MEMBRANE SKELETONS AND CYTOSKELETAL-MEMBRANE ASSOCIATIONS

Vann Bennett, Carl M. Cohen, Samuel Lux and Jiri Palek, Organizers

March 9 — 15, 1985

Membrane Skeletal Proteins: Characterization. Association and Ultrastructure

0411 SYNTHESIS OF SPECTRIN AND ITS ASSEMBLY INTO THE RED BLOOD CELL CYTOSKELETON OF NORMAL AND MUTANT MICE. Jane E. Barker, David M. Bodine, and Connie S. Birkenmeier, The Jackson Laboratory, Bar Harbor, ME 04609. The definition of defects in mice with hereditary spectrin deficiencies has led to speculations on cytoskeletal protein assembly. Four mutations (nb, ja, sph, and sph^{ha}) cause severe hemolytic anemias in homozygotes. Spectrin synthesis and binding have been studied in the reticulocytes of mice heterozygous and homozygous for the various mutations (1), and the results compared with data from phenylhydrazine treated normal mice. Reticulocytes of normal mice, like erythroid cells of chicken embryos (2), synthesize more alpha than beta spectrin but bind equivalent amounts to the membrane. Similar analyses combined with analyses of the electrophoretic profiles of the mutant mice have allowed us to ascribe the spectrin deficits to specific protein anomalies. In nb/nb miee, there is an ankyrin deficiency and only 1/2 the normal levels of alpha and beta spectrin are found in the membrane. In ja/ja mice, beta spectrin is lacking and alpha spectrin, while synthesized at near normal levels and bound initially to the membrane, is not present in electrophoretic profiles of ghost proteins. In sph/sph mice, no alpha spectrin is synthesized and membrane bound beta spectrin is decreased. In sph^{ha}/sph^{ha} mice, alpha spectrin synthesis is 6 × normal and 2 × more alpha spectrin is bound to the membrane. On the basis of binding studies in sph^{ha}/sph^{ha} mice, it is difficult to explain the paucity of alpha spectrin seen after electrophoresis of the ghost proteins. Double label experiments have been performed and suggest a rapid turnover of membrane bound alpha spectrin.

From the study of mutant mice, we can deduce some salient features of membrane assembly. Beta spectrin must be present to stabilize the bound alpha spectrin. The converse also appears to be true, although more beta spectrin can bind stably in the absence of alpha spectrin. Spectrin also appears to be incorporated stably but at reduced levels in the absence of ankyrin.

Assignment of the mutations to sites on specific chromosomes has been accomplished using biochemical and morphological markers. The sph and sph^{ha} loci, but not the ja locus, map to Chromosome 1. If, as we suspect, sph and sph^{ha} are mutations in the alpha spectrin gene and ja is a mutation on the beta spectrin, then the spectrin determinants are not linked. Mapping studies and analyses of the various mutants at both the cellular and molecular level continues.

1. Bodine, D. M., IV, Birkenmeier, C. S., and Barker, J. E. 1984. Cell 37:721.
2. Bilkstad, I., Nelson, W. J., Moon, R. T. and Lazarides, E. 1983. Cell 32:1089.

0412 STRUCTURE AND FUNCTION OF PROTEIN 4.1, *Thomas L. Leto, Department of Pathology, Yale University School of Medicine, New Haven, CT 06510.*

Protein 4.1 is an essential component of the membrane cytoskeleton which participates both in the association of spectrin with actin filaments and in the attachment of the cytoskeleton to transmembrane glycoproteins. In erythrocytes this protein is composed chiefly of two chemically very similar polypeptide chains (MW ~ 80,000 dal) which appear to differ only by 2,000 daltons. Recently, we have combined detailed chemical analyses with various functional assays in an effort to describe the functional topology of protein 4.1. Several methods for specific cleavage of purified 4.1 have been developed and characterization of these cleavage products has revealed several striking features (1). Protein 4.1 appears to be a highly polarized molecule. Mild treatments of 4.1 with α-chymotrypsin cleave the molecule at central locations and generate very basic, protease resistant, and acidic, protease sensitive, domains. Amino acid sequence analysis indicates that the basic domain is amino terminal and that the region of variability between 4.1 a and b components lies near the carboxyl terminus. Sulfhydryl reagents have revealed a cluster of cysteine residues within the basic amino terminal domain. Methionine residues appear to be clustered as well, based on the mapping of cyanogen bromide cleavage sites and amino acid compositional analysis of α-chymotryptic fragments. Tyrosine residues are also highly localized.

Studies exploring functional sites suggest that two separate functions are spatially segregated into distinct regions of protein 4.1. Proteolytic fragments of protein 4.1 which exhibit glycophorin binding are unable to promote the association of spectrin and F-actin, while those peptides which complex with spectrin and actin do not bind to glycophorin. A chymotryptic fragment as small as 5 kilodaltons is capable of promoting spectrin-F-actin associations (2).

Several phosphorylation sites within protein 4.1 have been observed. The occurrence of ^{32}P within the substructure of protein 4.1 apparently depends on solution conditions and may involve several kinases. Phosphorylation occurs at sites near the carboxyl terminus when protein 4.1 is associated with spectrin depleted membranes, while other unique sites closer to the amino terminus are labeled on soluble protein 4.1 by a kinase isolated from erythrocyte membranes. The effect of these various phosphorylation states on protein 4.1 cytoskeletal associations is currently under investigation.

References

1. Leto, T.L. and Marchesi, V.T. (1984) J. Biol. Chem. 259, 4603.

2. Correas, I., Leto, T.L., Speicher, D.W. and Marchesi, V.T. (1984) J. Cell Biol. 99, 300a.

0413 STRUCTURE AND FUNCTION OF BRAIN AND ERYTHROCYTE SPECTRIN: IMPLICATIONS FOR CYTOSKELETAL REGULATION, Jon S. Morrow, Department of Pathology, Yale Medical School New Haven, CT 06510

The antigenic, morphologic, and functional similarity of the non-erythroid and erythroid spectrins has fostered the belief that their roles may be similar to that in the erythrocyte, i.e. support of the plasma membrane. Detailed analysis of the specific functions and structural domains preserved in human brain spectrin suggest quite a different role for this protein. Brain spectrin (Fodrin) differs from its erythrocyte counterpart at specific structural and functional domains within each subunit. By peptide mapping, limited tryptic digestion, sequence, and antigenic criteria there is a significant preservation of the interior domains of both subunits (analogous to erythrocyte spectrin domains alpha II-V and beta II and III). Conversely, regions of each molecule specific for self-association, covalent phosphorylation, ankyrin and calmodulin binding appear functionally, structurally, and antigenically distinct. These changes render brain spectrin most suited for a role as a specific linker of surface receptor molecules to cytoplasmic actin, rather than as a participant in the formation of an elaborate membrane skeleton. Additional isotypes of human spectrin also appear to be present in skeletal muscle, with properties intermediate between the erythrocyte and brain forms. All of the spectrins appear subject to regulation. Covalent phosphorylation or ankyrin binding alters the self-associative properties of erythrocyte spectrin. All of the spectrins bind calmodulin, and all are covalently phosphorylated. In cell culture studies SV40 transformed fibroblasts exhibit spectrin patching and redistribution away from the membrane as one of the earliest manifestations of transformation. Together these data support the concept that as a family of related proteins the spectrins have been adapted to two fundamental roles: i) membrane support, by the formation of an elaborate "membrane skeleton"; and ii) signal transduction, by participation as a modulatable and specific link between organizational events involving membrane receptors and the cortical and cytoplasmic matrix.

0414 STRUCTURAL FEATURES OF HUMAN ERYTHROCYTE SPECTRIN: FUNCTIONAL AND EVOLUTIONARY IMPLICATIONS, David W. Speicher, Pathology Department, Yale University School of Medicine, New Haven, CT 06510.

Erythrocyte spectrin is a heterodimeric protein (M. W. = 460,000) which is a major component of the membrane skeleton. Recently, closely related proteins have been identified in most cell types by many research groups. The number of distinct proteins which belong in this class has not been determined. Erythroid spectrin is the most extensively characterized member of this group, and it serves as a model for many structural properties of these proteins. Amino acid sequences of more than 1500 residues have been determined for human erythrocyte spectrin. The two subunits are about 35% identical, and both subunits are comprised of homologous non-identical segments with a periodicity of 106 amino acids. Approximately 35 of these repetitive segments exist in the heterodimer, comprising more than 90% of the total protein structure. This existence of many homologous segments has facilitated construction of a detailed conformational model representative of essentially the entire molecule. Each 106 amino acid segment forms a rigid triple helical segment approximately 5nm in length. These rigid segments are connected by short non-helical regions which impart flexibility to the molecule. Connecting regions are not susceptible to mild proteolysis and might be shielded by the ends of the helices. The flexible interface between triple helical segments is a potential site for modulating overall flexibility of the molecule. The second helix in the triple helical segment is the longest and is also the least well conserved. The two shorter helices are better conserved and are probably involved in subunit association. Stabilization of subunit association potentially involves the following interactions: dipole moment, hydrophobic, hydrogen bonding and electrostatic.

Several interesting evolutionary features are indicated by available sequence information. The protein evolved to its current size long ago and has undergone extensive mutation (average homology between segments is about 20%). Enigmatically, the many repeats in spectrin have retained precisely 106 amino acids per segment. Apparently there is a strong selective pressure to maintain sequence length, although the reasons for this conservation have not yet been elucidated. This protein probably evolved from an ancestral 12,000 dalton peptide through a series of intermediate stages. During early stages of development the smaller-sized intermediates may have been antiparallel homodimers. A number of important evolutionary events probably occurred when the protein was half its current size. Actin and Protein 4.1 binding capacity might have been acquired at this stage. This was probably also the stage where two independently mutating genes developed giving rise to a heterodimer. Finally, this stage is a likely site for divergence to multiple tissue type spectrins.

Characterization and Assembly of Membrane Skeletal Proteins

0415 SPIN-LABEL STUDIES OF MEMBRANE-SKELETAL PROTEIN INTERACTIONS IN HUMAN ERYTHROCYTES, D. Allan Butterfield, Troy M. Harmon, and Bennett T. Farmer, II, University of Kentucky, Lexington, KY 40506-0055

Spectrin, the predominant protein of the erythrocyte membrane skeleton, normally exists in the tetrameric state of aggregation. Increased amounts of dimeric spectrin and increased membrane skeleton instability. The polyamine, spermine, and the polyphosphate, 2,3-diphosphoglycerate 2,3-DPG, retards or accelerates respectively, the lateral diffusion of transmembrane proteins. The effects of increasing the amount of dimeric spectrin in membranes and separate investigations of the effects of spermine and 2,3-DPG on the physical state of erythrocyte membrane skeletal proteins were studied by protein, lipid, and carbohydrate-specific spin labeling methods. Increasing the proportion of dimeric spectrin and disruption of the skeletal protein interactions by 2,3-DPG greatly alters the physical state of skeletal proteins ($P<0.002$ and $P<0.01$, respectively), but does not alter the physical state of membrane phospholipids or cell surface carbohydrates. In contrast, spermine, which is thought to interact with the skeleton, significantly decreases the mean ESR parameter reflective of protein segmental motion by nearly 40% ($P<0.0001$), slightly increases the order of membrane phospholipids ($P<0.02$), and increases the rotational rate of cell surface sialic acid, most of what is on glycophorin A ($P<0.0005$). These findings demonstrate that agents which differentially affect lateral diffusion of transmembrane proteins by interaction with the skeleton result in differential alterations of membrane components. Supported by NIH (AG-00084) and the Alzheimer's Disease and Related Disorders Association.

0416 ASSOCIATION OF 5'-NUCLEOTIDASE WITH MICROFILAMENTS: DEMONSTRATION BY PHALLOIDIN SHIFT ON VELOCITY SEDIMENTATION SUCROSE GRADIENTS. C. Carraway, C. Sindler and M. Weiss, Univ. of Miami School of Medicine, Miami, FL 33101

An emerging paradigm in cell biology is that organization of cell surface molecules is controlled by interaction of the cytoskeleton, particularly microfilaments (MF), with plasma membrane components. Evidence for the association of the surface enzyme 5'-nucleotidase (5'N) with the cytoskeleton has been presented for lymphoid cells and for isolated microvilli from ascites tumor cells. The enzyme remains with the "cytoskeletal residue" prepared by nonionic detergent extraction and centrifugation, a technique used to suggest the association of a growing number of cell surface glycoproteins with the cytoskeleton. Artifacts arising from trapping and cosedimentation cannot be ruled out in such studies. We have recently extended this methodology to demonstrate the association of a surface glycoprotein (CAG, cytoskeleton-associated glycoprotein) with MF. The method utilizes the discriminatory capability of velocity sedimentation sucrose density gradients and the specificity of phalloidin as a MF-stabilizing agent for analysis of MF-associated proteins. In a variety of Triton X-100-containing buffers most of the microvillar actin is found as filaments in the denser sucrose fractions, coincident with a large fraction of the 5'N activity. When phalloidin is added to the extraction buffer to stabilize MF, both filaments and 5'N activity are shifted concomitantly further into the gradient. These results provide evidence for a specific association of 5'-nucleotidase with microvillar microfilaments.

0417 FIBRONECTIN POTENTIATES ACTIN POLYMERIZATION IN THROMBIN-ACTIVATED PLATELETS, C.S. Cierniewski, J.Karczewski and M.A. Kowalska, Medical School of Lodz, POLAND

Thrombin-activation of platelets causes the cells to acquire the ability to bind fibronectin to cell-surface receptors. Studies in fibroblasts indicate that fibronectin molecules associated with membranes communicate either directly, or through some transmembrane linkages, with actin microfilaments. Therefore, we decided to study the effect of fibronectin on the course of actin polymerization in platelets.

Triton X-100 insoluble cytoskeleton was prepared from thrombin-activated platelets. Total actin was determined by scanning SDS-PAGE gels. The conversion of G-actin into F-actin was monitored by an assay involving DNAse I inhibition by G-actin. It was found that fibronectin decreased the level of G-actin in Triton X-100 extracts of thrombin-activated platelets, which suggests that a large amount of F-actin was present in the Triton X-100 insoluble cytoskeleton. At the same concentration, fibrinogen only slightly increased actin mobilization while bovine serum albumin at a much higher concentration, caused a small inhibition of this reaction. Our data suggests that fibronectin, through interaction with the platelet actomyosin fibrillar system, facilitates actin polymerization into cytoskeleton.

0418 ACTIN MAY BE INVOLVED IN SYNAPTIC PLASTICITY, Eva Fifkova, Jeffrey A. Markham, and Karen Cullen-Dockstader, University of Colorado, Boulder, Colorado 80309.

A central problem in neurosciences concerns the mechanism of neuronal plasticity. Since plasticity allows for adaptive changes in the brain in response to experience, its mechanism is likely to be an integral part of normal neuronal functions. Functional capacity of the brain is determined by the capacity of its synaptic contacts because they subserve communication between neurons. A number of neurons form synapses on dendritic spines which are appendage-like outgrowths of dendrites. Various experiments have shown that increased neuronal activity can be translated into morphometric changes that involve enlargement of the spine head and widening and shortening of the spine stalk. The latter change would decrease longitudinal resistance of the stalk and thus may enhance neuronal activity. Since we assumed that such a change could involve contractile proteins, we used the S-1 fragment to demonstrate the presence of actin filaments in spines. Indeed we have observed that actin filaments form a dense network in the spine head and longitudinally oriented strands in the spine stalk. The filaments are associated end-on and laterally with the spine membrane and the postsynaptic density. Both modes of association were also observed with vesicular structures and with the SER. Since myosin and actin-associated proteins are also present in dendritic spines and since free cytosolic calcium increases with synaptic activity, a sequence of reactions is likely to be triggered which might induce solation of the actin network in the spine head and contraction of the filaments in the spine stalk. Both these activities would result in the morphometric changes observed in stimulated dendritic spines.

0419 EVIDENCE OF EXTENSIVE MOVEMENT OF GC, A VITAMIN D- AND ACTIN-BINDING PROTEIN IN THE CELL MEMBRANE, Robert M. Galbraith, David L. Emerson and Andre E. Nel, Medical University of South Carolina, Charleston, S.C. 29425

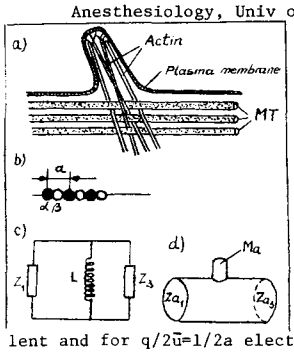
Gc is the major vitamin D-binding protein in serum, and is also known to interact at high affinity with G-actin, facilitating depolymerization of F-actin and stabilizing G-actin in the monomeric form. Small amounts of Gc have been found in the cytoplasm of most nucleated cells together with a large excess of unoccupied specific binding sites. In addition, we have recently documented an indistinguishable protein on the plasma membrane of certain cells, particularly peripheral blood mononuclear cells; by immunofluorescence and physiochemical methods. Warming of monocytes and B cells to 37^{o} showed little intrinsic lateral mobility of Gc, but clear co-mobility (co-patching and co-capping) was evident if cells were first reacted with specific antisera known to induce mobility of other membrane proteins. This was associated in the case of B cell membrane immunoglobulin (MIg) with physicochemical evidence of interaction contemporaneously between Gc, MIg and membrane actin. By contrast, similar experiments in T-enriched cells showed little or no endogenous membrane Gc detectable by fluorescence. However, primary reaction with monoclonal Pan T antiserum (Leu 1) led to a clear increase in Gc fluorescence intensity and availability for surface radioiodination, indicating the possibility of vertical displacement in the lipid bilayer. Similar enhancement of Gc fluorescence was seen following other primary antisera of different specificity. These results suggest that in addition to any possible roles in vitamin D3 metabolism, Gc may become spacially associated with certain membrane proteins, including actin, with resulting movement both laterally, and in a vertical dimension.

0420 Crosslinking of Fibronectin To Human Platelet Cell Surfaces: Evidence That The Glycoprotein IIb-IIIa Complex Is A Receptor For Fibronectin. John M. Gardner and Richard O. Hynes. The Center For Cancer Research, M.I.T. Cambridge, MA 02139

Fibronectin binds to the surface of activated platelets in a manner which is time and dose dependent, but it does not interact with resting platelets. To identify the surface membrane component which mediates the interaction of FN with platelets, we have utilized a bifunctional, cleavable crosslinking agent (DTSSP), to covalently link candidate receptor molecules to FN or the 12kd pepsin resistant fragment of FN. Binding of the 12kd fragment was specific and competed by a synthetic peptide containing the cell attachment site of FN. Binding of the 12kd fragment or native FN to ^{125}I-labeled, activated platelets followed by crosslinking, lysis and immunoprecipitation with anti-Fn antibodies resulted in the specific co-precipitation of several labeled platelet surface components with MW=90,000-140,000 Daltons upon SDS-PAGE analysis. Direct binding of ^{125}I-labeled 12kd fragment to thrombin activated platelets followed by crosslinking resulted in transfer of label into bands of MW=110,000 and 130,000 Daltons. In addition, when these crosslinked complexes were subjected to immunoprecipitation with monoclonal antibodies directed against the glycoprotein IIb-IIIa complex present on platelet surfaces, they were specifically precipitated. These lines of evidence implicate the glycoprotein IIb-IIIa complex as a fibronectin receptor on stimulated platelets. There already exists a strong body of evidence that glycoprotein IIb-IIIa also functions as a fibrinogen receptor on platelets and given our results it appears that this novel membrane protein complex has evolved to interact with more than one extracellular ligand.

0421 SUBSTRUCTURE OF THE α-ACTININ MOLECULE. N. Arakawa, Darrel E. Goll, R.M. Robson, and William C. Kleese, University of Arizona, Tucson, Arizona 85721.
The exact physiological function of α-actinin is still unclear. In vitro, α-actinin binds to and cross-links actin filaments. But, other proteins such as filamin and spectrin also cross-link actin filaments, and spectrin presently seems more likely to have a role in linking actin filaments to the plasma membrane than α-actinin. α-Actinin also increases the Mg^{2+}-modified ATPase activity of reconstituted actomyosin suspensions, but the physiological significance of this effect is unknown. Mixtures of α-actinin and actin are difficult to study because they are heterogeneous and thixotropic. Therefore, we have attempted to learn whether "active" fragments of α-actinin could be produced by proteolytic digestion. As determined by SDS-PAGE, rigorous tryptic digestion of turkey gizzard α-actinin produces 72-kDa and 32-kDa fragments from the 100-kDa α-actinin polypeptide. The 72-kDa fragment breaks down quickly to a 65-kDa fragment and then to fragments of 54- and 50-kDa. The 32-kDa fragment breaks down more slowly to a 15-kDa fragment. In nondenaturing solvents, the 72-, 64-, 54-, and 50-kDa polypeptides are assembled into dimers like the native α-actinin molecule, whereas the 32-kDa fragment is monomeric. The N-terminii of the 32-kDa and 15-kDa fragments are blocked. The 32-kDa fragment binds weakly to actin filaments but does not increase the viscosity of F-actin solutions. The 65, 54, and 50-kDa fragments do not bind to actin filaments. The results are consistent with but do not prove an anti-parallel, two-chain structure for α-actinin with a 32-kDa polypeptide extending from either end of a highly α-helical, two-chain core. (Supported by NIH Grant No. AM-19864).

0422 ELECTRO-ACOUSTIC EFFECTS IN MICROTUBULE-ACTIN INTERACTIONS CAN EXPLAIN MEMBRANE SURFACE ALTERATIONS, Djuro L. Koruga, Ph.D. and Stuart R. Hameroff, M.D., Dept of Anesthesiology, Univ of Arizona HSC, Tucson, AZ 85724



Microtubules (MT) possess crystal characteristics since the α and β subunits are arranged on a cylindrical surface by sphere packing of the Oh (6/4) symmetry group law. MT tubulin subunits contain more acidic amino acids than basic, thus surplus electrons can lead to capacitance behavior. With available thermal energy α and β tubulin subunits should oscillate. Since the masses of α and β tubulin are approximately equal, the photon-optical and phonon-acoustical frequency (ω^+ and ω^-) characteristics will be equal when the frequency wave vector ($q/\bar{u}$) is ½a (where a is the distance between α and β tubulin centers fig.b). Interactions among ions (Mg^{++}, Ca^{++}...) and MT subunits may result in functional electric fields in MT which may be induced in other proteins like actin. In figure c), these are viewed as an LC circuit. Since α and β tubulin masses are equivalent and for $q/2\bar{u}=1/2a$ electro-acoustical analogies may be used. Thus the electrical circuit $[Z_2=j\omega L]$ has an analogous acoustic form $[Za_2=j\omega Ma]$ with a physical component as in fig.d). Thus electro-acoustical properties of cytoskeletal proteins can induce perpendicular bulges and other shape alterations in cell membranes.

0423 REGULIN, A SPECTRIN-ASSOCIATED PROTEIN IN RABBIT RETICULOCYTES. Gisela Kramer, Jennifer Tipper, Wieslaw Kudlicki, Susan Fullilove and Boyd Hardesty, Clayton Foundation Biochemical Institute, Dept. Chemistry, University of Texas, Austin, TX 78712

Preliminary characterization of regulin is given in Fullilove et al., J.Biol.Chem. 259, (1984) 2493-2500. Regulin can be extracted together with spectrin from the membrane fraction of lysed rabbit reticulocytes by the low ionic strength, high pH procedure developed for the extraction of spectrin from erythrocytes. Separation of regulin from spectrin can be achieved by chromatography on DEAE-cellulose at pH 8.5. Regulin is identified by the use of monoclonal antibodies as a 230,000 dalton protein. Upon lysis of reticulocytes, proteolytic degradation products of regulin are found in the postribosomal supernatant. They cochromatograph with phosphoprotein phosphatase activity and with a protein kinase that phosphorylates specifically peptide initiation factor two (eIF-2) in its alpha subunit and thereby controls protein synthesis. Regulin appears to be present in erythrocytes at a greatly reduced level.

0424 SPECTRIN AS AN INTEGRATOR OF INTRACELLULAR FILAMENT NETWORKS. Robert C. Langley, Jr. and Carl M. Cohen, Dept. of Biomedical Research, St. Elizabeth's Hospital, Boston, MA and Depts. of Medicine and of Molecular Biology and Microbiology, Tufts Univ. Med. School. We studied the association of spectrin with intermediate filaments (IF) by measuring IF induced sedimentation of spectrin at physiological pH and salt conditions. Spectrin binding to both desmin and vimentin filaments increased in a concentration dependent manner and appeared to saturate at approximately 20 ug of spectrin per 500 ug desmin. Spectrin binding to vimentin was more variable, plateauing in the range of 20 to 70 ug spectrin bound per 500 ug vimentin. Band 4.1 which enhances the binding of spectrin to actin, had little effect on spectrin-IF binding. Low angle rotary shadow electron microscopy of the spectrin-IF complexes showed infrequent associations of spectrin randomly distributed along the filament. Desmin and vimentin filaments bound to spectrin reconstituted inside out red cell vesicles (IOVs) forming multiple associations which resulted in aggregates of IOVs and filaments. IOVs that were not reconstituted with spectrin did not form complexes with the IF indicating the specific dependence on the presence of spectrin for filament-membrane interaction. Intermediate filaments have been shown to span the cytoplasm connecting the nuclear and cytoplasmic membranes, but the molecular mechanism of the interaction of IF with membranes is unknown. The binding of spectrin to IF demonstrates a possible mechanism by which IF can associate with membranes. Since spectrin also binds actin it may mediate associations of IF and actin. Spectrin's ability to bind actin, IF and a membrane associated protein, ankyrin, suggests a role for spectrin as a mediator of the interaction of the different cytoskeletal systems and their association with plasma membranes. Supported by NIH 24382.

0425 MOLECULAR SHAPE AND SELF ASSOCIATION OF VINCULIN AND METAVINCULIN, Leslie Molony Milam and Keith Burridge, Dept. of Anatomy, UNC, Chapel Hill, NC 27514

Vinculin is a 130,000 dalton protein localized to adhesion plaques, and thought to be involved in microfilament-membrane attachments. Metavinculin, a 150,000 dalton protein closely related to vinculin, and vinculin can be purified by low ionic strength extraction and ion exchange chromatography from chicken gizzard smooth muscle. Electron microscopy of purified vinculin and metavinculin which had been rotary shadowed has revealed two proteins with identical molecular shapes. Vinculin and metavinculin both have globular head regions attached to rod-shaped tail domains.

Both proteins have a tendency to form complexes. Examination of rotary shadowed vinculin and metavinculin complexes obtained by dialysis into a high ionic strength buffer showed multimers resembling umbrellas or "bunches of balloons". The multimers consisted of two to six individual vinculin or metavinculin molecules aligned head to head and tail to tail. Electron microscopy of talin, another protein thought to be involved in actin membrane attachment and known to interact with vinculin, shows a globular protein in low ionic strength buffer, and an elongated flexible rod in buffers close to physiological ionic strength. Proteolysis of talin results in a 190 Kd vinculin binding fragment which is elongated at both low and high ionic strength, as seen by electron microscopy and shown using biophysical techniques. We are currently investigating the interaction between vinculin and talin using electron microscopy and biophysical methods.

Supported by a Muscular Dystrophy Postdoctoral Fellowship and Grants from NIH (GM 29860) and MDA.

0426 CHARACTERIZATION OF A 190 K DALTON VINCULIN-BINDING PROTEIN FROM SMOOTH MUSCLE. T. O'Halloran and K. Burridge, Dept. of Anatomy, UNC-CH, Chapel Hill, NC 27514

From low ionic strength extracts of chicken gizzard we have purified a major protein with an apparent molecular weight of 190 kd in SDS gels. When transferred to nitrocellulose the purified protein binds iodinated vinculin. In addition its localization in chick embryo fibroblasts is similar to that of both vinculin and talin, staining adhesion plaques as well as fibrillar streaks which underlie the extracellular protein fibronectin. Western blots of chick embryo tissues (cardiac muscle, smooth muscle and skeletal muscle) probed with anti-190 reveal a 215 kd band corresponding to talin as well as the 190 kd band. To determine the relationship between the 190 kd protein and talin, we compared partial peptide maps. Talin, a 190 kd fragment of talin, and purified 190 kd protein were digested with V-8 protease or trypsin. Comparison of the peptides generated shows that the 190 kd polypeptide is identical to the 190 kd fragment of talin. These digests are identical to the partial digest of talin except for a 40 kd fragment generated by V-8 protease which we infer to be the difference between the 2 proteins. Although talin changes from a globular protein in low ionic strength buffers to an elongated rod in high ionic strength, the 190 kd polypeptide is an elongated rod in both buffers. We infer that the 40 kd polypeptide must be a necessary component for changes in shape of the talin molecule.

Supported by a grant from NIH (GM 29860), Muscular Dystrophy Association, and American Cancer Society.

0427 ACTIN POLYMERIZATION AND DEPOLYMERIZATION IN HUMAN NEUTROPHILS, R.G. Painter*, G.M. Omann and L.A. Sklar, Univ. of Texas Hlt. Ctr., Tyler, and Scripps Clinic & Res. Fdn., La Jolla, CA.

When exposed to the N-formylated chemoattractant peptides, neutrophils undergo a transient ruffling followed by a polarization that involves the redistribution of F-actin. The cells also undergo a biphasic right angle light scattering response whose first phase is maximal 10-15 seconds after exposure to the stimulus and a second phase which is longer in duration and maximal only after a minute or more. We now report that the first phase is accompanied by a transient polymerization of actin and the second phase is accompanied by a more sustained polymerization of actin. Based on correlative measurements of ligand binding and intracellular Ca^{2+} elevation, under conditions where we use the fluorescent Ca^{2+} chelator, Quin-2, to modulate intracellular Ca^{2+}, we conclude that the first phase requires less than 100 receptors/cell (out of 50,000) and does not require release of intracellular stores of Ca^{2+}. In contrast, the second phase requires both the occupancy of thousands of receptors (an estimated 10% of the total surface receptors occupied per minute) and the elevation of intracellular Ca^{2+}. When ligand binding is interrupted, F-actin rapidly depolymerizes with a half-time of 15 seconds. The right angle light scatter response also returns toward its initial value with kinetics that parallel the depolymerization of actin. Partial disaggregation of the cells follows these responses.

Based on these observations and their Ca^{2+} dependence, we describe a simple model to unify diverse observations concerning the relationship between cell morphology, actin polymerization and cell aggregation.

0428 THE BINDING OF PHOSPHOFRUCTOKINASE TO FILAMENTOUS ACTIN, Susan Roberts and George Somero, Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093

The inventory of proteins with which actin interacts has increased dramatically with the current surge in cytoskeletal research. Actin's interaction with proteins may function not only in cytoskeletal structure and motility but also in the organization of cytosolic enzymes. Here we present evidence that phosphofructokinase, a key regulatory enzyme in glycolysis, also binds to filamentous actin in vitro and that this association may significantly affect the allosteric properties and stability of PFK in vivo.

PFK and actin were purified from rabbit skeletal muscle and used in binding assays based upon the sedimentation of paracrystalline actin during low speed centrifugation. The binding of PFK is concentration dependent, reversible, and not saturated at a PFK/actin ratio 50 times higher than the estimated physiological ratio in muscle. Addition of tropomyosin does not interfere with the binding of PFK. Electron microscopy of actin paracrystals and PFK shows that PFK binds to specific sites on actin filaments forming a regular banding pattern. These bands are the width of a PFK tetramer and may be either perpendicular or at a 30^{0} angle relative to the actin filament axis. The repeat distance of the PFK bands averaged over 62 measurements is 37.2 ± 1.0 nm. This is close to the repeat distance of the actin helix; 36.2 ± 1.0 nm (n=16) as measured on micrographs calibrated with catalase crystals. Conditions which destabilize PFK, probably involving dissociation of tetramers to dimers, can be partially offset by the inclusion of filamentous actin in the medium. Thus actin may be important in determining both the localization and stability of phosphofructokinase.

0429 Association of Thrombospondin with the Triton-insoluble Cytoskeleton of Thrombin Activated Platelets.G.P Tuszynski, H.I.Switalska, S.Strivastava, C.Cierniewski, and S.Niewiarowski. Thrombosis Research Center and Lankenau Medical Research Center, Philadelphia, PA.

We have previously shown that fibrin associates specifically with the Triton-insoluble cytoskeleton (CK) of thrombin activated platelets (Tuszynski et al. J. Biol. Chem. 259, 5247-5254, 1984) and that thrombospondin (TSP) associates specifically with fibrinogen and fibrin immobilized on Sepharose (Tuszynski, et al., submitted for publication). Based on our previous findings, we wondered whether TSP could associate with the platelet CK through bound fibrin. To answer this question, the TSP and fibrinogen levels determined by radioimmunoassay were measured in the CK and total platelet lysates of control platelets(C) and platelets known to be deficient in fibrinogen ie platelets from patients suffering Thrombasthenia(T) and Afibrinogenemia(A). The total fibrinogen antigen levels of C ,T and A platelets were 4.0, 2.3, 0.23 ug/ 10^{8} platelets, respectively and the CK prepared from these platelets contained 2.2, 1.7, 0.036 ug fibrinogen, respectively. The total TSP levels of C,T, and A platelets were 1.7, 3.4, and 1.18 ug/10^{8} platelets, respectively and the CK from these platelets contained 0.20, 0.23, and 0.007 ug TSP, respectively. Since A platelet CK contained 64 fold less fibrinogen antigen and 29 fold less TSP than C and T CK despite the fact total TSP in A platelets was nearly equal to that of C and T platelets, we conclude that the CK receptor for TSP is fibrin and not $GPII_{b}$-GPIII since T CK contained the same levels of TSP as controls.

0430 MOLECULAR COMPOSITION OF STRESS FIBRE-MEMBRANE ASSOCIATIONS AND THEIR RELATION TO COMPONENTS OF THE EXTRACELLULAR MATRIX IN SITU, J. Wagner, D. Drenckhahn, University of Marburg, FRG.

Human vascular endothelial cells in situ contain actin filament stress fibres which extend underneath the abluminal (basal) plasma membrane. The stress fibres have a specific affinity for antibodies to actin, myosin, α-actinin and tropomyosin. Immunoblotting studies revealed actin, myosin and α-actinin to be major proteins of isolated endothelial cells (20-30% of total cellular protein). Ultrastructurally stress fibres were found to be composed of thick myosin-like filaments (10-15 x 300 nm) and thin actin-like filaments (5-8 nm in diameter) in parallel alignment. Actin-like filaments could be decorated with myosin cross-bridges (S-1 subfragment) and displayed changes in polarity which may allow a sliding filament mechanism. At their plasmalemmal attachment sites actin-like filaments exhibited uniform polarities with the S-1 arrowhead complexes pointing away from the plasma membrane. Antibody staining demonstrated vinculin at the cytoplasmic aspect of the attachment sites while fibronectin, laminin and different types of collagen were concentrated at the extracellular side of these plaques. As endothelial stress fibres can contract in response to MgATP and cause significant changes in cellular shape it is concluded that the plasmalemmal anchorage of stress fibres is tight enough to effect cellular morphology and to resist the fluid shear forces permanently acting on the cell membrane.
Supported by DFG Dr 91/4-1.

0431 G/F ACTIN EQUILIBRIUM: EFFECT OF INDUCERS AND INHIBITORS OF CELL MOVEMENT. P.J. Wallace, R.P. Wersto, C.H. Packman, M.A. Lichtman. U. of Rochester, Rochester, NY. Changes in the dynamic equilibrium of G- and F-actin accompany the onset of cell motility. We examined the specificity of these changes in human neutrophils (PMN) stimulated by a) initiators of ameboid movement: f-met-leu-phe (FMLP) and zymosan-activated serum (ZAS), b) initiators of localized membrane movement, the oxidative burst and degranulation: PMA and Con A, and c) inhibitors of cell movement: cytochalasin B or D, and N-ethyl maleimide (NEM). PMN F-actin content was measured by the uptake and binding to F-actin of NBD-phallacidin using flow cytometry. ZAS or FMLP induced a reversible increment in F-actin to a peak level of 220% of baseline at 30 sec, accompanied by a reversible increase in cytoskeletal actin measured by SDS gel electrophoresis. Cytochalasin B or D blocked the FMLP-induced increment in F-actin, but did not decrease the F-actin content of unstimulated cells. PMA or Con A induced a gradual increase in F-actin to 150% of baseline level at 10 min. 1 mM NEM induced a rapid non-reversible increase in F-actin of similar magnitude to that seen with maximal FMLP or ZAS stimulation; however, there was no increase in actin in cytoskeletons. NEM did not affect the conformational state of purified G-actin or F-actin in solution, suggesting it interfered with cytoplasmic stabilizers of monomeric G-actin and polymeric F-actin. Thus, inhibitors of cell motility may operate by at least two mechanisms: inhibition of F-actin formation (cytochalasins) and non-reversible formation of unpolymerized F-actin (NEM). Stimulators of localized membrane movement cause a slow increase in the proportion of F-actin, which does not parallel the kinetics of degranulation or oxidative burst. In contrast, inducers of ameboid motility produce rapid, large and reversible increases in F-actin.

0432 ALUMINUM-DISRUPTED BINDING OF MELITTIN TO CALMODULIN. Christopher Weis and Alfred Haug, Departments of Physiology, Microbiology and Pesticide Research Center, Michigan State University, East Lansing, MI 48824.

Upon binding stoichiometric amounts of aluminum ions, pronounced conformational changes are produced in calmodulin (CaM) (M = 17000), a key regulatory protein. Physico-chemical changes have been reported to involve the alpha helical content, the hydrophobic surface exposure, and the electrophoretic migration and are reflected in calmodulin dependent enzymatic processes. To get further insight into the nature of the changes which occur in the aluminum-induced calmodulin, we used the small protein melittin (M = 2800) as a structural probe. Melittin is known to bind tightly and specifically to Ca-calmodulin and has been suggested as a model for investigating the interaction between calmodulin and its target enzymes (Biochem. J. (1983) 209, 269).

Our investigations have shown that the specificity of melittin binding to aluminum-induced calmodulin is altered. Circular dichroism studies indicate that the induction of alpha helix in the melittin molecule produced by native calmodulin is considerably reduced upon association of melittin with the aluminum induced CaM. Tryptophan fluorescence emission spectra of the melittin molecule show an increased intensity and blue shift upon complex formation with native calmodulin. However, melittin titrated with the aluminum-induced calmodulin shows neither a blue shift nor an increase in fluorescence intensity. Polarization anisotropy of tryptophan fluorescence is found to be reduced in the Al-CaM/melittin complex and the relative strength of melittin binding as calculated for these experiments is considerably reduced. (This work supported by NSF grant #PCM-8314662 and a gift from Pioneer Hibred Inc.).

Membrane Skeletal Proteins: Characterization, Association and Ultrastructure

0433 ANKYRIN AND SYNAPSIN: SPECTRIN-BINDING PROTEINS OF BRAIN MEMBRANES, Vann Bennett, Anthony J. Baines, and Jonathan Q. Davis, Department of Cell Biology and Anatomy, Johns Hopkins School of Medicine, Baltimore, MD 21205

Brain membranes contain an actin-binding protein closely related in structure and function to erythrocyte spectrin. The proteins that attach brain spectrin to membranes are not established, but, by analogy with the erythrocyte membrane, may include ankyrin and protein 4.1. In support of this idea, proteins closely related to ankyrin and 4.1 have been purified from brain and demonstrated to associate with brain spectrin. Brain ankyrin binds with high affinity to the spectrin beta subunit at the mid region of spectrin tetramers. Brain ankyrin also has binding sites for the cytoplasmic domain of the erythrocyte anion channel (band 3), as well as for tubulin. Ankyrins from brain and erythrocytes have a similar domain structure with protease-resistant domains of M_r = 72,000 that contain spectrin-binding activity, and domains of M_r = 95,000 (brain ankyrin) or 90,000 (erythrocyte ankyrin) that contain binding sites for both tubulin and the anion channel. Brain ankyrin is present at about 100 pmol/mg membrane protein, or about twice the number of copies of spectrin beta chains. Brain ankyrin thus is present in sufficient amounts to attach spectrin to membranes, and has the potential to attach microtubules to membranes as well as to interconnect microtubules with spectrin-associated actin filaments.

Another spectrin-binding protein has been purified from brain membranes, and this protein cross-reacts with erythrocyte 4.1. Brain 4.1 is identical to the membrane protein synapsin, which is one of the brain's major substrates for cAMP-dependent and Ca/calmodulin-dependent protein kinases with equivalent physical properties, immunological cross-reaction and peptide maps. Synapsin (4.1) is present at about 60 pMoles/mg membrane protein, and thus is a logical candidate to regulate certain protein linkages involving spectrin.

0434 ASSEMBLY OF SPECTRIN-ACTIN-BAND 4.1 NETWORKS: REGULATION BY BAND 4.1 PHOSPHORYLATION. Carl M. Cohen, Dept. of Biomedical Research, St. Elizabeth's Hospital, Boston, MA and Depts. of Medicine and of Molecular Biology and Microbiology, Tufts Univ. School of Med.

Red cell band 4.1 promotes and stabilizes the cross-linking of red cell F-actin by spectrin tetramers. Analogues of all of these proteins are present in non-erythroid cells and the regulation of their assembly may play an important role in numerous cellular or membrane phenomena. Factors which may be involved in the regulation of network formation by these proteins include the level of free Ca^{++}, the stoichiometry of the proteins within the complex and phosphorylation of band 4.1 and spectrin. We have previously shown that only a single band 4.1 molecule is needed to promote spectrin binding to F-actin, and that each monomer of an actin filament can bind a spectrin molecule in the presence of band 4.1. Ca^{++} has been reported to modulate these associations and our studies suggest that this may take place via Ca sensitive band 4.1-F-actin association. In recent studies we have found that complex formation is also sensitive to the degree of phosphorylation of band 4.1. Purified band 4.1 contains an average of one mole phosphate per mole protein. When purified band 4.1 is treated enzymatically resulting in a 25-50% average dephosphorylation the ability of band 4.1 to promote the binding of spectrin to F-actin is enhanced by as much as two fold. Autoradiography of ^{32}P-band 4.1 electrophoresed on slab gels suggests that all of the identifiable band 4.1 species are dephosphorylated in parallel. This finding suggests that the integrity or stability of the red cell membrane skeleton may reflect the level of band 4.1 phosphorylation. Dephosphorylation of band 4.1 during cellular energy depletion may result in a rigid, indeformable cell membrane. Supported by NIH HL 24382.

0435 ENZYMATIC RECYCLING OF CLATHRIN FROM COATED VESICLES Sandra L. Schmid and James E. Rothman Department of Biochemistry, Stanford University School of Medicine, Stanford, CA. 94305

Coated vesicles mediate selective intracellular membrane transport. Delivery of the membrane and lumenal contents of the clathrin-coated vesicle is accomplished by fusion with a target membrane. In order to facilitate close membrane apposition and fusion, the bulky clathrin coat must be shed. Released clathrin triskelions, the assembly units of the coat, are freed to recycle for repeated rounds of vesicle budding. We have recently reported the purification of an enzyme from bovine brain which catalyzes the release of clathrin triskelions from coated vesicles and empty cages. The uncoating enzyme is a clathrin cage-dependent ATPase. Triskelions are released in a near stoichiometric complex with uncoating protein. Here we describe the dissection of this complex overall process into two distinct, separately measurable partial reactions. These stages involve distinct activities of the uncoating enzyme which have differing requirements both for recognition of their clathrin substrate and for utilization and specificity of their nucleotide substrate. A comparison of the requirements for these two stages and for the overall reaction has allowed us to develop a model for the mechanism of uncoating ATPase catalyzed cage dissociation.

Proteins that Modify or Connect Actin Filaments

0436 Ca^{++} REGULATED PROTEINS OF THE INTESTINAL BRUSH BORDER, John R. Glenney, Jr. and Phyllis Glenney, The Salk Institute, Post Office Box 85800, San Diego, California.

The intestinal epithelial cell and specifically the cytoskeleton of the brush border are thought to be controlled by micromolar levels of free calcium. Calcium-binding proteins of this system include intestinal calcium binding protein (CaBP), calmodulin (CaM), villin and a 36 Kd protein substrate of tyrosine kinases. In order to assess the sequence of events as the intracellular Ca^{++} level rises, we have determined the amount of CaM and CaBP in the intestinal epithelium by western blotting, and tested the Ca^{++} binding of CaM and CaBP by equilibrium dialysis. The Ca^{++}-dependent actin severing activity of villin was analyzed in the presence of physiological CaM levels and increasing calcium concentrations. In addition we analyzed the Ca^{++} levels required for interaction between CaM and the microvillus 110 K protein as well as fodrin and the interaction between P-36 K and actin. The results suggest that CaBP serves as the predominant Ca^{++} buffer in the cell, but CaM can effectively buffer ionic calcium in the microvillus and thus protect actin from the severing activity of villin. CaM binds to its cytoskeletal receptors, 110 K and fodrin differently, governed by the free Ca^{++} and pH. The interaction between P-36 K and actin, however, appears to require an unphysiologically high calcium concentration (10^{-4} to 10^{-3} M) to be meaningful. The results provide a coherent picture of the different Ca^{++} regulated events occurring when the free calcium rises into the micromolar level in this unique system. This study would suggest that as the Ca^{++} rises in the intestinal epithelial cell an ordered sequence of Ca^{++} saturation of intracellular receptors occurs with the order from the lowest to highest Ca^{++} requirements being CaBP<CaM<villin<P-36.

Journal of Cell Biology (in press)

0437 3-D ORGANIZATION OF ACTIN GELS AND OF LEUKOCYTE CORTICAL CYTOPLASM, John H. Hartwig and Richard Niederman, Massachusetts General Hospital, Boston, MA 02114.

The 3-D organization of actin in the cortex of rabbit lung macrophages and human polymorphonuclear leukocytes (PMNs) has been studied in detergent-extracted cytoskeletons which have been critical point dried and metal coated with platinum. Reaction of macrophage cytoskeletons with anti-actin, anti-actin-binding protein (ABP), and anti-myosin IgGs and with anti-IgG bound to colloidal gold allowed the identification of the location where these components reside in the cytoskeleton.

A dense network composed of actin filaments branching with striking perpendicularly is the dominate structure found in the macrophage cytoskeleton. The periphery of cytoskeletons prepared from PMNs protruding anterior lammellae prior to extraction with detergent is also organized into a dense network of branched filaments, but filaments composing the network branch more acutely than those composing macrophage cytoskeletons. In both cells, the filament network fills cortical lammellae then bifurates to form 200-500 nm thick layers on the top and bottom of the cell bodies. The average length of actin filaments between points of intersection is 118±72 nm in macrophages and 90±50 nm in PMNs. The average actin filament concentration is 6.0 mg/ml. 45% of the total actin in the cells but all of the filamentous actin, 90% of the actin-binding protein, and 50% of the myosin, remains in the detergent prepared cytoskeletons.

The architecture of actin in the cell cortex resembles the structure of actin in gels made with ABP. When actin is assembled in the presence of ABP, a contiguous, perpendicular network is formed, the spacing of which is dependent on the molar ratio of ABP to actin. Reaction of the gel with anti-ABP IgG and gold-coated with anti-IgG deposits clusters of gold exclusively at points where the filaments branch indicating that ABP resides at filament intersections and interacts with actin to generate filament branching. The density of gold label in the gels equals the number of ABP molecules added demonstrating that gold labelling was an efficient process. In macrophage cytoskeletons, anti-ABP IgG and gold-coated with anti-IgG also label only filament branch points or intersections. However, only about half of the total number of filament intersections decorate with gold particles. Since the network spacing determined by 3-D morphometry was half of that predicted from the molar ratio of ABP to actin, it is likely that many of the filament intersections result from filament entaglements not crosslinks.

Macrophage myosin, on the other hand, localizes to the cell margins and throughout the cytoskeleton. Gold labelling of myosin with anti-myosin IgG and anti-IgG coated gold is most consistent with the assembly state of myosin in cytoskeletons of dimers or tetramers.

0438 ACTIN ASSEMBLY AND ACTIN BINDING PROTEINS FROM ACANTHAMOEBA, Thomas D. Pollard, Dept. of Cell Biology & Anatomy, Johns Hopkins Medical School, Baltimore, MD 21205.

Actin polymerization in $MgCl_2$ and KCl involves several steps: (1) Starting with monomers having bound ATP and Ca^{++}, there is a moderately fast (k_+ = 0.05 s^{-1}) first order activation reaction that is thought to be the exchange of Mg^{++} for bound Ca^{++}. Activated monomers form nuclei and elongate filaments more rapidly than unactivated monomers. (2) The overall polymerization reaction is limited by the slow, unfavorable formation of nuclei consisting of 3 actin molecules. (3) The filaments then grow rapidly in both directions. At the barbed (B) end the association rate is very fast with a large forward rate constant ($10^7 M^{-1}s^{-1}$) and a low activation energy (8-9 kcal/mol) that both suggest that the rate is limited by diffusion. The association rate constant at the pointed (P) end is 5-10 times lower. (4) If the overall process is sufficiently slow, filaments can break slowly into shorter fragments. The ATP bound to the monomers is hydrolyzed subsequent to the incorporation of the actin into filaments in a first order reaction (k_{ATPase} = 0.07 s^{-1}). Even at steady state, ATP-actin occupies the end of most filaments and during rapid elongation there may be hundreds of ATP-actins at the ends of a polymer.

Acanthamoeba has a variety of actin-binding proteins that may regulate polymerization and cross-link actin filaments in the cell. The total actin is about 200 umoles/kg cells. Profilin is a small (11,700 MW) protein present in high concentration (100 umoles/kg) that is distributed throughout the cytoplasmic matrix. Profilin inhibits polymerization in 2 ways: First, it binds to actin monomers with K_d of about 5 uM. The complex cannot form nuclei or bind to the P-end of filaments. Second, both profilin and the actin-profilin complex can bind to and cap the B-end of actin filaments but the affinity is so low (K_d=100 uM) that profilin only weakly inhibits elongation at the B-end. Capping protein is a heterodimer of 28,000 & 31,000 MW subunits that is present in low concentration (2 umoles/kg) and is concentrated in the cell cortex with the bulk of the actin filaments. It binds to actin filaments and blocks monomer addition at the B-end, but also stabilizes actin oligomers and thereby promotes nucleation. Gelation protein is a 50 nm long rod with globular regions at both ends and in the center. It is composed of 2 90,000 MW polypeptides and is present at a concentration of 4 umoles/kg. It is found throughout the cytoplasmic matrix, but is concentrated in parts of the cortex. Although it binds only weakly to actin filaments, it effectively cross-links the filaments into 3-D networks. A 260,000 MW subunit of spectrin has been purified from soluble extracts of the amoeba. The isolated molecule is a slender rod 70 to 100 nm long that can cross-link actin filaments. Antibodies to the purified protein also react with an insoluble 240,000 MW polypeptide in the whole cell that may be a second spectrin chain associated with membranes. By fluorescent antibody staining, the spectrin is concentrated very near the plasma membrane in the cell. (This work was supported by grants from the NIH and the Muscular Dystrophy Association of America).

Association of Cytoskeletal Proteins with Membrane Components

0439 BIOCHEMICAL CHARACTERIZATION OF THE TRITON INSOLUBLE PLASMA MEMBRANE MATRIX OF MURINE LYMPHOID CELLS, John R. Apgar and Matthew F. Mescher, Medical Biology Institute, La Jolla, CA 92037

Triton extraction of murine tumor cell plasma membranes leaves behind a detergent insoluble protein matrix which is believed to form a structural meshwork located on the cytoplasmic face of the membrane. The proteins which form this matrix can be seperated into a minor, EGTA soluble (36K, 38K, 69K, 70K) and a major, EGTA insoluble fraction (20K, 40K, actin). The EGTA insoluble fraction, which accounts for almost 15% of the total plasma membrane protein, is only soluble in SDS or ionic detergents at high pH. These proteins have been seperated and purified by Sepharose CL-4B gel filtration and high pressure liquid chromatography in SDS. Amino acid composition and peptide mapping indicates that the 40K dalton protein is a dimer of the 20K dalton protein and that this aggregation can take place even in the presence of SDS and reducing agents. The ability of these proteins to form higher molecular weight aggregates may explain why these proteins fail to enter SDS polyacrylamide gels. The 20K and 40K proteins are probably integral membrane proteins which do not appear to have any regions exposed on the outer cell surface. However, these proteins do interact with other cell surface proteins. The association of the EGTA soluble proteins and actin, which are located on the inner surface of the plasma membrane, suggests that the 20K and 40K proteins have a cytoplasmic domain. (Supported by the Leukemia Society of America and NIH CA-30381)

0440 ACTIN-ASSOCIATED MEMBRANE GLYCOPROTEIN OF TUMOR CELL MICROVILLI, K. Carraway, G. Jung and C. Carraway, Univ. of Miami School of Medicine, Miami, FL, 33101

Microvilli isolated from ascites tumor sublines are being used as a model system to study the molecular details of microfilament-membrane interactions. Microvilli of the MAT-C1 ascites subline contain a stable transmembrane complex composed of actin, a cell surface glycoprotein (CAG, cytoskeleton-associated glycoprotein) and a 58,000 dalton polypeptide. The complex, isolated from microvillar membranes in Triton X-100, is large and heterogeneous with respect to size. Velocity sedimentation studies in the presence and absence of phalloidin have demonstrated that a fraction of the complex is associated with microfilaments. When microvilli are extracted with Triton in neutral buffer and fractionated by velocity sedimentation, the fraction of the complex not associated with filaments is found as a large (sedimentation coefficient >25S), heterogeneous species. When microvilli are similarly fractionated under denaturing conditions in Triton-carbonate (pH 11) or dodecyl sulfate (SDS), CAG is released from the complex and sediments as a 20S species. If 50 mM mercaptoethanol is included in SDS extracts, CAG sediments at approximately 5S, the size expected from its monomeric molecular weight on SDS electrophoresis under reducing conditions. These results indicate that CAG is a disulfide-linked oligomer in the microvillus membrane. We suggest that the stable oligomeric structure of the CAG permits it to act as the membrane association site for several microfilaments and plays an important role in the formation and stabilization of the microvillus structure.

0441 INTERACTIONS OF SPECTRIN and BAND 4.1 WITH PHOSPHOTIDYLSERINE VESICLES. A.M. Cohen, S.C. Liu, J. Lawler and J. Palek. Dept. Biomedical Research, St. Elizabeth's Hospital of Boston, Tufts University School of Medicine, Boston, MA 02135

Spectrin and band 4.1, two of the major red cell membrane skeletal proteins, are thought to bind to membrane proper via protein-protein linkages, i.e. spectrin-band 2.1-band 3 and band 4.1-glycophorin, respectively. Less known are their interactions with inner membrane phospholipids, such as phosphotidylserine (PS). To gain further insight into these interactions, we have characterized the binding of spectrin, band 4.1 and the major proteolytic fragments of band 4.1 to PS vesicles. Spectrin (0.8-2.0 mg/ml) or band 4.1 (0.1-2.0 mg/ml) was incubated with PS vesicles isotonically and the vesicles were pelleted by centrifugation through dextran cushion to separate bound and unbound protein. The amount of spectrin and band 4.1 bound per nmole PS were 0.5 ug and 2.68 ug, respectively. Electron microscopy of rotary shadowed platinum replicas of spectrin-PS complexes revealed that individual liposomes were decorated by spectrin which was attached to one of the ends of spectrin dimers, while band 4.1-PS complexes showed globule-decorated liposomes. Among peripheral membrane proteins tested for binding with PS, band 4.1 showed the highest level of binding, wheras band 2.1 and actin showed very little binding. To identify the domain of band 4.1 which binds to PS, we have incubated PS with chymotryptic digests of band 4.1. A 30,000 dalton fragment of band 4.1 was selectively bound. Based on its molecular weight and isoelectric point (pI=7.8), this fragment was identified as the terminal fragment which has been reported to contain the spectrin binding site. The data indicate that both spectrin and band 4.1 are capable of interacting with PS which may be important to the membrane structure and function.

0442 DOES A BAND 3-LIKE MOLECULE PARTICIPATE IN TRANSMEMBRANE LINKAGE OF SURFACE RECEPTORS TO THE CYTOSKELETON, D. Drenckhahn[1], K. Engel[1], P.S. Low[2], U. Schauer[1], K. Zinke-Schlüter[1], [1]University of Marburg, FRG and [2]Purdue University, West Lafayette, USA.

Polyclonal antibodies specific for either the cytoplasmic or the external domain of human erythrocyte band 3, the main anchoring site of the actin-spectrin cytoskeleton in the red cell membrane, decorated the plasma membrane of human lymphocytes. Immunoblots revealed a polypeptide of 60-65,ooo daltons which comigrated with a major fragment of band 3 in the red cell membrane. In viable lymphocytes, antibodies to the external domain redistributed into patches and caps. This indicates that the band 3-like molecule is mobile in the plane of the plasma membrane. In order to examine if the band 3-like molecule might participate in lateral mobility of cell surface receptors, lymphocyte immunoglobulin (Ig) receptors were induced to aggregate by FITC-labelled antibodies to Ig's. The cell were then stained for band 3 by rhodamine--labelled antibodies. Staining specific for band 3 (cytoplasmic as well as external domain) correlated with caps and patches of Ig surface receptors. Moreover, there was a clear correlation between Ig caps, band 3-like staining, and immunostaining for the cytoskeletal proteins actin, spectrin, vinculin and myosin. In view of our previous observation that vinculin (a protein found at microfilament-membrane associations) can bind the cytoplasmic domain of band 3 at a K_D of 10^{-5} it is suggested that a band 3-like molecule is involved in transmembrane linkage of surface receptors to the cytoskeleton. Supported byDFG Dr 91/5-1, NIH GM24417, GM o7211.

0443 RECONSTITUTION OF THE MEMBRANE-CYTOSKELETON IN A UNICELLULAR ORGANISM. Ron R. Dubreuil and G. Benjamin Bouck, University of Illinois at Chicago, Chicago IL 60680

The unicellular flagellate Euglena gracilis undergoes rapid and extreme shape changes. We have shown that cell shape is preserved in cell ghosts and isolated fragments of the cell surface which consist of only the plasma membrane and a thin submembrane cytoskeleton. When the plasma membrane is removed from surface isolates, the membrane skeleton retains both the gross shape of the cell and the microscopic ridge and groove organization characteristic of euglenoids. The membrane skeleton consists of narrow strips individually bridged along their lateral margins. Two polypeptides account for about 50% of the total protein in the skeleton but they do not appear to be related to spectrins. The plasma membrane can be separately isolated and retains neither the original surface form nor the paracrystalline array of intramembrane particles (IMPs) usually found in the region of each surface ridge in untreated surface isolates. When a solublized fraction of the membrane skeleton is incubated with the membrane, reassociation is found on only one side of the bilayer. Coincident with cytoskeletal protein rebinding, the IMPs become partially reordered. This binding is not inhibited or mimiced by incubation of membranes with an excess of non-specific protein, such as BSA. However, binding is eliminated by proteolysis of presumably exposed domains of the putative cytoskeleton binding sites on the membrane. These and other findings suggest that the euglenoid cell may provide an excellent model not only for examining membrane-cytoskeleton interactions, but also for resolving other facets of surface behavior such as cell motility and surface replication. Supported by NSF Grant #PCM8203442 to G.B.B.

0444 THE PLASMA MEMBRANE-BOUND CYTOSKELETAL NETWORK AND ITS RELATION TO CELL-SURFACE LECTIN RECEPTORS, Constance A. Feltkamp, Ed Roos, Dick A.M. Mesland and Hermina Spiele, The Netherlands Cancer Institute, Amsterdam, The Netherlands

With the dry-cleaving method, preparations can be obtained of large sheets of plasma membrane together with the adjacent part of the cytoskeleton. These preprations, thin enough to be studied with the electron microscope at normal voltages, can be obtained from both the substrate-attached and the medium-facing surfaces of cells grown in monolayer and from suspended cells. The plasma membrane-bound part of the cytoskeleton consists of a network of filaments of different size and diameter. We found differences in its morphology between different cell types, between normal cells and their transformed counterparts, and between suspended and attached cells of the same type.

Using immunogold labeling before dry-cleaving we studied the topographical relation between concanavalin A receptors and the filamentous network. In stereomicrographs the receptors were seen to be exclusively localized at relatively electron dense domains of the membrane, usually associated with denser parts of the filamentous network. The domains are interconnected and thus form a second network at the plane of the membrane. Stereomicrographs also show that short filaments, connecting the filamentous network with the plasma membrane always point towards these dense membrane domains.

The observations suggest that cell surface proteins are localized in specific membrane domains that are associated with the submembranous part of the cytoskeleton.

0445 SPECIFIC TX-100 RESISTANT SURFACE GLYCOPROTEINS OF MURINE T-LYMPHOMA CELLS, Daniel Hoessli and Elisabeth Rungger-Brändle, University of Geneva, Switzerland.

We have isolated a Triton X-100 resistant complex of membrane proteins and surface glycoproteins from murine T-lymphoma cells in the absence of lectin or antibody-induced remodeling of the cell surface (cf. Abstract Rungger-Brändle and Hoessli). Two plasma membrane fractions are obtained by upward flotation. The lighter fraction contains a select set of surface-labeled glycoproteins of 20-30 and 55 kd. The heavier fraction contains the complete spectrum of surface glycoproteins that can be vectorially labeled on intact cells. Upon extraction of both plasma membrane fractions and gel filtration of the extracts in the presence of detergent, we obtain a high M_r peak containing the two surface glycoproteins characteristic of the unextracted light density membrane fraction. The 20-30 kd glycoprotein is the Thy-1 antigen, a specific marker for murine T-lymphocytes. The 55 kd glycoprotein occurs in a fixed molar ratio with respect to Thy-1 and is different from H-2 glycoproteins of similar electrophoretic mobility expressed on this lymphoma cell. The association of the Thy-1 and 55 kd glycoproteins in a detergent-resistant membrane complex may reflect a functional association between two surface glycoproteins involved in specific ligand binding.

0446 MODULATION OF THE CELL SURFACE ASSOCIATION OF SV40 LARGE TUMOR ANTIGEN. Ulrich Klockmann and Wolfgang Deppert, Dept. of Biochemistry, University of Ulm, F.R.G.

A small percentage of SV40 large T antigen (large T) in SV40 transformed cells is specifically associated with plasma membranes. This subclass of large T can be distinguished from the bulk of nuclear large T by a specific posttranslational modification, the fatty acid acylation. Large T exhibits properties of a transmembrane protein, since it is exposed on the cell surface and tightly associated with a detergent resistant plasma membrane lamina.

The SV40 transformed Balb/c mouse tumor cell line mKSA can be grown in suspension culture as well as in monolayer attached to a substratum. Analysis of the plasma membrane association of large T in mKSA cells kept under these different growth conditions showed that about 90% of the cell surface associated large T was anchored in the plasma membrane lamina in cells from suspension culture. Only about 10% could be recovered in the NP40 soluble fraction of the plasma membrane. However, in cells attached to a substratum the amount of lamina associated large T was reduced to only about 30% whereas about 70% of the cell surface associated large T was NP40 soluble. This may indicate that the lamina association of large T is modulated depending on different growth conditions.

Suspension cells and cells attached to a substratum differ in their cytoskeletal architecture. To analyze whether cytoskeletal elements may be involved in modulating the lamina association of large T, mKSA suspension and monolayer cells were treated with drugs known to affect the cytoskeleton. Whereas actin-depolymerizing agents like cytochalasin B had no effect on the lamina association of large T, treatment with the microtuble-depolymerizing drug vinblastine induced a significant shift from lamina associated to NP40 soluble surface T. This suggests that the cytoskeleton participates in the modulation of the lamina association of large T.

0447 BAND 3 IS FOUND AT CYTOSKELETON-MEMBRANE JUNCTIONS OF NUCLEATED CELLS AND BINDS VINCULIN, P.S. Low[1], D. Allen[1], J. Otto[1], K. Zinke[2] and D. Drenckhahn[2], [1]Purdue University, West Lafayette, IN 47907 and [2]University Marburg, FRG.

Antibodies to the cytoplasmic domain of erythrocyte band 3 were found to cross-react with membrane proteins of various nucleated cells. A major site of immunostaining in cultured fibroblasts and tissue sections of exocrine glands, heart muscle and intestinal epithelium corresponded to adhesion plaques and related adhaerens-type intercellular junctions (terminal bar, intercalated disc, etc.). Anti-vinculin IgG stained the same sites as anti-band 3 IgG when cells were reacted with both antibodies. Immunoblots with antiserum to the cytoplasmic domain of band 3 revealed cross-reactive polypeptides in fibroblasts and isolated intestinal brush borders of ∿60,000 and/or 95,000 daltons. The smaller polypeptide corresponds to the molecular weight of the major fragment of whole band 3 (Mr ∿95,000) seen in red cell membranes. Because of the observed co-localization of band 3 and vinculin, the affinity of the two polypeptides for each other was examined. Chicken gizzard vinculin was found to bind the cytoplasmic domain of human erythrocyte band 3 with a $K_D \sim 10^{-5}$ at physiological pH and ionic strength. These findings suggest that band 3 may participate in anchoring cytoskeletal elements of nonerythroid cells to the plasma membrane, analogously to its role in erythrocytes. Supported by NIH GM24417, GM 07211, Amer. Cancer Soc. CD108A and Deutsche Forschungsgemeinschaft 91-5-1.

0448 CELL-SUBSTRATUM ADHESION SITES OF RSV TRANSFORMED FIBROBLASTS ARE FORMED BY SHORT PROTRUSIONS OF THE VENTRAL MEMBRANE CALLED PODOSOMES, P.C. Marchisio, D. Cirillo, F.G. Giancotti, P.M. Comoglio and G. Tarone, Inst. of Histology, Univ. of Torino Med. School, 10126 Torino, Italy.

RSV transformed BHK cells (RSV/B4-BHK) and RSV transformed chick embryo fibroblasts (SRD-CEF) adhere on fibronectin-coated dishes with protrusions of the ventral membrane forming discrete close contacts. These protrusions contain actin, vinculin, fimbrin and α-actinin spatially organized with a pattern different from that of adhesion plaques. EM analysis suggests an analogy with cellular feet justifying the denomination of podosomes. Screening of a large number of avian and mammalian transformed cell lines has shown that podosomes appear only in RSV and Fujinami sarcoma virus (FSV) infected cells whose oncogenes encode tyrosine phosphokinases with a specific tropism for the inner face of the membrane. Podosomes in fact concentrate phosphotyrosine containing molecules. Podosomes and adhesion plaques, although similar in cytoskeletal protein composition, have different formation mechanisms since 1) they do not require serum in the medium, 2) protein synthesis and 3) protein secretion for their formation as adhesion plaques do. Moreover they form within 1h on fibronectin. Podosomes represent a variant adhesion structure which does not allow the organization of microfilament bundles. Supported by CNR-Progetto Finalizzato "Oncologia".

0449 THE PLASMA MEMBRANE SKELETON OF MURINE TUMOR CELLS. PREPARATION, MORPHOLOGY AND COMPOSITION OF TRITON SHELLS, Matthew F. Mescher, John M. Robinson and John R. Apgar
Medical Biology Institute, La Jolla, CA 92037 and Harvard Medical School, Boston, MA 02115

It was previously shown that a Triton X-100 insoluble protein matrix could be isolated from the plasma membranes of P815 tumor cells and murine lymphoid cells (Mescher et.al. (1981) Nature 289:139). The properties of this matrix suggested that it might form a membrane skeleton, similar in some respects to the spectrin-containing skeleton of erythrocytes. We have now demonstrated that detergent extraction of intact P815 cells, using the same extraction conditions, results in recovery of Triton shells which consist of a layer of protein at the periphery, a largely empty cytoplasmic space and a nuclear remnant. 5'-Nucleotidase, a cell surface enzyme that remains associated with the matrix upon its isolation from plasma membranes, was quantitatively recovered with the shells. Despite removal of the lipid bilayer and most of the membrane proteins the peripheral layer persists as a continuous macrostructure surrounding the nuclear remnant. Morphological and biochemical evidence strongly suggests that this peripheral layer consists of the previously described set of matrix proteins which interact to form a membrane skeleton. The composition, location and appearance of this membrane skeleton clearly distinguish it from previously described cytoskeletal structures. Further biochemical characterization of the components of the membrane matrix are described in a separate abstract (Apgar,J. and M.F.Mescher).

0450 THE RED CELL SKELETAL PROTEIN BAND 4.1 BINDS TO PAS 2 IN THE MEMBRANE, Thomas J. Mueller, Dept. Biochemistry, St. Jude Hospital, Memphis, TN 38101

Following extraction of human erythrocyte membranes with Triton X-100, the transmembrane sialoglycoprotein PAS 2 (glycoconnectin; glycophorin C; SGP-β) is preferentially retained in the Triton-insoluble residue, whereas nearly all of PAS 1 and PAS 3 are Triton soluble. This suggests that PAS 2 associates with the membrane skeleton. The following data indicate that PAS 2 interacts with the skeletal protein band 4.1. Extraction of stroma with cold NaOH, pH 11.5, releases all of the peripheral proteins from the membrane except band 4.1, implying that band 4.1 interacts directly with the bilayer domain. Extraction of these NaOH-stripped membranes with Triton solubilizes PAS 2 and band 4.1, both of which can be immunoprecipitated with antibodies directed against PAS 2. In addition, analyses of these Triton extracts on sucrose gradients reveals that PAS 2 and band 4.1 cosediment. Furthermore, the sedimentation rate of PAS 2 is decreased if band 4.1 is released from the membrane prior to Triton extraction of PAS 2. Further support for the notion that band 4.1 binds to PAS 2 comes from analysis of membranes devoid of PAS 2 (Gerbich-negative, Ge-, donors; Anstee et al. (1984) Biochem. J. 218: 615-619). Extraction with cold NaOH (pH 11 or 11.5) releases **all** of band 4.1 from Ge- membranes, whereas no 4.1 is released from Ge+ membranes which contain PAS 2. Band 4.1 is also more readily released from Ge- membranes by 0.1 mM EDTA. These data provide convincing evidence that PAS 2 is an attachment site in the bilayer domain for the skeletal protein band 4.1, thus providing additional sites for anchoring the membrane skeleton to the bilayer domain. (supported by grant HL 30489).

0451 CHARACTERIZATION OF A MEMBRANE-MICROTUBULE COMPLEX, John M. Murray, Department of Anatomy, University of Pennsylvania, Philadelphia, PA 19104

A membrane-microtubule complex isolated from the unicellular algae Distigma proteus is being characterized in order to provide a convenient model system for membrane-microtubule interactions in other cells. The complex isolated from Distigma proteus is sufficiently ordered to permit 3D reconstruction by Fourier techniques from electron micrographs of negatively stained specimens. An integral membrane protein organized into a 2D crystalline array is included in the complex. A correspondence between the microtubule surface lattice and the membrane protein lattice predicts a likely arrangement of binding siges. Microtubules can be removed from the complex, then replaced by using purified brain tubulin. The readded microtubules occupy the same positions and have the same orientation as the original endogenous microtubules.

Partial purification of the protein which links the microtubules to the membrane has been achieved. Efforts are underway to reincorporate the solubilized microtubule binding sites into artifical membranes in order to have a complete membrane-cytoskeleton complex which can be reconstructed from individually well characterized components.

0452 HERPES SIMPLEX VIRUS MORPHOGENESIS/INTERACTION BETWEEN HSV GLYCOPROTEINS AND CYTOSKELETAL ELEMENTS STUDIES IN HUMAN FIBROBLASTS. B. Persson[$], L.-I. Larsson[*], B. Norrild[$]. *Histochemical Laboratory, Rigshospitalet, Frederik V's Vej, DK-2100 Copenhagen, [$]Institute of Medical Microbiology, Juliane Mariesvej, DK-2100 Copenhagen, Denmark.

Herpes Simplex Virus (HSV) infection of cultured human fibroblasts leads to production of more than 50 proteins. At least five glycoproteins named gB, gC, gD, gE and gG have been identified. The maturation and processing of gB, gC and gD was followed in HSV-1 infected cells by use of indirect immunofluorescence and previous studies showed that the glycoproteins were processed through the Golgi apparatus. Tubulin was shown to aggregate in infected cells in a juxtanuclear position and double immunofluorescence staining of the cells with antibodies to tubulin and glycoprotein D respectively demonstrated codistribution of these proteins. The transport of the HSV glycoproteins to the plasmamembrane was dependent on the presence of intact microtubules. Disruption of these with demecolcine led to a scattered distribution of the viral glycoproteins and also prevented their insertion into the plasmamembrane. Purification of cytoskeletal fibrillar structures and analysis of possible association of HSV proteins to microtubules, actin filaments and vimentine type of intermediate filaments are under current investigation and preliminary data will be presented. Current studies using immuneelectronmicroscopy have been designed to further demonstrate the possible association of viral glycoprotein carrying vesicles with cytoskeletal structures and preliminary data will be discussed.

0453 SKELETAL PROTEINS OF T-LYMPHOMA CELL PLASMA MEMBRANES, Elisabeth Rungger-Brändle and Daniel Hoessli, University of Geneva, Switzerland.

The non-ionic detergent-resistant proteins have been isolated from purified plasma membranes of murine T-lymphoma cells. Plasma membranes were separated from other subcellular components by upward flotation to their equilibrium density. Two fractions were obtained at 1.06 and 1.11 gm/cc and extracted with Triton X-100. The detergent-resistant components were recovered by sedimentation at 280,000 xg or separated from detergent-solubilized components by gel filtration in the presence of detergent. Both plasma membrane fractions yielded an identical detergent-resistant complex of high M_r (15×10^6 dalton) consisting of mostly acidic (pI 4.5-6.0) polypeptides in the 30 to 80 kd range. The column eluted complex banded at a density of 1.02-1.04 gm/cc after isopycnic sedimentation in sucrose suggesting a higher lipid to protein ratio than that of unextracted membranes. Actin is a major polypeptide of the sedimentable residue after membrane extraction, but is no longer present in the detergent-resistant, high M_r complex obtained by gel filtration. This is in keeping with the insensitivity of the column-eluted complex to extraction with low ionic strength, actin-depolymerizing buffers. The complex is otherwise sensitive to high salt buffers. It may represent the skeletal backbone of a plasma membrane domain endowed with specific ligand binding properties (cf. Abstract Hoessli and Rungger-Brändle).

0454 Human Erythrocyte Band 4.1 is a Phosphatidylserine-Binding Protein. Anne Rybicki and Robert S. Schwartz. Children's Hospital Medical Center-Research Department, Oakland, CA. Band 4.1 (4.1) interacts with spectrin and actin and presumably binds to a subpopulation of membrane glycophorins. We examined the interaction of 4.1 with membrane phospholipids (PL) and the extent to which this interaction contributes to maintaining PL asymmetry. Purified 4.1 or vesicles from 4.1-deficient (4.1d) red cells in combination with PL vesicles (liposomes) were used. Oxidized 4.1 and 4.1 from sickle cells (S-4.1) were also studied. Inside-out (IOV) and right side-out (ROV) vesicles were mixed with radiolabeled liposomes and the unreacted species separated. Normal IOV bound significantly more phosphatidylserine (PS) than did normal ROV (IOV/ROV=1.3) whereas 4.1d-IOV and ROV had no preference for PS liposomes (IOV/ROV=0.92). Pure ^{125}I-4.1 was mixed with liposomes and the ^{125}I 4.1-liposome complex separated. ^{125}I-4.1 bound to liposomes in direct proportion to their PS content. At saturation ~2 nmol PS bound/µg 4.1, a value strikingly similar to the 2.4 nmol PS/µg 4.1 ratio in normal red cells. Oxidation of 4.1 resulted in a 4-fold decrease in PS liposome binding. Binding of S-4.1 to 4.1d-IOV was decreased 3-fold from normals suggesting a defect in the S-4.1 molecule. We speculate that S-4.1 may be oxidized *in vivo* resulting in decreased PS binding and loss of PL asymmetry in sickle cells. We conclude that 4.1 is a PS-binding protein and that structural alterations in 4.1 (i.e., oxidation) affects the interaction between PS and 4.1.

0455 CYTOSKELETAL REORGANIZATION RESULTING FROM LIGAND BINDING AND RECEPTOR CROSS-LINKING IN RAT BASOPHILIC LEUKEMIA (RBL-2H3) CELLS. JeanClare Seagrave, Bruce H. Davis, Janet R. Pfeiffer, Grace G. Deanin, and Janet M. Oliver. University of New Mexico School of Medicine, Albuquerque, New Mexico and SUNY, Upstate Medical Center, Syracuse, New York.

Crosslinking of the IgE-receptor complexes on RBL-2H3 cells results in a series of morphological and biochemical changes culminating in the fusion of the basophilic granules with the cell membrane and the release of their contents of serotonin, histamine, and other inflammatory mediators. Very rapidly (within 30 sec) after ligand binding, dramatic changes in the cell surface morphology from microvillous to lamellar can be observed by scanning electron microscopy. These changes are associated with the polymerization of actin, as indicated by the increased binding of rhodamine-phalloidin and NBD-phallacidin measured by flow cytometry and by SDS-PAGE of Triton-X-100-insoluble cytoskeletons.

0456 IN VITRO REFORMATION OF THE MARGINAL BAND OF AVIAN ERYTHROCYTES: PERIPHERAL DETERMINANTS OF MICROTUBULE PATTERN, Judith A. Swan and Frank Solomon, MIT, Cambridge, MA 02139

The microtubules of nucleated erythrocytes are confined to a marginal band which is located at the edge of the cell, just under the peripheral cytoskeleton. The band is in one plane and curves with the elliptical shape of the cell. We have shown previously that the reformation of the band in vivo after microtubule depolymerization appears to depend upon determinants at the cell periphery. We now have been able to reform the marginal band in vitro by a reconstitution experiment: the components are detergent extracted cytoskeletons of erythrocytes which hace been depleted of microtubules in vivo, and phosphocellulose purified calf brain tubulin. The pattern of microtubules formed in vitro recapitulates several features of the intact marginal band microtubules, including position under the peripheralcytoskeleton,curvilinear microtubules and possibly the number of microtubule profiles. This in vitro recapitulation of an in vivo arrangement of microtubules allows us to assess the contribution of the various components of microtubules--organizing centers (MTOCs), associated proteins (MAPs), and tubulin -- to pattern formation. We show that marginal band formation is not dependent on MTOCs, on specific erythrocyte tubulins, nor, surprisingly, on some previously identified MAPs. However, we have identified proteins whose properties suggest that thet may specify the unique geometry of the marginal band.

Cytoskeletal-Membrane Contacts

0457 MEMBRANE - ASSOCIATED PROTEINS WHICH INTERACT WITH ACTIN. Susan S. Brown, Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109

Evidence is accumulating that there are other proteins in addition to spectrin which may mediate attachment of actin filaments to membranes. We are studying several such proteins.

Connectin is a high affinity receptor for laminin which has been isolated by affinity chromatography from several cell types (1-3). Since Sugrue and Hay (4) have documented that laminin can induce a rearrangement of actin filaments in cells, we asked whether connectin might provide the link between the extracellular matrix component laminin and the intracellular actin filaments. We found several indications that connectin interacts with actin (5): Connectin reduces actin viscosity, causes the formation of actin filament bundles, cosediments with actin and binds to actin on nitrocellulose disks. Scatchard analysis of the cosedimentation data indicates a submicromolar affinity of actin for connectin. If connectin interacts with both laminin and actin, a third piece of evidence needed is to show that connectin is an integral membrane protein which can connect the two. Initial evidence which supports this is that connectin can be incorporated into lipid vesicles.

A second approach we have taken is to identify actin - binding proteins in Dictyostelium which are membrane - associated, using the gel overlay technique (6). A number of proteins labeled with this procedure, including a 24 kd protein which was very resistant to detergent extraction. We have purified this 24 kd protein, and shown that the purified protein binds actin using a "two-phase" binding assay (7). The affinity of this protein for actin is again in the sub/micromolar range.

Finally, we are working to identify other putative membrane proteins which interact with actin in intact membranes by antibody blocking of actin low shear viscosity effects of these membranes.

1. Lesot H, Kuhl U, von der Mark K: EMBO 6:861, 1983.
2. Malinoff HL, Wicha MW: J Cell Biol 96:1475, 1983.
3. Rao NC, Barsky SH, Terranova VP, Liotta LA: Biochem Biophys Res Commun 111:804, 1983.
4. Sugrue SP, Hay ED: J Cell Biol 91:45, 1981.
5. Brown SS, Malinoff HL, Wicha MS: Proc Natl Acad Sci USA 80:5927, 1983.
6. Snabes MF, Boyd AE, Bryan J: J Cell Biol 90:809, 1981.
7. Stratford CA, Brown SS: J Cell Biol, in press.

0458 CYTOSKELETAL-MEMBRANE ORGANIZATION AT FIBROBLAST FOCAL CONTACTS. K. Burridge, T. O'Halloran, B. Paschal, L. Milam, and M. Beckerle. Dept. of Anatomy, UNC-CH, Chapel Hill, NC 27514

Nonerythrocyte spectrin, vinculin, talin and a 190k dalton protein are all candidates for a role in the attachment of microfilaments to the plasma membrane. Nonerythrocyte spectrin is not concentrated where microfilament bundles terminate at focal contacts, but it may be involved in attachment of the cortical actin network to the plasma membrane at other sites. Binding studies to membrane vesicles stripped of actin support this possibility. However, intracellular precipitation of fibroblast spectrin following antibody microinjection does not affect most of the motile properties of the cell, leading us to question the importance of nonerythrocyte spectrin in the attachment of microfilaments to the plasma membrane. By contrast, vinculin and talin are concentrated at specialized plasma membrane sites where there is cytoskeletal interaction with cell surface receptors or extracellular components: at focal contacts, underlying cell surface fibronectin and at the neuromuscular junctions of skeletal muscle. These two proteins interact with a Kd of 10^{-8} M and are both found at many of the same locations in the cell. However, at certain cell-cell junctions such as the zonula adherens of epithelial cells, vinculin is present with little or no talin. A 190 K vinculin-binding protein has been purified from chicken gizzards. Antibodies against this protein show reciprocal cross-reactivity with talin and give identical staining patterns on cultured fibroblasts. Partial proteolytic peptide maps of talin and 190 K are very similar and suggest that 190 K is a proteolytic fragment of talin. Proteolysis of talin generates two fragments of approximately 190 K and 40 K. The 190 K fragment contains the vinculin-binding site and has a rod-like appearance in the EM. Talin has either a globular or rod-like appearance depending on the ionic strength and it would seem that this shape change is regulated by the 40 K fragment. Supported by NIH GM 29860, the Muscular Dystrophy Association and the American Cancer Society.

0459 EXPRESSION OF MEMBERS OF THE HSP70 GENE FAMILY OF YEAST, Elizabeth A. Craig, Michael R. Slater and Kurt Jacobsen, Department of Physiological Chemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706.

The genome of S. cerevisiae contains a family of genes related to the heat inducible 70K gene of higher eucaryotes and to the dnaK gene of E. coli. Eight genes have been isolated and designated YG100-YG107. There are two pairs of highly homologous genes. YG100 and YG102 are 97% identical in the protein coding region, while YG101 and YG103 are ~94% identical. Homologies outside the protein coding regions are negligible. The homology amongst the less similar members is ~60-80%.

The expression of YG100-YG103 has been analyzed most extensively. Although the four genes are expressed at the optimal growth temperature of 30°C, their regulation differs. The abundance of both YG101 and YG103 transcripts decreases upon an increase in temperature from 23°C to 37°C. However, the abundance of YG100 transcripts increases after a temperature shift, while that of YG102 changes only slightly. Using the method of gene replacement, strains containing mutations in the four genes have been constructed. Although no phenotype of any of the single mutants as detected, haploid strains containing both the YG100 and YG102 mutations were not able to form colonies at 37°C. As the temperature is lowered, the effect on growth rate diminishes. These results indicate that YG100 or YG102 protein product is needed to obtain normal growth rates at all temperatures, but is essential at higher temperatures. Strains containing both the YG101 and YG103 mutations display altered growth properties at all temperatures. The cells grow fastest at 37°C; the severity of the effect increases with decreasing temperature, although colony forming ability is retained even at low temperatures. At 37°C the YG101 YG103 double mutant grows 22% slower than wild-type; at 23°C it grows 116% slower. These results suggest that the Hsp70 multigene family of yeast contains genes which have specialized but similar functions. Some of the gene products (YG100 and YG102) are required for sustained growth at high temperature and others (YG101 and YG103) at low temperatures.

Within the Hsp70 family, we have analyzed the heat inducible promoter of YG100 in most detail. A protein fusion to E. coli β-galactosidase was constructed through the tenth amino acid of YG100. This construction shows heat inducible β-galactosidase activity in yeast whether present as single-copy integrants or present in multicopy plasmid vectors. A 5' deletion series was constructed using Bal31. Sequences upstream of -340, where +1 is the first base of the initiation codon, are necessary for constitutive expression of YG100. A deletion at -246 is heat inducible whereas a deletion at -212 is not. A deletion at -236 has an intermediate behavior. Therefore, sequences between -246 and -212 are necessary for heat shock induction. A YG100 fragment from -320 to -190 was used to replace the upstream activating sequence of the yeast CYC1 promoter. This construction demonstrated that sequences from -320 to -190 contain a heat shock specific upstream activating sequence and those sequences are sufficient to elicit the heat shock response.

Cytoskeletal-Membrane Contacts

0460 BIOCHEMICAL ANALYSIS OF ACTIN-MEMBRANE INTERACTIONS,
Elizabeth J. Luna, Catherine Goodloe-Holland, Hilary M. Ingalls, Linda J. Wuestehube, and Derek Barkalow, Department of Biology, Princeton University, Princeton, NJ 08544.

A high capacity F-actin affinity matrix is constructed by binding fluorescyl-actin to anti-fluorescein IgG that is covalently bound to Sephacryl S-1000. When stabilized with phalloidin, the actin remains associated with the Sephacryl beads during repeated washes, activates the ATPase of myosin S-1, and specifically binds ^{125}I-HMM and ^{125}I-tropomyosin (1). These F-actin beads also bind highly purified, sonicated Dictyostelium discoideum plasma membranes. In low-speed sedimentation assays, ^{125}I-membrane binding to F-actin beads is specific, saturable, rapid, and apparently of reasonable affinity. Sonicated plasma membranes, after extraction with chaotropes, still bind F-actin beads. Heat-denatured membranes, proteolyzed membranes, and D. discoideum lipid vesicles do not bind F-actin beads (2). These results indicate that integral membrane proteins are responsible for much of the binding between F-actin and D. discoideum plasma membranes and agree with previous observations made using low-shear viscometry (3). Most of the binding between ^{125}I-membranes and F-actin beads is competitively inhibited by myosin S-1. This inhibition is MgATP-sensitive, exhibits a K_i of ca. 5 x 10^{-8}M, and is reciprocal (4). These results demonstrate that membrane binding and S-1 binding to F-actin beads are mutually exclusive and, therefore, that the membranes bind predominantly to the sides, rather than to the ends, of the actin filaments. Using F-actin beads as an affinity column for actin-binding proteins, we are looking for integral membrane polypeptides that bind F-actin directly or indirectly as part of a protein complex. Further work will focus on the correlation between these polypeptides and membrane structures responsible for endocytosis, chemotaxis, and cell-cell and cell-substrate adhesion.

References: (1) Luna et al., 1982, J. Biol. Chem. 257:13095-13100;
(2) Luna et al., 1984, J. Cell Biol. 99:58-70;
(3) Luna et al., 1981, J. Cell Biol. 88:396-409;
(4) Goodloe-Holland and Luna, 1984, J. Cell Biol. 99:71-78.

Regulation of Cytoskeletal Assembly and Function; Disease States

0461 ERYTHROCYTE SPECTRIN IS PARTIALLY DEFICIENT IN HEREDITARY SPHEROCYTOSIS, Peter Agre, James F. Casella, William H. Zinkham, Campbell McMillan, and Vann Bennett, Johns Hopkins Univ., Baltimore, MD 21205 and Univ. of North Carolina, Chapel Hill, NC 27514
Hereditary spherocytosis (HS) is a common, clinically heterogeneous hemolytic anemia. While the primary erythrocyte defect in most cases of HS has not been identified, it is thought to be some abnormality in the spectrin-actin membrane-skeleton which leads to loss of surface membrane. Recessively inherited spectrin deficiency with extreme erythrocyte fragility and spherocytosis has been identified in certain mutant mice and two severely anemic humans. While suspected, deficiency of spectrin has not been demonstrated in less severe forms of human HS by analysis of Coomassie blue stained SDS-PAGE gels. A better method for quantitation of erythrocyte spectrin is the spectrin radioimmunoassay. Normal erythrocytes were found to contain 240,000 copies of spectrin, whereas erythrocytes from 14 patients with a variety of HS types were all found to be partially deficient in spectrin (range 74,000 to 200,000 copies), and the magnitude of the deficiency correlated closely with disease severity and median osmotic fragility. Spectrin deficiency was not found in patients with several other types of anemias and was not affected by splenectomy. Spectrin deficiency of varying degrees is common to HS and probably represents the principal structural defect leading to loss of surface membrane.

0462 PHOSPHORYLATION OF A TROPOMYOSIN-LIKE (30KD) PROTEIN DURING PLATELET ACTIVATION, Lilly Y.W. Bourguignon, S. Field and G.J. Bourguignon, University of Miami Medical School, Miami, Fl. 33101

In this study we have used a tumor promoter, phorbol ester-TPA (12-o-tetradecanoylphorbol-13-acetate),as well as its biologically inactive analogue, 4α-PDD (4α-phorbol 12,13-didecanoate), to investigate platelet protein phosphorylation and its possible correlation with platelet activation. Our data show that TPA, but not 4α-PDD, induces a preferential phosphorylation of a 30,000 dalton (30KD) protein. This phosphoprotein is physically associated with an actomyosin-containing platelet membrane-cytoskeleton complex. Further analyses using both two-dimensional (isoelectric focusing and SDS) gel electrophoresis and an one-dimensional urea-SDS gel electrophoresis show that this 30KD protein displays tropomyosin-like properties. Most importantly, the degree of TPA-induced 30KD protein phosphorylation is directly proportional to the extent of granule release and shape change of the platelet as well as platelet aggregation. This phosphorylated tropomyosin may play a pivotal role in the regulation of actomyosin-mediated contractility which has been implicated in many platelet functions.

0463 IS PROTEOLYTIC DEGRADATION RESPONSIBLE FOR THE PARTIAL DEFICIENCY OF SPECTRIN IN HEREDITARY PYROPOIKILOCYTOSIS?T.L. Coetzer* and J. Palek. Department of Biomedical Research, Division of Hematology/Oncology, St. Elizabeth's Hospital of Boston, Tufts University School of Medicine, Boston, MA.

In hereditary pyropoikilocytosis (HPP) the erythrocyte membrane skeletons are markedly unstable when subjected to shear stress. This instability correlates with alterations in the spectrin (Sp) molecule, which exhibits defective Sp heterodimer (SpD) self-association and an altered limited tryptic digestion pattern indicating a molecular defect of the α I domain, which represents the SpD-SpD contact site. We have reported that HPP red cell membranes contain less Sp than normal as evidenced by a decreased Sp/band 3 ratio. One of the underlying causes of this Sp deficiency in HPP may be that the Sp molecule is unstable which renders it more susceptible to denaturation or proteolysis in the cell. Immunoblotting experiments using an affinity purified antispectrin antibody indicated that there is no loss of Sp during membrane preparation and also that whole HPP red cells as well as the ghosts and cytosol do not contain abnormal Sp degradation products. The Sp deficiency is already manifested at the reticulocyte level and does not change during red cell maturation since the Sp/band 3 ratio is decreased by the same amount in age fractionated HPP red cell preparations. Sp denaturation also does not appear to occur in HPP red cells since an overnight in vitro incubation of such cells at 37°C in a buffer that allowed the cells to maintain adequate GSH and ATP levels had no effect on the Sp content of the membrane. These findings indicate that the partial HPP Sp deficiency is not due to degradation of Sp in circulating red cells but that it might either be the result of degradation in red cell precursors in the bone marrow or to a synthetic defect.

0464 CHARACTERIZATION OF A MOUSE α-SPECTRIN cDNA CLONE. by Livia Cioe and Peter Curtis. The Wistar Institute, Philadelphia, PA 19104

A cloned segment of mouse α-spectrin mRNA has been identified by immunological techniques. Double-stranded complementary DNA derived from mouse anemic spleen was introduced into a bacterial expression vector, pUC, and transformed colonies were screened using an anti mouse erythrocyte membrane ghost proteins serum. Of seventeen positive colonies, two bound anti-mouse spectrin antibody, and these two colonies contained 750 bp inserts which cross hybridized. Transfer of the 750 bp insert to an expression vector containing the P_L promoter of lambda produced larger amounts of peptides which bound anti-mouse spectrin antibody. The spectrin-like peptides made in E.coli raised antibody which reacted only with the α-spectrin subunit of red cell membranes. This clone will be useful for the study of the structure and expression of the spectrin gene, particularly in understanding the role of spectrin in human inherited hemolytic anemias.

0465 A "MILLING CROWD" MODEL FOR THE LATERAL MOBILITY OF MEMBRANE PROBES. J. Eisinger, J. Flores and W. P. Petersen, AT&T Bell Laboratories, Murray Hill, NJ 07974.

The long range mobility of probes in membranes, usually measured by fluorescent photobleaching recovery (FPR), represents a value averaged over a distance on the membrane (~1 μm) in which many proteins are disbursed. In the "milling crowd" (MC) model, the mobility of probe molecules is simulated by a regular trigonal array of phospholipids in which a fraction, x, of the lattice points, is occupied by probes. The probes migrate by changing positions at random with one of six nearest neighbors at a frequency f. It is shown in computer simulations that if, as in erythrocytes, 50% of the membrane area is occupied by a random distribution of proteins (with diameters ~2 nm) the probe's diffusability is greatly reduced, and the FPR rate is a function of the disbursed proteins as much as of the local phospholipids' fluidity. The latter can be measured by the use of excimeric membrane probes. The experimentally determined ratio of excimeric and monomeric emission yields is combined with other measurable parameters and n, the average number of spatial exchanges required to form the excimer; n is obtained with the aid of the MD model. The local lateral mobility of the excimeric probe 1-pyrenehexadecanoic acid has been measured for lecithin vesicles, RBC's and ghosts. The local fluidity of biological membranes obtained in this manner was found to be several times larger than the long range fluidity, as determined by FPR.

0466 DIFFERENTIAL ACTION OF THE ANTIMICROTUBULAR DRUG NOCODAZOLE ON THE SECRETION OF PROCOLLAGEN BY CELLS IN CULTURE AND IN WHOLE TISSUES, John H. Fessler and Jane P. Petschek, and Liselotte I. Fessler, University of California, Los Angeles, CA 90024

Colchicine blocks procollagen secretion. To investigate the role of microtubules in procollagen secretion more specifically, cultured chick embryo fibroblasts were treated with nocodazole and the secretion of biosynthetically labeled procollagen I into the cell culture medium was monitored. This was slowed, but not abolished. Separate experiments showed that nocodazole does not inhibit the enzymes which process, extracellularly, procollagen to p-collagen and collagen. Therefore, the action of nocodazole on the transport of procollagen made by cells in whole calvarial bones could be monitored by following the appearance of p-collagen and collagen in these embryonic tissues. Nocodazole slowed procollagen transport within cells of whole tissues much more than in cultured cells. This suggests a model in which microtubules help to maintain a directionally organized export of procollagen in whole cells that may be largely lost in cell culture. The destruction of microtubules would then have a smaller effect on procollagen export by cultured cells.

0467 THE ROLE OF ACTIN MICROFILAMENTS-MICROTUBULES-TUBULIN INTERACTION IN CYTOTOXIC LYMPHOCYTES (CTL) TARGET CELL LYSIS .Anwar A. Hakim and Charles M. Siraki. Loyola University Medical Center. Maywood. Illinois 60153.

The CTL biofunctions are regulated by numerous factors including environmental factors, i.e. hormones, interferons, glycoproteins and metabolic agents. Earlier studies (Hakim and Joseph, Exp. Cell Biol. 51, 96-108, 1983) showed that adenosine 5'-triphosphate (ATP) changed the morphology of human fibroblasts from long-stretched attached elliptical to round floating cell cultures. The present investigations examined the action of ATP and plasma cold insoluble globulin (CIg) on the cytotoxic activities of Natural Killer (NK) cells and CTL against a series of target cells human mammary carcinoma (HMCC), human malignant melanoma (HMMC) and human hepatocellular carcinoma (HHCC). When incubated with neuraminidase (VCN) NK and CTL cytotoxic activities was increased by ten folds. VCN.NK and VCN.CTL were used as the effector cells. ATP inhibited the cytotoxic activities of these effector cells. The inhibition was ATP-concentration dependent, mediated by an intramembrane glycoprotein, causing depolarization of intr - cellular actin filaments inducing microtubular depolymerization into tubulin and release of an inhibitor of trypsin-like enzyme. CIg binds the intermembrane glycoprotein protecting the actin microfilament from ATP action. Destruction of the microtubules by ATP was monitored by Colcemid uptake and loss of cytotoxic activities of CTL , and NK .

0468 EVIDENCE FOR REGULATION OF FORMYL PEPTIDE INDUCED ACTIVATION OF GRANULOCYTES BY ASSOCIATION OF PEPTIDE RECEPTOR COMPLEX WITH CYTOSKELETON, A.J. Jesaitis, J.O. Tolley, R.A. Allen, and C.G. Cochrane, Scripps Clinic and Res. Fdn., La Jolla, CA 92037

N-formylated peptides induce a transient burst of superoxide production in isolated suspensions of human granulocytes. The rate of production is maximal between 0.5-1 min after exposure of cells to a saturating dose of F-Met-Leu-Phe. The response is terminated by 3-5 minutes. If dihydrocylochalasin B (dhCB) is added to the cells at different times after stimulation, then the rate of superoxide production increases to a level which depends on the time between stimulation and addition of dhCB. This effect is maximum when dhCB is added before stimulation, increasing the rate of production by 1.7±0.5. Addition of the dhCB after 4 min. of stimulation rejuvenates the terminated superoxide production back to its maximal unperturbed rate. By 8 min. no rejuvenation is observed. Analogous experiments utilizing ^{3}H-FMLP indicate that dhCB inhibits formation of the tertiary complex containing cytoskeleton and the "high affinity" form of the occupied receptor. These dhCB-effects are evident during the first minute after stimulation with FMLP. Consequently, receptor mediated endocytosis cannot control these processes. These results are consistent with the mobile receptor hypothesis as interpreted by Vale and Shooter (J. Cell Biol. 94:710, 1982). This interpretation suggests that conversion of occupied receptors to the high affinity form is induced in a reciprocal fashion by enhanced binding of occupied receptors to the cytoskeleton. This immobilization might then sequester receptors to cytoskeletal domains in the plasma membrane thus preventing them from performing required interactions with cellular components involved in the signal transduction.

0469 MOLECULAR CLONING OF MURINE ERYTHROCYTE BAND III, Ron R. Kopito and Harvey F. Lodish, Whitehead Institute, Cambridge, MA 02142

We have isolated and sequenced a full-length Band III cDNA clone from a λgt11 cDNA library of RNA from anemic mouse spleen using a polyclonal antibody against murine erythrocyte Band III protein. This clone contains a 2700 bp open reading frame constituting the Band III coding region, a 1600 bp 3' untranslated region containing the poly A tail and polyadenylation consensus sequence, and a 250 bp 5' untranslated region. Hybridization of this clone to Northern blots containing total anemic mouse spleen RNA or murine erythroleukemia cells (MEL) at various stages of differentiation reveals a single 4.5 kb band, transcription of which appears to be co-regulated with other erythroid markers in MEL cells. Analysis of the predicted amino acid sequence reveals substantial homology with regions of published sequence of human Band III peptides. Hydrophobic analysis of this sequence suggests the existence of 10 potential membrane-spanning helices. The transmembrane orientation of Band III in the RBC plasma membrane is being probed with antibodies against peptides predicted from the cDNA sequence.

0470 Polarization of the cytoskeleton and the Golgi apparatus inside CTL bound to their targets. Abraham Kupfer, and S. J. Singer, University of California at San Diego, La Jolla, CA 92093.

We are concerned with the mechanisms responsible for the unidirectional killing of target cells by cytotoxic-T lymphocytes (CTL). Mixing of two CTL lines, an A anti-B and a B anti-C, results in a specific and unidirectional destruction of the B anti-C CTL, and the A anti-B remains intact. By immunofluorescent microscopy in cell couples between such CTL, we observed that, shortly after cell binding, the Golgi apparatus (GA) and the microtubule organizing center (MTOC) in the A anti-B CTL (but not in the B anti-C CTL) became oriented toward the contact area with the target. Using affinity purified rabbit antibodies specific for talin, a 215 Kd cytoskeletal protein, the immunolabeling in the A anti-B CTL (but not in the B anti-C CTL) seems to be largely concentrated in the contact area. Antibodies specific for α-actinin and filamin did not display a similar concentration. This suggests that the plasma membranes and underlying cytoskeletal structures in the effector and target cells may be differentially specialized in the contact area. It is proposed that the function served by the coordinate reorientation of the GA and the MTOC is to direct the flow of Golgi derived vesicles, containing cytotoxic components, from the effector cell to its bound target. A specialized reorganization of the membrane in the contact area may provide a means of protecting the effector cell from being lysed by its own secreted cytotoxic material.

0471 HEMIN INDUCES INSTABILITY OF RED CELL MEMBRANE SKELETONS. Shih-Chun Liu and Jiri Palek. Dept. Biomedical Research, St. Elizabeth's Hospital, Tufts University School of Medicine, Boston, MA 02135

Spectrin tetramers and oligomers in normal red cells are crosslinked by actin oligomers and band 4.1 to form a two dimensional membrane skeletal network. We have recently shown that hemoglobin enhanced the self-association of spectrin dimers to tetramers, whereas hemin, a breakdown product of hemoglobin, disrupted the self-association of spectrin. We now report that hemin diminishes the stability of membrane skeletons upon mechanical shearing and alters the conformation of band 4.1. Membrane skeletons prepared by Triton X-100 extraction of red cell ghosts were sheared in a cylinder-rod shearing apparatus (2-4°C, shear stress 36 dynes/cm^2) and visualized without staining by phase contrast light microscopy with an attached contrast enhancement video camera. Control samples of Triton shells without hemin showed a slow rate of disintegration upon shearing (T_{50}, time for 50% disintegration, 30-40 min). In contrast,the addition of 10 and 50 uM hemin to Triton shells reduced T_{50} to 20 and 10 min, respectively. Hemin not only diminished the ability of spectrin to self-associate but also altered the conformation of band 4.1 as revealed by the change of band 4.1 mobility on non-denaturing gel electrophoresis. Since hemin may be liberated from oxidized or unstable mutant hemoglobin under pathological conditions, these hemin-induced effects on spectrin, band 4.1 and membrane skeletal stability may play a role in the membrane lesion of these red cells.

0472 CELL SURFACE-CYTOSKELETAL ASSOCIATIONS DURING MYOGENESIS, Alex A. Lowrey and Stephen J. Kaufman, University of Illinois,Urbana,Il.61801

Monoclonal antibodies have been used to study surface antigenic determinants (Det) on myogenic L8E63 rat cells (Lee and Kaufman 1981 Dev. Biol. 81:81; Kaufman and Foster 1984 Exp. Biol. Med. 2:57). Two of these Det, A5 and H36, were studied using indirect immunofluorescence (IIF) and photometry. Treatment of myogenic cells at different stages of development with 1% Triton X-100 extracted H36 from myoblasts (Mb) and from small or unbranched myotubes (Mt), but not from large, branched Mt. H36 resisted extraction also when 1° and crosslinking 2° antibodies were used. A5 persisted after Triton treatment throughout development. Using actin filaments (AF) labelled with rhodamine conjugated phalloidin and IIF, we found a limited coincidence between A5 and AF, and a more apparent coincidence between H36 and AF, especially on the attached cell surface,that increased greatly with development. Treatment of Mt with cytochalasin B resulted not only in the disruption of AF and H36, but also in the formation of large aggregates, which contained actin, A5, and to a much lesser degree H36. Triton failed to extract A5 from these aggregates, but did extract H36. These experiments indicate that different associations exist between myogenic cell surface Det and AF during development. The significance of specific interactions between such Det and the cytoskeleton during myogenesis will be discussed. This work was supported by grant DHHS GM 28842 to SJK.

0473 The Role of the Cytoskeleton during the Development of Polarity in Mouse Early Blastomeres. Bernard Maro and Martin H Johnson.

During compaction, the blastomeres of the 8-cell mouse embryo flatten against one another and become polarized, both at the surface and in the cytoplasm. The role of microtubules and of microfilaments during this process was investigated using specific drugs (Taxol, Nocodazole and Cytochalasin D) and specific antibodies to tubulin and actin to follow the distribution of these structures during compaction. Microtubules were shown to be necessary for cytoplasmic polarity and to exercise a constraining role on cell flattening and on surface polarity. Microfilaments were mainly involved in flattening and to a lesser extent in the setting up of polarity. Antibodies directed against cell adhesion molecules did inhibit flattening but were unable to inhibit polarization. Intercellular adhesion seems to be required to provide a reference for the normal positioning of the cytoplasmic and surface poles. These results plus the fact that surface polarity,which is the only feature of compaction which is stable during division, is necessary for reformation of the cytoplasmic poles leads us to propose that the cell cortex is able to provide an organising focus for the cytoplasmic reorganisation of the cytoskeleton at the 8- and 16-cell stage.

0474 CHARACTERISTICS OF TARGET CELL RECEPTORS FOR MALARIA SPOROZOITE SURFACE PROTEIN (*PLASMODIUM BERGHEI* Pb44 PROTEIN), Samuel J. Pancake, Michael R. Hollingdale and Alan L. Schwartz, Biomedical Research Institute, Rockville, MD 20852

Malaria is initiated in the mammalian host by the bite of an infected *Anopheles* mosquito releasing malaria sporozoites into the circulation. These rapidly and specifically invade liver parenchymal cells to initiate the exoerythrocytic phase of the infection. Monoclonal antibodies to the major surface protein of *Plasmodium berghei* sporozoites block target cell invasion and indicate that this protein likely mediates invasion. Sporozoite surface proteins derived from human malaria species are currently being tested for use in the development of anti-malarial vaccines. The present work was initiated to isolate and characterize the putative cellular receptors for the Pb44 protein. Using an *in vitro* assay, sporozoites were observed to bind as frequently to the surfaces of fixed as to those of live target cells. Fixed target cells oxidized with periodate, heated with 0.1N H_2SO_4, or exposed to neuraminidase and galactose oxidase showed little change in sporozoite binding activity. Exposure to proteases could be shown to largely remove target cell reactivity. Live cells extracted with organic solvents and cytoskeletons prepared by high salt and detergent extraction remained reactive with sporozites. Antisera are being prepared to the major protein components isolated by SDS-PAGE and are being tested for inhibitory effects on the binding of sporozoites to target cells.

Supported by the Agency for International Development Contract No. DPE-0453-C-00-3051-00

0475 HEREDITARY HEMOLYTIC ANEMIA WITH DEFECTIVE SPECTRIN SELF-ASSOCIATION AND NORMAL THERMAL PROPERTIES. Y. Ravindranath and Robert M. Johnson, Detroit Children's Hospital and Wayne State Medical School, Detroit MI 48201

Spectrin extracted from normal erythrocyte membranes at 0° is nearly all tetrameric, while in hereditary pyropoikilocytosis (HPP) and type 1 hereditary elliptocytosis (HE), a substantial amount of the extracted spectrin is dimeric. We here report the case of a family in which the child has moderately severe hemolysis, with extreme microcytosis (MCV=44) and poikilocytosis. The spectrin extracted at 0° is predominately dimer. Parents had levels of dimer intermediate between patient and controls:

	controls	patient	father	mother
% dimeric spectrin	11 ± 6 (7)	77 (2)	27 (2)	49 (2)

The case is not HPP, since in all three individuals the temperature dependence is normal for (a) erythrocyte fragmentation; (b) spectrin extractability; and (c) circular dichroism of purified spectrin. It is not HE, since neither parent had elliptocytic red cells as judged from smears and scanning electron microscopy. The spectrin variant therefore appears to be a new type. The presence of substantial amounts of dimeric spectrin in the parents is consistent with a model in which each parent is heterozygous for a different mutant non-associating spectrin, while the child has inherited a non-associating molecule from each parent. In each member of this family, the mechanical stability of the erythrocyte membrane, determined by ektacytometry, is correlated with the amount of tetramer found in the membrane.

0476 IMMUNOGLOBULIN HEAVY CHAINS ASSOCIATED WITH THE LYMPHOCYTE CYTOSKELETON ARE PHOSPHORYLATED, Allen J. Rosenspire and Yong Sung Choi, Sloan-Kettering Institute, Rye, NY

On B lymphocyte surfaces, membrane immunoglobulin (mIg) functions as antigen-receptor. The cross linking of mIg receptors by either ligand or anti-receptor antibodies has been shown to generate transmembrane signals that lead to mitogenesis, and under proper circumstances, eventually to differentiation. Although most mIg can normally be solubilized from cell membranes by nonionic detergents, when mIg is cross linked by anti-Ig antibodies, much of it becomes insoluble so that the mIg co-isolates with the lymphocyte cytoskeleton. When the (cytoskeletally-associated) insoluble detergent pellets are extracted with low salt buffers, several membrane proteins are solubilized, including mIg. In cells which have been biosynthetically labeled with ^{32}P orthophosphate prior to lysis, we find that some of the Ig which is extracted from the cytoskeletal pellet with low salt has phosphorylated heavy chain. On the other hand, we can find no evidence that any Ig not associated with the cytoskeleton is phosphorylated. The implication is that the phosphorylation of mIg may be essential for the association of mIg with the cytoskeleton and in the generation of transmembrane mitogenic signals.

0477 TRANSPORT OF HERPES SIMPLEX VIRUS GLYCOPROTEINS IN NEURONS AND ASTROCYTES.INTERACTION WITH CYTOSKELETAL ELEMENTS,Christence Stubbe-Teglbjærg and Bodil Norrild, Institute of Medical Microbiology, University of Copenhagen,Denmark.

Herpes Simplex Virus (HSV) is a neurotropic virus which establish life-long infections of the nervous system. Reactivation occurs at intervals with synthesis of viral proteins of which the glycoproteins become inserted into the plasma membrane of the infected cell. Five glycoproteins designated gB. gC,gD,gE and gG have so far been identified. We have studied the synthesis of gB,gC and gD in immature and mature mouse neurons and astrocytes. As these cells mature specilization of the plasmamembrane occurs with formation of branching cell processes of the astrocyte and formation of dendrites, axons and synapses of the neurons. The difference in ability to express the viral glycoproteins gB,gC and gD in immature and mature membranes of neurons and astrocytes will be discussed. HSV infection of human fibroblasts which is a non-polarized cell results in alteration of the cytoskeletal elemets which leads to co-distribution of HSV glycoproteins with both actin and tubulin. Intermediate filaments remains unaltered. Whether similar interactions of viral proteins with cytoskeletal elements take place in neurons which is a highly specialized and polarized cell harbouring the latent HSV in vivo is beeing investigated and will be discussed.

0478 MOLECULAR CLONING OF CYTOSKELETAL GENES FROM THE HUMAN ERYTHROCYTE, Jonathan Tyler Department of Genetics, University of Alberta, Edmonton, Alberta T6G 2E9 Canada

Genes coding for cytoskeletal proteins of the human erythrocyte were cloned to permit their detailed molecular analyses. Polyadenylated messenger RNA (mRNA) was isolated from rabbit reticulocytes by phenol extraction and oligo-(dT) chromatography. When translated in an mRNA-dependent reticulocyte lysate system, all major cytoskeletal proteins were visible in ^{35}S-methionine fluorographs. These included spectrin (SDS bands 1 and 2, 240,000 and 220,000 daltons, respectively) ankyrin (band 2.1, 210,000 daltons), band 4.1 (82,000 daltons) band 4.2 (76,000 daltons) and erythrocyte actin (band 5, 43,000 daltons). The extracted mRNA was fractionated on methyl mercury agarose gels, yielding 8 subfractions specific for mRNAs ranging in size from 2 to 8 kilobases. cDNA clones produced to relevant subfractions were identified by hybrid selected translation of complementary mRNA. Appropriate clones were analyzed by restriction digests and genomic Southern blotting. Messenger RNAs complementary to selected cDNA clones were visualized by Northern blotting techniques.
Nick translated dCT^{32}P cDNA has been used to probe rabbit and human genomic libraries to obtain structural genes with associated regulatory domains. Probes typically yield strongly positive genomic clones in a clean background even across species boundaries.

0479 RUBELLA VIRUS ASSOCIATED WITH CYTOSKELETON (RUBELLA VACS) PARTICLES - A NEW INFECTIOUS AGENT? D. Van Alstyne, P. Sunga, E.M. Smyrnis and M. DeCamillis, The University of British Columbia, Vancouver, B.C., Canada.

This laboratory has been studying persistent rubella virus (RV) infection in the central nervous system (CNS). To further these investigations, we have produced hybridomas which secrete monospecific anti-rubella antibodies. In the course of their characterization, it was observed that an immunosorbent column composed of monoclonal IgG (F1-6E) linked to Sepharose 4B beads could be employed to selectively remove all RV polypeptides simultaneously from infected L cell lysates. On electron microscopic examination it was determined that the viral polypeptides were eluted as filamentous structures averaging 50 nm in length. In addition, RNA'ase sensitive RNA was detected in association with these structures. In view of their filamentous nature and the identification of one polypeptide component (p44) as actin, the structures have been identified as a combination of rubella virus (RNA and protein) associated with cytoskeleton (Rubella VACS) fragments. De novo RNA and protein synthesis were measured following VACS infection and results indicate that these particles are infectious and demonstrate some unique biological properties unlike those of the virus stocks from which they were derived. These data suggest that the rod-like rubella VACS particles may share some features characteristic of the slow viruses, or prions, associated with some disorders in the CNS.

0480 REGULATION OF THE ACTIN-ACTIVATED ATPase OF THYMUS MYOSIN, Paul D. Wagner, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, MD 20205

While the actin-activated ATPases of both vertebrate smooth and nonmuscle myosins are regulated by phosphorylation of their 20,000 Da light chains, both the order of light chain phosphorylation and the mechanism of activation are quite different from these two types of myosin. We have found that native gel electrophoresis can be used to separate thymus myosin with two phosphorylated light chains from unphosphorylated myosin and myosin with one phosphorylated light chain. This technique was used to show that the light chains of both monomeric and filamentous thymus myosin are phosphorylated randomly. This apparent lack of cooperativity contrasts with the ordered phosphorylation of smooth muscle (gizzard) myosin light chains. Also unlike with smooth muscle myosin, we have found a linear correlation between the extent of light chain phosphorylation and stimulation of the actin-activated ATPase of thymus myosin. Since thymus myosin is phosphorylated randomly, this linear relationship shows that phosphorylation of one head of thymus myosin stimulates the actin-activated ATPase of that head independently of the phosphorylation of the other head. In contrast both heads of smooth muscle myosin must be phosphorylated for actin to activate the ATPase of either head.

Regulation of the actin-activated ATPase of smooth and nonmuscle myosins by light chain phosphorylation does not preclude other regulatiory systems. Indeed, it will be shown that it is possible to stimulate the actin-activated ATPase unphosphorylated thymus myosin.

Synthesis in Regulation of Membrane Skeletal Components in Normal and Disease States

0481 Glycophorin-Protein 4.1 Associations: The Role of Polyphosphoinositides, Richard A. Anderson, Department of Pathology, Yale University School of Medicine, New Haven, CT 06510.

The polyphosphoinositides, phosphatidylinositol-4-phosphate (DPI) and phosphatidylinositol-4,5-diphosphate (TPI) are asymmetrically located only on the cytoplasmic half of the bilayer. Recent evidence has demonstrated that these trace phosphorylated phospholipids are important in trans-membrane message transduction. In the erythrocyte, and perhaps in other cells, the polyphosphoinositides may also play a role in regulating the association of membrane proteins with membrane skeletal proteins. These associations may in turn modulate the assembly of the membrane skeleton.

Protein 4.1 is a red cell membrane skeletal protein which binds to spectrin and plays a pivotal role in the assembly of the spectrin-actin complex. The association of protein 4.1 with the membrane involves two binding sites; a high affinity site which has been identified as glycophorin (1) and a lower affinity site which is band 3 (2). Recent evidence has demonstrated that glycophorin has a requirement for bound polyphosphoinositides before it will act as a binding site for protein 4.1. Glycophorin in a soluble micellar form or reconstituted into phosphatidylcholine liposomes shows an absolute requirement for either DPI or TPI for its association within protein 4.1. Micellar glycophorin, reconstituted with DPI will bind protein 4.1 and inhibits protein 4.1 binding to inside-out membrane vesicles (IOVs), however, micellar glycophorin reconstituted with TPI shows a higher affinity for protein 4.1 binding and is a better inhibitor of protein 4.1 binding to IOVs. In comparison, glycophorin which is reconstituted into phosphatidylcholine liposomes shows a specific TPI requirement for the glycophorin-protein 4.1 association, whereas DPI does not appear to promote protein 4.1 binding to glycophorin.

In the erythrocyte membrane, and in other cell membranes, the monoester phosphates on the myo-inositol ring are rapidly turned over. Metabolic depletion or increased cytoplasmic Ca^{++} (3) causes a drop in the polyphosphoinositol content of the membrane (largely TPI) and a concurrent cell shape change. The molecular basis for such a change may lie in the polyphosphoinositide modulated glycophorin-protein 4.1 association.

References

1. Anderson, R.A. and Lovrien, R.E. Nature 307: 655-658 (1984).

2. Pasternack, G.R., Anderson, R.A., Leto, T.L. and Marchesi, V.T. J. Biol. Chem. In press.

3. Ferrell, J.E. and Huestis, W.H. J. Cell Biol. 98: 1992-1998 (1984).

0482 PROTEIN KINASE C AND CALCIUM ION IN TRANSMEMBRANE SIGNALING
Yoshimi Takai, Department of Biochemistry Kobe University School of Medicine, Kobe 650, Japan

Many extracellular signals elicit Ca^{2+}-mobilization and phosphoinositide turnover in their target cells. Ca^{2+} induces diverse effects and many of these are mediated through the activation of calmodulin and other Ca^{2+} receptor proteins. The function of phosphoinositide turnover has been unclear, but we have found that diacylglycerol, one of the initial products of the receptor-induced phosphoinositide turnover, serves as a second messenger for the activation of protein kinase C in the presence of Ca^{2+} and membrane phospholipids. We have synthesized unique diacylglycerols such as 1-oleoyl-2-acetyl-glycerol and 1-acetyl-2-oleoyl-glycerol which induce the activation of intracellular protein kinase C when added exogenously to intact cells. These diacylglycerols activate selectively protein kinase C without Ca^{2+}-mobilization, whereas a low concentration of Ca^{2+} ionophore A23187 induces Ca^{2+}-mobilization without activation of protein kinase C. Using these synthetic diacylglycerols and Ca^{2+} ionophore, it has been shown that both protein kinase C and Ca^{2+} are involved in and play a synergistic role in exocytosis from various secretory cells and superoxide generation in macrophages. Another series of experiments have revealed that protein kinase C is activated by tumor-promoting phorbol esters in a manner identical with that of diacylglycerol, and that the enzyme complexed with Ca^{2+} and phospholipids may serve as a receptor for phorbol esters. Since phorbol esters have been shown to act as a potent mitogen or comitogen in various cells, the result suggests that protein kinase C may play a role in the regulation of cell proliferation. In fact, evidence has been obtained that protein kinase C is activated by the action of platelet-derived growth factor and fibroblast growth factor and may be involved in the activation of c-*myc* gene in Swiss 3T3 fibroblasts. Moreover, synthetic diacylglycerols and phorbol esters are capable of stimulating DNA synthesis in the presence of insulin in this cell line. Available evidence thus far obtained from our and other laboratories strongly suggests that protein kinase C as well as Ca^{2+} may play a role of crucial importance in the actions of a wide variety of extracellular signals including hormones, neurotransmitters, growth factors, tumor promoters and other biologically active substances.

Molecular Defects of the Membrane Skeleton

0483 EVOLUTION OF THE α SPECTRIN GENE FAMILY: SEQUENCE CONSERVATION IN PROTEIN STRUCTURAL DOMAINS. Connie S. Birkenmeier, Jane E. Barker, and David M. Bodine, The Jackson Laboratory, Bar Harbor, ME 04609. Biochemical and immunological studies have identified three distinct types of spectrin. These spectrins have a tissue specific distribution and can be referred to as erythroid, non-erythroid and intestinal brush border types. It is likely that these proteins are coded for by a family of related genes. We are investigating this possibility at the molecular genetic level.

A 1.4Kb cDNA clone, designated 8-13a, was isolated by direct immunological screening of an expression cDNA library prepared from embryonic chicken gizzard and stomach poly(A)$^+$RNA (1). The antiserum was raised against adult chicken erythroid α spectrin.

Southern blots of chicken genomic DNA probed with 8-13a show a simple pattern of bands which appear with single copy intensity. Northern blot analysis supports the conclusion that our clone is from smooth muscle and is most closely related to the non-erythroid type spectrins, yet the amino acid translation of the DNA sequence of 8-13a shows significant homology to the amino acid sequence of human erythroid type spectrin. The 8-13a sequence is organized into homologous repeating domains similar to those reported by Speicher and Marchesi (2) for human erythroid α and β spectrin. Our data lends support to the possibility that the spectrin gene family arose by gene duplication of an ancestral spectrin gene which itself arose by duplications of a gene for the repeating domain.

(1). Birkenmeier, C. S., Bodine, D. M., Repasky, E. A., Helfman, D. M., Hughes, S. H., and Barker, J. E. 1984. J. Cell Biol. 99:(4) part 2:301a.
(2). Speicher, D. W. and Marchesi, V. T. 1984. Nat. 311:177.

0484 MOLECULAR DEFECTS OF α I DOMAIN OF SPECTRIN IN HEREDITARY ELLIPTOCYTOSIS, Jiri Palek, Jack Lawler, and Shin-Chun Liu, Department of Biomedical Research and Division of Hematology, St. Elizabeth's Hospital/Tufts Medical School, Boston, MA 02135

Hereditary abnormalities of the α I domain of spectrin, which contains the spectrin heterodimer (SpD) self-association site, are found in about 30% of patients with hereditary elliptocytosis (HE) and all patients with hereditary pyropoikilocytosis (HPP). Thus, these variants represent the most common hereditary defects of spectrin in man. They are detected by quantitative analysis of spectrin dimers (SpD), tetramers (SpT) and oligomers (SpO) in ghosts, extracted by a low ionic strength buffer at 0^{o}C. Under these conditions the SpT-SpD + SpO equilibrium is kinetically immobilized so that the percentage of these species in the extract represents their relative proprotion in the membrane. These abnormal spectrins are characterized by defective SpD self-association in solution, ghosts and inside out vesicles and by mechanical instability of membrane skeletons, exposed to sheer stress. At a structural level, four distinct abnormal α spectrins have been identified by limited tryptic digestion followed by two-dimensional isolectric focusing sodium dodecylsulfate polyacrylamide gel electrophoresis (IEF-SDS PAGE) of the tryptic peptides. In all of them, the normal, 80,000 dalton α I domain is markedly decreased in amount and a new group of peptides is present suggesting an altered conformation of the α I domain, allowing exposure of previously masked trypsin resistant sites. Based on the size of the most prominent abnormal peptides, these abnormal spectrins are designated Sp α I/74, Sp α I/46, Sp α I/65a and Sp α I/65b. In the latter two variants, the abnormal peptides have the same size, but they differ in isoelectric points. Although the amounts of abnormal spectrin can not be accurately measured, the % of SpD (out of the SpD+SpT pool) appears to approximate the fraction of abnormal spectrin in the cells. Heterozygote carriers who have approximately 21-34% of SpD are either asymptomatic or have elliptical red cells with minimal or absent hemolysis, except at conditions of increased micro-circulatory stress, when their red cells exhibit frank fragmentation and hemolysis. HPP patients, who presumably are compound heterozygotes, contain 48-62% of SpD and exhibit severe hemolytic anemia with in vivo red cell fragmentation (micro-poikilocytosis). In addition, their red cells have a decreased spectrin content to 60-70% of normal. In 6 out of 12 unrelated families, one of the parents of an HPP offspring carries the abnormal spectrin but is either asymptomatic or has mild HE. The other parent, who is both clinically and biochemically normal, must carry a silent genetic defect which, when transmitted to the offspring, leads to enhanced expression of the abnormal spectrin and a concomitant partial spectrin deficiency. Of particular interest are three unrelated HPP/HE patients whose red cells contain, in addition to normal spectrin, two non-linked spectrin variants (including those of α I, α II and/or α III domains respectively). These data demonstrate that alterations of the spectrin α I domain are associated with defective SpD-SpD interaction, skeletal and whole cell instability leading to hemolysis and red cell fragmentation in vivo. The apparent coexistence of more than two α spectrins in some of the patients suggests that more than two genes may code for αspectrin synthesis.

Synthesis and Assembly of Membrane Skeletal Proteins

0485 BIOGENESIS OF THE ERYTHROCYTE MEMBRANE, Harvey F. Lodish, Vikram Patel and Ron Kopito, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142, and Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139

The erythrocyte membrane contains several proteins not present, or present in low amounts, in precursor cells, such as spectrin, "Band III", and ankyrin. During erythropoiesis, plasma membrane proteins of the precursor cells are gradually replaced by mature membrane proteins. Recently we showed that one or more plasma membrane molecules that mediate cellular adhesion to fibronectin is specifically lost during erythropoiesis. Undifferentiated MEL cells, reticulocytes from splenectomized mice, and stress reticulocytes from anemic rabbits all bind tightly and specifically to immobilized fibronectin, not to laminin, collagen or other extracellular matrix proteins. Monoclonal antibodies specific for the cell-binding domain of fibronectin, but not those that bind to other parts of the molecule, block adhesion of MEL cells, reticulocytes, and fibroblasts. Adhesion to fibronectin is lost upon differentiation of these cells. Our studies indicate that some of the fibronectin-adhesive molecule(s) is lost in the bone marrow, the rest largely in the spleen as the reticulocyte membrane is remodeled to the erythrocyte membrane. We are now purifying these fibronectin-binding molecules.

We have also cloned a full-length cDNA encoding mouse erythrocyte "Band III", the principal integral membrane protein. The level of mRNA for this protein increases about 10 fold during differentiation of MEL cells. The sequence of this protein has illuminated its orientation within the phospholipid bilayer, and has provided insights into the mechanism of anion transport, a principal function of Band III.

0486 EXPRESSION AND BIOSYNTHESIS OF BAND 4.1 PROTEIN IN NEURAL TISSUE

Ling, E., Cohen, C. and Sapirstein, V.S.
E.K. Shriver Center and Tufts University

We have examined the distribution of Band 4.1 protein in the central and peripheral nervous system of the rat and have investigated its expression, distribution and biosynthesis in tissue culture. Band 4.1 was demonstrated in isolated membrane and cytoskeletal fractions from brain. In frozen sections of cerebellum band 4.1 was found in significant quantities in several neuronal cell types such as the granule cells absent in others such as the purkinje cell. The protein appears to be wide spread in neuronal processes such as the parallel fibers and basket cell axons of the cerebellum and the axons of the dorsal roots. Oblique sections of the dorsal roots indicate that the protein is restricted to the periphery of the axons subjacent to the axolemma, but absent from the central regions of the axoplasm. Tissue culture studies reveal the presence of band 4.1 in both neuroblastoma and glioma cells. An in depth analysis of its subcellular distribution levels and rate of synthesis during differentiation of neuroblastoma cells indicate that the expression of this protein is regulated during the cell differentiation process. Induction of differentiation of NB2A cells by db cAMP produces axon like processes and synaptic profiles and results in a significant increase in the level of bands 4.1 in membrane fraction and isolated cytoskeletal. Of the two major molecular weight forms of this protein in NB2A cells, eg. 145 K and 95 K daltons, only the steady state level of the 95 K was found to increase. The increase in steady state level was found to be correlated with an increase in the rate of synthesis of both the 145 K and 95 K species. The fact that the synthesis of both molecular weight forms increased while the level of only the 95 K was elevated suggests that the 145 K protein may be processed to the lower molecular weight form. Immunocytochemical localization of 4.1 in undifferentiated and differentiated cells indicate that the differentiation process brings about a relative localization of the protein to specific areas of the cell. Specifically, band 4.1 was found to be present in the axons like processes of differentiated cells with particular concentration in nerve endings especially where contacts had been made with other cells or processes. The data suggest that the expression of band 4.1 may be integral to events controlling axon elongation and synapse formation. (Supported by NS16186, HD05515 and HL24382).

Function of Membrane Skeletal Proteins

0487 CORTICAL ACTIN BINDING PROTEINS: THEIR ROLE IN CAPPING AND GROWTH OF PROTRUSIONS FROM THE CELL SURFACE,

J. Condeelis[1] and J. Wolosewick[2], [1]Albert Einstein College of Medicine, Bronx, NY 10461 and [2]University of Illinois Medical Center, Chicago, IL 60612

The cortex of Dictyostelium amoebae is packed with a dense meshwork of branched actin filaments. We have hypothesized that growth and rearrangement of filaments in the meshwork are responsible for motility at the cell surface such as pseudopod extension, and capping. Myosin, α-actinin and 120K are actin binding proteins that are present in large amounts in Dictyostelium amoebae. Their presence in the cortex during cell surface motility suggests that they could regulate growth and distribution of cortical filaments. In vitro, α-actinin cross-links actin filaments to form lateral arrays and stimulates the MgATPase of actomyosin. In vitro, 120K promotes the polymerization of F actin to form branched filaments similar to those seen in the cell cortex in situ and inhibits the actin stimulated MgATPase of actomyosin. Immunocytochemical studies demonstrate that 120K usually follows the position of actin in the cell. It is associated with actin containing protrusions that are extended from the cell surface during amoeboid movement and capping but is excluded from regions containing actomyosin such as the cap. In the electron microscope 120K is associated with meshworks of branched actin filaments. Unlike 120K, α-actinin is associated with regions of the cytoplasm containing actomyosin such as the cap. In the electron microscope, α-actinin is found in lateral arrays of filaments while myosin is associated with lateral arrays of filaments but is also found on the cytoplasmic surfaces of vesicles. These results are consistent with the proposal that 120K assembles meshworks of branched actin filaments that exclude myosin while α-actinin is involved in the assembly of lateral arrays of actomyosin. (Supported by NIH 25813 and Hirschl Trust).

0488 HUMAN ERYTHROCYTE MYOSIN: SOME NEW POSSIBILITIES FOR CYTOSKELETAL CONTROL OF CELL SHAPE. Velia M. Fowler* and Vann Bennett+. Department of Anatomy, Harvard Medical School, Boston, MA, and +Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, MD.

The human erythrocyte cytoskeleton is currently depicted as a network of short actin filaments cross-linked by long, flexible spectrin molecules that underlies and is closely apposed to the cytoplasmic surface of the membrane. This model does not account for the ATP-dependent biconcave shape of the normal erythrocyte, nor for the dynamic, calcium-sensitive discocyte-echinocyte shape transformations of the cells. Recently, we have identified on the erythrocyte membrane a non-muscle form of tropomyosin that is present in sufficient quantities to almost completely coat all of the short actin filaments in the membrane cytoskeleton (1). The only known function of tropomyosin is, together with troponin, to confer calcium-sensitivity on the ATP-dependent interaction of myosin with actin, but erythrocytes have not been thought to contain myosin. However, we have reinvestigated this question (2) and have found that human erythrocytes contain a M_r 200,000 polypeptide that cross-reacts specifically with affinity purified antibodies to the heavy chain of human platelet myosin. Immunofluorescence staining of formaldehyde-fixed erythrocytes demonstrated that the immunoreactive myosin polypeptide was present in all cells and was thus not derived from reticulocytes or contaminating platelets or neutrophils . Between 20-40% of the immunoreactive myosin polypeptide remained associated with the membrane after hyopotonic hemolysis and preparation of ghosts, suggesting that it may be bound to the membrane as well as being present in the cytosol. We have purified the erythrocyte myosin to homogeneity from the hemolysate and have found that it is a typical vertebrate myosin with two globular heads at the end of a rod-like tail 150 nm long, and with two light chains (M_r 25,000 and 19,500) in association with the M_r 200,000 heavy chain. The erythrocyte myosin forms bipolar filaments 0.3-0.4 um long at physiological salt concentrations, and exhibits a characteristic pattern of myosin ATPase activities with EDTA, Ca^{++}, and Mg^{++}-ATPase activities in 0.5 M KCl of 0.38, 0.48, and <0.01 umol/min/mg. The erythrocyte myosin is present in about 6,000 copies per cell, in a ratio of 80 actin monomers for every myosin molecule, which is an amount comparable to actin/myosin ratios in other non-muscle cells. We propose that the erythrocyte myosin could function together with tropomyosin in a membrane-associated actomyosin contractile apparatus responsible for the ATP-dependent biconcave shape of the cell as well as for their calcium-sensitive discocyte-echinocyte shape transformations.

1. Fowler, V.M. and V. Bennett. 1984. Erythrocyte membrane tropomyosin. Purification and Properties. J. Biol. Chem. 259:5978-5989.
2. Fowler, V.M., J.Q. Davis, and V. Bennett. 1985. Human erythrocyte myosin. Identification and purification. J. Cell Biol. 100(1):000.

0489 REGULATION OF CYTOSKELETAL STRUCTURE AND CONTRACTILITY IN THE INTESTINAL BRUSH BORDER, Mark S. Mooseker, Yale University, New Haven, CT 06511

The brush border (BB) cytoskeleton has two structural domains, the microvillus (MV) core and the terminal web (TW). In addition to providing membrane support, this cytoskeleton must have other roles given the functional diversity of its constituent proteins. For example, the MV core contains villin, a Ca^{++}- and actin-binding protein which at various $[Ca^{++}]$ can bundle (10^{-7}M Ca^{++}), cap (10^{-6}M), or sever (10^{-5}M) actin filaments. The core also contains 110K-calmodulin (CM) which comprises the lateral linkers between the core and membrane. The CM within the complex binds the 110K ± Ca^{++}, yet this CM is "available" to activate CM-dependent enzymes. The 110K-CM binds actin only in the absence of ATP and like myosin has EDTA-ATPase activity (Collins et al. 1984) suggesting that it may be myosin-like. The BB does contain "orthodox" myosin, localized in two regions within the TW--in the circumferential actin bundle at the zonula adherens (ZA) junction and within the inter-rootlet zone where it is present as filamentous strands which crosslink adjacent MV core rootlets. Myosin mediates the Ca^{++} and ATP-dependent contraction of the ZA bundle observed in isolated BBs. Much of the inter-rootlet myosin can be extracted from the TW by ATP treatment at 0°, without inhibiting contraction, suggesting the presence of two functional domains of myosin in the TW. The inter-rootlet zone also contains TW 260/240, a BB-specific form of spectrin which like myosin forms crosslinks between adjacent rootlets. Studies on TW 260/240 indicate that this protein can crosslink actin, potentiate actomyosin interaction and bind to inside-out vesicles from red cell membranes, but with much lower affinity than erythrocyte (human or chick) spectrin.