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Cytoskeleton–Plasma Membrane Interactions

Elizabeth J. Luna* and Anne L. Hitt

Proteins at the boundary between the cytoskeleton and the plasma membrane control cell shape, delimit specialized membrane domains, and stabilize attachments to other cells and to the substrate. These proteins also regulate cell locomotion and cytoplasmic responses to growth factors and other external stimuli. This diversity of cellular functions is matched by the large number of biochemical mechanisms that mediate the connections between membrane proteins and the underlying cytoskeleton, the so-called membrane skeleton. General organizational themes are beginning to emerge from examination of this biochemical diversity.

The first definition of a "membrane skeleton" (*1*) originated with the observation that the nonionic detergent, Triton X-100, disrupts hydrophobic, but not polar, protein-protein and protein-lipid interactions in the membrane of the human red blood cell (*2*). Interconnected cytoskeletal proteins, including actin and spectrin, and tightly associated integral membrane proteins co-pellet as Triton-insoluble structures with the approximate size and shape of the unsolubilized cells. The possible generality of this approach was suggested by the observation that most of the surface-labeled proteins in tissue culture cells also remain associated with a thin cytoplasmic layer after Triton extraction (*3*). However, Triton insolubility is an insufficient criterion for cytoskeletal attachment because other interactions also confer resistance to nonionic detergents (*4*). Thus, an association with cytoskeletal elements must now be directly demonstrated in order to conclusively identify a protein as a component of the membrane skeleton.

In this review, we will focus on systems in which molecular information is available on the attachments between membrane and cytoskeletal proteins. Because this is a large and fast-moving field summarized previously (*5–7*), we will emphasize recent advances in understanding how the membrane skeleton governs basic cell processes. Thus, we primarily describe specialized membrane domains that have been isolated and dissected biochemically. Most cells coordinate the functions of these domains so that they act in concert with each other and with other membrane and cytoskeletal proteins.

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Control of Cell Shape, Membrane Stability, and Domain Organization

Erythrocytes. Because of its comparative simplicity and the relative ease with which large amounts of homogeneous membrane can be obtained, the best understood membrane skeleton is that of the human red blood cell (*8*). These highly specialized cells are biconcave disks that lack internal organelles and transcellular filament systems. The membrane stability and deformability required during the erythrocyte's 120-day, tortuous journey through the bloodstream are maintained solely by the underlying meshwork of spectrin, actin, and associated proteins (Fig. 1). This meshwork is attached to the membrane by ankyrin and protein 4.1. Ankyrin links band 3 (the anion exchanger) to the β subunit of spectrin near the middle of the extended spectrin tetramer. Protein 4.1 binds to both spectrin subunits near the ends of the tetramer, enhancing the affinity of spectrin for actin. Protein 4.1 also binds in vitro to the transmembrane proteins, band 3 and glycophorin C. Binding between protein 4.1 and band 3 may involve an interaction between the amino acid sequence, LEEDY, near the NH_2-terminus of protein 4.1 and an oppositely charged motif (IRRRY or LRRRY) in the cytoplasmic domain of band 3, although other sites on band 3 also have been implicated (*9*). A sequence (YRHKG) present in glycophorin C contains the same charge distribution and hydrophobicity as the motif in band 3. Finally, spectrin and band 4.1 bind with low affinity to negatively charged phospholipids (*10*), interactions that may help to stabilize the lipid bilayer.

Proteins not bound directly to the membrane also add to the stability of the membrane skeleton (*11*). For instance, a heterodimeric calmodulin-associated protein, adducin, enhances spectrin binding to actin. Tropomyosin and dematin (protein 4.9) bind along the sides of actin filaments, and the tropomyosin-binding protein, tropomodulin, may control the lengths of the short actin filaments bound to the spectrin tetramers.

Studies of mice and humans with hereditary defects in erythrocyte shape and stability confirm the role of the membrane

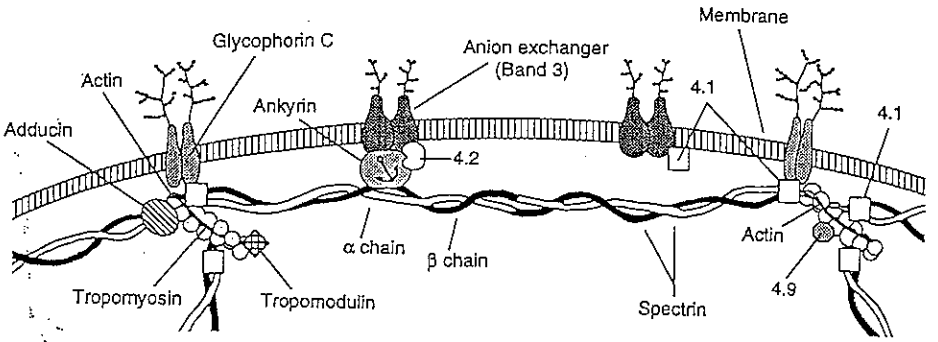

Fig. 1. Membrane-cytoskeleton attachments in the erythrocyte. Diagram shows the major interactions among spectrin (260 and 225 kD), ankyrin (215 kD), adducin (105 and 100 kD), band 3 (90 to 100 kD), protein 4.1 (78 kD), protein 4.2 (pallidin, 72 kD), dematin (protein 4.9, 48 kD), glycophorin C (~25 kD), actin (43 kD), and tropomyosin (29 and 27 kD). Protein sizes quoted here and in the text are apparent sizes based on migration positions in SDS–polyacrylamide gels.

skeleton in maintaining structural integrity (*12*). Deficiencies or defects in spectrin α- or β subunits, ankyrin, band 3, protein 4.1, and protein 4.2 (pallidin) are associated with increased fragility and lysis of erythrocytes. These cells also tend to be round, ellipsoidal, or spiculated rather than normal biconcave discs. Cells from patients lacking glycophorin C also are ellipsoidal, but their membranes exhibit normal mechanical properties.

Major unanswered questions are how the components of the erythrocyte membrane skeleton are organized and how this organization changes during deformation. One idea is that spectrin, a highly flexible elongated molecule, is elastic and stretches reversibly in response to local forces (*13*). Another possibility is that the connections among the skeleton components change in response to mechanical stress and metabolic conditions. For instance, spectrin on the membrane may exist largely as hexamers and octamers, but is usually extracted as dimers and/or tetramers, suggesting that interactions at the heads of the dimers are dynamic. Connectivity of the spectrin meshwork also could be regulated by the accessory proteins at the spectrin-actin junction. For instance, adducin-promoted binding of spectrin to actin is regulated by calmodulin and calcium ions, and phosphorylated protein 4.1 exhibits decreased affinities for spectrin and band 3. Furthermore, binding of protein 4.1 to glycophorin C requires the presence of phosphatidylinositol 4,5-bisphosphate (PIP_2) (*14*).

Proteins closely related to spectrin, ankyrin, band 3, adducin, protein 4.1, protein 4.2, and dematin are associated with the plasma membranes of many cells in organisms ranging from humans to amebae (*7, 8, 15*). Immunolocalization of these proteins, especially ankyrin and non-erythroid spectrin (fodrin), indicates that they often are concentrated in membrane domains which, as in the erythrocyte, may be specialized for mechanical stability and restricted diffusion of associated membrane proteins. Such "flexible holdfasts" include post-synaptic densities in brain and neuromuscular junctions, infoldings of basolateral membranes in epithelial cells, and the nodes of Ranvier and axon initial segments in myelinated nerves. Although spectrin and ankyrin are usually found together in these specialized domains, they are associated with a number of different integral membrane proteins. In addition to band 3, ankyrin also binds the two other members of the anion exchanger gene family as well as the voltage-gated Na^+ channel in brain, a 205-kD brain glycoprotein (ABGP205), the α subunit of Na^+,K^+-ATPase (adenosine triphosphatase) in kidney, an 85-kD GTP (guanosine triphosphate)-binding lymphoma glycoprotein (CD44, GP85), and a 116-kD CD44-like protein in endothelial cells. A fraction of the cell adhesion protein, E-cadherin (uvomorulin), also has been reported to co-sediment with ankyrin and spectrin (*16*), but direct binding has not been demonstrated.

The structural relationships between the erythroid and homologous non-erythroid proteins are complex (*17*). There are at least two genes for the spectrin α subunit and at least five genes encoding β subunits. The similarly sized α subunits each contain a series of 22 repeats of 106 amino acids (the "spectrin repeat") involved in dimerization, an *src*-homology 3 (SH3) domain in the middle of the molecule, and a predicted Ca^{2+}-binding region near the COOH-terminus. The β spectrins vary in size from 246 to 430 kD, but all presently available sequences consist of an NH_2-terminal domain with actin- and protein 4.1–binding sites, 17 spectrin repeats, and variable COOH-termini. The actin-binding site is apparently located within an ~250-residue sequence that is highly conserved among many actin filament crosslinking and bundling proteins. The ankyrin-binding site is contained within a 15.3-kD domain corresponding to the 15th spectrin repeat. An ankyrin-independent membrane-binding site also is present in non-erythroid β spectrin, located perhaps near repeats 5 and 6.

Ankyrin also is encoded by a multi-gene family; two genes and alternatively spliced isoforms have been characterized. The ankyrins all contain an NH_2-terminal ~90-kD domain containing 22 to 24 copies of a 33–amino acid motif similar to multi-copy motifs found in many other proteins that form macromolecular complexes, including cell cycle and transcriptional regulators (*18*). This NH_2-terminal domain is followed by a ~62-kD spectrin-binding domain and a ~55-kD regulatory domain at the COOH-terminus. The NH_2-terminal domain of ankyrin contains distinct binding sites for band 3 and the Na^+,K^+-ATPase. In addition, the cytoplasmic domains of these two integral membrane proteins contain no regions of significant similarity, suggesting that at least two different mechanisms for ankyrin-membrane attachment exist. Similarly, the proteins encoded by the band 3 gene family of anion exchangers are more divergent (59 to 84% identity) in their ankyrin-binding cytoplasmic domains than in their transmembrane regions (80 to 98% identity). However, both nonerythroid anion exchangers do contain a sequence (VRRHL) similar to the motif (LRRRY) implicated in protein 4.1 binding.

Platelets. Like erythrocytes, unactivated platelets are anucleate blood components with a well-defined shape. The flattened disc of the resting platelet appears to be maintained by an actin-based membrane skeleton and an equatorial coil of microtubules (*19*). Although spectrin and associated proteins also are present, a major link between the cytoskeleton and the membrane of the unactivated platelet appears to be ABP-280 (filamin), a flexible, elongated, actin-binding protein that is a homodimer of 280-kD subunits. ABP-280 has an NH_2-terminal actin-binding domain, a backbone of 23 repeats of 96 residues, and a COOH-terminus that is critical for dimerization (*20*). Although the actin-binding domain in ABP-280 is similar to that found in spectrin, the ABP-280 repeats are structurally unrelated to the spectrin repeats, and ABP-280 forms a tail-to-tail dimer, rather than a spectrin-like head-to-tail dimer. A membrane attachment site is located near the COOH-terminus of ABP-280, suggesting that this protein may function as a direct link between actin filaments and the plasma membrane.

ABP-280 binds directly to a transmembrane glycoprotein complex known as GP Ib-IX (*21*). GP Ib-IX mediates platelet attachment to an extracellular glycoprotein

(von Willebrand factor) immobilized on damaged vascular walls. The GP Ib-IX complex consists of two disulfide-linked subunits, GP Ibα (145 kD) and GP Ibβ (24 kD); and a third, noncovalently bound subunit called GP IX (17 kD). Whereas efficient expression of the complex requires all three subunits, GP Ibα contains both the ligand-binding site for von Willebrand factor and the ABP-280–binding site, which is located in the center of the cytoplasmic domain (22).

The pathophysiology of Bernard Soulier syndrome, an inherited bleeding disorder, is consistent with a role for the GP Ib-IX interaction with ABP-280 in the determination of platelet shape and function. Bernard Soulier patients have a defect or deficiency in the GP Ib-IX complex and bleed easily with long clotting times. Their relatively few blood platelets are large, polymorphic cells that exhibit decreased adhesion to vascular tissue because of an inability to bind von Willebrand factor.

An essentially ubiquitous component of cortical cytoplasm, ABP-280 also may be involved in stabilizing the membrane skeletons of other cells. Melanoma cell lines lacking ABP-280 migrate poorly in response to external stimuli and exhibit blebbing of the plasma membrane around the circumference of the cell. Restoration of normal ABP-280 levels by transfection with ABP-280 cDNA restores cell locomotion and reduces blebbing (23). As GP Ib-IX is not present in these cells, other membrane proteins also may bind ABP-280. One candidate is the immunoglobulin G (IgG) Fc receptor. This protein binds directly to ABP-280 in vitro (24), but the nature of the binding interactions and the importance of this binding in vivo are not yet known.

Striated muscle. Membrane stability and flexibility also are important in the extremely large (10 to 100 μm wide, up to 50 cm long) multinucleated cells of striated muscle. Stress-induced damage to the striated muscle plasma membrane (the sarcolemma) and leakiness to external calcium appear to be early hallmarks of Duchenne and Becker muscular dystrophy (25). Another flexible, elongated membrane-associated protein, called dystrophin, has been identified as the X-chromosome gene product that is characteristically absent (Duchenne) or altered (Becker) in these diseases. One of the largest members of the spectrin superfamily (427 kD), dystrophin contains four distinct domains: (i) an actin-binding NH_2-terminus homologous to the actin-binding domains of spectrin and ABP-280, (ii) a central rod-like domain with 24 spectrin-like repeats of 109 amino acids and four proline-rich "hinges," (iii) a cysteine-rich segment similar to the Ca^{2+}-binding domain of the actin cross linking protein, α-actinin, and (iv) a unique COOH-terminal domain of 420 amino acids (26). By analogy to spectrin, the central rod-like domain is thought to promote dimerization of dystrophin. The COOH-terminal tail may be the site of membrane attachment. The importance of the two domains near the COOH- terminus is indicated by the very high sequence conservation (95% identity) of these domains between chicken and human dystrophin. By comparison, the actin-binding and rod-like domains are 80 and 75% identical, respectively.

Dystrophin is localized at the cytoplasmic surface of the sarcolemmal membrane and in the infoldings of myotendinous and neuromuscular junctions (specialized sites of attachment to tendons and neurons) where it appears to link the plasma membrane to the sides of actin filament bundles (27). Dystrophin also may be enriched along with muscle β-spectrin in a subsarcolemmal latticework that contains both periodic transverse elements and strands extending the length of the cell (28). This lattice may stabilize the plasma membrane during muscle contraction by inducing an ordered folding of the membrane as the myofiber length decreases. Stress-induced ruptures of membrane undoubtedly contribute to the decreased osmotic stability of muscle cells lacking dystrophin and to the increased influx of calcium ions observed in dystrophic muscle fibers (29).

In addition to skeletal muscle, dystrophin is expressed in heart and smooth muscle and at low levels in a number of non-muscle tissues, including kidney, lung, placenta, and brain. In brain, dystrophin is enriched at post-synaptic densities in neurons of the cerebral and cerebellar cortices (30). The major transcription product from the dystrophin gene in non-muscle tissues encodes a 70- to 75-kD protein that contains the cysteine-rich and COOH-terminal domains of muscle dystrophin (31). In this protein, six amino acids encoded by an alternatively spliced exon replace the actin-binding region and spectrin repeats of dystrophin. Although the function of this small dystrophin isoform is unknown, its existence may help explain the extramuscular symptoms, such as mental impairment, that sometimes accompany Duchenne muscular dystrophy.

An autosomal homolog of the dystrophin gene encodes a ~400-kD dystrophin-related protein (DMDL) that is found in varying amounts in most tissues (32). Although most of this gene is distinct from dystrophin, DMDL is predicted to consist of 490 amino acids that are 73% identical to the dystrophin COOH-terminus. DMDL is localized at neuromuscular junctions in both normal and dystrophin-negative muscle. Although DMDL does not localize to the subsarcolemmal latticework in normal muscle, weak staining is observed at non-junctional plasma membrane in dystrophic muscle, implying that DMDL may bind to unoccupied membrane attachment sites for dystrophin.

Dystrophin solubilized from sarcolemma with 1% digitonin is part of a large complex containing six other proteins (33) (Fig. 2). A peripheral laminin-binding glycoprotein (156 kD) apparently provides a linkage between the complex and the extracellular matrix (34). The core of the complex appears to be a group of integral membrane proteins (50, 43, 35, and 25 kD) that react with a hydrophobic photolabel, remain associated with membranes after alkaline extraction, and co-sediment in the absence of peripheral proteins; the 156-, 50-, 43-, and 35-kD proteins are glycosylated. The 43-kD glycoprotein is synthesized as part of the COOH-terminus of a precursor polypeptide (dystroglycan) that is post-translationally processed into both the 156- and 43-kD proteins. Analysis of the cDNA encoding dystroglycan predicts that the 156-kD glycoprotein is extracellular and consists of ~457 amino acids. The 43-kD glycoprotein is predicted to contain a single membrane-spanning region and a 121-residue cytoplasmic domain. An unglycosylated 59-kD protein that co-extracts with dystrophin at pH 11 may help anchor dystrophin at the cytoplasmic surface of the complex. Expression of all the major dystrophin-associated proteins is dramatically decreased in dystrophic muscle (35), suggesting that dystrophin is required for stability of the entire complex.

Analogs of the dystrophin-associated proteins may be present in many cells (36). After overexpression in COS cells, dystrophin predominantly localizes to the plasma membrane, even though these cells are not of muscle origin and normally contain very little dystrophin. Furthermore, the electric ray contains a dystrophin-associated 58-kD protein that is involved in acetylcholine receptor clustering at postsynaptic membranes in electric tissue (and which may be homologous to the 59-kD muscle protein). Thus, the dystrophin-associated proteins from muscle may constitute just the first example of a general molecular mechanism for anchoring dystrophin-like proteins at specialized membrane domains.

Euglenozoa. Not all membrane skeletons are based on actin filaments and elongate actin-binding proteins. Cell shape in two related, but quite different, flagellated eukaryotes, *Trypanosoma brucei* and *Euglena gracilis*, is maintained solely by membrane skeletons that are based on microtubules and articulins, respectively. In *T. brucei*, the plasma membrane is stabilized by a

corset of closely spaced microtubules encasing the entire cell. MARP-1 (microtubule-associated repetitive protein), a 320-kD protein that contains >50 tandem microtubule-binding repeats of 38 amino acids, is one of several proteins that may link the microtubules to each other or to the plasma membrane (37). Studies with antibodies against MARP-1 suggest that it abuts the plasma membrane, but the microtubule-membrane linkage is not known.

The membrane skeleton in *E. gracilis* is divided into ~40 strips that change their lateral positions relative to one another during cell movement (38). Although microtubules are present at strip intersections, the membrane skeleton consists mostly of equimolar amounts of two proteins (80 and 86 kD) called articulins. These proteins form 17-nm filaments that apparently assemble perpendicular to the membrane surface. Binding of the 86-kD articulin to the membrane is dependent on the presence of the 80-kD protein. Each articulin contains a core domain with >30 repeats of a 12–amino acid motif. Another region containing four repeats of seven hydrophobic amino acids is found at both the COOH-terminus of the 80-kD articulin and the NH_2-terminus of 86-kD articulin. Since the 80-kD articulin binds to a 39-kD integral membrane protein (IP39), IP39 may bind one end of filaments containing head-to-tail articulin heterodimers. Additional articulin-associated proteins apparently crosslink or otherwise stabilize the skeleton.

Although the membrane skeletons discussed above are all quite different, there are at least two recurrent themes. First, all contain proteins with repetitive structures that may function both as spacers and as elastic elements to provide flexibility. Second, there appears to be more diversity in the cytoplasmic domains of the integral membrane proteins than in the cytoskeletal proteins that they anchor. In cells containing more than one type of specialized membrane skeleton, the location of a particular membrane domain thus may be determined mostly by the locations of the integral proteins. On the other hand, the apparent modularity of the actin-based cytoskeletal components should facilitate interactions between these specialized areas of membrane and the rest of the cytoskeleton.

Adhesion and the Membrane Skeleton

There are four major families of receptors that mediate cell adhesion (39): (i) cadherins; (ii) members of the Ig superfamily, including N-CAM and ICAM-1; (iii) carbohydrate-binding proteins called selectins; and (iv) integrins. The first three families primarily mediate cell-cell interactions, whereas most integrins are involved in attachments to the substratum. Although ICAM-1 recently was shown to bind α-actinin in vitro (40), relatively little is known about the intracellular interactions of members of the immunoglobulin superfamily, and the selectins are thought not to be attached to the cytoskeleton. We will focus here on junctional assemblies in which the cadherins and the integrins are mixed and matched with components of the actin and intermediate filament networks to form four of the best understood junctions mediating cell-cell and cell-substrate attachments.

Cell-to-cell adherens junctions. The cadherin superfamily is a group of proteins that mediate Ca^{2+}-dependent homophilic (like-with-like) adhesion between cells (41). Differential expression of cadherins appears to be involved in cell sorting because cells transfected with a particular cadherin adhere preferentially to other cells expressing the same protein. The so-called "classical" cadherins include E- (epithelial, also called uvomorulin), N- (neural, also called A-CAM), and P- (placental) cadherin, but at least 12 different members of the family are known. The classical cadherins are glycoproteins with a common structure. Each has an extracellular domain containing four major repeats (which have 37 to 46% identity among classical cadherins), a single transmembrane domain, and a highly conserved cytoplasmic domain (56 to 80% identity). The extracellular self-recognition sequence, which differs among cadherins, is located in the NH_2-terminal 113–amino acid residues. The cytoplasmic domains of the classical cadherins mediate interactions with at least three cytosolic proteins, called α-, β-, and γ-catenin (102, 92, and 85 kD, respectively).

In the presence of millimolar extracellular Ca^{2+}, cells expressing both cadherins and catenins form belt-like sites of cell-cell attachment (called the *zonulae adherens* or adherens junctions), which contain cytoplasmic "undercoats" associated with actin filaments (41). Cadherin-mediated cell adhesion is not observed in cells that lack α-catenin (42) or in cells expressing cadherins lacking the cytoplasmic domain. The α-catenin is structurally similar to vinculin (30% identity), an adherens junction protein that does not bind cadherin. Both α-catenin and vinculin have five domains of which domains one, three, and five are similar. As the fifth domain of vinculin is involved in oligomerization, a similar function has tentatively been assigned to this region of α-catenin. Oligomerization of α-catenin may induce clustering of cadherins, which could foster junctional assembly. Of the three catenins, β-catenin binds the tightest to cadherin. The β-catenin from *Xenopus* is similar to the *Drosophila* protein encoded by the segment polarity gene, *armadillo* (70% identity), and to human plakoglobin (63% identity), a protein found at the cytoplasmic surfaces of both adherens junctions and desmosomes (43). Although plakoglobin and β-catenin are structurally distinct, plakoglobin appears to be the same as γ-catenin (44).

Although it is not known how cadherins and catenins are linked to the cytoskeleton, actin is a principal component of the adherens junction undercoat. Actin-associated proteins in the undercoat include α-actinin (a 90-kD protein that crosslinks actin filaments), zyxin (an 82-kD protein that binds the NH_2-terminus of α-actinin), vinculin (a 116-kD protein that binds the COOH-terminus of α-actinin), and radixin (an 82-kD protein that caps the barbed ends of actin filaments). Radixin is related to ezrin and moesin (~75% identity); all three proteins contain 234 amino acids near their NH_2-termini that are similar to a region near the NH_2-terminus of protein 4.1 (~30% identity) (45). However, the amino acid sequence (LEEDY) postulated to mediate protein 4.1 binding to band 3 is not found in radixin, ezrin, or moesin. Although radixin has been postulated to link barbed ends of actin filaments to the undercoat, interactions with proteins other than actin have not been characterized. Other proteins immunolocalized to the adherens junction undercoat include the rod-like protein tenuin (~400 kD) and a 220-kD globular protein that binds α-spectrin (46).

Junctional integrity appears to be regulated by protein tyrosine phosphorylation (47). Elevated levels of phosphotyrosine generated either by transformation with Rous sarcoma virus or by inhibition of phosphotyrosine phosphatases rapidly, but reversibly, induce junctional disassembly. Although the tyrosine kinases encoded by *c-src*, *c-yes*, and *lyn* have been localized at the adherens junction, the corresponding protein tyrosine phosphatases have not yet been identified.

Desmosomes. The desmosome (*macula adherentes*) is a spot-like site of intercellular contact that, like the adherens junction, mediates Ca^{2+}-sensitive cell-cell adhesion (48). Intercellular attachment is mediated by transmembrane proteins: desmoglein I (150 kD) and the closely related splice variants, desmocollins I (120 kD) and II (110 kD). Desmoglein I and the desmocollins are each members of a gene family within the cadherin superfamily. The desmoglein family also includes the gene for the 130-kD pemphigus vulgaris antigen (PVA), a protein recognized by autoimmune sera in the blistering disease, pemphigus vulgaris (49). Antibodies against des-

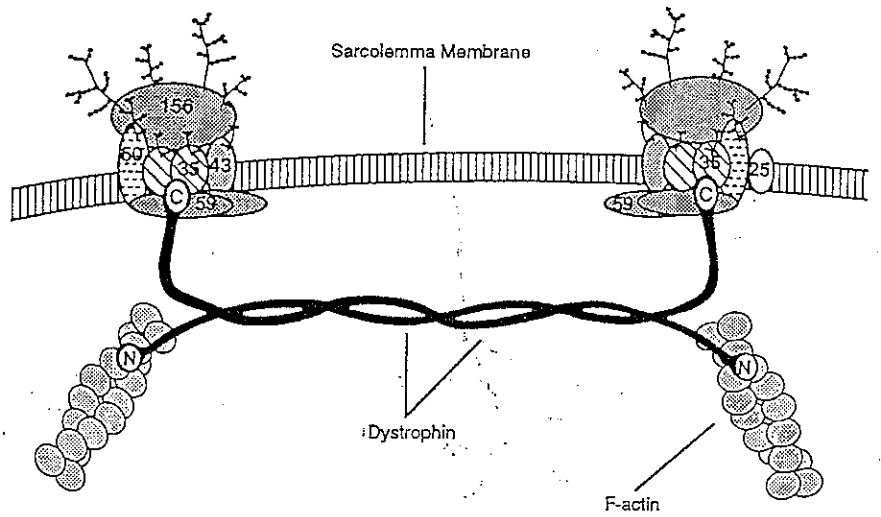

Fig. 2. Working model for the dystrophin-glycoprotein complex. Component stoichiometry is estimated as 1:2:1:1:2:~0.35 for dystrophin monomer:p59:gp50:gp43:gp35:p25, with an indeterminate amount of gp156. Protein interactions are speculative, based on the known locations of the proteins with respect to the bilayer. Diagram is modified from Ervasti and Campbell (*33*) with permission.

moglein I or PVA induce blistering of the skin by interfering with the adhesion of the cells that express these antigens at different positions within the epidermal layers. The desmogleins and desmocollins are Ca^{2+}-dependent cell-cell adhesion proteins with four external domains that are ~29 to 39% identical to the corresponding domains in the classical cadherins. However, their cytoplasmic domains possess unique characteristic features not found in the classical cadherins, undoubtedly because the desmosome is not associated with actin filaments, but is linked to intermediate filaments at a characteristic trilayered cytoplasmic structure, called the desmosomal plaque.

Components of the desmosomal plaque include plakoglobin (83 kD) and desmoplakins I (240 to 285 kD) and II (210 to 225 kD) (*50, 51*). Plakoglobin appears to bind directly to the cytoplasmic domains of desmoglein I and PVA through disulfide bonds. The desmoplakins, which are probably splice variants of a single message, are members of a gene family involved in intermediate filament organization. This family also includes the 230-kD bullous pemphigoid antigen of hemidesmosomes (BP230) and plectin, a cytoplasmic protein associated with intermediate filaments. Desmoplakin I appears to be a homodimer with an ~130-nm-long central rod-like domain separating globular domains on either end. The periodicity of acidic and basic residues within a repeating motif in the COOH-terminus of this protein suggests a possible site for interaction with intermediate filaments. Although direct binding of intermediate filaments to desmoplakin has not been observed, an overexpressed 90-kD COOH-terminal fragment of desmoplakin colocalizes with and eventually appears to disrupt the intermediate filament cytoskeleton (*52*), suggesting at least an indirect interaction. Other proteins present in at least some desmosomes include an extracellular 22-kD glycoprotein, a 140-kD plaque protein antigenically similar to the major intermediate filament-binding protein in the nuclear envelope (lamin B), and the plaque proteins, band 6 (77 kD), desmocalmin (240 kD), and desmoyokin (680 kD) (*53*).

Focal adhesions. Focal adhesions are regions of the plasma membrane that are so closely adherent to the substratum (separated by only 10 to 15 nm) that they appear as dark areas in interference reflection microscopy (*54*). Although the number of focal adhesions is inversely correlated with the rate of cell translocation, small areas at the leading edge of the cell may be required for traction during fibroblast locomotion (see below). The transmembrane adhesion proteins called integrins link extracellular matrix proteins on the substratum, such as fibronectin and vitronectin, to a dense cytoplasmic plaque associated with actin filament bundles (stress fibers).

Integrins are a family of transmembrane αβ heterodimers. The extracellular domains of the ≥14 α subunits (120 to 180 kD) and the ≥8 β subunits (90 to 110 kD) associate into at least 20 different heterodimers (*55*). Although any α should be able to pair with any β chain, not all combinations are co-expressed in vivo. Most integrins have cytoplasmic domains of 40 to 50 residues that can be alternatively spliced for even greater variability. Although several integrins localize at focal adhesions, most biochemical studies of integrin-cytoskeletal interactions have been based on the $\alpha_5\beta_1$ integrin (fibronectin receptor). This integrin binds directly to cytoskeletal proteins, apparently through the β_1 cytoplasmic domain (*55, 56*). Chimeric receptors containing the β_1 cytoplasmic domain fused to the extracellular and transmembrane domains of the human interleukin-2 receptor localize to focal adhesions, and deletion of all or part of this domain in αβ heterodimers abrogates localization. Sequences throughout the β_1 cytoplasmic domain appear to be involved. Although the α subunit also is important, its main role may be regulatory and related to its contribution to the ligand-binding site. Binding of extracellular ligand appears to induce a redistribution of the $\alpha_5\beta_1$ heterodimer from a diffuse membrane localization into clusters at focal adhesions, implying a ligand-induced conformational change that exposes one or more cytoskeleton-binding sites on the β_1 cytoplasmic domain (*57*). Conversely, disruption of integrin binding to the extracellular matrix results in the dissociation of cytoskeletal proteins from the focal adhesion. Changes in receptor conformation and affinity also can be induced by cytoplasmic events, indicating that focal adhesion structure is controlled from both sides of the plasma membrane (*55*).

The interactions among the proteins at focal adhesions appear to be very complex (*54, 58*). Figure 3 illustrates the major protein-protein interactions observed in vitro. The cytoplasmic domain of the integrin β_1-subunit binds talin (215 to 235 kD) and α-actinin (90 to 100 kD). Talin also binds to vinculin, apparently binds and nucleates actin assembly, and self-associates at high protein concentrations (*59, 60*). Vinculin (116 kD) binds paxillin (68 kD), the COOH-terminus of α-actinin, and may oligomerize with itself (*61*). Zyxin (82 kD) and actin both bind the NH_2-terminus of α-actinin (62). Tensin (150 and 200 kD) may bind vinculin, binds and caps the barbed ends of actin filaments, and contains an *src* homology 2 (SH2) domain at the COOH-terminus that might mediate binding to phosphotyrosine-containing peptides (*63*). At least one member of the gene family that includes radixin (a protein that caps the barbed ends of actin filaments), ezrin, and moesin also appears to be at focal adhesions (*45*). Other proteins localized to focal adhesions include tenuin (~400 kD) and VASP (vasodilator-stimulated phosphoprotein, 46 to 50 kD), a protein that binds actin in co-sedimentation assays (*64*). Finally, α-actinin, vinculin, and talin have been reported to bind directly to acidic phospholipids (*10, 65*).

The physiological functions of the focal adhesion proteins are being tested by experimental alterations of the amounts of indi-

vidual proteins in living cells (66, 67). Although neither genetic deletion of α-actinin nor microinjection of antibody against this protein results in an obvious phenotype in non-muscle cells, stress fibers and focal adhesions are disrupted in cells containing microinjected proteolytic fragments of α-actinin and in cells expressing truncated α-actinin that lacks ~10 kD of sequence from the COOH-terminus. Focal adhesions and stress fibers also are disassembled by microinjection of antibodies against either talin or vinculin. Overexpression of vinculin drastically reduces the movement of individual fibroblasts and suppresses the tumorigenicity of transformed cells, probably by increasing the size or stability of focal adhesions or adherens junctions. These results are consistent with the general observation that cell locomotion is inversely related to the number and extent of focal adhesions. On the other hand, fibroblasts microinjected with antibodies against talin exhibit both decreased motility and decreased numbers of focal adhesions, a result consistent with a requirement for talin at the leading edges of moving cells.

Changes in protein phosphorylation apparently regulate the structure and function of focal adhesions in vivo (68). The α isoform of protein kinase C, a 125-kD focal adhesion kinase ($pp125^{FAK}$), $pp60^{src}$, and other protein kinases all appear to be concentrated here. Tyrosine phosphorylation of paxillin and $pp125^{FAK}$ accompanies both v-src–mediated cell transformation and adhesion to extracellular matrix. Phosphorylation of $pp125^{FAK}$, in turn, up-regulates the kinase activity of this protein. Other phosphorylated substrates include β-integrin, talin, and vinculin. Herbimycin A, an inhibitor of cellular protein tyrosine kinases, also inhibits the formation of focal adhesions, suggesting that integrin-mediated tyrosine phosphorylation may be required for establishment of these structures.

Hemidesmosomes. Hemidesmosomes are relatively small domains (~0.4 μm in diameter) of the basal plasma membrane in epithelial and mesenchymal cells that mediate attachment to the underlying basal lamina (48). Ultrastructurally, they look like half of a desmosome and contain a trilayered cytoplasmic plaque associated with intermediate filaments. Biochemically, however, hemidesmosomes and desmosomes are distinct. Attachment to the basement membrane may be through fine strands (anchoring filaments) that contain three nonidentical, disulfide-linked proteins of ~165, ~155, and ~140 kD (in a complex called kalinin or epiligrin) (69). Antibodies against this complex cause cells to round up and detach from the basement membrane.

One of two transmembrane proteins known to localize at hemidesmosomes is the $\alpha_6\beta_4$ integrin (70). Antibodies against the extracellular surface of this integrin, but not antibodies against the β_1-subunit, block assembly of hemidesmosomes, suggesting that the $\alpha_6\beta_4$ integrin is involved in hemidesmosomal attachment. As expected for a molecule that directly or indirectly associates with intermediate filaments rather than with the actin-based cytoskeleton, the β_4 subunit is quite different from the β_1- and β_3-chains found at focal adhesions. The large cytoplasmic domain (>1100 residues) of the β_4 subunit could easily extend through the 12- to 15-nm-thick hemidesmosomal plaque into the underlying region of intermediate filaments.

Other components of the hemidesmosome have been identified as autoantigens in patients with bullous pemphigoid, a severe blistering disease (51, 71). A 180-kD transmembrane protein (BP180) with a ~55-kD NH_2-terminal cytoplasmic domain and an external COOH-terminus with collagen-like domains is one of these proteins. Another is a cytoplasmic 230-kD protein called BP230. The NH_2- and COOH-termini of BP230 are structurally similar to the corresponding regions of desmoplakin (identities of 35 and 38%, respectively), and BP230 also is predicted to contain a central rod-like domain. Thus, BP230 in the hemidesmosome and desmoplakin in the desmosome may have similar functions in their different intermediate filament-associated plaques. Finally, a 200-kD plaque component also has been identified immunologically.

Of the four membrane skeletons considered here, two (adherens junctions and desmosomes) are involved in cell-cell adhesion and two (focal adhesions and hemidesmosomes) mediate cell attachment to the substratum. The adherens junctions and focal adhesions are associated with the actin-based cytoskeleton, whereas intermediate filaments bind to the cytoplasmic plaques associated with desmosomes and hemidesmosomes. Comparison of these adhesive structures suggests that the extracellular domains of the integral membrane proteins mediating a particular type of adhesion (cadherins in cell-cell junctions and integrins at sites of cell-substrate attachment) are similar. Many of the cytoplasmic proteins at different junctions involving the same cytoskeletal system also are similar or identical. For example, α-catenin in adherens junctions and vinculin at focal adhesions contain some structural similarities, as do desmoplakin and BP230. Furthermore, α-actinin, tenuin, vinculin, and zyxin are found in the actin-associated undercoats of both adherens junctions and focal adhesions. However, as was observed above for the membrane skeletons involved primarily in cell integrity, the cytoplasmic domains of the transmembrane proteins anchoring these membrane skeletons appear to be quite different. No sequence similarity is apparent between the cytoplasmic domains of the classical cadherins and $\alpha_5\beta_1$ integrin, both of which are required for the formation of actin-based adherent structures, or

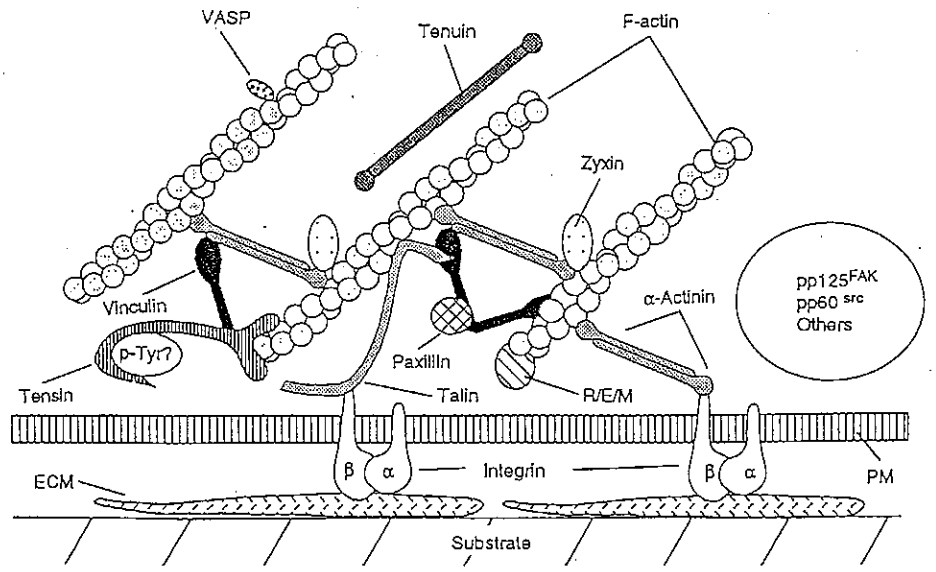

Fig. 3. Working model of the protein-protein interactions in focal adhesions determined by in vitro–binding experiments and immunolocalization. Most associations have not yet been verified by in vivo studies. In addition, several interactions are of relatively low affinity in solution, but may be enhanced due to the reduction in dimensionality at the membrane surface (*100*). Abbreviations are: ECM, extracellular matrix; PM, plasma membrane; p-Tyr-?, unknown phosphotyrosine-containing protein; R/E/M, member of the radixin/ezrin/moesin family; VASP, vasodilator-stimulated phosphoprotein. Diagram is modified from Simon *et al.* (*58*) with permission.

between the cytoplasmic domains of $\alpha_6\beta_4$ integrin and the desmosomal cadherins, which both anchor plaques containing intermediate filaments. Thus, it appears that membrane-cytoskeleton associations developed independently many times during evolution.

Movement and Cell Surface Protrusions

Cell surface protrusions at the leading edge appear to be involved in the movement of most cells (72). The names of these structures vary with the morphology of the protrusion: filopodia are thin and usually long, lamellipodia are broad, and pseudopodia are of moderate width with respect to the dimensions of the cell. Constantly forming, these protrusions either lift and fold back into the cell or spread and extend after contacting the substratum or another cell. While the mechanisms by which these protrusions are generated are not well understood (73), both membrane-cytoskeleton interactions and actin accessory proteins appear to be involved (Table 1). This part of the cell is hard to isolate because interactions at the leading edge tend to be transient. Thus, most of the proteins in Table 1 were first identified by their interactions with actin, the principal component of the protrusions, and then immunolocalized to this region of the cell.

Proteins apparently enriched at the leading edge include particular isoforms of actin and proteins that nucleate actin filament assembly, such as hisactophilin, ponticulin, and talin (60, 74). Hisactophilin is a histidine-rich peripheral protein that nucleates actin polymerization in a low salt solution; interactions at the membrane have not been characterized yet. Ponticulin is a transmembrane protein required for the binding and nucleation of actin filaments by isolated *Dictyostelium* plasma membranes. Unlike most actin-nucleating proteins that cap one end of the filament, the cytoplasmic domain of ponticulin promotes nucleation of actin filaments with both ends free for actin monomer addition or for interactions with other proteins. Thus, ponticulin apparently constitutes a direct actin-membrane link that could be involved in generating actin filaments in pseudopodial meshworks (75, 76). High concentrations of talin also have been reported to promote actin assembly. Because talin binds integrin and other focal adhesion proteins, actin nucleation mediated by talin or an associated protein could be an important step in the assembly of the actin filament bundles involved in cell-substrate attachments. Profilin, which also is concentrated near the leading edge, may accelerate filament elongation by promoting the formation of assembly-competent ATP-actin monomers and may even promote addition of these monomers to the fast-growing barbed ends of elongating filaments (77).

Rapidly polymerizing actin becomes assembled either into bundles of filaments in filopodia or into crosslinked meshworks in pseudopodia and lamellipodia. Filament organization at the leading edge is controlled by actin accessory proteins (5, 78), which include filament crosslinking proteins [ABP-280, spectrin, ABP-120, α-actinin, and MARCKS (a myristoylated alanine-rich C kinase substrate)], filament bundling proteins (caldesmon, fimbrin, p30a, and villin), calcium-dependent filament severing proteins (villin, gelsolin, and fragmin/severin), and proteins that stabilize filaments (caldesmon and tropomyosin). Some of these proteins also may help link actin to the membrane. For instance, ABP-280, spectrin, and α-actinin bind integral membrane proteins, and dephosphorylated MARCKS binds to an unknown membrane site (79). Although only the genetic deletion of ABP-280 has been shown to dramatically decrease cortical stability and cell

Table 1. Proteins at the leading edge.

Name	Size (kD)	Distribution
β- and γ-actin	43	Filopodia, pseudopodia, and lamellipodia in retinal pericytes and endothelial cells
Proteins controlling Actin Nucleation and Polymerization		
Hisactophilin	17	Pseudopodia in *Dictyostelium*
Ponticulin	17	Nascent pseudopodia in *Dictyostelium*; cell extensions in neutrophils
Talin	215	Platelets, smooth muscle, many non-neuronal tissues
Profilin	12–15	Isoforms in most cells; fibroblast lamellipodia
Actin Filament Accessory Proteins		
ABP-280 (filamin)	250	Isoforms in most cells; leading edges of chick gizzard and skin fibroblasts
Spectrin	240/235	Isoforms in most cells; ruffling membranes in EGF-stimulated A431 cells
ABP-120	120	Pseudopodia and lamellipodia in *Dictyostelium*
α-actinin	90–100	Most cells, including lower eukaryotes
MARCKS	68–87	Punctate localization at tips of filopodia and pseudopodia in macrophages
Caldesmon	70–80	Essentially all non-muscle cells; larger isoforms in smooth muscle
Fimbrin (plastin)	68	Microvilli and ruffling membranes in essentially all non-muscle cells
p30a	30	Filopodia and membrane ridges in *Dictyostelium*; ruffles and stress fibers in fibroblasts
Villin	95	Microvilli in brush borders of intestine, kidney pancreatic acinar cells and visceral endoderm
Gelsolin	90	Mammalian cells
Fragmin/severin	42	*Physarum, Dictyostelium*
Tropomyosin	30–40	Most cells; at least some isoforms in peripheral ruffles of rat kidney cells
Motor Proteins		
Myosin I	110–140	Called 110K-calmodulin in brush border microvilli; other isoforms at neuronal growth cones and pseudopods of *Acanthamoeba* and *Dictyostelium*
Myosin II	~200	Established protrusions in fibroblasts; neuronal growth cones
Other Actin-Binding Proteins		
p58	58	Peripheral cytoplasmic protein associated with a large glycoprotein complex in microvilli
Coronin	55	Cell surface projections in *Dictyostelium*
MAb 2E4 antigen	43	Platelets; fibroblasts lamellipodia; growth cones in PC12 cells
Other Potential Regulatory Proteins		
Ezrin	80	Microvilli and surface structures in many cells; marginal band of avian erythrocytes
GAP-43	43–57	Neuronal growth cones and processes

locomotion, functional overlap among the membrane skeletal proteins may obscure individual roles in the membrane skeleton (*23*, *66*). Alternatively, the actin filament–crosslinking activities of these proteins may be their primary contribution to cortical stability at the leading edge.

Isoforms of single-headed myosin I and the myosin I kinase that activates them are also enriched at membrane protrusions (*80*). These myosins contain an ATP-independent, as well as an ATP-dependent, binding site for actin and can slide filaments relative to each other in the presence of ATP. These proteins also bind to acidic phospholipids (and probably to uncharacterized membrane proteins) and move membrane vesicles toward the barbed ends of actin filaments. As these are the filament ends in actin bundles that are generally associated with filopodial tips, myosin I might mediate the movement of freely-diffusing membrane receptors toward the leading edge (*81*). Similarly, myosin I anchored to the substratum through transmembrane linkages could pull actin filaments rearward. Both types of movements have been reported (*7*, *82*). By contrast, conventional dimeric myosin II appears only in established protrusions and is apparently not essential for pseudopod expansion (*83*). Myosin II, however, is required for optimal cell movement and is probably responsible for cortical contractions that pull the rear of the cell forward.

Several other proteins that bind actin (p58, coronin, and the MAb 2E4 antigen) or become phosphorylated on tyrosine (ezrin) or serine (GAP-43) also may contribute to the structure and function of the leading edge (*5*, *84*). GAP-43, a protein enriched in nerve growth cones, has been shown to activate trimeric G proteins and to induce the formation of long, thin processes after transfection into non-neuronal cells (*85*).

Membrane-associated actin assembly has often been proposed as a driving force behind pseudopod extension because actin filaments appear to assemble at the membrane of the leading edge (*73*, *86*). Alternatively, actin polymerization at the membrane may stabilize protrusions generated by osmotic forces (*87*). Myosin I attached to large "rafts" of membrane components (*88*) also could propel membrane into the tips of extending protrusions. A better understanding of the membrane skeleton at the leading edge may help us to distinguish among these possibilities.

Regulation of Membrane Skeleton Assembly

The mechanisms by which the cytoskeleton is assembled at the cell surface are at least as varied as the number of different membrane skeletons. Regulated gene expression, protein synthesis, protein degradation, and post-translational modifications all are important (*89*). More recently, changes in compartmentalization of regulatory proteins have become increasingly appreciated (*90*). Lateral segregation at the cell surface appears to be involved in processes as disparate as clustering of acetylcholine receptors at synapses, adhesion-dependent cell signaling, and desensitization of a chemotactic receptor. Recruitment of components from a compartment near the Golgi apparently functions in assembly of desmosomes and in spectrin rearrangements accompanying cell stimulation and development (*91*, *92*). Regulatory enzymes such as protein kinase C and the tyrosine kinase, $pp60^{c\text{-}src}$, also may be found in similarly located internal stores (*92*, *93*).

Modular "src homology domains" known as SH2 and SH3 appear to be responsible for at least some of the intracellular shuffling (*94*). For instance, the SH2 domain present in tensin may allow this focal adhesion protein to bind phosphotyrosine-containing proteins as well as actin filaments. The SH3 domains found in spectrin and myosin I may represent potential binding sites for GTPase-activating proteins (GAPs) (*95*). Although highly speculative, this possibility is exciting because GAPs regulate the activities of small GTP-binding proteins–such as Ras, Rac, and Rho—that stimulate actin reorganization in vivo (*96*). Thus, recruitment of spectrin or myosin I to the site of Ras-mediated actin assembly at the leading edge could affect the local rate of actin polymerization as well as other aspects of cortical structure. Agonist-regulated capping proteins on actin filaments are other possible downstream targets in this signaling pathway (*97*).

Actin polymerization at the membrane also may be mediated by phosphoinositides or their metabolites (*98*). For example, PIP_2 can potentiate both the release of assembly-competent actin monomer from a complex with profilin and the uncovering of barbed filament ends blocked by gelsolin or other filament-capping proteins. The formation of new actin nucleation sites at the membrane is also triggered by diacylglycerol, a second messenger produced by hydrolysis of PIP_2 and other phospholipids. Interplay between Ras- and profilin-mediated actin assembly is suggested by the observation that overexpression of profilin largely rescues the morphological defects associated with the loss of a Ras-mediated signal pathway—a pathway that also is regulated by diacylglycerol and phospholipids (*99*).

Conclusion

The current challenge in the diverse field of membrane-cytoskeleton interactions is to integrate the emerging information on the numerous proteins and their localizations with studies of protein function in vivo. Although these analyses are complicated by the multiplicity of isoforms and overlapping functions of many membrane skeleton components, such studies are requisite for understanding the large number of fundamental cell processes controlled at the membrane-cytoskeleton interface.

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1. Citations are not comprehensive, but are intended only to provide an entry into the literature. Single-letter abbreviations for amino acids are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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101. We thank A. Werme for the graphics in Figs. 1 and 3. We also thank B. Bouck, K. Burridge, C. Cohen, K. Campbell, D. Fishkind, V. Fowler, J. Fox, K. Green, J. C. R. Jones, K. Knudsen, Y.-L. Wang, and M. Wheelock for their comments on the manuscript and all the other members of the membrane-cytoskeleton community who helped with preprints and advice. Work cited from this laboratory was supported by the National Institutes of Health.

Selectins: Interpreters of Cell-Specific Carbohydrate Information During Inflammation

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Although a bewildering array of cell surface carbohydrate structures have been described, the physiological relevance of any of these complex molecules has often eluded biologists. A family of cell surface glycoproteins, the "selectins," has a characteristic ability to use some of these carbohydrate structures in adhesive mechanisms that help localize leukocytes to regions of inflammation. This article will review the biology of these carbohydrate-binding adhesive proteins and discuss the potential for developing anti-inflammatory antagonists that could inhibit binding events that are selectin-mediated.

Leukocytes are the major purveyors of host defense during a pathogenic onslaught. Neutrophils provide for rapid, relatively nonspecific defense mechanisms, after which a more long-lived, antigen-specific response is determined by macrophage-monocytes and B and T lymphocytes. These circulating cells are constantly scrutinizing the organism for potentially threatening situations, and it is this surveillance that prevents a rapid and wholesale destruction of the individual by the plethora of pathogens that are routinely encountered. Unfortunately, this highly regulated system can show an overzealousness that results in an attack on the host itself. The resultant tissue damage can range from mild psoriasis to multi-organ failure, asthma, or arthritis. An understanding of the various processes that mediate normal inflammatory responses may lead to novel drugs that could be effective in the treatment of diseases induced by abnormal inflammation.

One of the most important aspects of the inflammatory process involves cell adhesion events (*1–5*). Leukocytes must combine a high degree of vascular mobility with the ability to specifically adhere in a temporally relevant manner to endothelial sites that are adjacent to tissues destined to be invaded by the appropriate type of inflammatory cell. This type of endothelial adherence and tissue extravasation is encountered on a routine basis in the lymph nodes, where lymphocytes of diverse antigenic specificities constantly pass through so that they may encounter sequestered antigens appropriately presented by resident antigen-presenting cells (*6*). This category of lymphocyte–endothelial cell adherence is part of a chronic, normal inflammatory event that is critical for the rapid analysis of potentially deleterious antigens that have been encountered in the periphery.

The importance of the adhesive interactions between neutrophils and the vascular endothelium is underscored by a rare syndrome termed the leukocyte adhesion deficiency, or LAD, syndrome (*7*). Individuals suffering from this genetic disease have an abnormal degree of life-threatening bacterial infections because their leukocytes cannot adhere properly to endothelial cells. This lack of adhesion to the endothelium therefore results in a deficiency in the ability of neutrophils to extravasate to tissues that are threatened by bacterial invasion. Lymphocyte recognition of the vasculature and neutrophil extravasation toward infected tissues are two examples of the critical involvement of cell adhesive events in the immune-surveillance of leukocytes. Adhesive mechanisms similar to those utilized during these normal inflammatory responses are probably used during pathogenic inflammatory episodes as well.

Although lectin, or carbohydrate recognition, domains of proteins have been shown to be involved in a number of biological events (*8*), the relationship between carbohydrate-mediated cell adhesion and leukocyte inflammation was not always clear. The discovery of the selectins, a family of three cell surface glycoproteins that contain lectin domains that mediate regional inflammatory responses by recognition of cell-specific carbohydrates, has unified these divergent fields and has given rise to some observations that may ultimately prove to be clinically significant.

Leukocyte Trafficking

Early work by Gowans and colleagues (*6*) gave the initial indications that tissue-specific adhesive interactions could influence regional leukocyte trafficking. These investigators showed that lymphocyte populations that were derived from regional lymphoid sites, when reinjected into an animal, tended to migrate, or "home," back to the sites from which they were initially derived. One interpretation of these data was that tissue-specific adhesive interactions between lymphocytes and the endothelium directed these cells to different lymphoid sites (*9*). These findings were expanded upon when an in vitro system derived from frozen tissue sections was produced that enabled an examination of the adhesive interactions between lymphocytes and the endothelium in various lymphoid sites (*10*).

These in vitro assays supported previous in vivo data on tissue-specific mechanisms of lymphocyte homing and allowed for the production of monoclonal antibodies (MAbs) to lymphocyte surface antigens that specifically blocked these adhesive interactions. One such MAb, termed the Mel 14 antibody, blocked the binding of lymphocytes to post-capillary high endothelial venules (HEVs) of the peripheral lymph node (PLN) and recognized a ~90- to 100-kD antigen that was confined to leukocyte cell surfaces (*11*). This MAb was inefficient at inhibiting adhesion of lymphocytes to another lymphoid tissue, the Peyer's patches, which suggested a tissue-specific distribution of the ligands recognized by the Mel 14 antigen. These data were consistent with the ~90- to 100-kD leukocyte surface antigen being a cell adhesion molecule that interacted with a ligand on the HEVs and directed lymphocytes to migrate primarily to PLNs. Because of its apparent involvement in the homing of lymphocytes to PLNs, the antigen recognized by Mel 14 was termed the "homing receptor," although subsequent publications used a diversity of other names.

A similar frozen section binding assay was

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