Microfilament–membrane interaction

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One of the major topics of research in modern cell biology involves the structure and organization of the cytoskeleton and, in particular, the interaction of its various components with the plasma membrane. Studies carried out in many laboratories over the last several years pointed to the complex and heterogeneous nature of membrane–cytoskeleton interactions in different systems. This is manifested by a remarkable cell-type specificity as well as distinct differences between the mode of anchorage of the various cytoskeletal components. Moreover the assembly and maintenance of each of the cytoskeletal systems within individual cells appears to be a dynamic process which is probably spatially and temporally regulated and modulated.

Here, examples are presented to illustrate some of the common features of the interactions which occur at the membrane–microfilament interphase, and their physiological significance is discussed (for a recent review see Ref. 1). One of the most important consequences of membrane–microfilament interaction is the local perturbation of the membrane and the outcoming alterations in its biochemical and biophysical properties. The generation of molecular microdomains in biological membranes by such mechanisms has central roles in cellular physiology, as discussed below.

Microdomains in membranes: the involvement of the cytoskeleton

The widely accepted fluid mosaic model depicts biological membranes as two-dimensional lipid bilayers in which 'integral proteins' are embedded[2]. It was proposed that these membrane proteins are free to laterally diffuse through the fluid phase and thus reach a uniform distribution. It was, nevertheless, pointed out in the original formulation of the model that the lateral mobility of the integral membrane constituents may be restricted by interactions with immobile matrices at the cytoplasmic or extracellular interphases of the plasma membrane. Such interactions may be mediated by 'peripheral membrane proteins', namely proteins which are tightly bound to either surface of the membrane without being actually inserted into its bilayer. Furthermore, it was conceivable that if the peripheral elements form mechanically stable networks, their attachments to the membrane may have long-range effects on the dynamics and topology of the latter. Many studies, both biophysical and structural, have suggested that membrane constituents may sometimes display non-random distribution, forming specialized domains with distinct topology, structure and functions. Many such domains have been identified, for example, the synaptic clusters of acetylcholine receptors, receptors to low-density lipoprotein, LDL (or to other ligands) which are pre-clustered in coated pits, surface glycoproteins in budding enveloped viruses, and specific cellular junctions. Moreover, morphological specializations and irregularities of the free surface could often be seen by transmission or scanning electronmicroscopy[3].

In principle, there are several possible mechanisms for microdomain formation, some of which are outlined in Fig. 1.

The starting state is the cell depicted on the left of Fig. 1 with different types of homogeneously distributed surface molecules (the squares and circles). This cell might undergo various changes which could lead to the segregation of the 'squares' and 'circles' into specific, specialized and mutually exclusive microdomains (see caption to Fig. 1). The mechanisms shown in Figs 1c and d are of direct interest to the topic of this article; they may involve either a unilateral association of the membrane receptors with intracellular matrices, namely the cytoskeleton (c) or a transmembrane interaction with insoluble elements at the two faces of the plasmalemma (d). Examples of the former might be the association of band-3 protein with the cytoskeletal shell in erythrocytes which is mediated by ankyrin, the attachment of microfilaments to the membrane in epithelial microvilli (see below), etc. Transmembrane interactions which involve cytoskeletal elements are commonly found in adherens and desmosomal junctions, in surface patches and caps induced by multivalent ligands, etc. (see Ref. 1 and discussion below).

Surveys of a large variety of biological systems suggested that the induction of surface specialization due to the interaction between the cytoskeleton and the inner surfaces of the plasma membranes can play multiple roles of great physiological significance in processes such as cell motility, attachment, division and recognition.

Microfilaments are associated with the plasma membrane via specific linker proteins

Microfilaments constitute one of the major cytoskeletal systems in living cells[4]

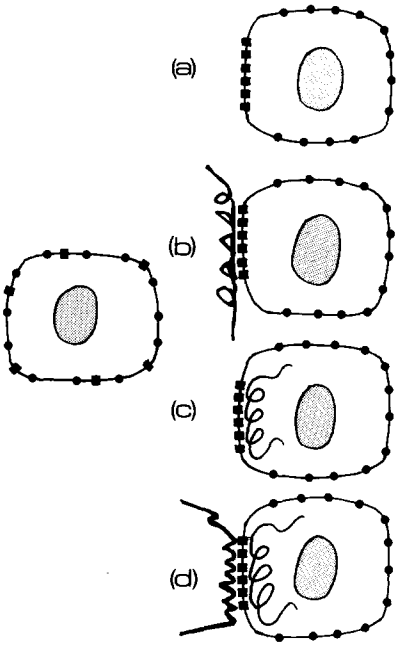

Fig. 1. Four different mechanisms for the generation of non-homogenous distribution of integral membrane proteins. The cell on the left has its 'square' and 'circular' receptors homogenously distributed. (a) Formation of a cluster of the 'square' receptors by intramembranal interactions. This aggregation may be triggered by interaction with another membrane protein, modification of the 'squares', changes in medium conditions (pH, ionic environment), etc. (b) Exofacial interactions with extracellular matrices leading to immmobilization and aggregation of a particular class of receptors. (c) Endofacial interactions, namely microdomain formation induced by an interaction of the 'square' integral proteins with cytoskeletal filaments. (d) Transmembrane interactions involving an integral membrane element attached to both extracellular and intracellular matrices.

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and are present predominantly in the cortical cytoplasm, subjacent to the plasmalemma. Many biochemical and ultrastructural studies indicated that microfilaments are not only enriched near the plasma membrane but are often actually attached to its endofacial surfaces (see Ref. 1). The cell types and subcellular assemblies in which membrane–microfilament interactions occur are however quite diverse in their origins and molecular properties. They include sites such as microvilli of polarized epithelia, lamellipodia and ruffling membranes, stereocilia of the sensory epithelium in the cochlea, contractile ring in dividing cells, end-on and lateral connections between myofibrils and the sarcolemma, dense plaques of smooth muscle, and intercellular junctions of the adherens type in a large variety of cells. In these locations, membrane–microfilament interaction probably affects such cardinal processes as cell morphogenesis, sensation, mobility, division, force generation, intercellular adhesion and cell communication. The involvement of membrane–microfilament interaction in such an important battery of cellular activities has motivated a general search for ubiquitous linkers of actin to the membrane.

However, as one might have anticipated on the basis of the apparent structural variability, direct biochemical and immunochemical data revealed considerable heterogeneity in the molecular mechanisms of membrane anchorage in the different systems. One of the common features, however, was the presence of specific linker proteins capable of bridging between F-actin and the membrane[5]. The necessity of accessory proteins for membrane anchorage has raised some controversy since the possibility has been considered over the years that actin filaments may be directly inserted into the lipid domain of the plasmalemma. This suggestion was supported by occasional reports on the presence of 'cell surface actin' or on the effects of anti-actin antibodies on the physiology of living cells. This view, however, is usually regarded as unlikely or, at least, physiologically irrelevant; spontaneous penetration of a hydrophilic protein such as actin into the core of the membrane seems to be extremely unfavorable thermodynamically and the incidental immunocytochemical detection of actin on the outer cell surface could be attributed, in most cases, to an artefactual binding of actin released from dead cells.

As pointed out, a more widely accepted mechanism for the anchorage of actin may involve peripheral association with the cytoplasmic faces of the membrane, mediated by specific integral receptor(s) and peripheral linking-protein(s).

An excellent example which may help to illustrate this mode of anchorage is the linkage between F-actin and the membrane in intestinal microvilli. The advantage of this system which attracted the attention of Mooseker and Tilney over a decade ago[6], lies in its relatively regular structure and the presence of only a few cytoskeletal components[7]. Electronmicroscope analyses of intestinal epithelium indicated that a bundle of

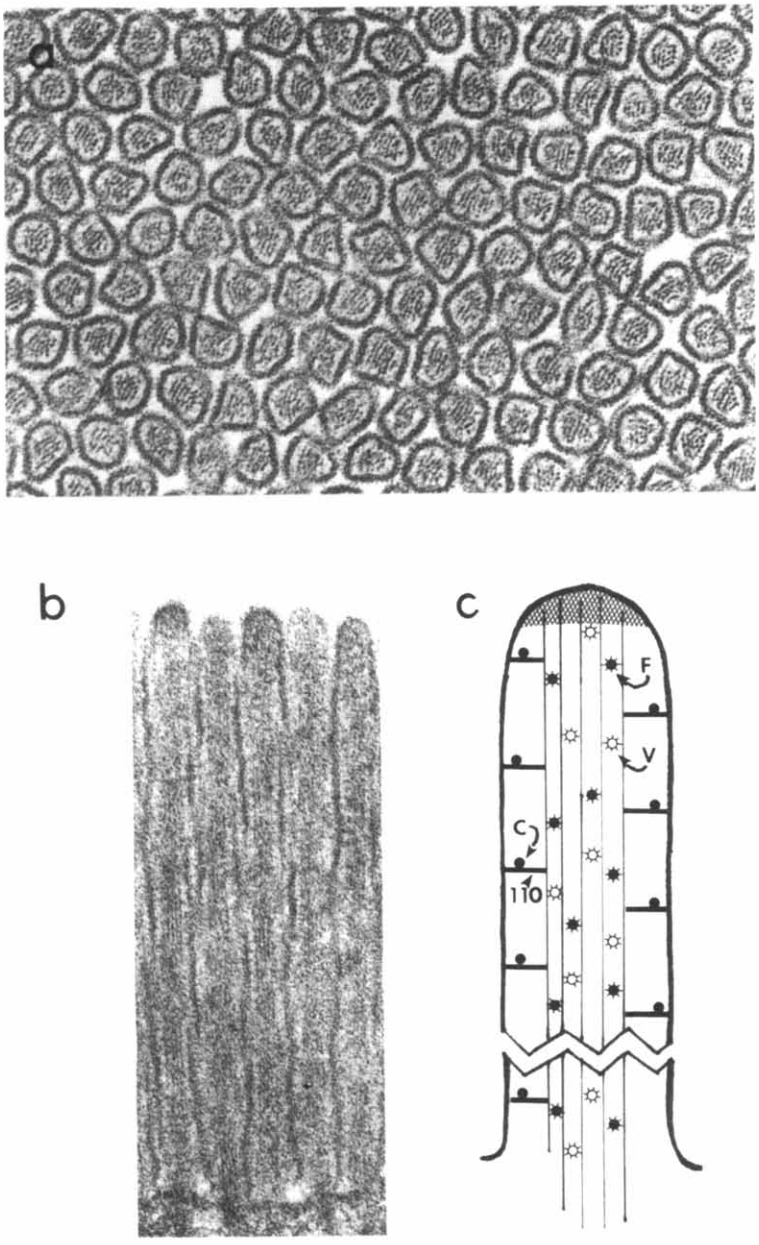

Fig. 2. The structure and molecular organization of intestinal microvilli. (a) and (b): Transmission electronmicrographs of cross-sections (a) and longitudinal sections (b) through chicken intestinal microvilli. (c) Scheme depicting the locations of the different cytoskeletal proteins of microvilli: F, fimbrin; V, villin; 110 kDa protein; C, calmodulin. The nature of the electron-dense material of the tip is not known.

microfilaments runs from the tip down to the terminal web along the core of each microvillus (Figs 2a and b). This bundle is associated with the membrane in two distinct modes; at the tip, microfilaments are apparently attached end-on to the membrane through an electron-dense plaque, while along the microvilli periodic cross-bridges extend from the core bundle to the lateral membrane. Biochemical analyses of isolated microvilli revealed that beside actin there are only four major cytoskeletal (detergent insoluble) proteins: villin (95 kDa), fimbrin (68 kDa), a 110 kDa protein and calmodulin. Selective extraction combined with immuno-electronmicroscope studies indicated that the lateral bridges connecting actin to the membrane consist of a complex between the 110 kDa protein and calmodulin. Villin and fimbrin, on the other hand, were localized within the core filament bundle where they are probably involved in the bundling of actin filaments or in their structural modulation (Fig. 2c). The exact mode of interaction of the 110 kDa protein with the membrane is not entirely clear yet; one view is that it associates with a specific integral membrane protein with an apparent molecular mass of 140 kDa (Ref. 8). An alternative possibility proposed recently is that the 110 kDa protein itself is an amphipathic molecule which is embedded in the lipid domain of the membrane and associated with the core actin filaments through a cytoplasmic extention (Ref. 9 and see below).

The molecular identity of the membrane linking proteins in intestinal microvilli seems to be quite unique to that cellular system but the principle also seems to hold for other cell types. One fine example is that of mammalian erythrocytes in which the cytoskeletal submembrane network is composed predominantly of spectrin filaments which are cross-linked by actin oligomers and several associated proteins. This dense web of filaments is attached to the cytoplasmic extension of integral membrane proteins (mainly band-3 protein) via the bifunctional membrane-linking protein, ankyrin. Other systems of membrane microfilament contact, such as junctional specializations, exhibit a similar general mechanism (see below) although the components involved are again quite different and their molecular topology is less clear (see below).

Interaction of microfilaments with membranes such as in intestinal microvilli belong to the third category of membrane microdomains formation depicted in Fig. 1. Actin, in this case, is attached to the cytoplasmic faces of the plasmalemma without any indication for corresponding local specialization at the exofacial cell surfaces. In these systems one may assume that the exact topology and timing of membrane–microfilament contact formation (of which we know nearly nothing) are intrinsically determined within the cells. In the next section I discuss other systems in which anchorage of actin filaments to the membrane is locally induced by an extracellular signal.

Transmembrane induction of membrane–microfilament association

Transmembrane induction of microfilament binding to the plasmalemma appears to be a common way of transducting signals into cells. Two apparently interrelated systems are briefly discussed here: the transmembrane interaction of ligand-clustered surface receptors with actin; and the formation of adherens junctions of various forms.

The former process is initiated by the specific binding of di-, oligo- or polyvalent ligands (an antibody, lectin, toxin, etc.) to an integral surface receptor. Subsequent to the binding, the individual ligand–receptor complexes become clustered and are eventually endocytosed. In lymphoid cells the 'patching' step is often followed by aggregation of the complexes into caps at one pole of the cell[10]. Early studies on the capping phenomenon (nearly 15 years ago), indicated that cytoskeletal structures and, in particular, microfilaments were somehow involved in the process. Initially it was shown that drugs which disrupt microfilaments such as cytochalasin b either alone or in conjunction with colchicine inhibit capping. Later, after immunocytochemical methods were developed for visualizing cytoskeletal proteins, the relationships between newly-formed caps and patches and the microfilament system could be more directly appreciated. Such studies confirmed (both visually and by quantitative methods such as fluorescence recovery after photobleaching) that membrane proteins which were initially free to diffuse laterally became immobilized after ligand-induced clustering and were apparently associated with submembrane microfilaments. Unfortunately, despite the efforts invested in the detailed characterization of the molecular processes involved in this type of transmembrane linkage, very little is presently known. The difficulties encountered are partially attributable to the limited spatial resolution of the methods used, to the possibility that different molecular interactions occur at the various phases of the process and to the many ligands and actin-associated proteins which might be involved. Nonetheless, it has been shown that several actin-associated proteins such as myosin and α-actinin accumulate under surface patches and caps, although the exact nature of their involvement is still not clear. It is noteworthy that in our experience α-actinin was the most prominent component of the subpatches and subcaps. The recent results which demonstrate the capacity of α-actinin to directly interact with natural and synthetic membranes (see below) may thus be of great significance for understanding the capping process and justify direct experimental examination. Among the many yet unanswered questions are: Do receptor–ligand complexes become attached to actin via additional linking proteins (integral, peripheral or both)? Do the various ligands perturb a normal flow of surface proteins? Are the different actin-binding proteins detected in subpatches and subcaps (α-actinin, myosin) involved in the anchorage to the membrane, or in the energy-dependent force-generating system involved in the mobilization of the surface clusters during capping and endocytosis?

Another system in which transmembrane linkage to actin occurs is a family of cell contacts known collectively as adherens junctions. These sites may be of two major classes: contacts with non-cellular surfaces such as basement membranes; or tissue culture substrates and direct intercellular attachments between neighbouring cells. Several examples for adherens junctions as visualized by electron microscopy are shown in Fig. 3. The distinctive property of all adherens junctions is their association with the microfilament system through a cytoplasmic, membrane-bound plaque. Attempts made by us and others to characterize the molecular nature of this anchoring plaque indicated that it contains a specific protein, named vinculin[11–13] and that α-actinin is usually enriched in its vicinity[14]. Extensive immunocytochemical studies indicated that while vinculin was ubiquitous in adherens junctions, it was not detected at or near any other site of actin-mem-

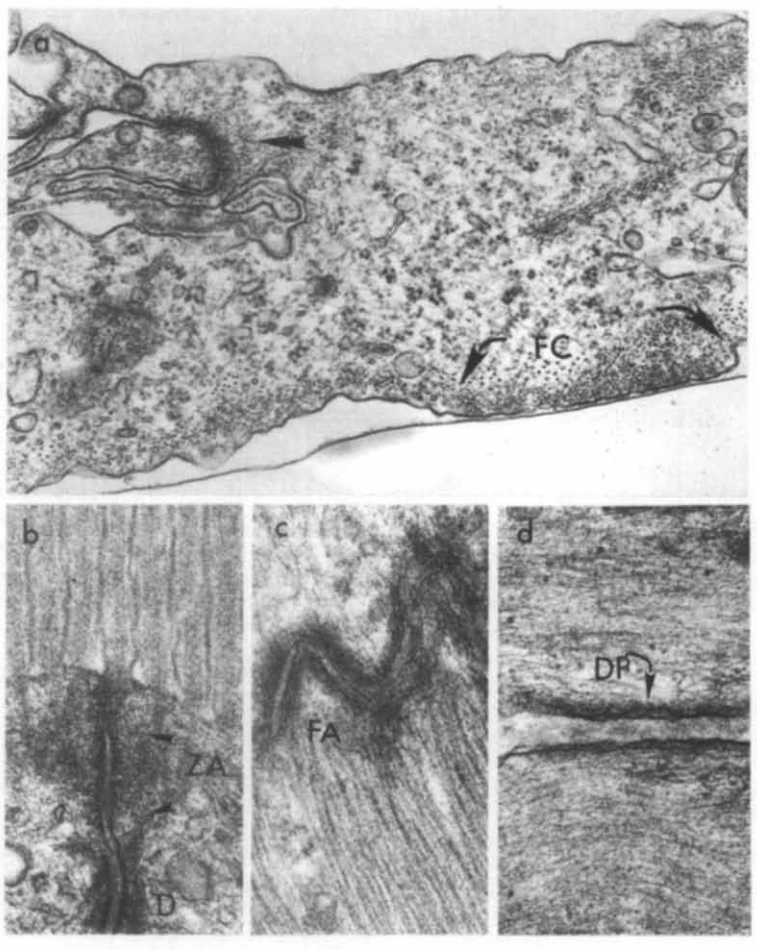

Fig. 3. Transmission electronmicrographs of different adherens junctions: (a) a cross-section through cultured chickens lens cells showing focal contact (FC) with the substrate with numerous submembrane microfilaments, as well as intercellular adherens junction (double arrowhead); (b) intercellular zonula adhaerens *(ZA) between neighbouring intestinal epithelial cells; (c) intercellular* fascia adhaerens *(FA) between adjacent cardiac myocytes; (d) membrane-bound dense plaques (DP) of chicken gizzard smooth muscle. Notice the intercellular material between the two smooth-muscle cells.*

brane association in which no contact with exogenous substrates or cells was present, such as microvilli, contractile ring, ligand-induced surface patches, etc. Unfortunately, not much is known about the biochemical properties of vinculin and even previous reports suggesting that vinculin can bind directly to actin raise controversy. In addition, vinculins can bind in the test-tube to another protein named talin, as well as to the junctional plaque, in an actin-independent manner.

Closer examination of adherens junctions has shed some light on the molecular substructure and dynamics of these sites. Experimental manipulation of adherens junctions suggested that beside adhesive extracellular surface molecules they contain three major molecular domains: (1) integral 'contact receptors' in the membrane; (2) peripheral, membrane-bound plaque at the endofacial surfaces of the contact area; and (3) a bundle of actin-containing microfilaments (Fig. 4). Only limited information is presently available on the molecular constituents of the various domains. It is not known, for example, whether there are ubiquitous exogenous components which induce adherens junction formation and whether all adherens junctions share the same 'contact receptors'. Neither is it clear how the different domains interact with each other at the molecular level. Recent studies, however, have shed light on additional components involved in the assembly of these sites and provided better understanding of the common features of different adherens junctions and of their molecular heterogeneity. Intercellular adherens junctions, for example, have recently been shown to contain a 135 kDa surface glycoprotein which is apparently absent from contacts with non-cellular surfaces[15]. In contrast, the recently-discovered protein, talin[16], is apparently present in the membrane-bound plaques of cell substrate contacts or in associations with connective tissues only and absent from intercellular junctions (for details see Ref. 17).

Although the information so far available is still incomplete, several general conclusions may be drawn from what we already know: (1) the linkage of actin to the membrane in adherens junctions involves peripheral linking-proteins which are the components of the junctional plaque, including vinculin and talin; (2) these proteins are unique to contact sites and differ from the linker proteins in non-junctional areas; and (3) on the basis of kinetic studies as well as dissection of the junction to its molecular domains, we proposed that the assembly of the various junctional elements is a vectorial process, triggered by local contact with extracellular surfaces. Contact-induced changes in the plasma membrane could lead to binding of vinculin to the membrane and to the subsequent induction of actin bundle assembly in the same areas.

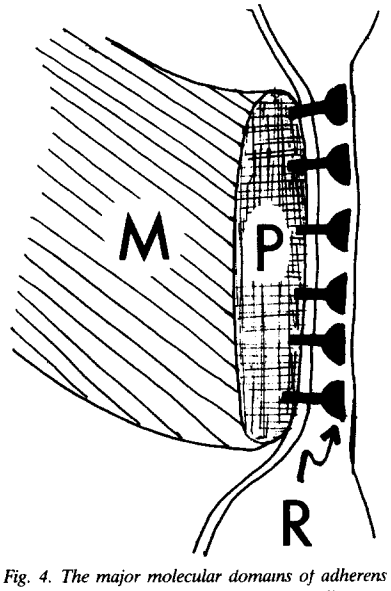

Fig. 4. The major molecular domains of adherens junctions including a microfilament bundle (M), membrane-bound plaque (P) and integral membrane receptors (R).

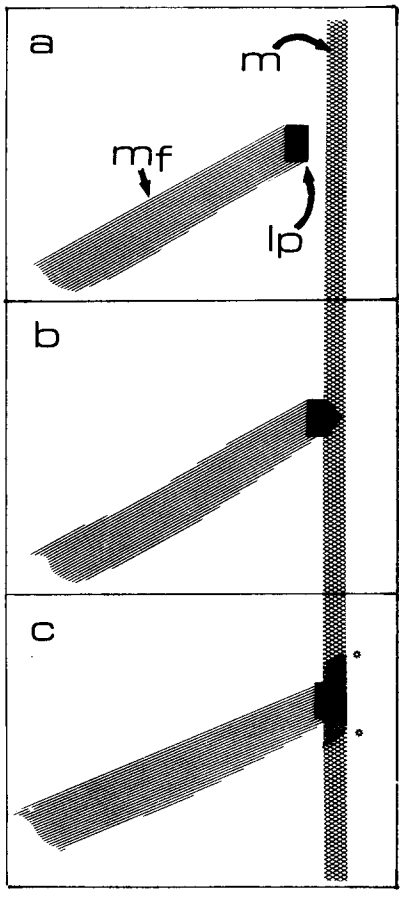

Fig. 5. Two alternative mechanisms for the incorporation of an actin binding protein into the lipid bilayer of the membrane. The starting state in (a) shows a microfilament bundle (mf) associated with a linker protein (lp), both residing in the cytoplasm. Interaction of the linker protein with the membrane can be triggered by a modification in the protein itself which renders it compatible with insertion into the bilayer (b). Alternatively local changes in the membrane may create membrane domains with a high affinity for the linker protein (c).

Direct insertion of cytoskeletal proteins into the lipid domain of the plasma membrane

The view that actin commonly interacts with membranes through other cytoplasmic 'linking proteins' which bind to integral membrane receptors implies that actin (a cytoplasmic component), the 'linker' protein (a cytoplasmic/peripheral membrane component) and the receptor (an integral membrane component) interact without being grossly translocated from their regular compartments within the cell.

An alternative possibility is that the 'linking protein(s)' or even actin itself may, under appropriate conditions, become embedded in the lipid domain of the membrane, without the requirement for additional integral membrane constituents. This view is still not widely accepted, nevertheless an increasing number of examples suggest that such a mechanism may indeed exist and that some actin-binding proteins might become integrated in the membrane. These include recent studies on α-actinin, metavinculin and the 110 kDa protein of microvilli.

A few years ago, attempts were made to identify integral membrane proteins in activated platelets using the hydrophobic photoactive probe, iodonaphthyl azide (INA). It was shown that beside known membrane constituents, INA became specifically bound also to a 100 kDa polypeptide identified as platelet α-actinin[18]. This suggested that during platelet activation, concomitantly with dramatic shape changes and polymerization of actin, α-actinin becomes available to the hydrophobic probes. One of the interpretations offered was that α-actinin might have become inserted into the lipid domain of the membrane. This possibility was later corroborated by important experiments in which pure α-actinin was shown to interact *in vitro* with lipid bilayers containing diacylglycerol and palmitic acid[19,20]. Moreover these lipids could promote the interaction between α-actinin and actin. Conceptually, these results imply, (though indirectly) that a cytoplasmic, water-soluble protein such as α-actinin may, under specific circumstances, be translocated from the cytoplasm and inserted into the membrane. It is still unclear what might trigger such translocation. Are there post-translational changes in the α-actinin molecule itself which lead to the exposure of hydrophobic domains on the surface of the molecule, or are there changes within the bilayer leading to the binding of α-actinin? (see Fig. 5.) At least in platelets the latter possibility is attractive in view of the documented turnover of phospholipids which occurs upon activation.

Another relevant system involves the smooth-muscle protein metavinculin which is a vinculin-like protein by antigenic and peptide map criteria with a molecular mass of about 160 kDa (larger than vinculin by 30 kDa). Biochemical studies indicated that metavinculin is quite resistant to extraction with conventional aqueous buffers. Unlike vinculin which is readily extractable in low-ionic strength buffers, the solubilization of the closely related metavinculin required the presence of both detergent and highly concentrated salt solutions suggesting that the latter might be embedded in the lipid bilayer[21]. This suggestion is still based on indirect observation and direct biochemical evidence to support the view is yet to be supplied.

The last example for an integral actin linker may involve the 110 kDa protein of intestinal microvilli. As discussed above, one view was that this protein mediates the binding of the core bundle of microfilaments to an integral membrane component. Recent studies, however, indicated that the 110 kDa, actin-binding protein has several biophysical properties typical of integral membrane proteins including partitioning into the detergent phase of Triton X-114 and incorporation into artificial lipid vesicles[9].

Concluding remarks

The detailed molecular structure of most of the systems studied so far, their diversity and the mechanisms of their assembly, are still poorly characterized. Nevertheless, the more we know about membranes and membrane dynamics, cell morphogenesis and mobility, etc., the more we appreciate the major physiological importance of these associations. Of particular interest and importance is the recent suggestion that some cytoskeletal proteins whose distribution was formerly believed to be restricted to the cytoplasm may, in fact, translocate and partition into the membrane proper. Hopefully, future research directed at the various questions discussed here, and the isolation of new 'linker proteins', will shed light on the mechanisms of membrane–microfilament interactions and their precise involvement in cellular process.

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Programmable messengers: a new theory of hormone action

M. Rodbell

Many hormone receptors are linked to GTP-regulatory proteins in membranes. When these proteins are activated by hormones and GTP, the α-subunits are released from the membrane as soluble proteins. It is proposed that these α-subunits are modified by kinases, proteases and other protein-modifying enzymes to give new forms with differing functions. This proviaes a way of explaining the multiple actions of a hormone on its target cell, and the released α-subunits of GTP-regulatory proteins can be called 'programmable messengers'.

Two ideas have dominated the field of signal transduction over the past 25 years. One is that hormone/neurotransmitter receptors interact with various effector enzymes in the plasma membrane to generate signals in the form of small molecules. The classical example is the receptor-controlled adenylate cyclase system in eukaryotic cells. The other is that receptors exist either in membranes or in the cytosol as 'mobile' elements which, when combined with the activating hormone, induce the receptor to collide with or move to the site(s) of the effector systems. Examples of theories that have evolved from the mobile-receptor theory are the 'collision-coupling'[1] and 'two-step'[2] theories proposed for the coupling of β-adrenergic receptors to the adenylate cyclase system. Another example is the estrogen receptor; it has been thought that the receptor first reacts with the steroid in a cytosolic compartment, and that the activated receptor then enters the nucleus where it regulates gene expression.

There is ample evidence that cyclic AMP and other small molecules (cyclic GMP and inositol trisphosphates are recent examples) mediate some of the effects of hormones. The question is whether the pleiotypical responses induced by a hormone are due solely to any of these molecules. If not, what type of molecule might be more closely linked to receptors that could serve as primary messengers of hormone action? As for the concept of receptor mobility, there is evidence that membrane receptors can be induced by agonists to move about in the plane of the membrane. However, there is no compelling evidence that mobility is necessary or causal for signal transduction to take place. Indeed, there is a report that increasing the fluid environment to enhance receptor mobility in membranes is detrimental to hormone action[3]. For the estrogen receptor, recent studies indicate that most of the receptors are bound to the nuclear matrix prior to their occupation by hormone; receptor release into the cytosolic compartment is an artifact of the methods used for isolating the nucleus[4].

This article proposes an alternative view of the function of membrane receptors and develops a logical framework for a theory that the primary messengers of hormones acting on membrane receptors are proteins that bind and degrade GTP. These are the so-called GTP-regulatory proteins (G) that are linked to numerous receptor types in eukaryotic cells. The fundamental aspects were presented five years ago in a theory called 'Disaggregation Theory of Hormone Action'[5]. This theory is now extended and modified in the light of information acquired recently.

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The disaggregation theory

Briefly, this theory suggests that various classes of receptors are complexed with a family of oligomeric GTP-regulatory proteins. When the receptors are occupied by agonists and the G units by GTP, the oligomers dissociate into monomers. In the process, the receptors are transformed from a high affinity state when they can bind physiological concentrations of hormones, into a low affinity state in which they are no longer active. At the same time, the G units are transformed to a 'monomeric' structure that reacts specifically with an effector unit (E) such as adenylate cyclase. The theory is thermodynamically sound[6]; it explains the apparent paradox of receptors undergoing transitions from high to low affinity states during concerted activation of G by hormone and GTP; it explains the findings of target analysis that the ground-state structure of receptors coupled to G exhibits a much higher molecular weight than the activated adenylate cyclase. This theory predicts that the putative monomeric form of G is the primary messenger of hormone action, whereas the product of the effector unit(s) is a secondary signal.

G units are oligomeric proteins

In recent years, G units have been purified and structurally analysed[7]. It is now clear that G units coupled to rhodopsin (termed transducin) and those coupled to receptors (R) that stimulate or inhibit adenylate cyclase (termed G_s and G_i, respectively), and a newly discovered G unit of unknown action (termed G_o) are composed of three distinct protein subunits, only one of which, the α-unit, binds GTP. The type of α-subunit coupled depends on the type of G unit (and associated R) to which it is attached. The other two sub-