages. Supported by NIH Grant GM29860.

1002 ERYTHROCYTE ADDUCIN: A CALMODULIN-REGULATED MODULATOR OF SPECTRIN-ACTIN INTERACTIONS. K. Gardner and V. Bennett, Dept. of Cell Biology and Anatomy, Johns Hopkins School of Medicine, Baltimore MD 21205.
A major calmodulin-binding protein in human erythrocyte membranes has been purified and characterized as a heterodimer that is present at 30,000 copies per cell and is tightly associated with the membrane skeleton (Gardner and Bennett, (1986) JBC 261, 1339). This protein (referred to as adducin from the Latin "adducere", to pull together or gather) binds with high specificity to spectrin-actin complexes with an apparent K_d of 80 nM, but binds poorly to either spectrin or actin alone. Evidence that adducin forms a ternary oligomer with spectrin-actin complexes includes the following observations: 1) Binding of adducin is saturable at a level close to the number of spectrin-actin complexes in solution; 2) Spectrin from pig erythrocytes, which binds to actin with a higher affinity than human erythrocyte spectrin, promotes formation of ternary adducin-spectrin-actin complexes with a 6 fold higher efficiency than human erythrocyte spectrin; 3) Negative stained samples of adducin-spectrin-actin mixtures contain ternary complexes when viewed under the electron microscope. Adducin promotes binding of spectrin to actin and it is likely that the additional spectrin molecules are binding to adducin-spectrin-actin complexes. Adducin-dependent spectrin addition occurs with a capacity of about 1 mole of spectrin per mole of adducin-spectrin-actin ternary complex and with an apparent K_d of 400 nM for spectrin. Calmodulin inhibits the adducin-dependent addition of spectrin with a K_I of 0.8 uM that is maximal at 1 uM calcium. Adducin and protein 4.1 are antagonistic since adducin will not bind to spectrin-actin complexes that contain protein 4.1, and since protein 4.1 inhibits binding of adducin to membrane skeletons. It is proposed that a function of adducin includes recruitment or "gathering" of spectrin onto spectrin actin complexes which is a critical step in assembly of a crosslinked spectrin network. Adducin-dependent binding of spectrin is inhibited by calmodulin and this interaction would be one site of action of calcium on the membrane skeleton. Adducin activity may be influenced by the appearance of protein 4.1 late in erythroid development, thus forming a large population of highly stable spectrin-actin complexes within the membrane skeleton that are not destabilized by calmodulin. A protein closely related to adducin has been isolated from brain, where it may perform an analogous function of modulating spectrin-actin interactions. Adducin in brain and the erythrocyte is a substrate for protein kinase C although functional consequences of phosphorylation remain to be examined.

1003 MACROMOLECULAR MOTION IN THE ACTIN CYTOSKELETON. Thomas D. Pollard. Department of Cell Biology and Anatomy, Johns Hopkins Medical School, Baltimore, MD 21205.

The cytoskeleton is a paradoxical structure. It is rigid enough to resist compressive and tensile deformations and elastic enough to recover from small deformations. It is also plastic enough to tolerate complete transformations of cellular shape over the period of minutes to hours. At the same time, two of its major components, actin filaments and microtubules form tracks for organelle movements and cables to transmit tension from place to place in the cell.

An important feature of this complex structure is that many of its constituent protein subunits bind to each other relatively weakly. This introduces a critical temporal element into many of the molecular interactions. The resulting high frequency molecular rearrangements may be responsible for many of the paradoxical properties of the whole system. For example, networks of actin filaments and the crosslinking protein alpha-actinin are very stiff when deformed rapidly and indistinguishable from actin alone when deformed slowly, because the alpha-actinin binds to and dissociates from the filaments rapidly, perhaps 20 times per second. When the network is stressed rapidly, it resists displacement of the filaments, but when the network is stressed slowly, the crosslinks can rearrange faster than the filaments are displaced.

I will review how weak, rapid interactions between proteins participate in the assembly of actin filaments, the crosslinking of actin filaments and the production and transmission of the forces that cause bulk contraction and the movement of some organelles.

1004 MICROTUBULE DYNAMICS IN VIVO, Eric Schulze and Marc Kirschner, Department of Biochemistry and Biophysics, University of California, San Francisco CA 94143-0448. Recent in vitro evidence has suggested that microtubules possess unusual dynamics. Individual polymer molecules grow and shrink extensively in vitro, while the entire population is at steady state. We have found that the binding of microtubule associated proteins to the polymer surface or structures such as kinetochores or centrosomes to the ends dramatically stabilizes the dynamic properties. Recently we have investigated polymer dynamics in vivo by microinjection of a modified form of tubulin (biotin tubulin) and examination of the pattern and kinetics of microtubule growth. The overall dynamics are consistent with the in vitro properties of dynamic instability. However, some microtubules were clearly isolated from the dynamic population and were more stable. Recently we have examined the posttranslational modification of tubulin in the stable and unstable populations. We have looked at both tyrosine addition to the C-terminus of α-tubulin and the acetylation of tubulin. Both of these modifications accumulate in the stable microtubule population. The role of rapid dynamics, associated protein binding, and posttranslational modification in the morphogenesis of microtubule arrays will be discussed.

Assembly of the Cytomatrix - II. Control Mechanisms in Assembly of the Membrane-Skeleton

NUCLEAR LAMINA AND REGULATION OF NUCLEAR ENVELOPE STRUCTURE DURING MITOSIS

1005 L. Gerace, B. Burke, F. Suprynowicz and U. Aebi, Johns Hopkins Univ. Sch. Medicine, Baltimore MD 21205.

The nuclear lamina is a protein meshwork that lines the nucleoplasmic surface of the nuclear envelope[1]. In higher eukaryotic cells during interphase, the lamina contains a polymer of 1-3 major polypeptides ("lamins") that form intermediate-type (10 nm) filaments organized in a quasi-tetragonal lattice2. The lamina is postulated to provide both a skeletal framework for the nuclear envelope and an anchoring site at the nuclear periphery for interphase chromosomes[1]. In higher eukaryotes, the nuclear envelope is reversibly disassembled during mitosis, concomittent with reorganization of numerous other cytoarchitectural elements. Our previous studies suggested that mitotic structural dynamics of the nuclear envelope may be regulated by reversible depolymerization of the nuclear lamina[1]. Lamina disassembly in turn may be controlled by reversible phosphorylation of the lamins. To further investigate the mechanisms by which the nucleus and nuclear envelope are restructured during mitosis, we devised cell-free systems based on mitotic Chinese Hamster Ovary (CHO) cells to study these processes. The nuclear assembly system[3](based on incubating total metaphase cell homogenates at 33°C) yields telophase-like formation of ultrastructurally normal nuclear envelopes around mitotic chromosomes, as well as chromatin decondensation. During nuclear envelope assembly in vitro, the three CHO cell lamins (A, B, and C) assemble into a Triton-insoluble structure around mitotic chromosomes and become dephosphorylated, resembling their behavior in vivo during telophase. For cell-free nuclear disassembly[4], a high-speed supernatant derived from mitotic CHO cells is incubated with isolated interphase CHO nuclei or an isolated rat liver lamina fraction in the presence of ATP at 33°C, resulting in nuclear envelope and lamin disassembly, lamin hyperphosphorylation, and chromatin condensation. In the future, these nuclear assembly and disassembly systems will provide powerful approaches for analyzing both nuclear structure and biochemical regulation of mitosis.

[1]Gerace, L. **Trends in Biochem. Sci.**, in press.
[2]Aebi, U., Cohn, J., Buhle, L. and Gerace, L. (1986). **Nature 323**, 560.
[3]Burke, B. and Gerace, L. (1986) **Cell 44**, 639.
[4]Suprynowicz, F. and Gerace, L. **J. Cell Biol.**, in press.

TRANSMEMBRANE SIGNALLING: A ROLE FOR CYTOSKELETAL LINKING PROTEINS,

1006 Vincent T. Marchesi, Department of Pathology, Yale University School of Medicine, New Haven, CT 06510. Cells respond to stimuli by a complex set of physical and metabolic responses which are often collectively referred to as transmembrane signals. This activation process is usually triggered by the binding of stereo-chemically specific ligands to surfaced-exposed receptors. Typical receptors are transmembrane glycoproteins of a common molecular design, consisting of multiple connecting modules. Receptor activation is believed to be accompanied by changes in the three-dimensional arrangement of the membrane-associated cytoskeleton, resulting in perturbations that are eventually relayed to the nucleus and other compartments of the cell. Many different macromolecules play some role in this network, among them a class of linking proteins that are able to bind both to membrane-bound glycoproteins and to cytoskeletal and nucleo-skeletal structures.

Protein 4.1 has a well-characterized linking function in red blood cells. It has both the capacity to promote spectrin-actin interactions and an ability to link this multi-protein complex to cytoplasmic segments of the glycophorins through a lipid-dependent association. Isoforms of 4.1 exist in all cells and are distributed throughout both nuclear and cytoplasmic compartments. A subset of these proteins bind to cytoplasmic microtubules and to parts of the mitotic apparatus in dividing cells. Circumstantial evidence is consistant with the idea that 4.1 isoforms play some role in regulating nucleo-cytoplasmic interactions.

1007 ASSEMBLY OF THE INTESTINAL BRUSH BORDER CYTOSKELETON DURING EMBRYOGENESIS, Mark S. Mooseker, Joan M. Carboni, Karen A. Conzelman and Toshiko Shibayama, Dept. of Biology, Yale University, New Haven, CT 06511.
The assembly of the intestinal microvillus (MV) cytoskeleton during embryogenesis in the chick was examined by ultrastructural and immunological techniques. Previous studies have shown that the assembly of the brush border (BB) is a complex and gradual process. In embryos between 5-9 days of incubation, the apical surface of the enterocyte is dome-shaped, containing a sparse population of irregularly shaped MV, the density of which increases during this time. By 9-10 days of incubation the apex of the cell has flattened and a dense lawn of short MV of uniform length are present. During subsequent developmental stages, the rootlet ends of the MV cores elongate, and the terminal web forms. MV remain short until the about the day of hatch which marks the onset of dramatic increases in length which occur during the next week. The expression and subcellular distribution of the 3 major proteins of the MV core, villin, fimbrin and the 110kD subunit of the 110K-calmodulin (CM) complex was examined by immunolocalization and immunoblot techniques which revealed that all 3 of these MV core proteins are expressed throughout development, from the earliest embryonic stages examined (day 6-7 incubation). However, there was a marked asynchrony in the movement of these proteins into the apical surface. Villin "arrives" first (day 7) at a time when very few MV are present. Fimbrin first displays apical staining in the 9-10 day embryo, a time when there is a marked increase in MV density. Surprizingly, the 110K protein displays a diffuse, cytoplasmic distribution, throughout development until 1-2 days before hatch, when it appears to move to the cell periphery (apical and basolateral surfaces). A restricted localization of the 110K to the BB surface was not observed until hatch. This timing roughly correlates with the maturation of the BB membrane, and also with the onset of MV elongation.

The potential significance of these observations with respect to MV assembly will be discussed in the context of the in vitro properties of these 3 core proteins and their interaction with actin. For example, recent studies in several laboratories have indicated that the 110K-CM complex may be a myosin-like mechanoenzyme. We have observed that 110K-CM exhibits actin-activated MgATPase; preliminary studies (done with M. Sheetz, Washington U.) using vesicle preparations containing 110K-CM on their outer surfaces have provided tentative evidence for 110K-CM-dependent movement using the Nitella bead movement assay. Given the possible motile properties of 110K-CM, perhaps it is involved in the targeting of newly synthesized membrane to the BB surface, a role consistent with its late re-distribution into the cell apex during development.

Mechanisms of Transmembrane Signal Transduction - I. Molecular Basis of Signalling

1008 PROTEIN KINASES AND SIGNAL TRANSDUCTION, Edwin G. Krebs, Christine P. Chan, Michael F. Cicirelli, Elizabeth A. Kuenzel, Kathyryn E. Meier, Steven L. Pelech, James M. Sommercorn, Donald A. Tinker, Howard Hughes Medical Institute and the Department of Pharmacology, University of Washington, Seattle, WA 98195. It has become increasingly apparent that almost all forms of signal transduction involve one or more steps in which the reversible covalent modification of proteins is implicated, and by all odds the most common form of such modification is phosphorylation. Protein phosphorylation-dephosphorylation reactions are catalyzed by an interacting network of kinases and phosphatases, the complexity of which has only begun to be appreciated. Many more protein kinases have been distinguished than protein phosphatases, and it appears that signals more commonly impinge upon the former set of enzymes than on the latter. In addition to modulation of protein kinase and protein phosphatase activities by messengers, however, the translocation of these enzymes in response to signals and variation in the rates of their biosynthesis and degradation are important regulatory factors governing their function. We have been investigating the role of protein kinases in several complex signal-response systems in an effort to broaden our understanding of these cascade mechanisms. Specific examples in mammalian systems include the coupling of insulin and growth factor receptor response to the phosphorylation-dephosphorylation of glycogen synthase, ribosomal protein S6, and acetyl-CoA carboxylase. With respect to these studies, it is assumed that a relatively simple link, i.e. one involving only a few steps, exists between protein-tyrosine phosphorylation and protein serine/threonine phosphorylation. Our approaches include the isolation and characterization of mitogen-activated serine/threonine kinases as a first step in understanding their mode(s) of regulation. Elucidation of the nature of this link may provide clues as to the molecular mechanism(s) involved in a broad range of cellular functions regulated via protein-tyrosine phosphorylation. A second set of studies is being carried out using Xenopus and starfish oocytes. It is hoped that the relationship between protein phosphorylation and the cell cycle M-phase promoting factor (MPF) can be established. These studies are designed to investigate phosphorylation mechanisms involved in amphibian and invertebrate oocyte maturation. In oocytes multiple serine/threonine kinases appear to be activated during maturation.

1009 CLONING OF Ca^{2+} ATPases AND CALSEQUESTRIN. David H. MacLennan*, Christopher J. Brandl*, Bozena Korczak*, Angel Zarain-Herzberg*, Larry Fliegel*, Mamoru Ohnishi**, Reinhart A.F. Reithmeier** and N. Michael Green+. *Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada. **Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada. +National Institute for Medical Research, The Ridgeway, Mill Hill, London, U.K.

In order to gain insight into the structure and function of Ca^{2+} ATPases of sarcoplasmic reticulum, we cloned cDNA encoding two Ca^{2+} ATPases. Neonatal rabbit muscle cDNA libraries contained nearly equal proportions of mRNAs encoding fast-twitch and slow-twitch/cardiac forms of the Ca^{2+} ATPase. These Ca^{2+} ATPases are encoded in two genes which, in humans, are located on separate chromosomes. In rabbit muscle the fast-twitch transcript is alternatively spliced at the 3' end of the coding region in a developmentally regulated fashion. The neonatal transcript encodes an extended, highly charged carboxyl-terminus whereas the adult transcript contains one additional exon and encodes a protein ending in the uncharged residue, glycine. The slow-twitch/cardiac transcript does not appear to be alternatively spliced.

The analysis of our sequence led to a model of the enzyme in which three cytoplasmic domains make up a headpiece structure. The transmembrane segment consists of ten alpha-helices. Five of these extend on the cytoplasmic side into alpha-helices which make up the stalk sector of the structure. We propose that the high affinity Ca^{2+} binding sites are made up on more than one alpha-helical stalk segment. We also suggest that the energy generated in the headpiece is utilized in rotating one or more of the stalk segments to carry bound Ca^{2+} into a channel formed in the interior of the stalk. This rotation would lead to disruption of the high affinity Ca^{2+} binding sites and to deposition of the Ca^{2+} into a channel connected to luminal spaces.

We have also cloned and sequenced the fast-twitch skeletal muscle form of calsequestrin. This protein of Mr 42,000 binds a high level of Ca^{2+} with low affinity and acts as a Ca^{2+} buffer in the terminal cisternae of the sarcoplasmic reticulum. The Ca^{2+} binding motif appears to be pairs or triplets of acidic amino acids. These are concentrated in the carboxyl-terminal half of the molecule.

1010 REGULATION OF CELL FUNCTION BY CALMODULIN, A.R. Means and C.D. Rasmussen, Department of Cell Biology, Baylor Coll. of Med., Houston, TX 77030.

Much of the signal transduction that results in a transient increase in intracellular Ca^{++}, requires calmodulin (CaM), to mediate the Ca^{++} dependent processes. The Ca^{++}-CaM complex exerts effects on membrane receptors, actin based microfilament bundles, microtubules and both ciliary and flagellar axonemes. In each case the effect requires protein modification by a Ca^{++}-CaM dependent enzyme. The solution of the 3 dimensional structure of CaM together with development of prokaryotic expression vectors capable of producing CaM and CaM mutants have allowed a better understanding of the molecular mechanisms by which enzyme activity is regulated. Different enzymes exhibit unique structural requirements for interaction with Ca^{2+}-CaM. In the case of myosin light chain kinases (MLCK), the CaM binding region interacts with the substrate binding region in the absence of Ca^{2+}-CaM. Thus CaM relieves inhibition of these enzymes rather than directly activating them. Since CaM exerts so many effects on cellular enzymes and processes we wished to determine the consequences of elevating the concentration of this regulatory protein in eukaryotic cells. A chicken CaM minigene driven by its own promoter was ligated into a bovine papilloma virus (BPV) based vector and used to stably transform mouse C127 cells. Cell lines were derived that contained equivalent copies of the BPV episome or BPV containing the CaM gene. The BPV-CaM genes were regulated during the cell cycle in an identical manner to the endogenous mouse gene. Transcription of the BPV-CaM genes was properly initiated but the derived mRNA was truncated by 200 nt at the 3' end due to preferential utilization of a cryptic polyadenylation site. All of the transcripts were processed properly resulting in polyribosome associated mRNA. The mRNA was translated into authentic vertebrate CaM. The most active cell line (CM-1) constitutively produced 6 x the amount of CaM present in the BPV transformed line BPV-1. So far four consequences can be attributed to the elevated CaM levels. These are: (i) the cells grow to a higher plateau density; (ii) the cell cycle is shortened by 2 hr. All of the difference is due to a decrease in G_1; (iii) the normal cell cycle regulation of MLCK mRNA is disrupted. However, cell cycle changes in CaM and histone H4 mRNA are unaltered; and (iv) there is a 5 x decrease in the level of both α and β tubulin mRNA in exponentially growing cells but no change in actin mRNA. Since the cytoplasmic microtubule network is not visably altered, these data may indicate that CaM affects the rate of dynamic instability of the microtubules. Current studies are designed to investigate the molecular nature of these effects of elevated CaM on cell growth and gene expression.

Mechanisms of Transmembrane Signal Transduction - II. Signal Discrimination

1011 MOLECULAR PROPERTIES OF DIHYDROPYRIDINE-SENSITIVE CALCIUM CHANNELS. William A. Catterall, Masami Takahashi, and Benson M. Curtis. Department of Pharmacology, University of Washington, Seattle, WA, 908195, USA.
Voltage-sensitive calcium channels are major regulators of the rate of entry of extracellular calcium into cells. These channels participate in electrical signaling in the cell surface membrane and mediate calcium influx to initiate excitation - contraction and excitation - secretion coupling. Multiple subtypes of voltage-sensitive calcium channels have been described by electrophysiological studies in a wide range of tissues. The L subtype mediates long-lasting calcium currents and is modulated by clinically important dihydropyridine calcium channel antagonists and agonists. It is the most widespread of the calcium channel subtypes. We have purified this voltage-sensitive calcium channel from skeletal muscle transverse tubules, a rich biochemical source.

Dihydropyridine-(DHP)-sensitive calcium channels were solubilized with digitonin and purified to a specific activity of 1950 pmol DHP receptor/mg protein. Three polypeptides termed α (162 kD), β (50 kD), and γ (33 kD) are noncovalently associated in the purified channel complex. The apparent size of α is reduced to 135 kD by reduction of disulfide bonds consistent with release of a small, covalently attached peptide. Calcium channels are regulated by cAMP and cAMP-dependent protein kinase. The α and β subunits of the channel are rapidly phosphorylated by cAMP-dependent protein kinase suggesting that they may be the sites of regulation by this enzyme. Incorporation of purified calcium channels into phospholipid vesicles restores their functional activity. DHP binding is allosterically modulated by verapamil and diltiazem. Bay K 8644 activates the reconstituted channels. The resulting $^{45}Ca^{2+}$ influx is blocked by inorganic antagonists and by the organic calcium antagonists PN200-110, D600, and verapamil with $K_{0.5}$ values of 0.2 μM, 1.0 μM, and 1.5 μM. Antibodies against the α subunit of the skeletal muscle Ca channels crossreact with DHP-sensitive calcium channels in brain and heart. The polypeptide recognized in each tissue has similar apparent molecular weight before and after reduction suggesting the presence of a homologous, but distinct, DHP-sensitive Ca channel in these three tissues.

1012 SIGNAL TRANSDUCTION IN CILIARY AND MICROVILLAR MEMBRANES OF CHEMOSENSORY NEURONS Doron Lancet, Dept. Membrane Research, The Weizmann Institute, Rehovot, Israel.
Chemosensory cells have a molecular machinery that receives and transduces chemical signals from the external world. As in other sensory cells, reception occurs in specialized cytoskeleton-rich organelles: the cilia of olfactory neurons and the microvilli of taste cells. Neurons in the vomeronasal organ, an accessory olfactory organ that may receive peptidergic signals, also bear microvilli. Like the cilia-derived visual rod and cone outer segements, the chemosensory organelles serve mainly to increase the effective sensory membrane area. In olfaction, odorants bind to yet unidentified olfactory receptor (OR) proteins in the ciliary membrane. We recently demonstrated that such interaction results in the specific activation of olfactory cilia adenylate cyclase, which has 100 times higher specific activity than that of respiratory cilia (Pace et al, Nature 316: 255-8,1985). Odorant-induced cAMP generation is GTP-dependent, and is shown to involve a stimulatory GTP-binding protein (G-Protein, Pace and Lancet, PNAS 83: 4947-51, 1986). Ciliary motility does not seem to be involved in olfactory transduction, since the chemosensory cilia of many species, including all mammals, lack dinein arms. It is, however, possible that transduction components interact with axonemal tubulin, thus undergoing specific seggregation into the sensory organelles. Olfactory cilia contain cAMP-dependent protein kinase that may mediate odorant activation of ion channels, leading to neuronal membrane depolarization. The main unresolved questions in olfactory research are: 1) What is the nature of olfactory receptor (OR) molecules: do they constitute a multiprotein family, like immunoglobulins and T-cell receptors, and do they bear homology to other G-protein-related receptors, such as rhodopsin, the β-adrenergic and muscarinc acetylcholine receptors. 2) Do the chemosensory ion channels (presumably specific for sodium) undergo modulation by cAMP, and does this happen through protein phosphorylation, or (as in retinal rod outer segments) via direct cyclic nucleotide gating (Lancet and Pace, Trends Biochem. Sci., in press).

Recently we have also begun a study of the role of cAMP in taste transduction (Striem, Pace, Zehavi, Naim and Lancet, Chemical Senses, in press). We find that sucrose at physiological concentrations (0.58M) enhances the activity of adenylate cyclase in a membrane preparation from rat tongue. This activation is guaninine nucleotide-dependent, and is not seen in membranes from muscle or from lingual epithelium devoid of taste papillae. Future studies on microvilli-enriched membrane preparations will help to substantiate and extend such findings.

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1013 DIFFERENTIATION OF A HUMAN ADENOCARCINOMA (HT29) IN CULTURE : A MODEL TO STUDY ASSEMBLY OF MEMBRANE SPECIALIZATION AND CELL SURFACE POLARITY IN EPITHELIA.

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We are studying the generation of cell polarity and the assembly of cell surface specializations in an intestinal cell-line (HT29) in culture. These cells remain undifferentiated in tissue culture media containing glucose whereas in the absence of hexose or upon replacement of glucose by galactose HT29 cells undergo terminal differentiation. We have been particularly interested in the assembly of the enterocyte brush border composed of thousands of stiff microvilli containing a bundle of microfilaments made of actin. We focused our interest on villin, one of the actin binding proteins found in intestinal microvilli. We have recently shown that villin can be used as a marker for normal and malignant undifferentiated cells as well as differentiated ones. Since, this protein is a good marker for intestinal cells and plays a structural role in the assembly of the brush border we have analyzed the expression and the localization of this protein using biochemical and immunocytochemical approaches. Differentiated cells contain a ten fold excess of villin compared to undifferentiated ones. In both conditions of culture villin is very stable. This large difference can therefore be explained by a much higher rate of synthesis in differentiated cells. Undifferentiated cells display a uniform distribution of villin throughout the cytoplasm. In contrast, villin is enriched at the apical borders of differentiated cells. The recruitment of this protein at the apical pole of the cells can be correlated with the existence of a well developed brush border. A cDNA probe (510 bp) encoding for the carboxy terminus of human villin has been isolated and sequenced (108 amino acids deduced from DNA sequencing). This primary sequence indicates 65% homology with a corresponding peptide of chicken villin, previously sequenced by protein sequencing located at the carboxy terminus and capable of binding to actin in vitro. In hybridization experiments using total RNA, we have demonstrated the presence of two RNA species (3.2 Kb and 4 Kb) in human intestine or human kidney. Interestingly the corresponding chicken tissues have only one mRNA (3.2 Kb) and rat display also one RNA (4.0 Kb). We have tested a variety of human epithelial cells in which villin cannot be demonstrated using very sensitive immunoassay, and have shown that they do not contain these RNAs. Finally, the abundance of RNA encoding for villin is ten fold higher in differentiated cells than in undifferentiated cells. These results confirm the tissue specificity of villin and suggest that regulation of the expression of this protein is under the control of transcriptional processes.

1014 **A NONMITOGENIC, BIOLOGICAL ACTIVITY OF pp60^{src} ON MULTICELLULAR EPITHELIAL STRUCTURES,** **W. James Nelson and Stephen L. Warren, INSTITUTE FOR CANCER RESEARCH, FOX CHASE CANCER CENTER, PHILADELPHIA, PA 19111.**

Madin-Darby Canine Kidney (MDCK) cells have retained the morphogenetic determinants required to form polarized multicellular epithelial structures in vitro, which exhibit many biochemical, functional, and architectural properties of transporting epithelia in situ. We are interested in defining the determinants involved in the intra- and intercellular organization of these cells. One approach that we are using is to study the role of plasma membrane-associated tyrosine kinases in regulating intercellular adhesion; as a model we used initially pp60$^{v\text{-}src}$. The v-src gene was stably introduced into MDCK cells, together with the neo^{r} gene, using helper-free, defective, amphotropic retroviruses. The vectors were designed for low-level expression of pp60$^{v\text{-}src}$, and clones of infected MDCK cells that had been selected in G418 exhibited very low levels of pp60^{src}-kinase activities in vitro (2-16 fold above the levels of endogenous c-src activity in normal MDCK cells). Detailed analysis of MDCK multicellular epithelial structures in vitro revealed that low-level expression of pp60$^{v\text{-}src}$ in MDCK cells elicits plasticity in these structures. Plasticity is revealed by the displacement of cells from mechanically-stressed regions of the epithelial monolayers; however, the 2-dimensional relationship between the cells in the remainder of the monolayer is maintained. Electron microscopy of multicellular structures revealed abnormal separation of the lateral membranes of adjacent cells and selective uncoupling of the junctional complex; the zonula adherens is disrupted, but the zonula occludens and desmosomes are retained. Significantly, this action is not accompanied by transformation of the cells, as judged by the absence of anchorage-independent growth potential. Since the v-src gene apparently arose from the c-src gene, which is expressed normally in post-mitotic differentiating tissues, we interpret these results to indicate that pp60$^{v\text{-}src}$ has retained the potential to act on morphogenetic determinants involved in modulating intercellular adhesion that may be controlled normally by related cellular tyrosine kinase(s); indeed, preliminary results show that elevated expression of a non-oncogenic variant of pp60$^{c\text{-}src}$ in MDCK cells elicits a similar non-transformed phenotype. Furthermore, we suggest that this trait resembles certain morphogenetic events in vivo, in which temporal and spatial variation of epithelial tension and plasticity are thought to be critical for the formation of 3-dimensiona tubular or cystic tissue patterns.

1015 ARG-GLY-ASP: A CELLULAR RECOGNITION SYSTEM FOR POSITIONAL SIGNALLING, Erkki Ruoslahti and Michael D. Pierschbacher, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Fibronectin is a large extracellular glycoprotein that can mediate adhesion of cells to the extracellular matrix. Our studies of the cellular recognition of fibronectin have recently led to the unexpected observation that, of the approximately 2,500 amino acids in the fibronectin polypeptide, three--an Arg-Gly Asp (RGD) tripeptide--are crucial for its interaction with its cell surface receptor. Moreover, analysis of the cell attachment sites of several other adhesion proteins including vitronectin, von Willebrand factor and fibrinogen has implicated the same amino acid triplet. The RGD sequences in the various adhesive proteins are recognized by cell surface receptors that are capable of distinguishing between the RGD sequences of individual proteins. So far, three such receptors have been identified: those for fibronectin and vitronectin, as well as a multifunctional RGD-directed receptor from platelets. These receptors are each composed of one alpha and one beta subunit. Amino acid sequences derived from cloned cDNAs show that the alpha and beta subunit sequences are unrelated to one another, but that the fibronectin and vitronectin receptor sequences are highly homologous. The RGD receptor family includes additional members. A collagen receptor recognizes the RGD sequences in type I collagen and subunits of leukocyte adhesion receptors LFA-1 and Mac-1 have NH_2-terminal sequences homologous with that of the vitronectin receptor alpha subunit. The recognition of an RGD sequence of individual proteins by specific receptors, therefore, appears to be a common mechanism of cell adhesion. The question as to how the receptors are capable of recognizing the RGD sequence in one protein but not in another has not been completely resolved, but available evidence points to the importance of the conformation of the RGD sequence in this regard. The RGD signals are likely to be important in directing cellular differentiation and migration during development, in remodeling of normal tissue, and perhaps also in the invasion of tissues by tumor cells.

1016 SIGNAL TRANSDUCTION IN STIMULUS-EXOCYTOSIS-COUPLING, Birgit H. Satir and Timothy J. Murtaugh, Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY 10461.

The ciliated protozoan Paramecium tetraurelia contains thousands of docked secretory vesicles (trichocysts) which can be induced to release synchronously when triggered with trinitrophenol (TNP). Upon release the trichocyst membrane fuses with the cell membrane and the trichocyst content called trichocyst matrix (tmx) undergoes a Ca-dependent irreversible expansion. In response to the secretagogue the most heavily in vivo phosphorylated protein M_r 63,000 undergoes a rapid dephosphorylation (1). Both secretion and dephosphorylation require extracellular calcium. In addition, a temperature sensitive secretory mutant, nd9, loses its ability to secrete and to dephosphorylate the 63kDa protein in response to TNP at the non-permissive temperature. These results suggest that the 63kDa phosphoprotein may be an important component in the pathway of stimulus-exocytosis coupling. Therefore, this protein has been purified and a polyclonal antibody against it produced. The initial characterization of the 63kDa phosphoprotein shows that it is a primarily cytosolic protein which, in the native state, is a 63kDa monomer with multiple isoelectric forms (pI's of 5.8-6.0), is normally kept phosphorylated on one or more serine residues and has hydrophobic regions capable of binding to phenyl-Sepharose in high salt. The affinity-purified antibody produced against the 63kDa was shown by Western blot analysis and by immunoprecipitation to bind to both the phosphorylated and nonphosphorylated protein. Western blot analysis, using the affinity purified antibody on other cells and mammalian tissues (such as Tetrahymena and a variety of rat tissues), showed that immunologically similar proteins are widely distributed in both ciliates and in rat tissues, all of which are in the range of M_r 60-65,000. This suggests that the 63kDa protein may be of general signifiance with regard to regulation of cellular function. Preliminary studies on subcellular localization of 63kDa protein in ciliates demonstrate that, while most of the 63kDa protein is soluble, some can be localized to structural components of the cell in certain circumstances. Subcellular fractionation and Western blot analysis showed that some 63kDa protein can bind to a microsomal fraction in a Ca^{2+}-dependent manner. Indirect immunofluorescence of Paramecium showed a pattern of rows of fluorescent rings or squares, reminiscent of the polygonal network characteristic of Paramecium cortex.

1. Gilligan and Satir (1982) J. Biol.Chem. 275, 13903-13906

Signalling During Motile Events - I. Cell Activation

1017 DOMAIN STRUCTURE OF GELSOLIN, Joseph Bryan and John A. Cooper*, Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030 and *Dept. of Biological Chemistry, Washington University School of Medicine, St. Louis, MO 63110.

Gelsolin is a calcium binding, actin associated protein that can sever actin filaments, nucleate filament growth and specifically cap the barbed end. Gelsolin binds two calcium ions and two actin monomers; EGTA releases one of the calcium ions and one actin monomer. The resulting complex will cap but not sever. Proteolysis with chymotrypsin and trypsin and the use of HPLC allows isolation of half and three quarter fragments. The N-terminal half nucleates, caps and severs, but shows no calcium regulation. The N-terminal half binds two actin monomers as judged by gel filtration and binding studies with radioactive actin. This complex has no severing activity but will cap depolymerizing filaments at subnanomolar concentrations. Equilibrium binding studies with calcium-45 show that the C-terminal of plasma gelsolin has two calcium binding sites, which presumably correspond to the sites mapped using monoclonal antibodies that recognize calcium induced conformations. In addition, there is a third calcium binding site on the N-terminal half. A three-quarter molecule, missing the N-terminal, shows no severing activity, but does have calcium dependent capping activity. The three-quarter molecule missing the C-terminal and one of the calcium binding sites has severing and capping activity, but is not calcium regulated. We conclude that the severing site is on the N-terminal and that actin binding is inhibited by the C-terminal unless calcium is present. Microinjection of cytoplasmic or plasma gelsolin does not affect the shape, actin distribution, deformability or ruffling activity of cells. In contrast, microinjection of the N-terminal half or the N-terminal half complexed with two actins has dramatic effects - cells round up, stop ruffing, become soft and lose their stress fibers. Efforts to increase the effects of injected gelsolin by raising the intracellular calcium concentration using calcium injections and ionophores have been unsuccessful. The results are compatible with a model in which gelsolin functions as a reversible cap on barbed filament ends while the N-terminal caps irreversibly. We cannot, however, rule out the existence of molecules that inhibit gelsolin, but not the N-terminal half.

1018 STRUCTURE, EXPRESSION AND FUNCTION OF CALPACTIN I HEAVY (p36) AND LIGHT CHAINS (p11) Tony Hunter*, Chris J. M. Saris*, Brian F. Tack+ and John R. Glenney, Jr*. *The Salk Institute and +Scripps Clinic and Research Foundation, La Jolla, Ca 92037

Many growth factor receptors and viral transforming proteins are protein tyrosine kinases (PTK). Tyrosine phosphorylation is therefore likely to be an important event in mitogenesis and malignant transformation. Among the identified PTK substrates are talin, vinculin, p81 (ezrin) and p36 (calpactin I heavy chain), all found in the submembraneous skeleton of cells. Phosphorylation of these proteins might be involved in the shape changes characteristic of transformed cells or cells treated with growth factors. Calpactin I exists as a $(p36)_2(p11)_2$ tetramer or a p36 monomer. p36 is phosphorylated by $pp60^{v\text{-}src}$ at Tyr 23 and by protein kinase C at Ser 25. p36 has 2 phospholipid-dependent Ca^{2+}-binding (Ca^{2+} K_d 1-5 μM) sites and more than 1 actin binding site, all present in the C-terminal 300 residues. The N-terminal 30 residues bind to p11. Calpactin I may thus act to link the plasma membrane to the cytoskeleton, or to crosslink intracellular vesicles. We have isolated and sequenced cDNA clones for murine and bovine p36. Both proteins are predicted to have 338 residues and are 98% identical. The C-terminal 300 residues can be arranged into a 4-fold 70 amino acid repeat structure, which in some way must reflect the multiple Ca^{2+}/phospholipid and actin binding sites. Calpactin II (p35) is also a Ca^{2+}/phospholipid and actin binding protein, which, although it exists solely as a monomer, may have a function analagous to calpactin I. p36 is 50% homologous with calpactin II (also known as lipocortin I, an inhibitor of phospholipase A_2), including the four 70 amino acid repeats. Calpactin I also has phospholipase A_2 inhibitory activity, although this may not be a physiological function. Residues conserved in all 4 repeats and between p36 and p35 have been chosen for site-directed mutagenesis to determine which regions of p36 are critical for Ca^{2+}/phospholipid and actin binding. Whether phosphorylation of calpactin I alters Ca^{2+} or phospholipid binding has not yet been examined. To complement in vitro studies on the effects of phosphorylation on the binding properties of purified calpactin I, we are planning to express p36 cDNAs which have been mutated by substitution of Tyr 23 and/or Ser 25. We have detected a single gene for murine p36, spanning about 30 kb, which has been isolated in a single cosmid clone. This clone is being used to determine the gene structure and characterize the promoter region. We have also obtained cDNA clones for murine and bovine p11. Again a single murine p11 gene is found, which covers about 5 kb. We have used the cDNA clones to measure the relative levels of expression of p36 and p11 mRNA in different cell lines and in mouse tissues. Although in most cases the two RNAs are expressed coordinately, there are exceptions where either p36 or p11 mRNA is present at a much higher level than the other. This may indicate that the two proteins have separate functions as monomers, in addition to acting together in the caplactin I tetramer.

I019 RECEPTOR G-PROTEIN INTERACTIONS AND DICTYOSTELIUM DEVELOPMENT, Peter Klein, Anne Theibert, Roxanne Vaughan, Maureen Pupillo and Peter Devreotes, Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Addition of GTP or GTP analogs to lysates of Dictyostelium amoebae leads to significant (up to 17-fold) and persistent activation of adenylate cyclase. Activations are enhanced 2 to 4 fold by cAMP, are specific for guanylnucleoside triphosphates, and are inhibited by GDP S. Guanylnucleotides are unable to stimulate the cyclase in cells which have become adapted by exposure to cAMP for longer than 10 min. This data suggests that adenylate cyclase in Dictyostelium is regulated by a guanylnucleotide protein. Characterization of an activation minus mutant, designated synag 7, demonstrates that guanylnucleotide stimulation of adenylate cyclase is not required for growth or differentiation but is essential for cellular aggregation. Guanylnucleotide stimulation of the adenylate cyclase in membranes of synag 7 can be reconstituted by addition of a high speed supernatant from wild-type cells.

The surface cAMP receptor in Dicytostelium appears as a doublet when analyzed by SDS/PAGE. The lower mobility band (MW=43000), designated D, appears to be a covalently modified form of the higher mobility band (MW=40000), designated R. In the absence of cAMP, only 10% of the receptors migrate as the R form. During 15 minutes of persistent stimulation with 1 μM cAMP, the precent D form increases to 80%. This transition occurs with a half-time of about 1 minute. Removal of the stimulus initiates a return to the basal state which occurs with a half-time of 5 minutes. The stimulus concentration which induces 50% of the maximal transition from R to D forms at steady-state is 10 nM, similar to the K_{50} for stimulation of cAMP production. There is little difference in the affinities for cAMP binding between the R and D forms. The pattern of modulation of the ligand-induced receptor modification suggests that it is associated with adaptation of the physiological responses. In the mutant FR17, cAMP does not induce receptor modification and cAMP production persists. Immunoprecipitation studies indicate that the transition from the R to D forms is associated with a increase in phosphorylation and that the D form contains about 4 mole/mole phosphate.

Western blots of membranes isolated at different times after starvation indicate that the appearance of cell surface cAMP binding sites during the aggregation stage of development (5-6 hours) is due to de novo synthesis of receptor protein. Pulse labeling with ^{35}S-methionine indicated that the receptor is most rapidly synthesized during the preaggregation stage of development (1-3 hours), prior to its maximal accumulation in membranes. The serum specifically immunoprecipitates a polypeptide of MW=37000 from an in vitro translation reaction using mRNA isolated from pre-aggregation stage cells but not mRNA from undifferentiated cells. The time course of expression of the mRNA coding for the MW=37000 polypeptide parallels that of the rate of in vivo receptor synthesis.

I020 THE PHOSPHATIDYLINOSITOL CYCLE AND CELL MOTILITY, Uno Lindberg and Ingrid Lassing, Stockholm University, S-10691 Stockholm, Sweden.

The activity seen in the cell surface in response to growth hormones and other external signals appear to reflect changes in the organization and activity of the microfilament (MF) weave associated with the inner face of the plasma membrane. Basic steps in this motile activity seem to be: 1) polymerization of unpolymerized actin to filaments, 2) cross-linking of these filaments to form the MF-weave, 3) translocation of the MF-assemblies and 4) depolymerization of the filaments to reform the unpolymerized precursor, which appears to be identical to profilactin (profilin:actin complex) abundant in many non-muscle cells. Profilin and profilactin in a buffer with 80mM KCl and 10^{-6}M Ca^{2+} interact specifically with polyphosphoinositides; PtdIns(4,5)P_2 being the most active. PtdIns(4,5)P_2 binds tightly to profilin and in the case of profilactin this causes the release of actin which then polymerizes; profilin remains bound to the phospholipid. Diolein and O-acetyl-diacylglycerol were inactive as were the zwitterionic phospholipids tested. These results together with the observed close correlation between activation of the phosphatidylinositol (PI)-cycle and induction of actin polymerization suggest a link between the PI-cycle and cell motility. Recent experiments on platelets support this hypothesis suggesting that generation of PtdIns(4,5)P_2 in the membrane recruits profilactin for actin polymerization, and indicate that there is no involvement of the products of PtdIns(4,5)P_2-hydrolysis (InsP_3 and diacylglycerol) nor Ca^{2+} in the induction of actin polymerization; these ligands instead appear to be linked to the regulation of subsequent steps in the cell motility cycle.

1021 POLYPHOSPHOINOSITIDE DEGRADATION AND ACTIVATION OF PHAGOCYTIC LEUKOCYTES BY CHEMOATTRACTANT RECEPTORS IS MEDIATED BY A NOVEL GUANINE NUCLEOTIDE REGULATORY (G) PROTEIN, Ralph Snyderman, Charles D. Smith, Ron Uhing, John Didsbury, A.P. Truett and Margrith W. Verghese, Howard Hughes Medical Institute and Division of Rheumatology and Immunology, Department of Medicine, Duke University Medical Center, Durham, N.C. 27710. Phagocytic leukocytes respond to chemotactic factors with readily quantifiable responses such as directed migration and the release of oxygen radicals and lysosomal enzymes. Receptors on membrane preparations from polymorphonuclear leukocytes (PMNs) and mononuclear phagocytes exist in high and low affinity states which are interconvertible by guanosine di- and triphosphates. This suggests that chemoattractant receptors interact with guanine nucleotide regulatory proteins (N or G proteins). However, chemoattractant receptors appear to utilize G proteins to regulate activation of phospholipase C rather than adenylate cyclase. Treatment of phagocytes with pertussis toxin (PT), which ADP-ribosylates and thereby inactivates certain G proteins, inhibits the activation of phospholipase C and abolishes the cells' responsiveness to chemoattractants. Moreover, chemoattractants require GTP to activate phospholipase C in PMN membranes and this GTP-dependent enzyme activation is absent in membranes prepared from PT-treated PMNs. Therefore, chemoattractant receptors utilize a PT-sensitive G protein to stimulate phospholipase C. This G protein, termed G_C, has a mwt. of ca. 40 kD and is a substrate for ADP-ribosylation by both PT and choleratoxin (CT). A cDNA clone potentially encoding G_C has been isolated. The deduced amino acid sequence of this clone is most similar to G_i but the 3' noncoding region is unique. Solubilization of membranes from myeloid HL60 cells yields a 40 kD CT substrate which elutes from heptylamine-sepharose prior to the major GTPγS binding protein (presumably G_i) as well as G_s. G_C is present in myeloid cells but not in lymphocytes, erythrocytes, or in brain. G protein activation by chemoattractant receptors initiates cellular responses by shifting the Ca^{2+} requirement for phospholipase C activation from supra-physiological levels to ambient cytosolic Ca^{2+} concentrations thereby allowing the hydrolysis of inositol 4,5-bisphosphate. Cleavage of this phospholipid results in the formation of Ca^{2+} mobilizing inositol 1,4,5-trisphosphate (IP_3) and the protein kinase C activator 1,2-diacylglycerol (DAG). Stimulation of the respiratory burst requires an additional step which leads to the prolonged accumulation of DAG without further IP_3 production. Activation of the secondary DAG response by formylpeptides appears to require extracellular calcium and is enhanced by cytochalasin B. The messengers involved in leukocyte activation also feed back to attenuate receptor stimulation of phospholipase C.

1022 BIOLOGICAL SIGNIFICANCES OF CYTOCALBINS (CYTOSKELETON-RELATED CALMODULIN-BINDING PROTEINS), Kenji Sobue Department of Neurochemistry and Neuropharmacology, Institute of Higher Nervous Activity, Osaka University Medical School, 4-3-57 Nakanoshima, Kita-ku, Osaka 530 Japan

The biological expression of all cells is governed by the signal transduction systems that translate and amplify external stimuli into the intracellular signals. It has been generally accepted that the Ca^{2+}-signal is one of the important intracellular messengers. Until recently, several proteins were discovered as intracellular mediators of the Ca^{2+}-signal in many biological processes.

Among them, calmodulin is a well-known Ca^{2+}-mediator. Our interests in this field are the molecular mechanism of signal transduction mediated by calmodulin. Several lines of evidence suggest that calmodulin activates the several intracellular enzymes in a Ca^{2+}-dependent manner. On the contrary, during the search for the targets of calmodulin besides the calmodulin-dependent enzymes, we have purified or identified more than ten species of calmodulin-binding proteins which also interact with cytoskeletal elements and regulate the cytoskeletal functions. These cytoskeletal elements are actin filaments and tubulin. We propose that these proteins are collectively termed "cytocalbin" (cytoskeleton-related calmodulin-binding protein). Cytocalbin includes caldesmon, calspectin (nonerythroid spectrin or fodrin), cytosynalin, MAP2, tau factor, 78/82K doublet protein and others.

We have most recently demonstrated that some cytocalbins involve in the formation and regulation of the submembranous cytoarchitectures, those are the main part of the signal transduction systems. Like erythrocyte spectrin, calspectrin is one of major submembranous constitutents and may play the control of cell surface receptor functions. Cytosynalin is a unique protein, which localizes with calspectin and also interacts with calspectin, tubulin and actin filaments. Therefore, there is a possibility that it will be a core protein in forming a network of cytoskeleton at the inner surface of cell membrane. Furthermore, we have purified the 78/82K calmodulin-binding protien which binds to calspectin and actin filaments.

In this symposium, I would like to demonstrate the involvement of cytocalbins in the regulation of the submembranous cytoarchitectures and in the biological processes.

Signalling During Motile Events - II. Control of Cell Function

1023 FLAGELLAR MOTILITY: REGULATION BY CALCIUM AND PHOSPHORYLATION, Charles J. Brokaw, Division of Biology, California Institute of Technology, Pasadena CA 91125

Study of the regulation of flagellar motility by phosphorylation has been led by investigations on spermatozoa. Demembranated sperm flagella are typically either totally (trout, *Ciona*) or partially (dog, sea urchin) non-motile unless they are activated *in vivo* before demembranation, or *in vitro* by incubation with cAMP after demembranation. Both *in vivo* and *in vitro* activation of *Ciona* and *Lytechinus* spermatozoa can be reversed by incubation of demembranated spermatozoa with protein phosphatase, or by including millimolar Li in the reactivation solutions. Li and phosphorylation both appear to regulate the flagellar oscillator, rather than the active sliding process. In many cases, *in vivo* activation involves increases in internal pH, which are beginning to be well understood, and increases in internal [cAMP]. The signal pathway leading to [cAMP] increase is still obscure.

Calcium has been found to be a regulator of motility in almost every system where reactivation of demembranated flagella has been tested at both high (>>1 μM) and low (<<1 μM) [Ca^{++}], and it is assumed that a similar regulation by calcium ion occurs *in vivo*. In *Paramecium* thigmotaxis, a nearly complete story involving mechanoreceptor membrane depolarization, electrical excitation of calcium channels in the ciliary membrane, and increased internal calcium in the cilia, has been available from the work of Eckert and others for several years. At the other end of the scale, the chemotactic response of sea urchin spermatozoa to an egg jelly peptide has been shown to be calcium-dependent, but the events linking the peptide receptor to properly-timed changes in internal calcium concentration have not been identified. In all of these cases, the mechanism by which calcium alters the motility of the axoneme remains unknown. In many cases, calcium alteration of motility can be equally well described in terms of changes in dynein-mediated active sliding, or in terms of an independent, calcium-driven, change in flagellar configuration, upon which normal dynein-mediated sliding is superimposed. A role for calmodulin has been indicated, especially in cases where addition of exogenous calmodulin alters motility, but in sea urchin sperm flagella, the relationship between bending pattern and calcium ion concentration suggests that another, more sensitive, mediator may be involved in addition to calmodulin.

(My work is supported by NIH grant GM 18711.)

1024 CALCIUM CHANNELS AND AMOEBOID CELL MOVEMENT, Mark S. Cooper and Manfred Schliwa, Department of Zoology, University of California, Berkeley, CA 94720.

To examine the relationship between Ca-channels and directional amoeboid cell locomotion, we investigated the effects of Ca-channel antagonists, Ca-ionophore and electric fields on the motility of the fish epidermal cell, or keratocyte. In situ, keratocyte migration is involved in the rapid closure of epidermal wounds. When placed in culture, these cells migrate at rates up to 40 μm/min, extending broad lamellipodia which are composed of a pervasive actomyosin network. The microtubules of migratory keratocytes are wrapped around the cell nucleus. Disassembly of these cytoskeletal elements with nocadozole and cold treatments has no effect on cell polarity or motility. When keratocytes are placed in a constant DC electric field of 1-15 V/cm, the cells migrate directionally toward the negative, depolarizing electrode (cathode). When 10 mM Co^{2+} (a Ca-channel blocker) is added to the medium, the cells become paralyzed within seconds. These paralyzed cells can be reactivated by the addition of 5-20 μM A23187 to the Co^{2+}-containing medium, indicating that a Ca^{2+} influx is required for spontaneous locomotion. These reactivated cells, however, reverse their direction of migration in the electric field, migrating instead toward the positive electrode. Cells treated with only A23187 do not reverse their direction of migration. The ability to reverse the polarity of migratory keratocytes by selectively blocking the cells' Ca-channels strongly suggests that these channels provide the dominant polarity cue for cathode-directed migration in the electric field. Since many Ca-channels are voltage-sensitive, the external electric field probably induces a gradient of Ca^{2+} influx across the keratocyte, with enhanced influx occuring on its cathode-facing (depolarized) side. A23187 is a mobile, lipophilic, negatively charged molecule. When keratocytes are exposed to an external electric field, A23187 on the upper surface of the cell membrane will be subjected to an electrophoretic force pulling it toward the anode-facing side (positive) side of the cell. A secondary gradient of Ca^{2+} influx, established by an asymmetric distribution of ionophore, could explain the reversed, anode-directed cells migrations once the Ca-channels of keratocytes are blocked with Co^{2+}.

1025 ACTIN-MEMBRANE INTERACTIONS AND THEIR REGULATION OF PLATELET FUNCTION, Joan E. B. Fox and Janet K. Boyles, Gladstone Foundation Laboratories, P.O. Box 40608, San Francisco, California 94140, USA.

Platelets are rich in cytoskeletal proteins that are involved in the cell's contractile responses. Approximately 50% of the platelet actin is polymerized into filaments, most of which are in the cytoplasm. However, a small pool of the actin filaments is found in a distinct structure that is linked to glycoprotein (GP) Ib on the plasma membrane. The GP Ib-filament interaction is mediated by actin-binding protein. This report describes the composition and organization of the membrane-associated filamentous structures and shows how the interaction of GP Ib with the actin filaments affects GP Ib function. Membrane-associated actin was isolated by centrifugation from platelets lysed with Triton X-100 in the presence of Ca^{2+}. This method depolymerizes the actin filaments of the cytoskeleton but not those associated with the membrane. SDS-polyacrylamide gels of the isolated material showed that three of the major components were actin, actin-binding protein, and GP Ib. Platelets also contain a small amount of spectrin, all of which was associated with the membrane-associated actin. Visualized by electron microscopy, the membrane-associated structures isolated from unstimulated discoid platelets had the appearance of empty shells, composed of amorphous material, that retained the outline of the platelet. The membrane-associated structures isolated from abnormally shaped platelets retained the abnormal outline of these platelets. By analogy to the red blood cell, these results suggest that the membrane-associated actin in platelets functions as a skeleton regulating the shape of the cell. The membrane-associated actin was in a filamentous form, as shown by its depolymerization by DNase I. However, as with the erythrocyte's membrane skeleton, actin filaments could not be visualized by thin-section electron microscopy, suggesting that the filaments may be only a few monomers long. To investigate additional functions of the membrane-associated actin, the linkage between the membrane skeleton and GP Ib was disrupted by exposing platelets to agents such as ionophore A23187 or dibucaine. This exposure activates the Ca^{2+}-dependent protease, hydrolyzing actin-binding protein, and thus releasing GP Ib from the membrane skeleton. The release of GP Ib resulted in the budding off of small vesicles from the plasma membrane, and correlated with a decreased ability of GP Ib to bind von Willebrand factor. The loss of GP Ib function resulted from activity of the Ca^{2+}-dependent protease, as indicated by the ability of leupeptin to inhibit this loss. We conclude that platelets contain a membrane skeleton that is distinct from the cytoskeleton and is composed primarily of actin and actin-binding protein linked to GP Ib on the plasma membrane. This membrane skeleton maintains platelet shape, stabilizes the membrane against fragmentation, and regulates the function of GP Ib.

Signalling During Motile Events - III. Amoeboid Chemotaxis

1026 *RAS* GENE FUNCTION AND EXPRESSION IN *DICTYOSTELIUM*, Richard A. Firtel, Center for Molecular Genetics, Dept. of Biology, UCSD, La Jolla, CA 92093

As in many of the eucaryotic systems, signal transduction mechanisms play a central role in regulating cellular processes. In *Dictyostelium*, folic acid is a chemoattractant which is used by vegetative and early developing cells to identify food sources. During development itself, cAMP acts as a chemoattractant mediating aggregation to form the multicellular organism. Both of these chemoattractants mediate their responses through cell-surface receptors. In addition cAMP/cAMP cell-surface signal transduction systems play central roles in regulating gene expression and cellular differentiation in this organism. During the preaggregation-aggregation stages, the stages prior to and during the formation the multicellular aggregate, pulses of cAMP mimicking those which produce normally during aggregation down-regulate the expression of a number of very early genes and induce the expression of other gene sets. During the multicellular stage, cAMP is one of the signals responsible for controlling the expression of prestalk and prespore cell-type specific genes. Interestingly, the regulation of these different classes of genes all appear to be mediated via the extracellular cell-surface receptor and do not require the rise in intracellular cAMP. *Dictyostelium* contains a single *ras* gene (*Dd-ras*) whose expression is developmentally controlled. It is expressed in vegetative cells and is apparently required for growth. The *ras* expression is turned off at the onset of development but is reinduced in a cell-type-specific manner coordinate with other prestalk-specific genes and is modulated by cAMP through the cAMP receptor. To examine the function of the *ras* gene in *Dictyostelium*, we transfected an over-expressed wild-type *Dd-ras*-Gly_{12} gene construct and a missense mutation, *Dd-ras*-Thr_{12}, in transformants. Transformants overexpressing the *ras*-Thr_{12} gene showed abnormal development under several conditions. In a submerged aggregation assay the cells did not stream. When cells are plated on an air-buffer interphase the cells aggregated. However, they formed aggregates containing multiple tips and/or formed aggregates which are appreciably smaller than either the parental or the *ras*-Gly_{12} transformed cells and which do not continue to differentiate. Results suggest an effect on signal transduction that is possibly mediated via the cell-surface receptors. Activation of the cAMP receptor by cAMP during aggregation results in a transient activation of guanylate cyclase. In association with the laboratories of Christophe Reymond, Peter Devreotes, Peter Van Haastert, and Roul Van Driel, we have been examining the effect of *ras* mutation on signaling in *Dictyostelium*. Experiments using a chemotactic assay showed that the *ras*-Thr_{12} transformants showed reduced and delayed chemotaxis toward either component. Analysis of the activation of adenylate cyclase and the cAMP response and its adaptation were shown to be normal in both types of transformed cells. Examination of the guanylate cyclase response of transient activation of guanylate cyclase both in response to cAMP and to folic acid showed that during the initial 5-7 seconds of the activation of guanylate cyclase the response is normal but that the response then terminates resulting in approximately a 1/3 reduction in the level of cGMP produced. These results suggested a precocious adaptation of the activation of guanylate cyclase in response to both folic acid and cAMP. The possibility that adaptation was altered in these cells was examined further and the results supported this hypothesis. Newell and collaborators have shown that the guanylate cyclase response can be activated in permeabilized cells using IP_3 or Ca^{++}. We are presently examining the effect of a number of other activated *ras* genes on signaling in *Dictyostelium* and the effect of the mutations on inositol phosphate metabolism.

1027 STIMULATION OF ACTIN NUCLEATION BY CHEMOTACTIC FACTORS IN VITRO, Sally H. Zigmond, Monica Carson, and Mary W. Wilde, Biology Dept. Univ. of PA, Phila. PA 19104. Addition of the chemotactic factors, fNleLeuPhe or C5a, to a crude membrane fraction isolated from PMNs increases the rate of polymerization of exogenously added pyrene actin. Since the actin concentration is controlled by the exogenously added actin, the increased rate comes from an increase in the number actin polymerization sites, or actin nuclei. The stimulated rate is inhibited by cytochalsin D suggesting that the filaments are growing from the high affinty, "barbed end". The stimulation by fNleLeuPhe seems to require a "G" protein. Thus the peptide stimulation requires the presence of GTP; neither GDP nor ATP can substitute. In the absence of chemotactic factors, GTP-γ-S can increase actin nucleation on the membrane fraction. Neither fNleLeuPhe or the GTP-γ-S can stimulate nucleation on a membrane fraction prepared from cells treated with *Bordetella pertussis* toxin, islet activating protein. Activation of phospholipase C does not seem essential for the stimulation of nuclei. Neomycin, which inhibits phosphatidyl inositol turnover, does not block the stimulation of nucleation. An increase in barbed-end actin nucleation sites could contribute to the increase in the proportion of polymerized actin and determine the site of actin growth.

Mechanochemical Force Transduction and Translocation

1028 STRUCTURE-FUNCTION STUDIES ON THE ACANTHAMOEBA MYOSIN ISOENZYMES, Edward D. Korn, Mark A.L. Atkinson, Hanna Brzeska, John A. Hammer III, Goeh Jung and Thomas J. Lynch, Laboratory of Cell Biology, NHLBI, NIH, Bethesda, MD 20892.

Three different myosin isoenzymes have been purified to homogeneity from Acanthamoeba castellanii. Myosin II contains a pair of heavy chains of 175 kDa and two pairs of light chains of 17.5 and 17 kDa. The amino acid sequence of the heavy chain deduced from the genomic DNA sequence shows a high degree of similarity in the globular head with vertebrate and invertebrate muscle myosins and a tail sequence predicting a helical, rod-like tail typical of other myosins. Sequence analysis and electron microscopy reveals a hinge about 35 nm from the C-terminus. The 30-residue C-terminal, non-helical tailpiece of each heavy chain contains 3 phosphorylatable serines and myosin II expresses actin-activated ATPase activity only when these sites are not phosphorylated. Transient electric birefringence studies indicate that monomers form parallel dimers with heads staggered by 30 nm. These could assemble into filaments containing 16 monomers with heads staggered by 15 nm. The phosphorylation state could regulate enzymatic activity either by inducing transitions in the secondary structure of the non-helical tailpiece, or by direct interactions between the phosphorylation sites and the hinge region, either of which could affect filament conformation and the flexibility of the hinge.

Myosin IA and IB are the first examples of a new class of myosins. They contain one heavy chain (130 or 125 kDa) and one light chain (17 or 27 kDa). Neither forms filaments and both require heavy chain phosphorylation for actin-activated ATPase activity. The deduced amino acid sequence of IB heavy chain shows 55% identity of sequence with muscle myosins in the globular head but a C-terminal 35-kDa that is greatly enriched in glycine, proline and alanine and clearly cannot form a coiled-coil rod. Myosin IA has been cleaved proteolytically into a 100-kDa N-terminal peptide that contains the catalytic, actin-binding and phosphorylation sites, and the associated light chain and retains full actin-activated ATPase activity, and a 30-kDa C-terminal peptide that has no catalytic activity but contains a high-affinity, ATP-insensitive actin-binding site. Controlled proteolysis of myosin IB heavy chain produces an N-terminal 80-kDa peptide with full, regulated actin-activated ATPase activity and suggests that the C-terminal 45-kDa region contains a second actin-binding site. Myosin IB heavy chain can be further degraded to a 60-kDa fragment that contains the catalytic, actin-binding and phosphorylation sites but has lost actin-activated ATPase activity and studies with myosin IA suggest that integrity of a region about 38 kDa from the N-terminal of the heavy chain is required for actin-activated ATPase activity. The existence of two actin-binding sites in the heavy chain (one ATP-insensitive) allows myosin I to cross-link actin filaments and support contractile activity although it lacks a tail and an S-2 region.

1029 SWITCHING MECHANISMS IN THE CONTROL OF DYNEIN ACTIVITY IN PARAMECIUM CILIA

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The swimming behavior of the ciliated protozoan Paramecium tetraurelia depends on the opening of voltage-sensitive Ca^{2+} channels in the ciliary membrane and subsequently on changes in intracellular Ca^{2+} (pCa) which induce changes in the direction of the effective stroke of the cilia. Recently, cyclic nucleotides (cAMP and cGMP) have been recognized as additional control factors that regulate swimming speed of the cell and frequency of ciliary beat. It has been suggested that such control factors may work via changes in protein phosphorylation of axonemal proteins that influence the switching on and off of dynein arm activity in the cilia, and hence determine which microtubules are actively sliding at which times during a beat. The final stages of this signal transduction process are retained in detergent treated permeabilized cells, where the cell, ciliary and outer alveolar membranes are greatly disrupted or missing entirely. Permeabilized cells with demembranated cilia swim forward at pCa7 and backwards at pCa5 when ATP and Mg^{2+} are present in the reactivation medium. Several lines of evidence suggest that calmodulin (CaM) is the axonemal Ca^{2+} sensor, and immunogold localization shows that CaM is retained in the axonemes after permeabilization. To identify which phosphoproteins may be important in the control of ciliary activity, axonemes have been prepared from paramecium and labelled with γ-^{32}P-ATP in the presence or absence of cAMP at various pCa's. In 1D SDS-PAGE, more than 20 polypeptide bands incorporate label at pCa~8 in the absence of cAMP. In the presence of 5μM cAMP, labelling in two polypeptides (Mr 65 kDa and 29 kDa) is remarkably enhanced. Ca^{2+} inhibits cAMP dependent phosphorylation of the 29 kDa polypeptide, but does not cause dephosphorylation. Labelling of the 65 kDa polypeptide is unaffected by pCa. The 29 kDa polypeptide is detached from axonemes in high salt incubation, rebinds to microtubules under conditions where dynein arms rebind and copurifies with dynein heavy and light chains in an alloaffinity release procedure with 1mM ATP. These features indicate that this polypeptide is a candidate for a role in the switching mechanism of dynein arm activity.

Signal Transduction in Cytoplasmic Organization and Cell Motility

1030 ORGANELLE TRANSPORT USING MICROTUBULE-BASED MOTORS, Ronald D. Vale, Cell Biology Program, Dept. of Pharmacology, University of California, San Francisco, CA 94143.
Although a great deal has been learned about the molecular basis of myosin or dynein-based movement in muscle or ciliated cells, very little is known about the mechanism of organelle transport and other forms of microtubule-based motility that are ubiquitous to all cells. Our ignorance of such processes stems in part from the fact that the molecules that are involved in generating or regulating such movement are present in smaller quantitites and because of the difficulties of using intact cells as experimental systems. However, within the last couple of years, it has been possible to reconstitute the movement of isolated squid axoplasmic organelles along purified microtubules, thereby opening up new possibilities for experimentally examining this process. Organelle movement in this in vitro system is enhanced by a soluble fraction from axoplasm, and suprisingly this soluble fraction also induces movement of latex beads along microtubules and microtubules along a glass coverslip. This result suggested that axoplasmic transport of organelles may be driven by a soluble motor that reversibly associates with organelles (possibly via a membrane receptor) or latex beads and coverslips (via a surface charge interaction). Using microtubule movement on glass as an assay for fractionation, the soluble motor (named kinesin) was purified. Purified kinesin, however, only supports movement of beads towards the plus end of the microtubule, and organelles travel in both directions along microtubules in native axoplasm. Such bidirectional movement indicates a fundamental difference between vesicle transport and muscle contraction, where myosin generates unidirectional movement along actin filaments, or ciliary motility, where dynein generates unidirectional movement along microtubule outer doublets. It was subsequently discovered that bidirectional movement of latex beads on centrosomal microtubules could be reconstituted using a crude supernatant fraction from axoplasm. The motor that induces this movement is distinct from kinesin as determined by its greater sensitivity to NEM and vanadate, its greater velocity of movement and the fact that it does not react with a monoclonal antibody against kinesin. These results suggest that axoplasm contains two distinct polarity specific motors. Since vesicles appear to be selective in binding of one motor versus the other, microtubule motors could play an important role in sorting organelles to different domains of the cell and in positioning large organelles in cytoplasm.

Integration of Signalling and Force Transduction During Embryogenesis

1031 CELL RECOGNITION DURING NEURONAL DEVELOPMENT IN DROSOPHILA: EXPRESSION AND MOLECULAR GENETICS OF THE FASCICLIN III GLYCOPROTEIN. Corey S. Goodman, Nipam H. Patel, and Peter M. Snow, Department of Biological Sciences, Stanford University, Stanford CA 94305.
During neuronal development, growth cones display a selective affinity for specific neuronal cell bodies and axons. The 7G10 monoclonal antibody (MAb) recognizes a surface antigen expressed on a subset of neuronal cell bodies and axon fascicles in the Drosophila embryo, and immunoprecipitates 4 highly related surface glycoproteins called fasciclin III. We isolated the fasciclin III gene by cDNA expression cloning using antisera raised against each of these related proteins; the gene maps on the 2nd chromosome to 36E1. Df(2L)H20/Df(2L)VA18 trans-heterozygote embryos are deficient for the fasciclin III gene and only a few other genes. Such embryos have a quite normal central nervous system at 12 hr, with the exception of subtle yet consistent abnormalities in the axon pathways of a small number of neurons. At the level of interactions between the growth cones, axons, and cell bodies of identified embryonic neurons which normally express fasciclin III, the phenotype of this small deficiency is consistent with the notion that fasciclin III is a neuronal recognition molecule which either modulates or mediates cell adhesion in the Drosophila embryo.

DEVELOPMENT OF NEURONAL POLARITY BY VECTORIAL GROWTH, Karl H. Pfenninger,
1032 UCSM, Dept. of Cellular & Structural Biology, Denver Colorado 80262.

After terminal mitosis, neurons develop a high degree of polarity by directed outgrowth of axons and dendrites. Neurite growth seems to occur by the distal insertion of new components of the plasmalemma and, probably, of cytoskeletal elements. The membrane components added at the growing tip, the nerve growth cone, are different from those present in other areas of the developing or the mature neuron. Thus, a unique membrane domain is established at the distal neurite; it is characterized by a low number of intramembrane particles, distinctive sets of glycoproteins and glycolipids, and the enrichment of growth-regulated proteins such as pp46/GAP 43 and the N-CAM-like 5B4 antigen (1). It is believed that these components serve the special functional needs of the nerve growth cone.

In the vertebrate peripheral nervous system neurite growth is known to be controlled, at least in part, by nerve growth factor (NGF). NGF is known to exert both trophic and chemotactic influences on the developing neurite, and the early signal transduction processes are known to occur in the distal neurite rather than the perikaryon. However, the mode of signal transduction for NGF is not understood. Therefore, the study of second-messenger systems in nerve growth cones is of great interest. Nerve growth cone fragments isolated from the fetal central nervous system contain a simple set of major phosphoproteins: pp46, a major membrane protein and the substrate of both a calcium/calmodulin-dependent kinase and protein kinase C; and pp80 and pp40, further substrates of protein kinase C. In view of the prevalence of protein kinase C activity it was not surprising to find a high level of phosphatidylinositol (PI) phosphorylation and turnover even under baseline conditions. Fetal brain contains a small, apparently novel polypeptide which stimulates dramatically inositol trisphosphate release from growth cones (2). While the influence of this factor on growth cone function is not yet known it is highly likely to regulate growth cone advancement and, possibly, chemotaxis. If this can be confirmed, growth regulation of the neurite resembles the regulation of leukocyte chemotaxis.

1) Pfenninger, K.H. (1986) in: R.K. Hunt (Ed.) Current Topics in Developmental Biology, Vol. 21, in press.
2) Pfenninger, K.H., Hyman, C. and Garofalo, R.S. (1986) in: W.H. Gispen and A. Routtenberg (Eds.) Progress in Brain Research, Vol. 69, Chapter 19, in press.
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Assembly of the Cytomatrix

I 100 **CALMODULIN AND ITS INFLUENCE ON THE RED BLOOD CELL CYTOSKELETON.** Lars Backman, Mats Strömqvist, Åsa Berglund & Vithaldas P. Shanbhag, Department of Biochemistry, University of Umeå, S-90187 Umeå, Sweden

Calmodulin interacts with components of the red blood cell cytoskeleton as well as with the membrane itself. Under physiological salt conditions, calmodulin has a calcium-dependent affinity for purified spectrin and isolated cytoskeletons. Since the affinity of calmodulin for these sites are similar and the number of sites for calmodulin in the cytoskeleton corresponds to the concentration of spectrin it appears possible that spectrin constitutes the major binding site for calmodulin in the intact cytoskeleton.

Although the concentration of calmodulin is high in the red cell (2-3 μM), the only function established is the activation of the membrane (Ca^{2+}+Mg^{2+})-ATPase. As nanomolar calmodulin saturates this enzyme, the bulk of calmodulin should be available for other functions.

Despite the relatively weak binding to spectrin (~10 μM), calmodulin may occupy a substantial fraction of available binding sites on the cytoskeleton due to the high spectrin concentration close to the membrane. Thus it seems rational to assume that any conceivable function of calmodulin (besides the activation of the calcium pump) would involve the membrane skeletal complex. In view of these considerations we have investigated whether binding of calmodulin to spectrin has any influence on the membrane cytoskeleton and its organization.

I 101 ABBERANT BRUSH BORDER ASSEMBLY IN HUMAN COLONIC NEOPLASMS. Kenneth W. Barwick, Brian West, Joan Carboni, Catherine Isaacs, Jon S. Morrow and Mark S. Mooseker, Yale University, New Haven, CT. 06510.

Villin and 110K-CM are differentiation specfic proteins of the brush border of the intestinal epithelial cell. In avians, these proteins assemble near the apical membrane at different times during embryonic development, villin localizing early and exclusively at the luminal surface, while 110K-CM only assembles at this location after hatching. Monoclonal and polyclonal antibodies prepared to villin or 110K-CM have been used by indirect immunoperoxidase and immunofluorescent technique to examine the assembly of these proteins in frozen sections of 10 normal human colons and in 19 human colonic neoplasms. We find that villin partitions to the luminal surface in most neoplasms, even those with scant microvilli. However, in higher grade carcinomas, the luminal distribution of villin becomes patchy, there is increased cytoplasmic villin, and some becomes abnormally organized at the basolateral surface. We conclude that the mechanisms directing polarized assembly of the brush border are altered in certain high-grade adenocarcinomas.

I 102 NUCLEOTIDE EFFECTS IN MICROTUBULE NUCLEATION IN VITRO. Peter Bayley, Felicity Butler, Paula Karecla, Stephen Martin & Jun Mei Zhou. National Institute for Medical Research, Mill Hill, London NW7 1AA.

The rate of assembly of tubulin dimer (bovine brain) at 37°C (0.1M Pipes, pH 6.5 plus 1M glycerol) shows only a small dependence on [GTP] up to 1 mM; by contrast, the nucleation of microtubule protein is strongly dependent on [GTP], as shown by assembly rate and mean length measurements. The addition of pyrophosphate ion (1mM) removes this dependence, and produces short MTs (mean length 5u) even with [GTP]=15uM. Likewise, short MTs are formed at [GTP]=15uM in the presence of a GTP regenerating system (acetyl phosphate + acetate kinase). The low rate and extent of assembly at low GTP is unaffected by GTP added after the initial part of the growth phase. These results show that it is the nucleation phase of microtubule assembly which is extremely sensitive to nucleotide conditions, particularly the presence of GDP, and that post- nucleation, the elongation rate (as with dimer assembly) is not markedly sensitive to the absolute [GTP] concentration. We deduce that GDP can suppress spontaneous nucleation of microtubule protein, even in the presence of excess GTP, a process with potential for regulating microtubule assembly.

I103 INVOLVEMENT OF LIPIDS IN THE REVERSIBLE INTERACTION OF CYTOSKELETAL PROTEINS WITH MEMBRANES.

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The simplest models for cytoskeleton-membrane associations propose interactions of microfilaments with specific integral membrane proteins via cytoplasmic linker proteins. The cytoskeletal proteins α-actinin, vinculin and talin have been implicated to mediate these interactions. Recent results showed an involvement of α-actinin, fatty acids and the second messenger diacylglyerol in the regulation of cytoskeleton-membrane interactions in blood platelets (P. Burn et al., Nature (1985), 314, 469). A covalent binding of lipid to vinculin, which is reduced in virally transformed cells has also been shown to occur (P. Burn and M.M. Burger, Science (1986), submitted). Even though α-actinin and vinculin are not thought to be integral membrane proteins they may belong to a separate class of membrane proteins that reversibly interact with lipids and membranes. An altered interaction of these proteins with lipids may alter their function, leading to a change in their association with membranes. Thus, the interaction of these proteins with specific lipids (covalently or noncovalently bound) could be a powerful mechanism involved in the regulation of cytoskeleton-membrane interactions, leading to cytoskeletal rearrangements as observed during cell shape changes and transformation.

I104 THE EXPRESSION OF A SPECIFIC TROPOMYOSIN ISOFORM LEADS TO THE RESTORATION OF MICROFILAMENT BUNDLES IN TRANSFORMED CELLS

Steve Cheley, Jeremy C. Lamb, James R. Feramisco, Yuriko Yamawaki-Kataoka and David M. Helfman. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

We have used established rat embryonic fibroblasts (REF-52 cells) and their adenovirus type 5-transformed counterparts (Ad5D.4A cells) to examine the role of major tropomyosin isoforms in cytoskeleton organization and cell shape. Normal cultured rat embryonic fibroblasts are well spread while the transformed fibroblasts are rounded, adhere poorly to culture substratum and lack actin microfilament bundles or "stress fibers". REF-52 cells contain five distinct tropomyosin (TM) isoforms. One of these isoforms, TM-1, has been shown to be absent from the transformed Ad5D.4A cells. We therefore examined the effect of forced expression of TM-1 on cytoskeleton organization and cell shape in these transformed fibroblasts. Microinjection of cloned SP6-derived TM-1 mRNA into transformed cells lacking TM-1 mRNA led to cell spreading and concomitant restoration of actin stress fibers characteristic of normal cultured fibroblasts. These effects were blocked by pre-treatment of cells with cyclohexamide. In addition, cell spreading and formation of the microfilament bundles was not seen after microinjection of two other major fibroblast tropomyosin isoform mRNAs present in Ad5D.4A cells (TM-2 and TM-4). Our results support the idea that selective loss of a major actin-binding tropomyosin isoform may be responsible for the changes in cell shape and microfilament organization that accompany transformation.

I105 PROTEOLYTIC ACTIVATION OF CALCIUM-DEPENDENT PROTEASES.

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Many proteins, including talin (P235), filamin (ABP), C-kinase, spectrin, 4.1, the EGF-receptor and fodrin, are susceptible to cleavage by the ubiquitous, intracellular, calcium-dependent proteases CDP-1 and CDP-2. With the exception of proteolysis of P235 and ABP during platelet activation, the physiological regulation of these calcium-dependent proteases and functional consequences of their activity are unknown. We have recently shown that CDP-2 exists as an inactive pro-enzyme which requires autoproteolytic cleavage of its small subunit (26kDa→24kDa) for enzyme activation in vitro (J Biol Chem 261, 12047). A similar mechanism appears to regulate CDP-I in vitro but the large subunit is also cleaved (84kDa→78kDa). This proteolytic processing can be identified and monitored in cellular extracts by immunoblot analysis and thereby provides an assay for the activation of the proteases in response to physiological stimuli in vivo. We have used CDP-loaded erythrocyte ghosts to demonstrate cleavage of spectrin and 4.1 (Biochim Biophys Acta 882, 287) and concomitant activation of the proteases by autoproteolysis. Analysis of the activation of CDPs in various cells in response to hormones and effectors which utilize calcium as part of their signal transduction mechanism will allow us to identify physiological regulators of CDP-activity. Subsequently we will try to correlate proteolysis of individual proteins with CDP-activation.

I106 ASSOCIATION OF A BAND 3-LIKE ANION EXCHANGER WITH THE SPECTRIN-BASED MEMBRANE CYTOSKELETON AT THE BASOLATERAL MEMBRANE OF THE DARK CELLS IN THE TURTLE URINARY BLADDER, D. Drenckhahn, M. Oelmann, S. Wagner, M. Schaaf and M. Wagner, University of Marburg, D-3550 Marburg, FRG.

The turtle urinary bladder serves as model for collecting duct functions in the mammalian kidney. Both the epithelium of the turtle bladder and of the mammalian collecting duct can generate a 1:1000 gradient for H^+ ions between blood and urine. Secretion of H^+ into the urine is coupled to a basolateral efflux of HCO_3^- that appears to be mainly exchanged against Cl^-. Here we show that about 80 % of the dark cells of the bladder contain a Mr = 110,000 analog of the turtle erythrocyte anion exchanger, band 3. The band 3 analog is confined to the basolateral cell surface and is absent from the apical membrane. The bladder band 3 protein is colocalized with actin and isoforms of ankyrin (Mr~200,000) and spectrin (Mr~230,000) along the basolateral membrane. Linkage of band 3 via ankyrin to the spectrin-actin lattice may restrict this anion exchanger to the basolateral membrane surface. In view of our previous observation of a band 3-like anion exchanger in the collecting duct epithelium of the rat kidney these findings point to a common molecular basis for acid-base transport in the mammalian collecting duct and the reptilian urinary bladder.

I107 PROTEOLYTIC DOMAIN STRUCTURE OF HUMAN BRAIN SPECTRIN (240/235) Alan S. Harris and Jon S. Morrow, Yale University School of Medicine, New Haven, CT 06510.

The complete domain structure of human brain spectrin (240,235) has been determined by limited proteolytic digestion and 2-D cellulose mapping. The alpha (240 kD) subunit is defined as four domains. The alpha I and alpha III domains, 33 kD and 24 kD respectively, are relatively sensitive to proteolytic digestion, whereas alpha II (80kD) and alpha IV (108 kD) are both found in significant abundance after 24 hours of digestion at O C with equimolar quantities of trypsin. The alpha subunit also contains a centrally located site that is exquisitely sensitive to the action of many proteases including calcium activated neutral proteases, trypsin, chymotrypsin and V-8 protease. This cleavage results in the production of two peptides of apparent molecular weights of 145 and 150 kD and which migrate anomolously on SDS gels. 2-D cellulose peptide mapping, IEF/SDS-PAGE and kinetic data demonstrate that these two peptides are complimentary halves of the alpha subunit. The beta subunit is comprised of four domains as defined by limited tryptic digestion ranging in size from 27 to 78 kD. Generally, the beta subunit is more sensitive than the alpha subunit to most proteases tested, with the exception of the 78 kD beta IV domain. The establishment of a domain map of each of the subunits of brain spectrin, and their orientation to each other will serve as a guide in refining the functional topography of this molecule. Supported by NIH grant GM 07562-09 and by the March of Dimes Foundation.

I108 CYTOSKELETAL ASSEMBLY IN CULTURED CHICKEN CELLS, W.B. Isaacs, R.K. Cook, and A.B. Fulton, Univ. of Iowa, Iowa City, IA 52242.

We are investigating two related questions pertaining to cytoskeletal assembly: 1) do nascent cytoskeletal proteins associate (assemble) directly with the cytoskeleton (csk) during translation, i.e. are csk proteins cotranslationally assembled?; and 2) how fast do newly completed csk proteins assemble? We have used pulse-chase labeling, cell fractionation, and immunoprecipitation to address these questions for a number of different proteins. In cultures of developing embryonic skeletal muscle, one-third of all nascent myosin heavy chains (mhc's) are associated with the csk via an RNase and/or puromycin resistant linkage indicating a direct interaction of the mhc's with the cytoskeleton before completion. The remaining two-thirds of the mhc's assemble rapidly upon completion (t1/2 = approx. 1 min). Similar results have been obtained for the assembly of vimentin. This fast, partially cotranslational mode of assembly is not the only mode of assembly for csk proteins, however. For example, newly synthesized alpha actinin is assembled much more slowly, primarily from a soluble precursor pool. Furthermore, the rate of assembly of a given protein varies with cell type: we observe a much slower assembly of vimentin in embryonic erythroid cells than in either muscle or fibroblasts. Thus, assembly is not solely a function of the physicochemical properties of a particular protein but also the cellular milieu.

I109 A NEURONAL PROTEIN (NP185) BINDS VESICULAR TUBULIN AND THE CLATHRIN COATED VESICLE ASSEMBLY COMPLEX, D. Stave Kohtz and Saul Puszkin, Mt. Sinai School of Medicine, CUNY, New York, NY, 10028.
Neurons use clathrin coated vesicles (CCVs) to mediate processes that do not occur in other cell types; therefore, CCVs isolated from brain would be expected to incorporate unique polypeptides that allow them to mediate these specialized functions. In the course of developing monoclonal antibodies (mAbs) to other brain CCV antigens, two mAbs were developed (S-6G7 and S-8G8) that reacted with a 185 kD neuronal vesicle polypeptide (NP185) that is abundant in brain CCVs, scant in synaptic vesicles, and absent from the plasma membrane. NP185 was found to be distinct from clathrin, the 180kD coat structural protein, by several criteria. Immunofluorescence analysis with mAbs S-8G8 and S-6G7 using both cultured cells and tissue sections revealed reactivity only in tissues of neuronal origin. S-8G8 and S-6G7 do not react with purified preparations of clathrin from either brain or adrenal gland, and NP185 immunoprecipitated by these mAbs does not contain clathrin light chains. Immunoblots of two dimensional gels using S-8G8 and S-6G7 and an anti-clathrin mAb also distinguish NP185 from clathrin. Similarly to the clathrin light chains, NP185 can be chymotryptically removed from the CCV without affecting the clathrin cage structure. NP185 can be extracted from the CCV by 1M NaCl but not by .5% Triton X-100, indicating that it is associated with the vesicle structure, but it is not a transmembrane cargo protein. When 1M NaCl CCV extracts are diluted and immunoprecipitated by S-8G8 or S-6G7, NP185 precipitates as a complex with a fraction of the CCV asssembly complex. Interestingly, immunoprecipitation experiments have revealed that NP185 and the assembly complex can be coprecipitated with tubulin by anti-tubulin mAbs when tubulin is added to the diluted 1M NaCl extracts. Further experiments have indicated that NP185 can simultaneously bind both tubulin and the assembly complex, and that its interaction with the CCV is mediated by these polypeptides.

I110 IMMUNOCHEMICAL CHARACTERIZATION OF A BAND 3-LIKE ANION EXCHANGER IN THE COLLECTING DUCT OF THE HUMAN KIDNEY, R. Koob, R. Vogel, R. Lietzke, S. Wagner and D. Drenckhahn, Department of Anatomy and Cell Biology, University of Marburg, Robert-Koch-Str. 6, D-3550 Marburg, FRG.
Poly- and monoclonal antibodies have been prepared against the cytoplasmic domain (43 kDa) and the 17-kDa and 35-kDa fragments of the membrane-spanning domain of the human erythrocyte anion exchanger, band 3. The antibodies were used to localize and further characterize the analog of band 3 in the human kidney. We report here that the intercalated cells of the cortical and medullary collecting ducts and connecting tubules contain a 100 - 110-kDa analog of the erythrocyte anion exchanger. The human kidney band 3 protein contains immunoreactive sites of both the cytoplasmic domain and the two main fragments of the membrane-spanning domain of erythrocyte band 3. While no immunological differences were detected between the membrane-spanning domains of erythrocyte and kidney band 3 there are at least three sites along the cytoplasmic domain of kidney band 3 that differ from erythrocyte band 3 in either amino acid composition or posttranslational modifications. Immunocytochemical studies showed that the kidney analog of band 3 is confined to the basolateral membrane of the intercalated cells where this membrane protein is probably important for reabsorption of bicarbonate and thus for the acidification of the urine.

I111 STABLE COMPLEX OF CALMODULIN WITH BRAIN ACTOMYOSIN CONFERS HIGH CALCIUM SENSITIVITY TO ATPASE AND PROTEIN KINASE ACTIVITIES. Roy E. Larson, Jesus A. Ferro and Domingos E. Pitta, FMRP, University of São Paulo, Ribeirão Preto, São Paulo, 14049 Brazil.

Rabbit brain actomyosin prepared in the presence of PMSF showed 3-5 fold stimulation of MgATPase activity by Ca^{2+}. Further activation (50-80%) occurred upon addition of purified bovine brain calmodulin. Fluphenazine, 100 µM, abolished these effects without altering the basal MgATPase activity. Sr^{2+} but not Ba^{2+} substituted for Ca^{2+}, paralleling their effects on calmodulin structure. Ca^{2+}-dependent endogenous phosphorylation of myosin heavy chain and light chains was observed. SDS-PAGE showed that actomyosin accounted for > 95% of Coomassie stained material and revealed a polypeptide which comigrated with purified calmodulin in 10%, 12% and 6-15% gradient gels. This peptide showed a Ca^{2+}-dependent shift in migration in 4M urea-PAGE identical with purified calmodulin. Ca^{2+}-dependent ATPase and protein kinase activities and the calmodulin-like peptide accompanied actomyosin when precipitated in 1% Triton X100 or 10 mM EGTA, when solubilized in 0.4 KCl and through a solubilization - precipitation (1M - 0.1M KCl) cycle. The calmodulin-like peptide eluted with a myosin fraction during filtration on Bio-Gel A15. We conclude that a salt-stable, Ca^{2+}-independent complex of calmodulin with brain actomyosin is responsible for the high Ca^{2+} sensitivity of ATPase and endogenous protein kinase activities.

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I112 STRUCTURE AND FUNCTION OF CYTOSKELETAL TROPOMYOSINS, Alexander R. MacLeod, Ludwig Institute for Cancer Research, Cambridge, U.K.
Cultured human cells contain fine distinct proteins which share the physical and chemical properties of tropomyosins. Molecular cloning and DNA sequence analysis of fibroblast tropomyosin mRNA's indicates that all five proteins are structurally related. They are the products of four distinct tropomyosin genes each of which is expressed by an alternative mRNA splicing mechanism. Only two of these proteins are unique to the cytoskeleton, the others being identical to tropomyosins normally associated with smooth and skeletal muscle. The occurrence of both muscle and non-muscle tropomyosins argues for a complex functional role for these proteins in the cytoskeleton. The proteins of the microfilament system play an important but as yet ill-defined role in the control of cellular proliferation. An example is the occurrence of actin and tropomyosin in the oncogenes fgr and trk respectively. We are currently investigating the functional role of tropomyosin in the cytoskeleton using constructs such as trk as biological probes.

I113 ORTHOVANADATE-TREATED BHK FIBROBLASTS SHOW CYTOSKELETON AND ADHESION PATTERNS SIMILAR TO THEIR RSV-TRANSFORMED COUNTERPARTS. Pier Carlo Marchisio, Nicoletta D'Urso, Paolo M. Comoglio, Filippo G. Giancotti and Guido Tarone. Dip. di Scienze Biomediche e Oncologia, Università di Torino, Torino, Italia.
RSV transformed hamster fibroblasts (RSV/B4-BHK) adhere to fibronectin by means of dot-like adhesion sites called podosomes in view of their shape and function of cellular feet (Exp.Cell Res.159,141,1985). Podosomes are sites of concentration of phosphotyrosine proteins and appear in cells transformed by oncogenes coding for tyrosine kinases. We correlated the level of tyrosine phosphorylation to cytoskeleton remodeling (including the formation of podosomes) occurring in transformed cells. To achieve this we exposed non-transformed BHK fibroblasts to orthovanadate which inhibits the dephosphorylation of phosphotyrosine thus increasing the activity of the c-src kinase. Orthovanadate treatment (10-100 uM) induces in a time- and dose-dependent manner the redistribution of microfilaments and the appearance of a transformed phenotype in BHK cells. In about 10% of the cells podosomes are also formed. Cytoskeleton remodeling occurs with a marked increase of phosphotyrosine proteins immunodecorated on Western blots by antibodies cross-reacting with phosphotyrosine. Orthovanadate effect is enhanced by simultaneous exposure of cells to phorbol myristate acetate (100 nM) also reported to enhance tyrosine kinase activity. Under the latter condition the proportion of BHK cells showing podosomes is close to 100%. We propose that, in normal cells, orthovanadate induces marked cytoskeletal changes typical of transformation by simply enhancing the kinase activity of c-onc-coded tyrosine kinases.
Supported by CNR special project "Oncology".

I114 ACTIN-ASSOCIATED CELL SURFACE GLYCOPROTEIN OF THE ASCITES TUMOR CELL MICROVILLI: BINDING TO ACTIN AND IMMUNOLOGICAL RELATIONSHIP WITH IgM HEAVY CHAIN. T.N. Metcalf III, C.A.C. Carraway and K.L. Carraway, Univeristy of Miami School of Medicine, Miami, FL 33101. Isolated microvilli from 13762 ascites tumor cells contain a transmembrane complex composed of actin and a cell surface glycoprotein, CAG (cytoskeleton-associated glycoprotein), which has been proposed to act as a microfilament binding site. CAG is a disulfide-linked multimer, molecular weight about 10^6, which can be purified using a two step density gradient centrifugation procedure in the presence of sodium dodecyl sulfate (SDS). The reduced CAG subunit appears slightly larger by SDS gel electrophoresis than the IgM heavy chain. Unreduced CAG migrates substantially slower than IgM in SDS agarose-acrylamide gels. Overlays of agarose-acrylamide gels with 125I-actin demonstrate binding of actin to CAG but not to IgM. By Western blots, reduced CAG will react with an antibody directed against the heavy chain of rat IgM, but not with antibodies against rat IgG. CAG can be immunoprecipitated by binding anti-IgM to sealed microvilli prior to detergent lysis, suggesting that the recognized epitope(s) are exposed at the cell surface. These results indicate the structural and immunological similarities and differences between CAG and IgM, which may reflect their functional similarities (requirement for stable multivalent structure) and differences (binding of actin vs antigens).

I115 THE 110/105 KDA CALMODULIN BINDING PROTEIN OF ERYTHROCYTES PROMOTES SPECTRIN-ACTIN BINDING. Sheenah M. Mische, Et-Tsu Chen, John P. Anderson and Jon S. Morrow, Yale University School of Medicine, New Haven, CT. 06510.

The erythrocyte membrane skeleton is composed predominantly of spectrin, protein 4.1, protein 4.9, and actin. An additional component has recently been recognized which is composed of two subunits of molecular weights of 110,000 and 105,000. The 105 KDa subunit binds calmodulin in a Ca++ dependent manner and both subunits of this 105/110 kDa protein are phosphorylated. Stable low ionic strength cytoskeletal extracts contain spectrin, protein 110/105, protein 4.1, protein 4.9 and actin in a molar ratio of 2:0.5:2:1:6-8 respectively. Invitro sedimentation assays, using purified protein components, demonstrate that protein 110/105 promotes the association of spectrin with F-actin in a protein 4.1 independent manner. It also binds actin independently of spectrin. These results suggest multiple levels of regulation of spectrin-actin association and provide additional data for the role of modulatory proteins in cytoskeletal assembly and regulation. Supported by NIH grant HL-28560.

I116 SPECTRIN OLIGOMERIZATION IS ALLOSTERICALLY COUPLED TO ANKYRIN AND PROTEIN 3, Jon S. Morrow, Mauro Giorgi, and Carol Cianci, Yale University School of Medicine, New Haven, CT. 06510.

Ankyrin links the erythrocyte cytoskeleton to the membrane by simultaneously binding to beta spectrin and the cytoplasmic domain of protein 3. Spectrin heterodimers self-associate into tetramers and larger oligomers. These interactions have generally been assumed to be independent binding events. We now find that the self-association process in spectrin, the binding of spectrin to ankyrin, and the binding of ankyrin to the 43 kDa cytoplasmic portion of protein 3 are coupled in a true allosterically regulated and positively cooperative fashion. The linkage between spectrin oligomerization and ankyrin binding must involve conformational rearrangements in beta spectrin, since ankyrin, which binds only to beta spectrin, enhances the affinity of spectrin dimer for a 80 kDa proteolytic fragment derived from the NH-terminal region of the spectrin alpha subunit (alpha -I domain). This domain, which binds only to beta spectrin, stimulates spectrin-ankyrin binding by nearly 10 fold. Similarly, the binding of the 43 kDa cytoplasmic protion of protein 3 to ankyrin stimulates its binding to spectrin oligomers and simultaneously stimulates oligomer formation in spectrin-ankyrin complexes. These results demonstrate a cooperative molecular mechanism whereby a putative membrane receptor may drive cytoskeletal assembly.

I117 SYNAPSIN-I IS AN ACTIN BUNDLING PROTEIN, Tamara C. Petrucci and Jon S. Morrow, Yale University School of Medicine, New Haven, CT. 06510.

Synapsin-I is a neuronal phosphoprotein comprised of two closely related polypeptides of 78,000 and 76,000 Mr. It is immunologically related to erythrocyte protein 4.1., and is found in association with the small vesicles clustered at the presynaptic junction. Its precise role is unknown, although presumably it participates in vesicle clustering and/or release. The association of synapsin-I with microtubules and neurofilaments has been reported. We have examined the interaction of purified phosphorylated and unphosphorylated bovine and human synapsin-I with tubulin and actin filaments, using co-sedimentation assays, viscometric, electrophoretic, and morphologic techniques. Unphosphorylated synapsin-I increases the steady state viscosity and sedimentability of F-actin solutions. When it is present, bundled actin filaments are observed by electron or dark field microscopy. Phosphorylation by cAMP dependent kinase has no effect on this interaction, but complete phosphorylation of the molecule at all sites inhibits its bundling activity. The region of synapsin-I responsible for this activity is present in selected NTCB peptides which map to the center of the molecule. Its microtubule binding activity is insensitive to phosphorylation. We conclude that synapsin-I is an actin bundling protein subject to phosphorylation control.

I 118 ASSAY OF THE DISTRIBUTION OF STOP PROTEIN IN VARIOUS MAMMALIAN TISSUES, Fabienne Pirollet*, Didier Job*, and Robert L. Margolis+ , * INSERM U 244, DRF/BRCE, Centre d'Etudes Nucléaires, 38041 Grenoble Cédex, France. + The Fred Hutchinson Cancer Research Center, Seattle, Washington 98104.

We have developed an assay of microtubule cold stability which is generally applicable to the detection of STOP (stable tubule only polypeptide) activity in various tissues. The assay involves dilution of recycled labile microtubules into a Mes-40% sucrose buffer containing small quantities of the protein fraction to be tested followed by exposure to cold temperature. As the assay does not require assembly of microtubules from the tested tissue, it allows quantitation of STOP in various crude tissue extracts. As it involves detection of protein activity under dilution conditions, ion exchange column fractions can be assayed without prior desalting. Using this assay we show STOP protein activity is most abundant in neuronal tissue but is detectable in all tissues tested with the exception of heart muscle. Furthermore, in all tissues that we have examined, the cold stabilizing activity elutes as a single peak from heparin-Ultrogel columns ; and, as in brain, all activity is Ca-calmodulin sensitive. We conclude that the STOP protein purified from brain tissue (Margolis R.L., Rauch C.T., Job D., Proc. Natl. Acad. Sci USA, 1986, 83, 639-643) or an analogue with several identical properties is widespread in mammalian tissues. The protein may therefore be of general importance to regulation of microtubule activity in various cells.

I 119 CALPACTINS I & II: CALCIUM, PHOSPHOLIPID AND ACTIN BINDING PROTEINS, Mark A. Powell & John R. Glenney, M.B.V.L.,Salk Institute, San Diego CA 92138

Calpactins are calcium, phospholipid, and actin binding proteins which appear to co-localize with spectrin in the membrane skeleton of cells. They were initially identified as ~36 kDa substrates of tyrosine kinases, and they are among a group of calcium and phospholipid binding proteins identified in a number of cell types. We have isolated two distinct calpactins (I and II) from bovine lung and human placenta. 2-dimensional gel electrophoresis, peptide mapping, antibody reactivity, and protein sequencing demonstrated they were distinct proteins. Calpactin I was isolated as a monomer and a 95 kDa complex with its 11 kDa light chain, whereas calpactin II was isolated only as a monomer. Equilibrium dialysis showed calpactin II bound 2 moles Ca per mole protein, only in the presence of phospholipids, as shown previously for calpactin I. Differences in the phospholipid (PL) specificity were found, calpactin I bound to PS and PI, but not PC and PE; calpactin II bound to PS, PI, PE, and PC. Monomer forms of both calpactins aggregate phospholipids, suggesting they have at least two binding sites for phospholipids. Antibodies have been raised to calpactins I and II, and western blotting has shown that they are not coordinately expressed, e.g. in intestine only calpactin I is found whereas both calpactins are detected in placenta and lung.

I 120 THE IN VIVO RELATIONSHIP OF MICROTUBULE STABILITY TO POST-TRANSLATIONAL MODIFICATIONS OF MICROTUBULES, Eric Schulze, David Asai, Jeannette Chloe Bulinski, and Marc Kirschner. University of California at San Francisco, San Francisco CA 94143.

By combining microinjection of biotin-tubulin with a novel immunocytochemical technique, we have probed the relationship of "stable" (non-exchanged) microtubules to post-translationally modified microtubules (acetylated, or detyrosinated). Our results indicate that in most SKNSH (human retinoblastoma) cells: 1) "dynamic" (exchanged) microtubules are not highly acetylated, 2) some "stable" microtubules are highly acetylated, 3) "stable" microtubules are detyrosinated, and 4) acetylated microtubules are also detyrosinated, and vice versa. This has allowed us to conclude that in most SKNSH cells "stable" microtubules are both highly acetylated, and detyrosinated. Since these modifications are believed to occur in a time-dependent fashion, it is likely that the above correlation is simply a reflection of the stable microtubules' long half-life which allows for their increased post-translational modification. Implications for cellular morphogenesis are discussed.

I121 CELL SURFACE EVENTS AND CYTOPLASMIC ORGANIZATION IN A SMOOTH MUSCLE CELL MODEL SYSTEM. Arthur R. Strauch and Mark D. Berman, Department of Anatomy, College of Medicine, The Ohio State University, Columbus, OH 43210.
Cell-cell contact and serum withdrawal are necessary for BC3H1 smooth muscle cell differentiation (JBC 259:3152, '84; 261:849, '86). Trypsin-sensitive cell surface proteins may control shape changes and smooth muscle alpha-actin expression in BC3H1 cells. Dispersion of confluent myocytes with trypsin:EDTA does not alter their bipolar shape or ability to express alpha-actin. However, myocytes dispersed with EDTA alone are de-differentiated as indicated by their morphology and expression of non-muscle actins. Undifferentiated myoblasts do not reattach to the substrate after trypsin treatment implying myoblasts and myocytes may express different cell surface proteins required for attachment. Myocytes treated with trypsin:EDTA may be depleted of cell surface molecules required for transmitting signals about cell density to the cytoplasm whereas EDTA used alone may preserve these surface groups. Responsiveness to extracellular conditions may be restored in trypsin-treated myocytes by treatment with fibroblast growth factor which promotes shape changes and alpha-actin down-regulation similar to that seen when myocytes are subcultured with EDTA alone. The importance of cell surface interactions in mediating BC3H1 cell phenotype is further indicated by two observations. First, mechanical perforation of myocyte monolayers caused myocytes on the periphery of torn areas to change shape and migrate. Second, undifferentiated myoblasts subcultured using EDTA alone differentiate into myocytes and express alpha-actin when seeded at low density onto myocyte extracellular matrices (ECM). Myoblasts that differentiate on the ECM do so without forming detectable cell-cell contacts indicating that this material may contain information necessary and sufficient for induction of smooth muscle differentiation. Supported by the AHA and March of Dimes.

I122 MOLECULAR STRUCTURE AND INTERACTION PARTNERS OF PLECTIN, Gerhard Wiche, Roland Foisner and Harald Herrmann, University of Vienna, 1090 Vienna, Austria
Plectin is a cytoskeletal high M_r protein of widespread and abundant occurrence in cultured cells and tissues. Its polypeptide chains are of apparent M_r=300kDa, but the M_r of native plectin molecules is over 1,000 kDa as shown by chemical and biophysical analysis. Rotary shadowed and negatively stained specimens have a dumb bell-like shape consisting of a 200 nm long rod and two globular end domains. Based on circular dichroism, hydrodynamic and ultrastructural data, it is suggested that plectin molecules consist of four polypeptide chains partially assembled into a coiled coil rod. Self association of plectin molecules was found to take place via their globular end domains yielding star-like clusters. Besides interacting with itself plectin interacts with vimentin filaments and microtubules as shown by immunoelectron microscopy and in vitro copolymerization. Using solid phase binding assays several binding partners were identified: vimentin, microtubule-associated proteins (MAP) -1 and -2, the 240 kDa component of brain fodrin and erythrocyte -spectrin. These data suggest that plectin is a multifunctional cytomatrix protein engaged in interlinking filaments of various types and connecting them to the membrane skeleton. This suggestion is supported by previous immunolocalization data.

Transmembrane Signal Transduction

I200 FUNCTIONAL HETEROGENEITY OF THE N-FORMYL PEPTIDE RECEPTOR. Rodger A. Allen, and Algirdas J. Jesaitis, Scripps Clin. & Res. Fdn., La Jolla, CA 92037
We have undertaken an analysis of N-formyl peptide receptor forms present in the human neutrophil. The investigation was carried out using the covalent affinity ligand CHO-Met-Leu-Phe-[2-(ϱ-azidosalicylamido)ethyl-1,3'-dithiopropionyl]-Lys-^{125}I (FMLPL-SASD) and SDS-urea-PAGE with autoradiography to visualize the receptor forms. Whole cells and the detergent-insoluble portion of the cell or the "cytoskeleton" were analyzed in the resting (unstimulated) state as well as after stimulation with FMLP or FMLP in the presence of dihydrocytochalasin B (dhCB). In the resting cell the characteristic diffuse band of Mr 50-60K was evident, which sometimes appeared to contain 2 or 3 discrete bands within the broader band. Stimulation of the cell with PMA or FMLP and dhCB yielded a significant increase in the amount of surface receptor of the same Mr. The association of the 50K and 60K components of the receptor to the cytoskeletal fraction appeared to be dependent on the stimulation state of the cell. Covalent affinity labelling of cytoskeleton fractions revealed a predominant Mr 55K receptor form, which was not accessible to surface labelling. When cytoskeletons prepared from FMLP/dhCB stimulated cells were covalently radiolabelled, the receptor showed a slight increase in Mr (~2K) and there was no evidence of depletion of the "cytoskeletal receptor pool" as a result of stimulation. Scatchard analysis of ^{3}H-FMLP binding to cytoskeletal receptors demonstrated a 10-fold higher affinity (1 nM V. 10 nM) than the cell surface receptor. The possible relevance of these findings will be discussed.

1201 RAPID ACTIVATION OF ORNITHINE DECARBOXYLASE DURING TRANSDUCTION OF GROWTH SIGNALS IN T LYMPHOCYTES

Leif C. Andersson, Tomas Mustelin and Hannu Pösö
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We have found that treatment of human T lymphocytes with mitogenic ligands, lectins or monoclonal antibodies, (but not with non-mitogenic ligands) induces an activation of ornithine decarboxylase (ODC) within 5 minutes. This early ODC induction is independent of active protein synthesis but requires energy and an intact cytoskeleton. Guanine nucleotide binding regulatory G-proteins are involved in the early ODC activation. Introduction of non-hydrolyzable GTP analogues (GTP S) into T lymphocytes by transient membrane permeabilization induces a promt activation of ODC. The receptor ligand interaction delivering activation signals to T lymphocytes induces membrane phosphoinositide breakdown. When T lymphocytes are incubated in an inositol free medium in the presence of 1 mM Li^+ which inhibits the inositol-1-phosphatase the ODC activation induced by mitogenic ligands is blocked. Addition of inositol or phosphatidyl-inositol restores the mitogen induced ODC activation within 3 min. Our recent observations indicate that ODC is covalently linked through inositol to the cytplasmic surface of the membrane phospholipids. The molecular mechanism of the early ODC activation during transduction of a mitogenic signal will be presented.

1202 INSULIN RECEPTOR CAPPING DURING LYMPHOCTE ACTIVATION, Lilly Y. W. Bourguignon and Gerard J. Bourguignon, Department of Anatomy and Cell Biology and Program of Physical Therapy The University of Miami Medical School, Miami, FL. 33101.

In this study we have used either mitogens [a ligand-dependent process] or electric stimulation [a ligand-independent process] to activate mouse splenic lymphocytes. Our results show that both ligand-dependent and ligand-independent treatments cause an immediate aggregation of insulin receptors into patched and capped structures (as early as 1-5 min after treatment) followed by protein synthesis (about 20 min after treatment) and DNA synthesis (approximately 24 h after treatment). Insulin receptor capping can be inhibited by: (1) cytochalasin D suggesting the involvement of microfilament; (2) sodium azide indicating a requirement for ATP production; (3) Ca^{2+} channel blockers, Disoldipine, Diltiazen, Flunarizine and D-600 suggesting the involvement of Ca^{2+} channel; and (4) trifluoperazine, W-2, W-7 and W-12 drugs implying a need for Ca^{2+}/calmodulin activity. Most importantly, treatment with these metabolic or cytoskeletal protein inhibitors causes a significant inhibition of the following protein and DNA synthesis. Further analyses indicate that actomyosin and Ca^{2+}/calmodulin/myosin light chain kinase are closely associated with the insulin capped structures. These findings strongly suggest that an actomyosin-mediated contractile system (regulated by Ca^{2+}, calmodulin and myosin light chain kinase in an energy-dependent manner) is not only important for the collection of insulin receptors into patched and capped structures but is also necessary for the subsequent protein and DNA synthesis. Therefore, we speculate that insulin receptor patching/capping is a required step for the onset of lymphocyte activation.

1203 A Calpactin-like Protein from Ascites Microvilli: Phosphorylation and Binding to Chlorpromazine. C.A. Carothers Carraway, Y. Liu, K.L. Carraway III and K.L. Carraway, Univ. of Miami School of Medicine, Miami, FL 33101

A major calcium-sensitive microfilament-binding protein (AMV-p35, ascites microvillarp35) was isolated from microvilli of 13762 ascites tumorcells. AMV-p35 shows the following similarities to calpactin I, a $pp60^{src}$ kinase substrate. 1) It binds to microfilaments and to liposomes in a Ca++-dependent manner. 2) Treatment with chymotrypsin yields a 33,000 dalton fragment. 3) It is phosphorylated by extracts of BHK/B4 cells, a Rous sarcoma virus transformed cell line. 4) It shows strong reactivity with antibody to calpactin I. Unlike calpactin I, AMV-p35 has no associated 10,000 dalton subunit,andis isolated as a monomer of 35,000 daltons rather than an 80,000 dalton heterotetramer. These observations indicate that AMV-p35 is closely related to the $pp60^{src}$ kinase substrate calpactin I, even though its native size and subunit structure are different. In the presence of Ca++ AMV-p35 binds 3-4 moles of chlorpromazine per mole of protein, similar to calmodulin. Chlorpromazine is a phenothiazine-type antipsychotic drug which binds to calmodulin and inhibits its modulation of a variety of calmodulin-dependent activities. These results suggest that calpactins may be alternative sites for action of phenothiazines.

1204 CALMODULIN'S ROLE IN DETERMINING NEUROTRANSMITTER REGULATION OF NEURONAL ADENYLATE CYCLASE. Dermot M.F.Cooper, Michael K.Ahlijanian and Edward Perez-Ripoll. University of Colorado Medical School, Denver, CO 80262

Studies of the adenylate cyclase activity of various brain areas have indicated that the enzyme can be stimulated by from 50% to 7-fold, depending on the source. Free Ca^{2+} concentrations between 0.1 and 1uM fully stimulate the enzyme in the presence of calmodulin. In most brain areas the stimulation by Ca^{2+}/calmodulin is essential for the observation of inhibition by opiates, dopamine, adenosine, acetylcholine and GABA. In contrast, stimulation of activity by for instance, VIP and norepinephrine is reduced under conditions of calmodulin stimulation. These results suggest that receptor-mediated regulation of adenylate cyclase activity may be dependent on the ambient free Ca^{2+} concentration in a neuron. This is in turn determined by the state of depolarization of a cell or by the activation of a receptor that is linked to the mobilization of Ca^{2+} via inositol phosphates. These findings indicate that calmodulin may play a central role in determining whether receptor occupancy can be translated into an alteration in the levels of second messengers within the neuron.

1205 ACTIVATION OF PHOSPHOINOSITIDE BREAKDOWN BY THE B CELL ANTIGEN RECEPTOR, Anthony L. DeFranco, Michael R. Gold, Dawne M. Page and Katherine A. Fahey, University of California, San Francisco, CA 94143.

B lymphocytes express membrane forms of immunoglobulins as cell surface receptors for antigens, which regulate cellular proliferation and differentiation. Crosslinkage of membrane IgM with anti-IgM antibodies causes the breakdown of phosphatidylinositol 4,5-bisphosphate, generating diacylglycerol and inositol 1,4,5-trisphosphate ($InsP_3$). This latter compound is apparently converted to two other inositol polyphosphate compounds, inositol tetrakisphosphate and inositol 1,3,4-trisphosphate. The potential second messenger roles of these compounds are as yet unknown, although in a B lymphoma cell line, WEHI-231, we have found that anti-IgM-induced growth regulation can be only partially mimicked by the combination of phorbol diesters and appropriate elevation of cytoplasmic calcium with Ca^{++} ionophore, suggesting a possible biological role for the inositol polyphosphates. We have recently isolated over thirty independent mutants of WEHI-231 cells in which the growth of the cells is no longer regulated by anti-IgM. Current experiments are aimed at determining the nature of the defect in these mutants, most of which have normal levels of membrane IgM.

1206 FIBRINOGEN INDUCES ADHESION AND SPREADING OH HUMAN ENDOTHELIAL CELLS IN VITRO BY INTERACTING WITH A MEMBRANE RECEPTOR SIMILAR TO PLATELET GPIIb-IIIa. Elisabetta Dejana, Silvia Colella, Lucia R. Languino and Pier Carlo Marchisio, Ist. M. Negri, Milano and Dip. di Scienze Biomediche e Oncologia, Università di Torino, Torino, Italia.

It has been shown that fibrinogen (fg) specifically binds to cultured human umbilical vein endothelial cells (HUVE) and promotes their migration (J. Clin. Invest. 75,11,1985). In this study we evaluated whether fg acts as a substratum for HUVE attachment. When HUVE were seeded on human fg or fibronectin (FN) coated coverslips, they adhered, spread and organized microfilament bundles and formed focal contacts as shown by immunofluorescence and interference reflection microscopy. In contrast HUVE did not adhere and spread on albumin. Antibodies to fg, but not to FN, prevented HUVE adhesion to fg, while antibodies to FN, but not to fg, prevented adhesion of HUVE to FN. Removal of FN contamination to less than 1 ng/mg fg from fg preparations by DEAE-cellulose did not change HUVE adhesion to fg. Inhibitors of protein synthesis and release did not impair HUVE adhesion to fg. The synthetic peptides GRGD and RGDS, containing the RGD sequence, responsible for the cell binding activity of FN, inhibited HUVE adhesion, spreading and cytoskeletal organization either on fg or on FN but not on gelatin. Conversely, the peptide RGG was ineffective. Antibodies raised against platelet gpIIb-IIIa bound to HUVE at focal contacts giving a typical "needle-eye" pattern and blocked HUVE adhesion and spreading on fg. We conclude that HUVE adhere and spread on a fg substratum and this occurs by recognition mechanisms similar to those reported for the interaction of platelets with fg and FN. Supported by CNR projects "Oncology" and "Genetic Engineering and Hereditary Diseases".

1207 MONOCLONAL ANTIBODY INTERFERENCE WITH CELL MEMBRANE SIGNALLING VIA PUTATIVE LIPID-BINDING PROTEINS. Donard S. Dwyer, Joachim Bartels, F. Pierre VanderVegt and George B. Brown, University of Alabama at Birmingham, Birmingham, AL 35294.

To study neurite outgrowth in PC 12 cells, monoclonal antibodies (Mabs) were raised against cell surface antigens. One of these Mabs, PC 11, inhibited neurite outgrowth in response to nerve growth factor. In separate studies, we discovered that a Mab against the P_2 protein of peripheral myelin (BC 18) would inhibit T lymphocyte activation. Although BC 18 did not affect neurite outgrowth, PC 11 did dramatically inhibit T cell activation, similar to BC 18. Both antibodies stain cell surface structures on PC 12 cells. We then examined which lymphocyte populations were stained by these Mabs. T cells and B cell hybridomas were negative whereas macrophages and dendritic cells were positive. Therefore, T cell activation is probably blocked by interference with antigen presentation. These antibodies inhibit the proliferation of antigen-specific T cells in response to both antigen and T cell mitogens such as concanavalin A. Preliminary experiments to characterize the cell surface antigen involved indicate that the epitope is carried by a protein with a molecular weight of 16-18,000 daltons. As mentioned, BC 18 recognizes the P_2 protein (m.w. 15,000 daltons) whose role in myelin structure is unclear. However, other anti-P_2 protein antibodies do not stain PC 12 cells or dendritic cells suggesting that the antigen is not simply P_2 protein. It has been proposed that the P_2 protein belongs to a family of lipid-binding proteins based on amino acid sequence homology. Perhaps, it is another member of this family which is being recognized by PC 11 and BC 18. We propose that lipid-binding proteins participate in signal transduction by linking extracellular events with cytoskeletal elements controlling membrane and cell movements. Antibody binding to these proteins may disturb antigen presentation and neurite outgrowth by disrupting the flow of signals delivered at the cell surface.

1208 HUMAN INSULIN RECEPTOR cDNA EXPRESSION : ALTERATIONS OF INSULIN CELLULAR ACTION BY DELETION AND POINT-MUTATIONS. M. Edery (*), L. Ellis, D. Morgan, E. Clauser, R.A. Roth & W.J. Rutter. Hormone Research Laboratory, UCSF, San Francisco, Ca.94143, USA and (*) Laboratoire de Physiologie de la Lactation, INRA, 78350 Jouy-en-Josas, France.

The human insulin receptor (hIR) is an integral transmembrane glycoprotein comprised of two α and two β subunits. An immediate consequence of insulin binding to the extracellular α subunit is the autophosphorylation, predominantly at tyrosine residues on the intracellular domain of the β subunit. The placental hIR cDNA has been cloned and sequenced, providing the primary structural features of the protein (Cell 40, 747-758, 1985). In order to investigate the functions of the β subunit and particularly the role of autophosphorylation and tyrosine phosphokinase (TPK) activity (a feature shared by other receptors and oncogenes proteins) in transmembrane signalling, we designed an expression system of the hIR cDNA in eucaryotic cells, as described by Southern & Berg (J. Mol. Appl. Genet. 1, 327 - 341, 1982). Superexpressing CHO lines, that contain about 10^6 functional hIR/cell. have been developed. In these cells half maximum stimulation of the glucose uptake occurs at $5x\ 10^{-12}$ M insulin, whereas normal CHO cells require $5x\ 10^{-10}$M insulin. In this expression system we have carried out directed mutagenesis experiments in which domains of the molecule have been deleted or particular amino acids have been replaced by others. The replacement of either or both the tyrosine residues 1162 and 1163 compromise an auto phosphorylated site that is important for kinase function and the insulin response. These studies show also that the C terminal 112 amino acids portion of the β subunit is important for the stability of this protein ; the truncated molecule is rapidely degraded though the α subunit remains intact and functioning.

Supported by NIH grants to W.J. Rutter & R.A. Roth

1209 ASSOCIATION OF Gc PROTEIN WITH THE LIPID BILAYER: INTERACTION OF HYDROPHOBIC DOMAIN WITH FATTY ACIDS. R.M. Galbraith, M. H. Williams, P. J. Goldschmidt-Clermont, and E. Merler. Medical University of SC, Charleston, SC 29425.

The actin-sequestering protein Gc is expressed on the membrane of certain cells including peripheral blood mononuclear cells and syncytiotrophoblast, and Gc is known to be the major carrier for Vitamin D metabolites. We therefore searched for a hydrophobic domain on this protein. Native Gc purified from normal human serum was extracted with chloroform/methanol. Reverse phase HPLC demonstrated large amounts of associated unsaturated fatty acid (62% arachidonate, 12% palmitoleate, 7% oleate), and defatted Gc clearly re-bound ^{3}H-arachidonic acid. Experiments with the hydrophobic probe toluidinylnaphthylene sulfonate (TNS) also showed strong binding to native Gc, but subsequent addition of purified human skeletal muscle or platelet G-actin reduced spectrofluorimetric evidence of TNS binding to negligible levels. Finally, experiments performed with ^{125}I-labeled native Gc in rabbits showed enhanced clearance of Gc after complexing with G-actin (t/2 20 hrs and <4 hrs respectively). These results indicate that circulating Gc features a hydrophobic domain and is partially or completely complexed in native state with hydrophobic ligands, particularly unsaturated fatty acids. This suggests that Gc could possibly become associated with fatty acids of cell membranes, and that sequestration of actin might facilitate such uptake by displacement of endogenous ligand.

1210 REGULATION OF LEUKOCYTE ACTIVATION BY MEMBRANE-RECEPTOR CYTOSKELETAL INTERACTIONS.
A.J. Jesaitis, C.A. Parkos, J.O. Tolley, and R.A. Allen, Scripps Clin. & Res. Fdn. La Jolla, CA 92037

Human granulocytes can be desensitized to the chemoattractant F-met-leu-phe (FMLP) by preincubation with 100 nM FMLP at 15°C for up to 60 min. Such desensitized cells are incapable of producing superoxide at 37°C in response to FMLP, but will produce maximally when stimulated with phorbol myristate acetate. C5a produces a partial response. The desensitization occurs more slowly at 4°C or in the presence of dihydrocytochalasin B. During this time occupied receptors convert to a form which dissociates very slowly, (t[1/2]~100 min), remains at the cell surface and is found to coisolate with the cytoskeletal fraction of the cell. The ability of preincubated cells to respond to chemoattractants at 37°C appears to be exponentially related to the number of receptors remaining uncomplexed with the cytoskeleton in this apparently down-regulated form. When subcellular fractions are prepared from such desensitized cells the occupied receptors are found in a membrane fraction of higher density (ρ=~1.16 g/cc) than from cells preexposed to ligand at 4°C but not desensitized (ρ=~1.14). Relative to plasma membrane markers, this fraction has a higher content of flavin and at least 3 polypeptides detected by SDS-PAGE (180,88, and 47 kD). Photoaffinity labeled FMLP receptors found in this fraction show no alteration in mobility by SDS-PAGE. These results are consistent with lateral segregation of FMLP receptors from the bulk plasma membrane components. Such a process could, therefore, result in desensitization of these receptors by sequestering them or their active complexes.

1211 PLATELET MEMBRANE GLYCOPROTEIN (GP) IIb-IIIa AND THE FIBRONECTIN RECEPTOR,
Thomas J. Kunicki, Randolph S. Piotrowicz, Diane J. Nugent, Susan M. Kristopeit, Kenneth M. Yamada, The Blood Center of Southeastern Wisconsin, Milwaukee WI 53233, Fred Hutchinson Cancer Research Center, Seattle WA 98104, National Cancer Institute, Bethesda MD 20892.

The fibronectin receptor (FnR) of chicken embryo fibroblasts is a complex composed of three glycoproteins (GP), the smallest component designated band 3. The FnR of human fibroblasts is a heterodimer with a smaller (beta) subunit thought to be analogous to band 3. Human platelets and chicken thrombocytes possess yet another heterodimeric receptor, GPIIb-IIIa, that, in the case of human platelets, binds fibronectin only when platelets are activated. By cytofluorimetry, we show that chicken thrombocytes and human platelets express analogs of both GPIIb-IIIa and the fibronectin receptor, based upon simultaneous binding of both rabbit polyclonal anti-band 3 and murine monoclonal anti-GPIIb-IIIa (AP2). By an immunoblot assay and immunoaffinity chromatography, anti-band 3 reacts with band 3 in chicken thrombocyte lysates and the beta subunit of the FnR in human platelet lysates. Neither band 3 nor the FnR beta subunit are antigenically or structurally identical to GPIIIa. These results demonstrate that both GPIIb-IIIa and the fibroblast-type fibronectin receptor are expressed by chicken thrombocytes and human platelets and that these receptors are composed of structurally and antigenically distinct glycoproteins.

1212 DIFFERENT SITES OF INHIBITION OF PIP2 BREAKDOWN FOR PGE1 AND PMA. PGE1 INHIBITS PIP KINASE, WHILE PMA INHIBITS PIP2-PHOSPHOLIPASE C.
Marianne Frolich Lafrance, Charles V. Olson and Pramod M. Lad, Kaiser Regional Research Laboratory, 4953 Sunset Blvd.,L.A. CA 90027.

Breakdown of PIP2 by activation of a specific phospholipase C (PhL C) to generate IP3 and thereby stimulate release of intracellular Ca is a common signal for many effectors of neutrophil stimulation such as PAF and f-met-leu-phe. Since a very limited pool of PIP2 exists in the membrane, regeneration of PIP2 by stimulation of PIP kinase is a necessary condition for continuous IP3 release and therefore part of the signal. We have followed the kinetics of PIP2 phosphorylation simultaneously with the Ca level (measured by the fluorescence of Quin2)of human neutrophils stimulated with PAF. A pattern of degradation and reappearance of PIP2 is observed. The 10-15 sec decrease represents PhL C activation occurring before PIP kinase can catch up. Preincubation with PMA abolishes both the PIP2 decrease and Ca mobilization. By contrast, preincubation with PGE1, does not abolish the decrease in PIP2, instead the recovery process is absent or delayed, indicating inhibition of PIP kinase, while the Ca mobilization is only partly inhibited. Our results imply: PIP2 PhL C and PIP kinase are enzymes of the PI cycle which are differentially regulated by PMA (kinase C) and PGE1 (kinase A).

1213 ROLE OF CYTOSKELETAL REARRANGEMENT IN HUMAN NEUTROPHIL LIGAND-RECEPTOR DYNAMICS, G. M. Omann, W. N. Swann, Z. G. Oades, C. A. Parkos, A. J. Jesaitis, and L.A. Sklar, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

The binding and dissociation of fluorescein-labeled N-formylhexapeptide (FLPEP) to human neutrophils (PMNs) and PMN cytoplasts were studied at 37°C using real-time fluorometric methods. For both PMNs and cytoplasts, dissociation of FLPEP (determined after quenching of non-bound FLPEP by anti-fluorescein antibody) after 15 sec of binding revealed two components, a rapidly dissociating component (t[1/2] ~ 10 sec) and a slowly dissociating component (t[1/2] ~ 3 min). After 3 min of binding, only the slowly dissociating component was seen. 1ug/ml cytochalasin B (CB) did not alter the rate of conversion of the rapidly dissociating form to the slowly dissociating form as defined by these fluorimetric assays, although it did inhibit FLPEP-induced actin polymerization. Thus it appears that the slowly dissociating form of the receptor observed at 37°C using the fluorometric assay may not be the same as the cytoskeletal-associated high-affinity form of the receptor (which could be inhibited by CB) previously reported using radioligand methods (A.J. Jesaitis, J.O. Tolley, R.A. Allen, J. Biol. Chem. 261:13662, 1986). When FLPEP binding was stopped by adding antibody to fluorescein, oxidant production decayed with a similar rate (t[1/2]~ 15 sec) in the presence and absence of CB (1ug/ml) although the oxidative burst was enhanced by CB. Thus the decay of oxidant production after inhibition of binding did not require actin polymerization. A model will be discussed in which these various forms of the receptor modulate cell activation.

1214 MUTANTS RESISTANT TO HEAT SHOCK-INDUCING ANION CHANNEL BLOCKERS IN DROSOPHILA HAVE AMPLIFIED INSOLUBLE CYTOSKELETON PROTEINS. M.M. Sanders and A.C. Sherwood, UMDNJ-Robert Wood Johnson Medical School, Piscataway, N.J. 08854

The heat shock response in Drosophila is characterized by profound changes in the regulation of gene expression and by a collapse of the intermediate filament cytoskeleton, decreased cell volume, decreased anion uptake and slowed growth. A signal generated at the plasma membrane can induce the heat shock response since all of the characteristics of the response can be produced at the normal growth temperature in Drosophila Kc cells by treating the cells with a class of impermeant drugs, typified by 4,4'-diisocyanostilbenedisulfonate (DIDS), which block anion exchange. Studies correlating anion transport with expression of the heat shock response induced by various anion channel blockers have led us to conclude that the anion transport system is multifunctional in these cells, since anion exchange per se is clearly not the entire signal for heat shock induction (Sherwood and Sanders, manuscript submitted). We took advantage of the growth inhibiting properties of the anion channel blockers to select mutants whose drug-sensitive target proteins were amplified. Stepwise selection protocols with two different anion transport inhibitors were used. The variants isolated in these two separate selections share similar phenotypes including altered growth properties, an altered heat shock response, reduced drug-sensitive anion transport and 5-10 fold increased amounts of three major polypeptides (46, 60 and 130 kD MW) present in the detergent insoluble cytoskeleton. The amplified 46 kD polypeptide has a peptide map identical to the major intermediate filament protein. This observation may establish a connection between the anion channel protein and the insoluble cytoskeleton in Kc cells.

1215 CONCANAVALIN A INDUCES A CYTOSKELETAL ASSOCIATION OF THE MEMBRANE GLYCOPROTEIN T200 IN CULTURED MURINE CYTOTOXIC T LYMPHOCYTES, Rolf E. Taffs and Sandra J. Ewald, Montana State University, Bozeman MT 59717.

The T200 family of membrane glycoproteins is believed to be involved in a variety of lymphocyte functions including the development and activity of cytotoxic T lymphocytes (CTL). We have examined immunoprecipitates of CTL labeled biosynthetically with ^{35}S-methionine using SDS-PAGE and fluorography. The CTL line was produced in allogeneic mixed lymphocyte culture and maintained in vitro with conditioned medium and weekly antigen restimulation prior to labeling and treatment with 50 ug/ml concanavalin A. We observed that in CTL treated with lectin, a significant amount of precipitable T200 was lost from the soluble fraction of cells lysed in 0.5% Triton X-100, but could be recovered by incubating the insoluble material under conditions allowing actin depolymerization. In contrast, very little T200 could be precipitated from the insoluble fraction of untreated CTL. This leads us to conclude that concanavalin A induces an association of T200 molecules with cytoskeletal components in cultured CTL.
(This work was supported by USPHS grant CA 43489.)

1216 A PROTEIN FROM YEAST MEDIATING THE LATE STAGE OF INTRAGOLGI PROTEIN TRANSPORT. Binks W. Wattenberg and Mary P. White. The Upjohn Company. Kalamazoo, MI 49001. Movement of a membrane protein through the compartments of the Golgi apparatus has been analyzed using a cell free system that reconstitutes this process (Fries, E. and Rothman, J.E. (1980) Proc. Natl. Acad. Sci. USA, 77, 3870.) Previous studies have established that this transport requires, in addition to ATP and physiological buffer conditions, at least two and possibly more soluble cytosolic proteins. Kinetic studies have subdivided this complex transport process into early and late stages and it was found that one cytosolic protein appeared to act at the late stage only. While initial studies were performed with mammalian cytosolic proteins, we report here the characterization of a yeast cytosolic protein that can replace mammalian cytosol in driving the late stage of intra-Golgi transport. Its size and ionic character are remarkably similar to its mammalian counterpart.

1217 SIGNAL-DEPENDENT RELEASE OF THE ACTIN-SEQUESTERING PROTEIN Gc (VITAMIN D-BINDING PROTEIN) FROM HUMAN T LYMPHOCYTES BY CALCIUM/IONOPHORE. M.H. Williams, G.M.P. Galbraith, D.L. Emerson, and R.M. Galbraith. Medical University of SC, Charleston, SC, 29425. Cell signaling, signal transduction and activation are frequently associated with increased concentrations of intracellular Ca^{2+} ions. Since the latter constitutes a major activation signal in lymphocytes, we further studied the effects of Ca^{2+}/ionophore on normal peripheral blood T lymphocytes, with particular emphasis upon Gc, an actin-sequestering protein which also binds Vitamin D metabolites. SDS-PAGE and isoelectric focusing of supernatants obtained from cells treated with 2mM Ca^{2+} and 0.05-5.0μM A23187 showed protein with properties similar to those of circulating Gc (MW 56K; pI 4.8-5.2). Transblotting with specific heteroantiserum provided further confirmation that this protein is indistinguishable from Gc. Release of Gc was dose-dependent, with a maximal at 5μM A23187, and was virtually undetectable if either Ca^{2+} or A23187 was omitted, or if both agents were used in the presence of 10mM EGTA. By immunofluorescence, cells clearly expressing membrane Gc decreased to <5% following Ca^{2+}/ionophore treatment. In parallel, cells prelabeled in vitro with ^{3}H-arachidonic acid demonstrated a similar dose-dependent loss of radioactivity in response to combined treatment with Ca2+/A23187. This apparent release of membrane Gc, possibly related to phospholipase A2 hydrolysis of fatty acid, may facilitate signal-dependent polymerization of cellular actin.

Signalling and Motility

1300 INOSITOL LIPID METABOLISM AND PHAGOCYTOSIS IN THE PROTOZOAN ACANTHAMOEBA CASTELLANII, Philip G. Allen Jr., Harvard Medical School, Boston Ma. A. castellanii is an avidly phagocytic soil amoeba capable of endocytosing a variety of particles. We have examined the turnover of the phosphoinositides (PI,PIP, PIP_2) in this cell in response to phagocytosis using heat killed yeast as stimuli. Within 30 seconds of the addition of yeast in the presence of 10 mM LiCl, we detect a decrease in PIP and PIP_2 and a concomitant increase in IP_3. In addition we detect an increase in a highly charged inositol containing compound tentatively identified as inositol hexaphosphate (IP_6). Addition of theophyline or cAMP to intact cells at concentrations reported to inhibit phagocytosis increase the incorporation of ^{32}P orthophosphate into PIP_2. These results suggest an involvement of phosphatidylinositol metabolism in the process of phagocytosis. Supported by NIH training grant 5 T32 GM 07226 and NSF DCB 8503856.

1301 PHOSPHORYLATION-DEPENDENT INTERACTION OF SYNAPSIN I WITH ACTIN, Martin Baehler and Paul Greengard, The Rockefeller University, New York NY 10021.

Synapsin I is a neuron-specific phosphoprotein localized to the cytoplasmic surface of synaptic vesicles. This phosphoprotein is a major substrate for cAMP-dependent and calcium/calmodulin-dependent protein kinases. Its state of phosphorylation can be altered both in vivo and in vitro by a variety of physiological and pharmacological manipulations known to affect synaptic function. In the nerve terminal, synaptic vesicles are embedded in a cytoskeletal network, consisting in part of actin. We report here the ability of synapsin I to interact with actin, as evidenced by its ability to bundle F-actin. Moreover, this activity is reduced when synapsin I is phosphorylated by cAMP-dependent protein kinase and virtually abolished when it is phosphorylated by calcium/calmodulin-dependent kinase II or by both kinases. These in vitro results support the possibility that, in vivo, the phosphorylation of synapsin I, by decreasing its affinity for one or more components of the cytoskeleton, may be involved in regulating the translocation of synaptic vesicles to their sites of release.

1302 REACTION WITH DANSYLAZIRIDINE BLOCKS THE ABILITIES OF CARDIAC TROPOMYOSIN TO POLYMERIZE AND TO BIND F-ACTIN. Leslie D. Burtnick and Lisa Veres, Department of Chemistry, University of British Columbia, Vancouver, B.C., Canada, V6T 1Y6.

Dansylaziridine (DAZ) reacts with rabbit cardiac tropomyosin (TM) primarily at Cys-190 to give a highly fluorescent product, DAZ-TM. The emission maximum of the bound probe (515 nm) and the susceptibility of the fluorescence to quenching by iodide suggest that the fluorescent chromophore is bound to an exposed site on the surface of the protein. The low level of observed fluorescence polarization indicates that the attachment site has considerable flexibility. Circular dichroism measurements show that the modification does not alter in a significant way the highly helical nature of the TM structure. However, thermal denaturation studies show a less distinct pretransition and a major melting transition a few degrees lower than for unlabelled TM. Viscosimetry demonstrates that the modification inhibits normal head to tail polymerization of TM in low ionic strength, neutral pH solutions. The lack of changes in fluorescence properties of DAZ-TM in response to ionic strength supports this conclusion. Ultracentrifugation fails to cosediment DAZ-TM with F-actin under conditions normally favorable to the binding of unlabelled TM to F-actin. However, inclusion of troponin T in the system restores the ability of DAZ-TM to bind to and cosediment with F-actin.

Supported by the B.C. Heart Foundation and the Natural Sciences and Engineering Research Council of Canada.

1303 REGULATION OF DISCOIDIN I PRODUCTION IN *DICTYOSTELIUM DISCOIDEUM*. Margaret Clarke and Samuel Kayman, Albert Einstein College of Medicine, Bronx, New York 10461.

Discoidin I is a lectin produced by *Dictyostelium* cells; it has some sequence homology to fibronectin and is believed to function in cell-substratum adhesion (Springer *et al.*, *Cell* 39, 557, 1984). One of a group of phagocytosis-defective motility mutants that we isolated proved to be defective in the regulation of discoidin I synthesis, leading us to examine the regulation of discoidin I production by wild type cells. Using suspension cultures so that the *Dictyostelium* cell density and the concentration of bacteria can be controlled, we are monitoring discoidin I by immunoblot analysis and indirect immunofluorescence. We find that wild type *Dictyostelium* cells produce discoidin I during exponential growth, but not constitutively. Discoidin I production is a function of the relative densities of *Dictyostelium* cells and food bacteria, and is initiated approximately three generations before the food supply is depleted. A soluble activity is present in the growth medium of cells producing discoidin I that causes low density cells to initiate discoidin I synthesis. This activity is destroyed by heat and is not dialyzable. A simple model suggested by our data is a regulatory system consisting of two interacting components, one elaborated by the *Dictyostelium* cells and the other by the food bacteria. These findings also suggest that discoidin I may function to facilitate the acquisition of bacteria when the food supply is suboptimal.

1304 *ROLE OF LEUKOTRIENES IN SUTURAL TISSUE FORCE TRANSDUCTION* J.W. Daniel, J.L. Collins, R. Cederquist and D.H. Enlow. Case Western Reserve Univ., Cleveland, OH 44106

Studies in cell culture and in animals suggest a role for prostaglandins (PG) in force-dependent bone development. Although their in vivo role is not well established Radulovich et al. (J. Dent Res 65, 29, 1986) observed a marked indomethacin (IM)-sensitive response of endogenous calvarial PGE_2 and cAMP to mechanical stress (MS). The related eicosanoid leukotrienes (LT) are also possible effectors of bone remodeling. Employing the 5'-lipoxygenase (5'-LO) inhibitor, piriprost (PP), depression of LT synthesis, without inhibiting PG synthesis, stimulated MS-dependent bone development. MS [20g tension (T) or pressure (P)] was applied across the midsagittal suture of osteotomized weanling rats with or without PP for 5 d. LTB_4, LTC_4 and PGE_2, extracted from osteotomy samples, were measured by RIA. Conditions favoring marked bone formation (T alone, T+PP and P+PP) elevated the PGE_2 sutural content and depressed LTB_4 and LTC_4 content. Marked stimulation of osteoblast formation and of calvarial medullary space occurred in parallel. Conditions favoring predominant resorption (P alone) elevated the LTC_4 content but not LTB_4 or PGE_2 content. In PP-treated sham controls where LTs were suppressed and PGE_2 unaffected, a mild resorptive response with some medullary space formation was observed. The congruity of metabolic and histologic responses suggests that inhibition of the 5'-LO pathway participates in transducing the force signal (normally T-dependent) and in converting the P-induced resorption signal to a T-like formation signal. Transduction processes may include the T-associated IM-sensitive PGE_2 increase and the implied LT induction of osteoclastic activity. Possible responding elements include monocytic osteoprogenitor and microvasculature cells.

1305 MODULATION OF ORGANELLE TRANSPORT IN NEURONAL GROWTH CONES, Paul Forscher, JoAnn Buchanan and Stephen Smith, Section of Molecular Neurobiology, Yale School of Medicine, New Haven, CT 06510.

We will present evidence that changes in intracellular cyclic AMP dynamically modulate directed organelle transport in Aplysia bag cell neuron growth cones. We have used video-enhanced DIC microscopy and digital image analysis techniques to analyze organelle motility. Under control conditions directed organelle transport is evident in growth cone central cytoplasmic domains but not in lamellae. Digital motion analysis also reveals wavelike motions in lamellae that propagate slowly (0.07 um/sec) in a retrograde direction. Application of forskolin and a phosphodiesterase (PDE) inhibitor at concentrations known to induce changes in bag cell protein phosphorylation resulted in: 1) rapid extension of directed organelle transport into lamellae, 2) cessation of growth cone extension and 3) inhibition of retrograde wave activity; in essence, transformation of lamellae into neurite endings packed with microtubules and organelles, a large proportion of which appear to be neurosecretory granules. The effects were reversible, dose dependent, potentiated by PDE inhibitors, mimicked by 6-N-butyl-8-benzyl-thio-cAMP and persisted in Ca-free/EGTA external solutions. The cyclic AMP induced changes in growth cone morphology we report here suggest a role for protein phosphorylation in promoting growth cone differentiation and structural changes accompanying secretion.

1306 ACTIN POLYMERIZATION DURING AMEBOID CHEMOTAXIS IN DICTYOSTELIUM DISCOIDEUM, Anne L. Hall, Anthony Schlein and John S. Condeelis, Albert Einstein College of Medicine, Bronx, NY 10461.

Ameboid cells are chemotactic toward cAMP during starvation. Video microscopy and scanning electron microscopy were used to observe changes in cell behavior during uniform upshifts in cAMP concentration. Three distinct changes in cell shape and activity were detected after the cAMP pulse: cell rounding at 25 sec, pseudopod extension and flattening at 50 sec, and normal ameboid movement by 90 sec. During the rounding and flattening responses the cells remained immotile.

Changes in cell behavior were correlated with F-actin content using an NBD-phallacidin binding assay. cAMP induces several rapid and large increases in F-actin content that correlate temporally with changes in cell behavior. Particularly interesting is the 50% increase in F-actin that peaks at 60 sec, which corresponds to the time during which cells extend ruffles and pseudopods. The cAMP-induced increases in F-actin are blocked by cytochalasin D (50% inhibition of the 60 sec peak at 2 μM).

In summary, these results indicate that (a) actin polymerization follows chemotactic stimulation, (b) the growth of pseudopods is temporally correlated with actin polymerization, (c) nucleation sites responsible for regulating actin polymerization must be globally distributed to permit the multidirectional extension of processes in uniform pulses of chemoattractant, and (d) actin polymerization induced by chemotactic stimulation involves polymerization at the barbed end of the filaments.

I 307 THE GRADATED SURFACE DISTRIBUTION OF PLASMA MEMBRANE GLYCOPROTEINS IN LOCOMOTING CELLS, Akira Ishihara, Bruce F. Holifield, and Ken Jacobson, University of North Carolina, Chapel Hill NC 27514.
Using monoclonal antibody labeling and digitized fluorescence microscopy, we have been making quantitative measurements of the surface distibutions of membrane proteins in locomoting cells and studying their relation with cell locomotion mechanisms. The distribution of a major plasma membrane glycoprotein, GP80, was gradated in locomoting mouse fibroblasts (higher concentration in the rear), whereas it was nearly uniform in stationary cells. The gradated distribution appeared to be a steady state condition while cells were locomoting. Similar gradated distributions of GP80 were observed also in motile macrophages and polymorphonuclear leukocytes. Gradated distribution of membrane proteins in locomoting cells can be quantitatively explained by either of two extreme models: in the retrograde lipid flow model, the flow carries slowly diffusing membrane proteins toward rear (Bretscher, Science 224:681); alternatively lipids and unanchored proteins could conceivably flow forward to form new leading edges, leaving transiently anchored proteins behind. The GP80 distribution was measured quantitatively and compared with the predictions of the two models. In contrast to GP80, the glycoproteins Thy-1 and H-2 exhibited nearly uniform distributions in locomoting fibroblasts. This can be explained by relatively rapid lateral diffusion of Thy-1 and H-2 compared with GP80. Both models predict shallower gradients when the lateral diffusion of the membrane protein is faster. Supported by NIH GM 39325 and ACS CD 181B.

I 308 TRANSMEMBRANE MODULATION OF ORGANELLE AND BULK TRANSPORT IN GOLDFISH RETINAL GANGLION CELL AXONS, Edward Koenig and Brian Edmonds, State University of New York at Buffalo, Buffalo, N.Y. 14214.
Ganglion cell axons of goldfish retinal explants have varicosities and intervening phase dense inclusions (IPDIs) that undergo bidirectional transport. These are aggregate structures composed of arrays of tubulo-vesicular elements embedded in an amorphous cytomatrix and represent a form of rapid bulk transport [Koenig, et al., 1985, J. Neurosci., 3:715]. Movements of varicosities and IPDIs appear to be passive, and powered by small and large hyperdense particles [Edmonds & Koenig; submitted]. The effects of surface binding by lectins on transport were studied by time-lapse video phase-contrast microscopy. Wheat germ agglutinin (WGA; 200 ug/ml) and Limax flavus agglutinin (LFA; 300 ug/ml) cause a rapid arrest of transport traffic, which is reversible in the case of WGA when N-acetylglucosamine (50 mM/ml) is added; succinylated WGA is not effective. On the other hand, succinylWGA (200 ug/ml) stimulates the dispersion of varicosities and redistribution of axoplasm. These results indicate that sialoglycoconjugated receptors are responsible for mediating inhibition of transport, while N-acetylglucosame-containing receptors facilitate axoplasmic redistribution in immature axons. Supported by grant NS21843 from the NINCDS.

I 309 MODULATION OF CURVATURE AND WAVE INITIATION IN TRITON X-100 EXTRACTED RAT SPERM BY Ca^{2+}, EGTA AND Ni^{2+}, C.B. Lindemann and J. Goltz, Oakland Univ., Rochester, MI 48063
Rat sperm from the cauda epididymis were extracted with .1% Triton X-100 in a medium containing: 0.13M sucrose, .024M K^+ glutamate, .02M Tris-HCl, 1mM DTT, and 2mM $MgSO_4$ (pH 7.9). ATP (0.3mM) and cAMP (3.0μM) were added to reactive motility. When 0.5mM EGTA or 0.5mM nitrilotriacetic acid (NTA) were included in the reactivation cocktail the sperm flagella uniformly exhibited a circular curvature in which the tip of the flagellum curved towards the tip of the asymmetric head. Inclusion of an excess of Ca^{2+} (>.5mM) reversed this curvature. The Ca^{2+} induced curvature often took the form of a sharp bend in the midpiece region that gave the cell the general shape of a "fish hook". These hooked cells were less motile than their straighter counterparts. The presence of the EGTA or NTA was not essential for induction of Ca^{2+} curves but seemed to improve the uniformity of the response. 0.5mM $NiSO_4$ inhibited the production of coordinated flagellar waves and antagonized the formation of hooks. Nickel induced an EGTA-like curvature pattern, even in the presence of 1mM Ca^{2+}. Although the hooked cells in Ca^{2+} were poorly motile, we discovered that a stepwise (1mM increments) transition to higher ATP concentration (5mM) often induced a very vigorous motility of the hooked midpiece region. This vigorous motility was sometimes limited to the midpiece but occasionally also entrained the principal piece in a unified wave. Our results suggest that the sharp Ca^{2+} dependent bends can participate in an oscillation mechanism but that establishment of oscillations under this condition may require a greater force development, necessitating that the level of Mg-ATP be high. [Supported by NSF Grant DCB-8510349.]

1310 GROWTH FACTORS INDUCE THE SYNTHESIS OF A CELL CONTRACTION-PROMOTING GLYCOSAMINOGLYCAN, A. Macieira-Coelho, I.J. Hiu and B. Azzarone, Laboratory of Cell Pathology, 94804 Villejuif, Cedex, France.

It is well known that membrane movements accompany the initiation of the division cycle but the relationship between the two events has not been elucidated. In order to study how growth factors trigger membrane movements, we measured the incorporation of aminosugars and sulfate into focal adhesion sites of resting stage human fibroblasts before and after adding FGF, PDGF and a growth factor produced during RSV infection. The incorporation of glucosamine and galactosamine decreased and that of sulfate increased. The material extracted from adhesion sites was precipitated and chromatographed with FPLC (Pharmacia). The change in the incorporation of the precursors occurred in a molecule containing glucuronic acid, with a molecular weight of 20,000 which could potentiate cell spreading and contraction. Its synthesis increases progressively during the G1 period and was directly related with the fraction of cells engaged in the division cycle. The molecules synthesized in resting stage and after the addition of growth factors, differed in the content of glucuronic acid, sulfate and aminosugars. Results show that growth factors induce the synthesis of this glycosaminoglycan in an active form. They supprt the previous conclusion (Int. Rev. Cyt., vol. 83, 1983, p. 183) that one of the first signals that trigger the division cycle is of a topological nature and that membrane movements are necessary for the initiation of the cycle because they are transmitted through cytoskeleton to the nuclear cage where they contribute to create the chromatin conformation favourable for gene expression.

1311 ACTIVATION OF PROTEIN KINASE C INCREASES F-ACTIN IN HUMAN BLOOD T LYMPHOCYTES. C.H. Packman, P.D. Phatak, M.A. Lichtman, Univ. of Rochester Medical Center, Rochester, NY.

Protein kinase C (PK-C) plays a key role in several forms of signal transduction. Actin has been proposed as a link in signal transduction in cells as well. Since phorbol myristate acetate (PMA) activates PK-C, we examined changes in the G/F-actin equilibrium of lymphocytes stimulated with PMA. Using the F-actin specific fluorescent probe NBD-phallacidin and flow cytometry, we observed that PMA caused a 2-3 fold increase in F-actin content in human lymphocytes. F-actin content peaked at 2 min with 1 μM, 5 min using 0.1 μM, and 10 min with 0.01μM PMA. Peak levels were similar with these doses and were sustained for at least 60 min. Neither phorbol-13-acetate, which does not activate PK-C nor f-Met-Leu-Phe, for which lymphocytes lack receptors, increased cellular F-actin. 1-oleoyl 2-acetyl glycerol (OAG), an analog of diacylglycerol, the proposed physiologic activator of PK-C produced a dose-dependent (5 to 25 ng/ml) increase in F-actin content. The increase in F-actin was followed by cell polarization in 3-8 min (depending on dose of PMA) with concentration of F-actin in the pseudopod and in long thin projections of cytoplasm. PMA- and OAG-induced increases in F-actin content were inhibited in a dose-dependent manner by cytochalasin B but the morphologic changes were not. Using fluorescent monoclonal antibodies, we determined that in response to PMA, F-actin increased almost 4-fold in T-helper cells (Leu 3+), 3-fold in T-suppressor cells (Leu 2+) and 1.3 fold in B cells (B4+). Thus, it is strongly suggested that activation of PK-C causes an increase in lymphocyte F-actin; this increase is much greater in T- than in B-lymphocytes. The temporal and chemical dissociation of cytoplasmic movement from the F-actin increase suggests this movement is not actin mediated. The increase in F-actin may have another function, perhaps as an early step in signal transduction.

1312 ACTIN IN THE PREPROPHASE BAND OF ALLIUM. Barry A. Palevitz, University of Georgia, Athens, GA 30602

The preprophase band (PPB) of microtubules (Mt) appears before mitosis in plant cells and marks the position at which the cell plate, led by the phragmoplast, joins the plasmalemma (PM) in telophase. Although the PPB disappears by midprophase, our evidence with time-lapse video microscopy shows that as the phragmoplast reaches the cortex it rotates and aligns with the PPB zone in Allium guard mother cells. Because of the importance of the positional information contained in the PPB or coincident "charmed" zones in the cortex or PM, additional data is needed on the composition of these elements. I now report that the PPB of Allium contains F-actin. Cells affixed to subbed slides were obtained from formaldehyde-fixed root tips digested in cellulysin. The cells were extracted with Triton, treated with rhodamine-phalloidin and rinsed briefly in PBS. Interphase cells contain a network of actin fibers that extend into the cortex. By preprophase, the meshwork is replaced by a band of fibers aligned in the position of the PPB. The actin band seems to consist of a thin layer of fibers next to the PM. Like the Mt PPB, the actin band becomes more narrow as preprophase progresses; it disappears by midprophase. Fluorescent bands are not seen in fixed cells treated with unlabeled phalloidin before staining. Roots treated with cytochalasin D do not have actin in their PPBs, but Mts at all stages of organization are present. Colchicine leads to the loss of both PPB Mts and actin. Thus, F-actin reorganization may be a result of Mt aggregation and not vice versa. New studies are aimed at a further understanding of the composition of the PPB zone and cytokinetic apparatus. Supported by NSF grant DCB84-05496.

1313 ALTERATION OF MACROPHAGE MORPHOLOGY AND FUNCTION BY 3-DEAZAADENOSINE. Karen L. Prus, Thomas P. Zimmerman and Gerald Wolberg, Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709.

3-Deazaadenosine (c^3Ado), a close structural analog of adenosine, exhibits anti-inflammatory and immunosuppressive activity in animal models. Although c^3Ado is both a substrate and inhibitor of S-adenosylhomocysteine (SAH) hydrolase, several reports have indicated that the biological activity of c^3Ado is not attributable to the inhibition of methylation reactions. We have previously shown that c^3Ado inhibits phagocytosis by mouse resident peritoneal macrophages through a mechanism independent of an interaction of c^3Ado with SAH hydrolase. Through the use of fluorescence microscopy with rhodamine-phalloidin or indirect immunofluorescence with an anti-actin antibody, it was demonstrated that c^3Ado alters the organization of microfilaments in these marcrophages. Scanning electron microscopy revealed a reduction of surface blebs and the conversion of microlamellae to large folds. The cessation of cell motility was confirmed by video-enhanced phase contrast microscopy. These various biological and morphological effects of c^3Ado were reversed rapidly (within 30 min) after washout of the drug from the cells. It is unlikely that c^3Ado affects microfilaments directly, as it has no effect on the viscosity of macrophage extracts or of purified skeletal muscle actin. 3-Deazaadenosine 5'-triphosphate (c^3ATP) is formed in these cells in small amounts (to approximately 1-2% of ATP levels), but data indicate that 5'-phosphorylation is not responsible for the expression of the biological effects of c^3Ado. The marked effects of c^3Ado on macrophage motility and function warrant further investigation of the interaction of c^3Ado with microfilaments.

1314 CYTOSKELETON, CYTOPLASMIC STREAMING, ELECTRICAL BEHAVIOUR AND LIGHT CONTROL ON *ACETABULARIA*, S. Puiseux-Dao°[+], A.C. Dazy[+], E. Garcia[+], A.M. Durrand[+], A. Moreau[+] and D. Schovaert*, (°) Unité INSERM 303 "Mer et Santé", Station Marine, BP 8, 06230 Villefranche-sur-mer, (+) Lab. de Cytophysiologie et Toxicologie cellulaire, Univ. PARIS VII, 2 Place Jussieu, 75005 Paris, (*) CHU, 94270 Kremlin-Bicêtre (FRANCE).

In *Acetabularia* giant cells, before cap formation, actin and tubulin-like proteins have been identified. Actin is abundant in the cytoplasmic strands where streaming occurs. Tubulin is diffuse in the cytoplasm and not organized in microtubules during the growth phase. Negative staining of cytoplasmic extracts, treated or not with Triton X 100, indicates that the cytoplasm of *Acetabularia* contains other filaments of different sizes. The most abundant and stable are very thin filaments, 1-4 nm in diameter, associated in bundles. These filaments may be responsible for the preferential association of poly(A)-RNA with the cytoskeleton (Triton resistant fraction of the cytoplasm) in *Acetabularia*.

Cytoplasmic streaming in vegetative cells is controlled by light : it ceases in the dark and recovers when cells are irradiated, showing rhythms and subrhythms, even in continuous light. Besides we observed that the electrical polarity of *Acetabularia* cells is also controlled by light in a manner very close to that operating for streaming : it drops around zero in darkness and resumes when the algae are illuminated again ; rhythms and subrhythms similar to those of streaming are detectable even with continuous irradiation also.

Simultaneous recording of chloroplast transport and electrical polarity seems to confirm the coupling of the two processes.

1315 INTRACELLULAR COMMUNICATION BETWEEN CULTURED CILIATED CELLS PROPAGATES CHANGES IN BEAT FREQUENCY INDUCED BY MECHANICAL STIMULATION, Michael J. Sanderson, Ida Chow* and Ellen R. Dirksen, Departments of Anatomy and Physiology* and the Jerry Lewis Neuromuscular Research Institute*, UCLA School of Medicine, Los Angeles, CA 90024.

Cultured, mammalian ciliated cells from the respiratory tract respond to mechanical stimulation by displaying a rapid, transient increase in beat frequency. This response is communicated to surrounding cells (Sanderson and Dirksen, Proc. Natl. Acad. Sci. USA, 82: 7302, 1986). To characterize the progression of the communicated response we have developed an automated, computer-assisted image analysis system to examine high-speed films of responding cells. We have measured the lag-phase, magnitude and duration of each cellular response, the velocity of propagation and the distances of transmission. The time it takes for the response to be transmitted from one cell to the next is slow (2-3 secs). This suggests that communication is not mediated by an electrical signal but is achieved by a diffusible messenger that moves via gap junctions. To support this hypothesis we have confirmed, with freeze-fracture techniques, that gap junctions exist between cells in both epithelial explants and outgrowths. Furthermore, we have demonstrated that adjacent or non-adjacent ciliated, as well as non-ciliated, cells are electrically coupled. We suggest that intracellular communication provides a mechanism to regulate beat frequency between ciliated cells in order to facilitate metachronal activity; a prerequisite for normal mucus transport. Supported by the Cystic Fibrosis Foundation, Smokeless Tobacco Research Council, Inc. and the National Science Foundation.

1316 STUDIES ON THE ROLE OF CELL MOTILITY IN GROWTH CONTROL USING AN AUTOMATED MICROSCOPE SYSTEM: METHODS AND PRELIMINARY RESULTS, Gavin Thurston, Bruno Jaggi and Branko Palcic, B.C. Cancer Foundation, Vancouver, B.C. V5Z 1L3 Canada.
Various lines of experimental evidence have implicated a role for the cytoskeleton in the regulation of cellular proliferation, but a coherent framework remains elusive. We have attempted to shed light on this issue by developing a flexible automated microscope system to study the motility and morphology of mammalian cells in tissue culture. The system has three modes of operation. One, the system performs fully automated tracking of a large number of individual motile mammalian cells, and records the movements of each cell centre. Two, the system is coupled to an optical memory disc recorder, and performs simultaneous cell tracking and image recording on many cells. Three, the system extracts morphological features of cells as they are tracked using real time image analysis. The first mode of operation has been used to look at the perturbation of cell motility by various agents, such as temperature and serum concentration. In many cases, but not all, there is a strong correlation between the average rate of cell movement and the average growth rate. This is in contrast to the performance of individual cells. The second mode of operation has enabled us to record serial images of cells with relatively little effort and cost, and has been used in the assessment of morphological and behavioural features. The third mode is currently under development: we are exploring a set of relevant morphological features, and measuring each feature in real time as cells are tracked. In this way, a quantitative relation may be established between morphology, patterns of motility, and certain features of in vitro cell behaviour.

1317 EVIDENCE FOR A TEMPORAL CHEMOTAXIS MECHANISM IN *DICTYOSTELIUM DISCOIDEUM* AMEBAE, Barbara Varnum-Finney and David R. Soll, Univ. of Iowa, Iowa City, Iowa 52242.
In an aggregation territory, waves of the chemoattractant cAMP originate at the center and sweep outwardly across each ameba. At the front of each wave, an ameba experiences a positive spatial gradient and an increasing temporal gradient. At the back of each wave, it experiences a negative spatial and a decreasing temporal gradient. Utilizing a perfusion chamber, we mimicked the temporal aspects of the wave in the absence of a spatial gradient. An increasing temporal gradient stimulated the rate of movement and reduced the frequency of pseudopod formation and turning. In addition, we examined chemotaxis in a relatively stable spatial gradient of cAMP in a Zigmond chamber. When amebae migrated toward the source, they were faster, extended fewer new pseudopods and turned less frequently than when they migrated away from the source. In addition, amebae extended pseudopods randomly with respect to the gradient and did so in a polarized fashion. However, pseudopods formed towards the source produced a turn more frequently than those formed away from the source. Finally, we perfused amebae with a shallow increasing temporal gradient which mimicked the temporal gradient generated by movement toward the source in a Zigmond chamber. The frequency of pseudopod formation and turning were depressed. These results indicate that there are two decision making systems, one localized in the pseudopods and one along the entire cell body, and although they do not rule out the involvement of a spatial chemotaxis mechanism, they strongly suggest that the temporal dynamics of a cAMP wave and the temporal gradient generated by movement through a spatial gradient may play a major role in eucaryotic chemotaxis.

1318 CALCIUM CHANNEL INVOLVEMENT IN EXOCYTOSIS IN PARAMECIUM, Alice L. Vuoso and Birgit H. Satir, Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, New York 10461.
A possible calcium channel involvement in TNP-induced exocytosis of trichocyst matrices in Paramecium tetraurelia was examined with the calcium channel blocker, verapamil. Verapamil (30-75 uM) inhibited secretion in a time and dose-dependent manner under standard buffer conditions (includes 20mM Mg^{2+}, >8mM Na^{+}, 1mM K^{+}, and 0.5mM Ca^{2+}) and likewise, in buffers lacking Na^{+} or K^{+}. The effect of the drug was reversible. Cadmium inhibited exocytosis, but nifedipine had no effect. In verapamil an intermediate stage of TNP-stimulated release response, termed "$A^{+}P^{-}$" (anterior positive for secretion, posterior negative) was observed for the first time in which inhibition began at the posterior end of the cell body, and subsequent recovery proceeded from the anterior end. This polarized response provides evidence for either a gradient of calcium channels in the plasma membrane or a differential sensitivity of the calcium channels to verapamil and supports the hypothesis for their involvement in exocytosis in Paramecium.

1319 REGULATION OF FLAGELLAR MOTION BY cAMP AND Ca^{2+}, W. Wasco, J.A. Horowitz, M. Paupard, J. MacLeod and G.A. Orr. Albert Einstein College of Medicine, Bronx NY 10461.
Sperm motility is stimulated by cAMP and inhibited by Ca^{2+}. The majority of cAMP-dependent protein kinases in rat sperm are located on the demembranated flagellum and are of the type II class. They exist in stoichiometric amounts with the dynein inner or outer arms. The regulatory subunit (RII) interacts with a limited number of flagellar polypeptides. It is released from the flagellum by thiol reducing agents along with a limited subset of flagellar polypeptides which does not include tubulin, the dynein ATPase, or any of the putative binding proteins. The site of interaction between RII and the flagellum appears to be within the proteolytically sensitive hinge region Evidence for the involvement of CaM in the Ca dependent regulation of motility has previously been lacking. We have found that rat flagella contain a sub-population of CaM which remains tightly bound despite numerous EGTA washings, and that there is a Ca^{2+}/CaM regulated cyclic nucleotide phosphodiesterase firmly attached to the flagella of rat and bovine sperm. ^{125}I-CaM overlay studies have revealed the presence of a one major CaM-binding protein in both rat and bovine flagella. This protein has a subunit M_r of approximately 65,000 by SDS-PAGE. In conclusion, we have shown that a Ca^{2+}/calmodulin-regulated cyclic nucleotide phosphodiesterase and the regulatory subunits (R) of cAMP-dependent protein kinase are integral components of mammalian sperm flagella. We propose that one mechanism whereby Ca^{2+} could serve to inhibit flagellar motion is by activation of the CaM regulated PDE, resulting in a reassociation of the cAMP-dependent protein kinase subunits and consequently in the inhibition of all cAMP-dependent phosphorylation.

1320 CALCIUM AND MITOTIC PROGRESSION IN STAMEN HAIR CELLS OF TRADESCANTIA.
Stephen M. Wolniak and Tung-Ling Chen, University of Maryland, Dept. of Botany, College Park, MD 20742, U.S.A.
The manipulation of extracellular Ca^{2+} activity or Ca^{2+} conductivity across the plasma membrane has a significant impact on mitotic progression in stamen hair cells of Tradescantia. A reduction of extracellular Ca^{2+} by treatment with quin-2 results in metaphase arrest that is reversible by the addition of the cation with timing that is inversely related to the Ca^{2+} level in the medium. A23187 lowers the Ca^{2+} concentration necessary for reversal and shortens the time interval for reversal. Nifedipine (a Ca^{2+}-channel entry blocker) also induces metaphase arrest. Photoinactivation of this drug by UV irradiation results in anaphase onset, the timing of which is inversely related to the free concentration of the cation added to the medium. We have also observed precocious entry into anaphase by treating cells with Bay K-8644 (a Ca^{2+}-channel agonist) $\leq$ 10 min after nuclear envelope breakdown. The 20-30% reduction in metaphase transit time with Bay K-8644 is loosely dependent on extracellular Ca^{2+} activity; at very low levels of the cation (pCa $\simeq$ 8), anaphase onset is forestalled, while at higher levels, mitotic progression is accelerated. Similarly, treatment with ruthenium red up to 10 min prior to anaphase onset promotes precocious entry into anaphase in a Ca^{2+}-related (though hard to define) fashion. Collectively, these results suggest that a rise in cytosolic Ca^{2+} is necessary for anaphase onset, and a likely source for at least some of this Ca^{2+} is outside the plasma membrane. (Supported by NIH grant GM-32552.)

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