ACTIN-BINDING PROTEINS–LIPID INTERACTIONS

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I. INTRODUCTION

Investigating biological interfaces has gained more interest recently since new biochemical and biophysical techniques have become available to cell biologists. The events at, near or through a plasma membrane are complex, in as much as one does not simply consider a membrane bilayer as a mechanical barrier. From the physicochemical point of view, we have to imagine a multilayered colloidal system consisting of proteins and lipids in various aggregation states. This tri- or tetralayer membrane (Figure 1) extends from inside of a cell to the extracellular environment and involves the cytoskeleton proteins (microfilaments, microtubules, intermediate filaments; cf. Niggli this volume), which are specifically anchored in the fluid mosaic lipid bilayer and interconnected transmembranously with extracellular matrix proteins. In this way signals are transmitted from outside to inside or vice versa. Signals, however, can be generated in the lateral plane by diffusion within the lipid layer or by chemomechanical transduction mechanisms through the interconnected fibrous protein network (plasmalemma undercoat). Furthermore, new microcompartments within or between these multilayers may be generated.

Although the binding of membrane components to the intracellular cytoskeleton and extracellular matrix proteins may be characterized by rapid exchange and low affinity, these may be of considerable importance in determining the order of magnitude of D, the apparent diffusion coefficient of the mobile fraction of membrane proteins (Zhang et al., 1993). The *transient* nature of interactions involving lipid–protein or lipid–protein/protein binding may thus give rise to divergent diffusion constants. A new aspect in this scheme of interactions involves the binding of ectodomains of membrane proteins, the pericellular matrix (PCM), to the large extracellular matrix molecules (Sheetz, 1993). Since some of these PCM proteins are lipid-binding proteins and, in addition, are intracellularly linked to the cytoskeleton, one is confronted with complex cooperative reaction schemes that are restrictive (by inhibiting diffusion) and permissive (by allowing signal transduction).

Before evaluating actin-binding proteins–lipid interactions at the plasma membrane interface in more detail, two aspects should be considered: (1) Generation of intracellular signals or second messengers—besides the classical receptor pathway—may involve mechanical stimuli that are produced by applying physical

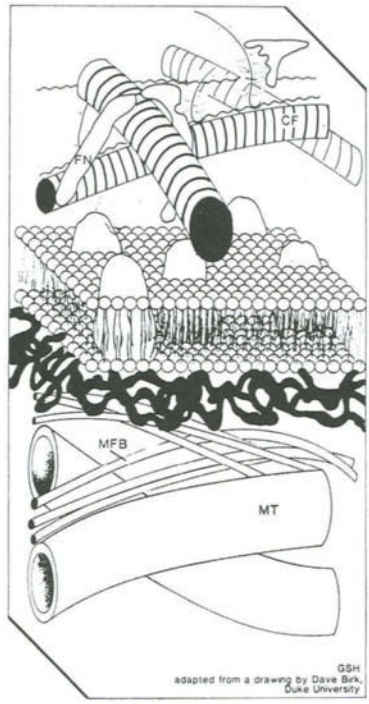

Figure 1. A multi-layer model of the membrane interface. Microfilaments (MF) microtubules (MT) and intermediate filaments (IF) are connected via lipid binding proteins (black) to the membrane bilayer. Pericellular matrix proteins and the extracellular fibronectin (FN)/collagen fiber system (CF) transmit forces through transmembrane receptor proteins (integrins). From Dave Birk, Duke Univ., North Carolina, USA, with permission.

forces to the cell surface. This process of "mechano-transduction" will allow function to follow a given form (P.A. Watson, 1991) and represents a new concept. (2) More than 10 years ago, Lazarides and colleagues proposed that the topogenesis of a membrane skeleton is determined by assembly limiting reaction steps; that is, the stoichiometry of binding would simply be governed by the availability of binding sites independent of the rate of synthesis (Lazarides and Moon, 1984). From these two examples, it is clear that important signals for cell behavior may be generated by specific architecture of the membrane itself.

Hence, actin-binding protein–lipid interactions may play a central role not only in (1) mediating the anchorage of the cytoskeleton in the lipid layer, but also in (2) defining a specific membrane topology. Moreover, the release of actin-binding proteins or certain lipids (e.g., inositolphosphates or diacylglycerol; DAG) from

their complex upon stimulation via receptors (see below) may represent a more direct way for a cell to trigger intracellular events through a transmembrane signaling mechanism.

II. THE ROLE OF LIPIDS

The general classification scheme of lipids that interact with actin-binding proteins follows simple criteria:

(1) Fatty acids are distinguished by their number of C atoms and double bonds.
(2) Phospholipids have a common glycerol backbone.
(3) The lipid head groups are linked by a phosphate ester to the glycerol backbone.

Most common phospholipids are: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and derivatives, phosphatidylinositol-4-monophosphate (PIP), phosphatidylinositol-4,5-biphosphate (PIP-2), inositoltriphosphate (IP3), phosphatidylglycerol (PG), (Figure 2). For the naturally occurring lipid composition of various mammalian cell membranes the reader is referred to the recent article by Zachowsky (1993).

Protein–lipid binding involves hydrogen bonds, van der Waals forces, hydrophobic interactions and electrostatic coupling (Dill, 1990). Different binding phenomena result from distinctly folded protein domains that are properly exposed in the appropriate membrane environment (i.e., after binding to several lipid molecules in protein–lipid complexes and lipid-protein–protein interactions). The actin-binding protein 4.1 serves as an example; its spectrin-binding domain of protein 4.1, which is accessible in aqueous solution, is masked after reconstitution into lipid vesicles, but regains its spectrin-binding capacity in the presence of actin (Cohen and Foley, 1982; Bennett and Stenbuck, 1979). In addition, binding constants for lipid components may change dramatically depending on whether measurements are performed in solution or after reconstitution into a lipid bilayer. For example, the binding constant of protein 4.1 for PIP-2 shifts from low to high affinity after transfer to its "natural" glycophorin-containing membrane environment (Anderson and Marchesi, 1985). Because lipids act as cofactors that modulate the interaction of proteins within the membrane plane and because a selective and high-affinity binding to the lipid bilayer may depend on more than two components, it is essential to study protein–lipid interactions in a system that mimics the natural environment.

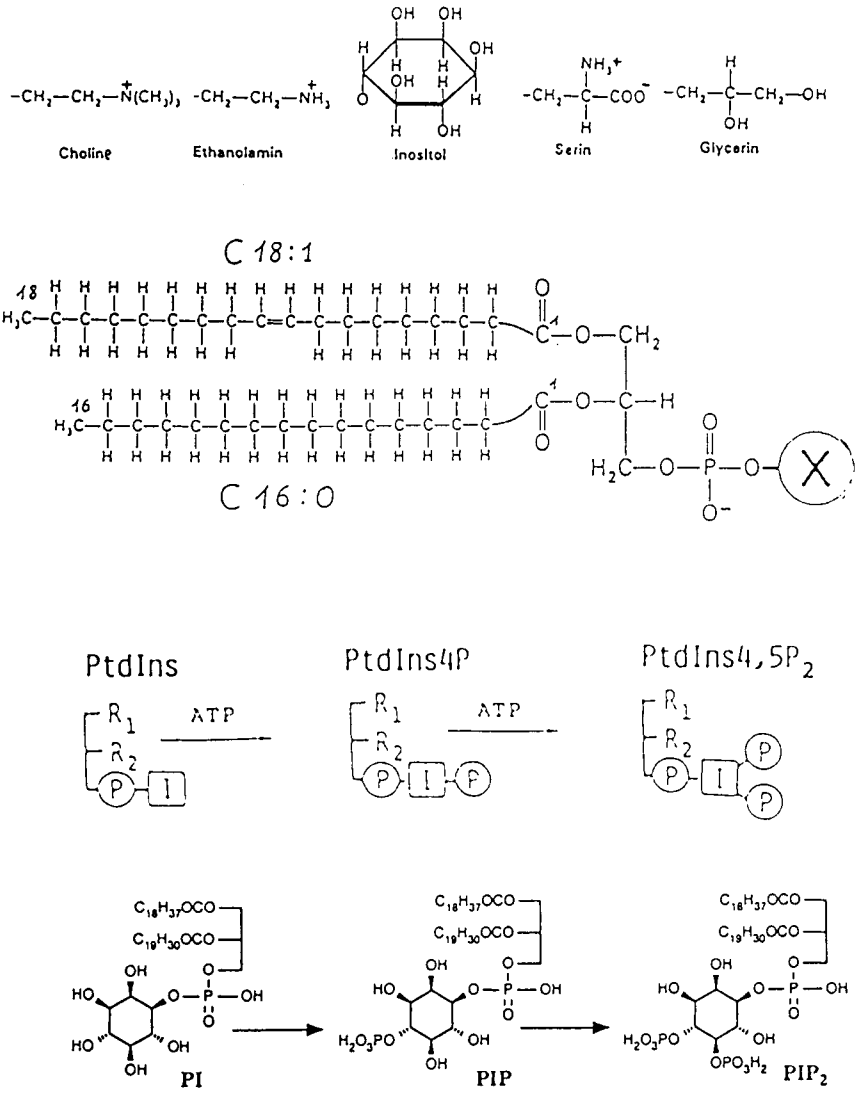

Figure 2. Major lipid components from animal cell membranes. For explanation see chapter II.

III. A SURVEY OF APPLICABLE TECHNIQUES FOR INVESTIGATING PROTEIN–LIPID INTERACTIONS

A number of biophysical techniques, some of these techniques are discussed below in more detail, are now available to investigate lipid–protein interactions within a membrane bilayer. Infrared spectroscopy (Siminovitch et al., 1987) and high-sensitivity differential scanning calorimetry (DSC) (Mason et al., 1981) applied together are powerful tools to provide the thermodynamic and structural information required to characterize lipid conformation and intermolecular interactions. Complementary techniques such as X-ray diffraction (Hui and Huang, 1986), NMR spectroscopy (Ruocco et al., 1985a; 1985b), Raman spectroscopy (O'Leary and Levin, 1984), electron spin resonance spectroscopy (ESR) (Marsh, 1990), and measurements of fluorescence anisotropy using diphenylhexatriene (DPH; Tendian and Lentz, 1990), are becoming routine techniques for studying lipid–protein interactions and to determine the degree of interdigitation in lipid bilayers.

In most laboratories multilayered vesicles, or even better, unilamellar, small (100 nm) and giant liposomes (> 500–1000 nm) serve as membrane models (New, 1990). It should be pointed out that the amount of protein incorporation into liposomes depends on the size of the molecule and the degree of membrane curvature.

Advancement has been made in developing a miniaturized Langmuir technique (Heyn et al., 1991). Two-dimensional lipid monolayers, spread on an air–water interface, can serve as model systems for biological membranes. In mixed lipid–protein films, the lateral packing density of molecules, temperature, ionic strength and pH of the subphase can be controlled in a precise manner. By introducing a small fraction of fluorescent dye molecules, domain formation and mobility of lipids and proteins can be traced individually by epifluorescence light microscopy (Möhwald, 1990; Heyn et al., 1991; Dietrich et al., 1993). An additional advantage is the procedure of transferring lipid–protein monolayers onto planar solid supports, which in the near future, will allow the investigation of pattern formation of lipid–protein complexes in two dimensions by scanning tunneling (Hörber et al., 1988) and atomic force microscopy (Egger et al., 1990; Ohnesorge et al., 1990; Weisenhorn et al., 1991).

IV. ARTEFACTS OF LABELING TECHNIQUES

Interpretation of measurements of actin-binding protein–lipid interactions has to be done with caution: Binding of PI phosphates alone is insufficient to document a stable interaction with membrane lipids. Moreover, as pointed out by Niggli (1993), the labeling of proteins with ^{14}C-PI after SDS-PAGE could be due to oxidative deterioration of polyunsaturated fatty acids and a resulting covalent cross-linking of reactive lipid breakdown products. Application of antioxidants such as 50 mM mercaptoethanol may prevent such artifacts.

It is important to bear in mind that most of the membrane-anchoring proteins are lysed by using detergents and this may lead to structural distortion or an artificial exposure of hydrophobic protein domains. The elution of proteins together with lipid vesicles from gel filtration columns, is indicative of lipid interactions unless the insertion into the hydrophobic layer of lipid molecules has been documented by other techniques. In addition to DSC, hydrophobic labels, which partition into the hydrophobic part of the membrane, are useful tools to document protein–lipid interactions *in situ*. One class of these labels is brominated fatty acids that compete with fatty acids at the hydrophobic-binding sites of spectrin (Isenberg et al., 1981). INA (5-iodonaphtyl-1-azide) is an hydrophobic photoaffinity label used to label membrane-bound α-actinin (Rotman et al., 1982). More recently, the photoactivatable PC analogue [^{3}H]-PTPC-11 was used to document actin-binding protein–lipid interactions (Niggli et al., 1986; Goldmann et al., 1992; Niggli et al., 1994).

The usefulness of this new photolabeling and cross-linking protocol is discussed extensively in a review by Brunner (1993). However, these elegant techniques are not trivial and should be applied with caution. Control experiments are necessary to exclude any unintended labeling of peripheral or soluble proteins by chemical or radiolytic degradation products. Since these probes will also label hydrophobic domains of otherwise electrostatically coupled, hydrophilic proteins, labeling experiments should be carried out at various salt concentrations. Alternatively, scanning calorimetric measurements also help to give a clear answer (see below).

V. FOCAL CONTACT PROTEINS INVOLVED IN CYTOSKELETON–LIPID INTERACTIONS

Specialized adhesion zones of the plasma membrane, which a motile cell uses to establish a connection to a substrate, are called *focal contacts* (for review cf. Burridge et al., 1988; Jockusch and Füchtbauer, 1983; Dunlevy and Couchman, 1993). Contractile stress fibers (Isenberg et al., 1976) terminate in such transmembrane junctions (Samuelsson et al., 1993). Transmission of force requires linkage to the lipid bilayer. Many actin-binding proteins anticipated to play a role in anchoring have been localized in focal contacts (see Niggli, this volume), but only a few of them have been shown to interact with lipids directly. Three major actin-binding proteins, talin, vinculin and α-actinin bind to actin and interact with lipids by inserting into the hydrophobic part of membrane leaflets.

A. Talin

A special role for talin, a major protein of focal contacts (Burridge and Connell, 1983a,b; Hock et al., 1989; Beckerle and Yeh, 1990; Burridge et al., 1990), may be in coupling microfilaments to plasma membranes (Isenberg, 1991; Isenberg and Goldmann, 1992). Our laboratory became interested in talin when we applied

highly sensitive DSC to actin-binding protein–lipid mixtures. DSC allows the recording of phase transitions of lipids in the absence and presence of proteins. A lipid bilayer will, with rising temperature, undergo these phase transitions by changing its molecular structure from *crystalline* (L_c) in the frozen state to a gel phase ($L_{\beta'}$), the *ripple* phase ($P_{\beta'}$) and to the *fluid* phase (L_α; Figure 3). Phase transitions are endothermic processes, that is, heat is required from the environment. The calorimeter in its reaction chamber compensates for the additional energy by comparing the sample with the reference probe. This energy difference is recorded as a function of temperature. Phase transitions appear as peaks in the profile (thermogram). Integration over the area below the DSC signal yields the difference in enthalpy, ΔH. Assuming a reversible first order phase transition one can calculate the difference in entropy: $\Delta S = \Delta H/T_m$ (T_m = temperature of phase transition). The large change in enthropy resulting from the disorder of the C chains in the fluid phase ($T > T_H$) is thus essential for driving the phase transition (Figure 3). T_s* represents the solidus line, the onset of lipid chain melting, and T_l* represents the endpoint of chain melting in the fluid phase. Hydrophobic and electrostatic interactions are reflected by a shift of the transition states T_s* to lower temperatures or T_l* to higher temperatures, respectively.

DSC measurements have shown that there is a weak but stable hydrophobic interaction of talin with neutral dimyristoylphosphatidylcholine (DMPC) vesicles (Heise et al., 1991). The interaction of talin with DMPC, however, is greatly enhanced in mixed phospholipid bilayers containing negatively charged phospholipids, e.g., dimyristoylphosphatidylglycerol (DMPG) or, (DMPS) (Heise et al., 1991) (see Figure 3). While the weak hydrophobic interaction remains unsaturated up to high protein–lipid molar ratios, the electrostatic interaction reaches steady state conditions rapidly (Figure 3). These are some reasons to assume that talin, like vinculin, (Niggli and Burger, 1987) interacts with lipid membranes in a two-step mechanism: The protein may first be attracted to and fixed at the bilayer surface by electrostatic interactions before it is able to insert its hydrophobic portions into the hydrocarbon bilayer. This mechanism was also proposed for protein 4.1 by Kimelberg and Papahadjopoulos (1971). Covalently bound fatty acids may help to facilitate this insertion (Keenan et al., 1982). If we assume that the major portion of the talin molecule covers the bilayer surface, it would be expected that insertion is maximal at a protein concentration, that leads to steric hindrance at the bilayer surface. This would explain the difference in saturation behavior of hydrophobic and electrostatic interactions (Figure 3).

The selectivity of phospholipid binding of talin and other actin-binding proteins can be demonstrated by Fourier transform infrared spectroscopy (FTIR) (Heise et al., 1991) with one of the lipids being deuterated. Spectra can be taken from DMPC-d_{54} and non-deuterated DMPG in a 1:1 mixture and plotted against temperature (Figure 4). Incorporation of talin almost exclusively affects the DMPG spectra. Moreover, the position and width of the main transition of the noncharged component (i.e., DMPC) are only slightly changed by the protein, while for the

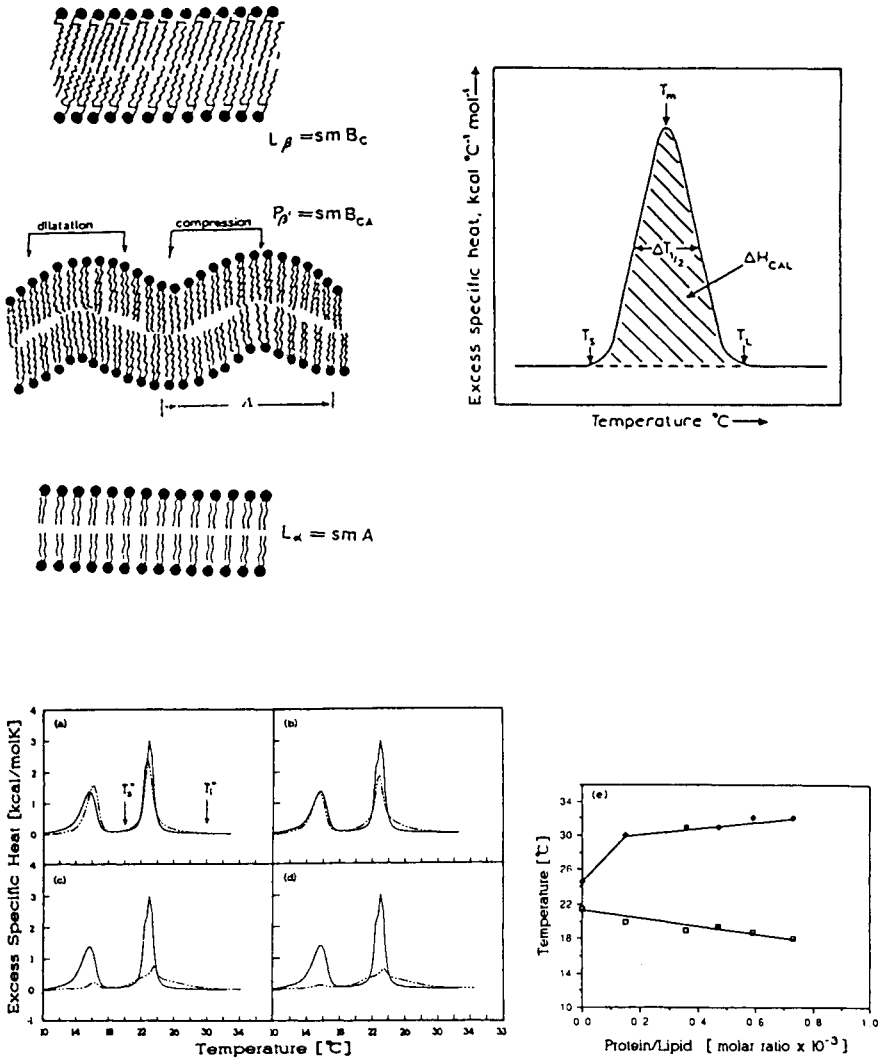

Figure 3. Above: Schematic thermogram of lipid chain melting and various stages of lipid orientation. Below: DSC-measurements of DMPC/DMPG with and without reconstituted talin. Note the shift of phase transitions with increasing protein/lipid ratios (a–d). In (e) the shift of solidus lines (▣) and liquidus lines (♦) are a function of protein–lipid molar ratios. Taken from Heise et al., 1991, with permission.

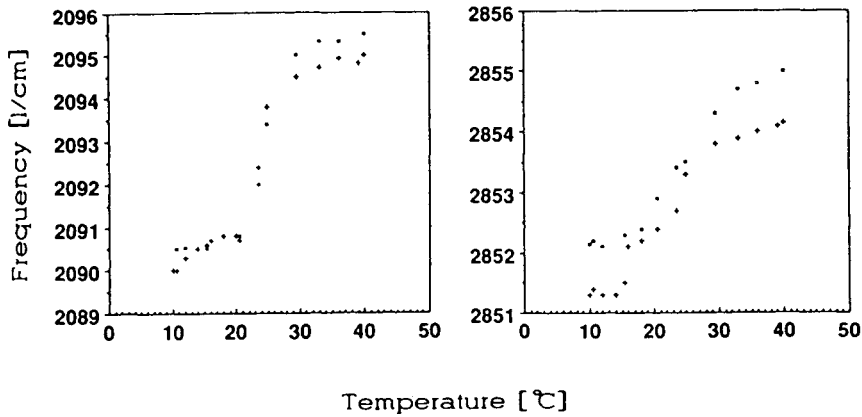

Figure 4. FTIR study of the interaction of platelet talin with 1:1 mixed vesicles of chain-deuterated DMPC (left panel) and DMPG (right panel). The shift in frequency numbers plotted against temperature is significant for DMPG spectra. Taken from Heise et al., 1991, with permission.

charged component T_l is shifted to a T_l* of 33°C and the solidus temperature T_s* is shifted to around 18°C (Figure 4). Both these shifts are in reasonable agreement with the calorimetric data. It is important to stress this point because only the combination of various techniques will allow us to obtain comprehensive answers to our questions. In line with this multimethodological approach is the application of photoactivatable lipid analogues for hydrophobic labeling of lipid-binding, membrane-associated proteins (Niggli et al., 1986; Goldmann et al., 1992; Brunner, 1993). [^{3}H]- PTPC/11, a photoactivatable PC derivative (Figure 5), has been used successfully to label talin and vinculin after reconstitution into lipid vesicles. Since these lipid probes selectively react with protein domains, which insert into the hydrophobic part of lipid membranes, it is concluded that talin and vinculin belong to this category of proteins (Niggli et al., 1986; Goldmann et al., 1992). A labeling efficiency of 0.004–0.01 mol of label/mol of protein suggests that only a minor portion of the talin molecule penetrates into the hydrophobic membrane core (see below).

Taking talin as a representative model protein for the studies of actin-binding protein–lipid interactions, the film balance technique remains to be discussed as a method to study the interaction of this protein with lipid monolayers (Dietrich et al., 1993). Lipid monolayers, spread on an air–water interface, are particularly suited to investigate the insertion behavior, surface pressure induction and pattern formation of proteins in two dimensions (Figure 6). Partitioning of talin was measured in mixed DPPC–DMPG lipid monolayers. For viewing with epifluorescence light microscopy, talin was labeled with NBD (Detmers et al., 1981). For

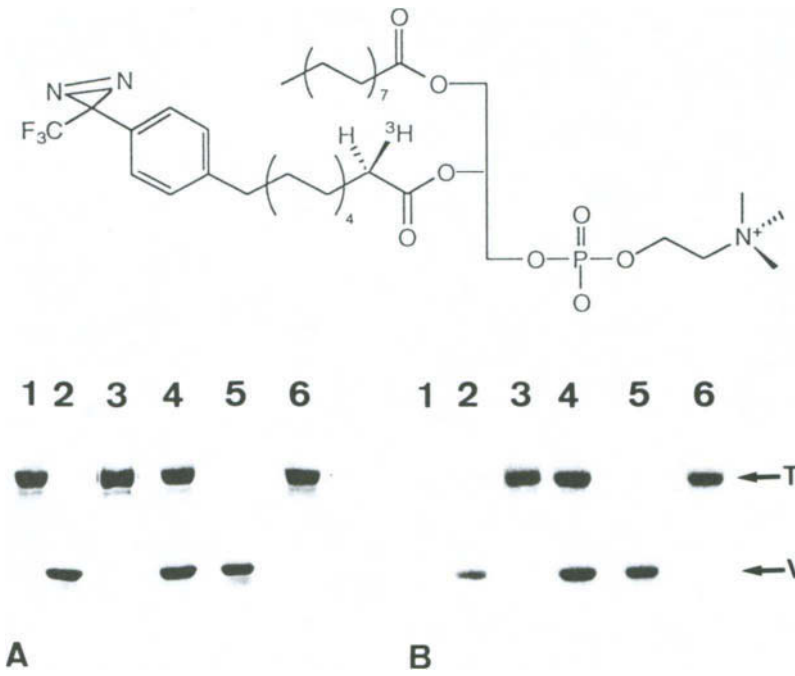

Figure 5. Above: Chemical formula of [^{3}H] PTPC/11, 1-palmitoyl-2-[11-[4-[3-(trifluoromethyl)diazirinyl] phenyl][2-^{3}H]undecanoyl]-*sn*-glycero-3-phosphocholine. Below: Hydrophobic photolabeling of talin upon incubation with phosphatidylserine liposomes (a) Coomassie blue stained gradient gel (b) the corresponding autoradiogram. (Lane 1) talin incubated with liposomes without photolysis; (Lane 2) vinculin incubated with liposomes in the presence of 130 mM KCl and 1 mM $MgCl_2$; (Lane 3) talin incubated with liposomes in the presence of 130 mM KCl and 1 mM $MgCl_2$; (Lane) talin and vinculin, incubated with liposomes in the absence of added salt; (Lane 5) vinculin incubated with liposommes in the absence of added salt; (Lane 6) talin incubated with liposomes in the absence of added salt. Arrows on the right indicate the position of talin (T) and vinculin (V). Taken from Goldmann et al., 1992, with permission.

lipid labeling, small amounts (~ 0.1%) of N-(texas-red-sulfonyl-dipalmitoyl-L-α-phospatidylethanolamine) were added to the lipid solution. Since binary lipid mixtures normally show a phase transition inducing partial separation of the two components (Frey et al., 1987), it is expected that dark domains (cf., Figure 6) are rich in components of a lower phase transition pressure (here, DPPC), while the more *fluid* regions are enriched in components with higher transition pressure (here, DMPG or DMPC). For DPPC–DMPG monolayers, which are composed of less charged, *crystalline* regions and negatively charged *fluid* regions, it was demonstrated that talin codistributes with lipids in the negatively charged region (cf., Figure 6 bottom row,

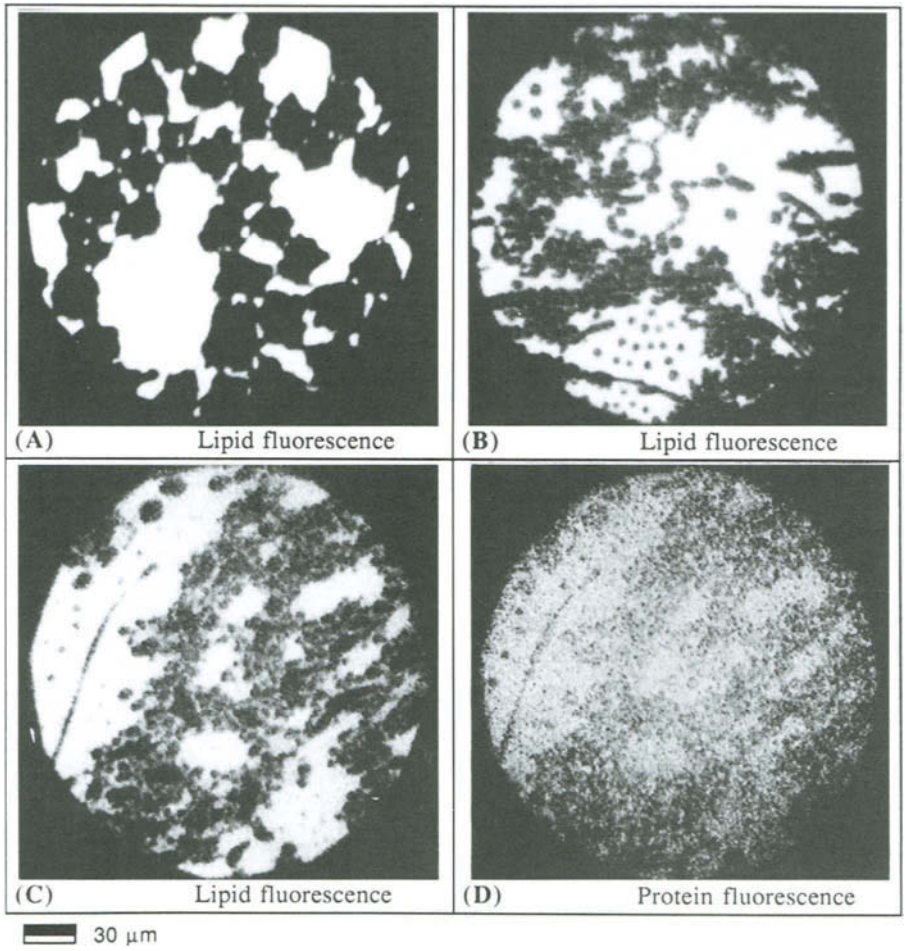

Figure 6. Film balance study of talin incorporation into mixed lipid monolayers: DPPC/DMPG at a molar ratio of 6:4. Lipid fluorescence in the absence (a) and presence (b) of talin; lipid fluorescence (c) and protein fluorescence (d) 50 min after injection talin into the subphase. Taken from Dietrich et al., 1993, with permission.

where lipid and protein fluorescence are displayed in the same image). The film balance apparatus, when equipped with high resolution optics and data acquisition, is thus of great potential in supplying data complementary to DSC and FTIR.

The next step is to map the lipid interacting domain of talin. The primary sequence of talin is known (Rees et al., 1990), and it has been speculated from the apparent sequence homology with the membrane and actin-binding proteins 4.1 and ezrin that the lipid-binding site is localized in the smaller (47 kDa) calpain II cleavage product (Fox et al., 1985; Beckerle et al., 1987; Rees et al., 1990). We have recently

confirmed (Niggli et al., 1994) that, out of a mixture of the 47-kDa and 190-kDa talin fragments, only the 47-kDa domain carrying the N terminus binds to lipid vesicles. We have additional reasons to believe that the actin-binding site is located in the 190-kDa C-terminal fragment. Hence, it is conceivable that both subunits can redistribute into focal contacts after microinjection (Nuckolls et al., 1990, 1992). So far, only the binding sites for vinculin (Burridge and Mangeat, 1984) have been identified on the tail portion of the talin molecule (Gilmore et al., 1993), and other binding sites, for instance, for actin (Muguruma et al., 1990), α-actinin (Muguruma et al., 1992) and integrin (Horwitz et al., 1986), await their precise characterization.

B. Vinculin

Vinculin was first identified and isolated by Geiger (1979) and soon discovered in a variety of cells and tissues (Geiger et al., 1987; Otto, 1990). The primary sequence of this 117-kDa protein (130 kDa on SDS gels) has been published for chicken (Price et al., 1987; Coutu and Craig, 1988), nematode (Barstead and Waterstone, 1989) and human species (Weller et al., 1990). The organization of the entire human vinculin gene, including its promotor sequence was reported recently (Moiseyeva et al., 1993). For many years vinculin was believed to be an actin-binding protein (Isenberg et al., 1982; Jockusch and Isenberg, 1982; Ruhnau and Wegner, 1988; Westmeyer et al., 1990), and indeed the vinculin sequence contains actin-binding sites that can be blocked by specific antibodies (Westmeyer et al., 1990). Binding domains for actin in talin have been mapped to residues 1–258 (Jones et al., 1989; Gilmore et al., 1992).

Although the molecular shape of vinculin can best be described by the "balloon-on-a-string" model (Eimer et al., 1993), the location of binding domains on the vinculin molecule has not been determined. Vinculin is a typical amphitropic protein (Burn, 1988), in that it exists both as a soluble cytoplasmic protein as well as a membrane-bound protein. Binding to negatively charged phosphlipids (PA, PI, PG) has been reported; however, neutral lipids (PC and PE) do not promote binding (Ito et al., 1983; Niggli et al., 1986). Vinculin was also reconstituted into lipid monolayers (Fringeli et al., 1986; Meyer, 1989). The latter study reports that the dissociation constant for vinculin–phospholipid interaction can vary dramatically (1.2×10^{-6} M–5.3×10^{-10} M) depending on temperature, surface pressure and different lipid composition and ratios. It is not clear if posttranslational modification of vinculin is important for lipid bilayer interactions because a minor fraction of vinculin has myristate (Kellie and Wigglesworth, 1987) and palmitate (Burn and Burger, 1987) covalently attached. The degree of posttranslational lipid modification may be related to the phosphorylation state: The phoshoplipid modified 10-fold increase of vinculin phosphorylation induced by the purified *src*-gene product (Ito et al., 1983) contrasts with a 3-fold lower level of palmitylated vinculin in Rous sarcoma virus-infected fibroblasts (Burn and Burger, 1987). Vinculin is one of the

rare examples for which hydrophobic labeling by a lipid analogue (TID) may be applied *in vitro* (Niggli et al., 1986) and *in vivo* (Niggli et al., 1988).

The binding characteristics, which have been determined for two components do not necessarily remain unchanged in a more complex system: In a ternary complex of vinculin/α-actinin (Wachsstock et al., 1987) and phospholipids, photolabeling of α-actinin is markedly suppressed, whereas α-actinin alone (see below) is well labeled (Niggli and Gimona, 1993). On the other hand, ternary complex formation of vinculin–talin (Nuckolls et al., 1990) and phospholipids *in vitro* did not influence the insertion of talin into lipid membranes (Goldmann et al., 1992). Clearly, more *in vivo* labeling studies will be needed to establish whether all the lipid binding capacities reported *in vitro* are manifest in more complex structures like focal contacts.

C. α-Actinin

The biochemistry of α-Actinin (MW ~100 kDa) has been reviewed recently (Blanchard et al., 1989; Vandekerckhove, 1990). Agreement exists that α-actinin (i) is a homodimer with its subunits orientated in an antiparallel fashion (Wallraff et al., 1986; Schleicher et al., 1988), (ii) cross-links actin filaments into a three-dimensional network (Jockusch and Isenberg, 1981; Jockusch and Isenberg, 1982) and (iii) is involved in linking the cytoskeleton to the plasma membrane (Geiger et al., 1980). The influence of α-actin on actin polymerization is still controversial (Muguruma et al., 1992; Colombo et al., 1993) as is its regulation by Ca^{++} in muscle and nonmuscle tissues. Generally, it was believed that the nonmuscle isoforms bind to F actin in a Ca^{++}-sensitive manner whereas binding of muscle α-actinin is Ca^{++} insensitive (Condeelis and Vahay, 1982; Duhaiman and Bamburg, 1984). More recent data, however, (Pacaud and Harricane, 1993) clearly demonstrate that macrophage α-actinin binds to actin independent of regulation by Ca^{++}. Interaction of α-actinin with lipids has been reported by several laboratories (cf. Fritz et al., 1993). In skeletal muscle, a high ratio of PIP-2 (20–30 mol/mol protein) is endogenously bound to α-actinin, whereas smooth muscle α-actinin strongly binds exogenously added PIP-2 (Fukami et al., 1992). α-Actinin-induced gelation of F-actin *in vitro* is greatly enhanced by PIP-2 but not by PIP or PI, as long as the added inositolphosphate is below the critical micelle concentration. Lipid interaction of α-actinin *in vivo* is likely with the finding that in platelets, when physiologically activated, a 30-fold increase in lipid binding was detected in the nondetergent-lysed cytoskeleton (Burn et al., 1985). Furthermore, when immunoprecipitated from prelabeled, activated platelets, α-actinin was found to have PA and DAG bound in a molar ratio of 1:1 (Burn et al., 1985). Consistent with this result, Meyer et al. (1982) reported that out of a total lipid extract from yeast only two lipids, PA and DAG, formed a stable 1:1:1 complex with α-actinin. In summary, α-actinin has potential lipid-binding capacity but as mentioned above, however, it has not been confirmed if this is of particular cell biological importance.

D. MARCKS

The myristoylated alanine-rich C kinase substrate (MARCKS) is a protein belonging to a family of signal-transducing proteins (for review cf. Aderem, 1992). The protein, which is amphitropic in nature, can be isolated in a cytoplasmic and membrane-bound form from the same source (Manenti et al., 1992; 1993). Sequence analysis shows, the protein to have an actual mass of 31 kDa but when myristoylated on the N–terminus the molecular weight on SDS gels is about 70 kDa. Myristoylation is necessary for membrane binding and is thought to occur cotranslationally. The interaction of MARCKS with lipids involves hydrophobic and electrostatic components (Taniguchi and Manenti, 1993), the latter being phosphorylation dependent. Incorporation of negatively charged phosphate groups leads to dissociation from PS-containing lipid bilayers. Irrespective of its phosphorylation state, MARCKS binds to F actin with different affinity (Hartwig et al., 1992). Since MARCKS is regulated by two important chemotactic signals, it serves as a PKC and calcium–calmodulin-regulated transducer during cell stimulation.

VI. ACTIN-BINDING PROTEIN–LIPID INTERACTIONS IN THE LEADING EDGE OF MOVING CELLS

The mechanisms by which a cell moves forward is not completely understood. We still believe that the unidirectional polymerization of actin can be utilized for vectorial force production (Isenberg et al., 1978). This hypothesis received support by the finding that the majority of actin filaments in the extreme outer edge of advancing lamellipodia is polarized with the fast polymerizing end directed towards the growing front (Small et al., 1978). Under physiological conditions the barbed end growth is favored over the pointed end growth, due to the difference in critical concentrations at each end (Wegner and Isenberg, 1983), and polymerization primarily occurs in the desired direction unless the filaments are blocked (e.g., by capping proteins, see below). Theriot and Mitchison (1991) showed that the rate of actin polymerization directly correlates with the advancement of lamellipodia (Cramer and Mitchison, 1993). Hypotheses that developed included the notion that actin alone may be sufficient as a driving force for polymerization once the reaction is nucleated at the membrane interface (Heath and Holifield, 1991a; 1991b; Rinnerthaler et al., 1991; Sheetz et al., 1992).

It is noteworthy that monomeric actin is sequestered by both profilin (see below) and thymosin β_4, the latter a highly potent actin-sequestering protein in higher eukaryotic cells (Safer, 1992). One interesting hypothesis is that release of actin monomers from thymosin β_4 is regulated by the ratio of ATP/ADP in this region, rendering actin–ADP molecules an unfavorable but available source for polymerization (Carlier, 1993; Carlier et al., 1993). An actin-nucleating protein could have a tremendous impact on these events.

A. Talin

From microinjection studies using rhodamine-conjugated actin (Wang, 1984; 1985) and from high-resolution immunofluorescent studies coupled with interference reflection contrast (Izzard and Lochner, 1980; DePasquale and Izzard, 1987; 1991), it was established that stress fiber assembly is coupled with actin polymerization, starting at discrete foci, so-called talin-rich nodes, which represent precursor structures for subsequent developing focal adhesions (Izzard, 1988). Therefore, talin is not only a focal contact protein but also involved in actin rib formation and polymerization events at the leading membrane. Talin is a true nucleating protein for actin polymerization *in vitro*: Talin binds to G–actin (Muguruma et al., 1990; Goldmann and Isenberg, 1991), it overcomes the rate limiting steps in actin assembly by facilitating actin nuclei formation and it enhances actin polymerization by favoring an increase in filament number concentration over filament length (Kaufmann et al., 1991, 1992; Goldmann et al., 1992). Although talin nucleates actin filament growth, it does not restrict assembly of actin monomers at either end, because it is not a capping protein (see below). All these features match the requirements that predict the essentials of pseudopod formation during cell locomotion (Stossel, 1989; Condeelis et al., 1992). In addition, viscoelastic measurements (Ruddies et al., 1993) show that talin induces an increase in actin filament stiffness. Such a reduction in chain dynamics may avoid repulsion between filaments due to undulation forces and thus favor their parallel arrangement (Goldmann et al., 1993).

Further evidence indicates that talin can nucleate actin filament assembly at the lipid interface by (i) driving polymerization and (ii) anchoring the newly formed filaments into the lipid bilayer (Kaufmann et al., 1992; Figure 7). Purified talin, when reconstituted into lipid vesicles, has been directly visualized by video-enhanced microscopy to facilitate polymerization of actin filaments starting at the bilayer surface and proceeding into the surrounding medium. From these experimental findings, a model was presented to explain how talin, with a minimum of binding parameters, functions in (i) driving the assembly of actin filaments, (ii) anchoring the cytoskeleton to the lipid bilayer and (iii) transmitting signals to the extracellular space via integrins (Isenberg and Goldmann, 1992).

B. Ponticulin

Ponticulin is a membrane-spanning glycoprotein that mediates actin binding and nucleation (Wuesthube and Luna, 1987; Wuesthube et al., 1989; Luna et al., 1990; Shariff and Luna, 1990; Chia et al., 1991). The 17-kDa protein is present in the outer membrane of *Dictyostelium* cells and is particularly enriched in cell–cell adhesions and arched actin-rich membrane regions, reminiscent of presumptive stages of pseudopod formation. Consistent with morphological observations, pon-

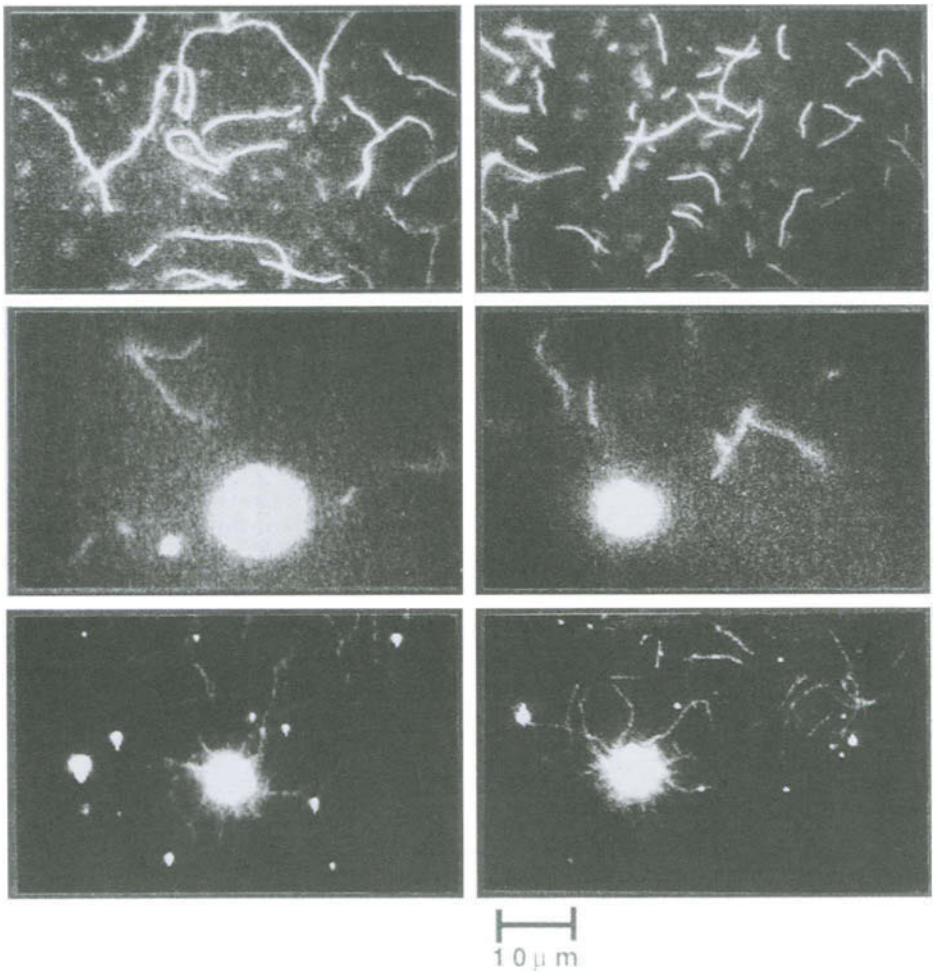

Figure 7. Fluorescent imaging of rhodamine-phalloidin-labeled actin filaments in the absence and presence of talin (top row; left to right). Polymerization of actin in the presence of lipid vesicles alone (middle row) and in the presence of lipid vesicles with reconstituted talin (bottom row). Taken from Kaufmann et al., 1992, with permission.

ticulin, like talin, triggers lateral association of actin filaments with the lipid bilayer, with both ends free for monomer assembly and disassembly (Chia et al., 1993). When ponticulin was added to commercially reconstituted lipid mixtures, it failed to nucleate actin polymerization (cf. talin, see above), but was active when incorporated into native *Dictyostelium* membrane vesicles (Chia et al., 1993). Thus, a specific lipid composition including DAG (Shariff and Luna, 1992) may be needed to effect actin nucleation by ponticulin. Alternatively, augmentation of nucleation may be achieved by dissociation of capping proteins or by activation of other

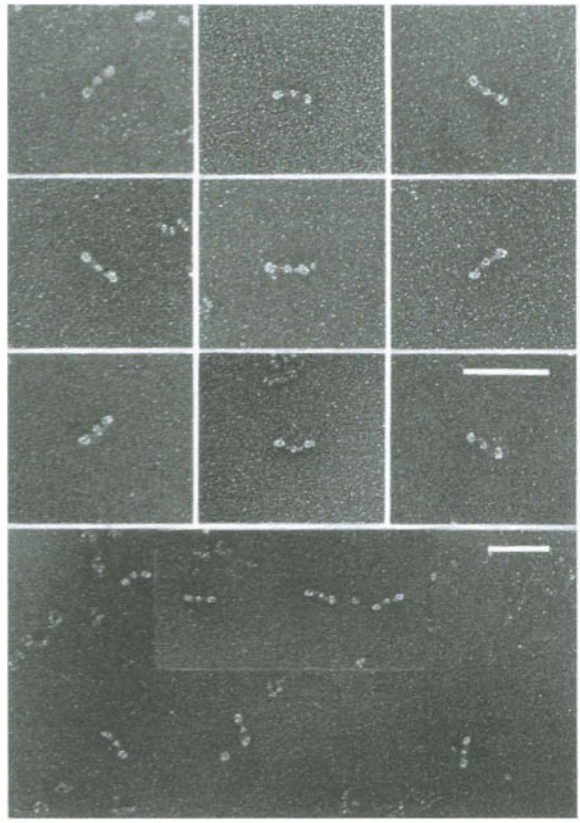

Figure 8. Glycerol-sprayed, rotary metal-shadowed human platelet talin in 50 mM Tris/HCl, 2 mM EDTA, 0.1 mM DTT, pH 8.0, at a final concentration of 0.1–0.3 mg/ml (in 30% glycerol). (**a**) Low magnification overview, and (**b**) gallery of selected talin molecules. Scale bars, 100 nm (a, b) For details see Goldman et al. (1994). Native Talin Is a Dumbbell-shaped Homodimer When it Interacts with Actin. J. Struct. Biol. 112, 3–10. Electron micrographs and montage courtesy of G. Isenberg, M. Häner and U. Aebi.

nucleating proteins. An analogue protein of ponticulin has been identified in the plasma membrane of polymorphonuclear leucocytes, which makes this protein an interesting candidate for triggering cell motility in general (Luna, 1990).

The protein hisactophilin (Scheel et al., 1989) is another protein from *Dictyostelium* involved in signal transduction by acting as a pH sensor. It is unusual that this low molecular mass (13.5 kDa) protein possesses 31 histidine residues out of 118 amino acids. Its structure was found to be similar to interleukin-1β and fibroblast growth factor (Habazettl et al., 1992). Though a lipid interaction of this actin nucleating protein is highly likely, this has yet to be demonstrated.

VII. CAPPING AND SEVERING PROTEINS

A. Capping Proteins: Cap 32/34; Cap 100; gCap 39

The first actin filament capping protein ever identified was isolated from *Acanthamoeba* (Isenberg et al., 1980). The protein consists of two polypeptides (32 and 34 kDa). It is ubiquitous and was isolated from bovine brain (Kilimann and Isenberg, 1982), *Dictyostelium* (Schleicher et al., 1984) and skeletal muscle (Casella et al., 1986). The skeletal muscle analogue of this protein was later renamed CapZ (Casella et al., 1989; Heiss and Cooper, 1991). Capping proteins bind to the fast-growing end of actin filaments and inhibit polymerization. Since this is the membrane facing end in the outer edge of protruding cells (lamellipodia) capping proteins have been investigated with respect to their potential interaction with phospholipids. Membrane localization, amphitropic behavior or reconstitution into lipid model membranes has not been reported for any of the known capping proteins. However, binding to PIP-2 is common to most capping proteins from various sources and is inhibitory to their function. The heterodimeric capping proteins (Cap 32/34) (Heiss and Cooper, 1991; Haus et al., 1991), Cap-100, a novel capping but nonnucleating protein from *Dictyostelium* (Hofmann et al., 1992), and g-Cap 39, the Ca^{++}-regulated phosphoprotein (Yu et al., 1990; Onoda and Yin, 1993) belong to this group.

B. Gelsolin

Gelsolin is an actin-binding protein that interacts with actin in several ways (Stossel et al., 1985; Yin, 1988; Stossel, 1990): (i) it binds to actin monomers and stimulates the formation of actin nuclei, (ii) it acts as a capping protein at the barbed ends and (iii) it severs actin filaments in a Ca^{++}-dependent manner. PIP-2, and to a less extent PIP, have been found to inhibit the Ca^{++}-dependent-severing activity of gelsolin specifically (Jamney and Stossel, 1987; Janmey et al., 1987). Moreover, it was shown that PIP-2 micelles dissociate the EGTA-resistent 1:1 gelsolin–actin complex and restore its severing activity (Janmey and Stossel, 1987; Yin et al., 1988). Since EGTA reacts with the Ca^{++}-sensitive actin-binding domain in the C-terminal half of gelsolin (Kwiatkowski et al., 1985; Chaponnier et al., 1986), it follows that the actin-binding site, which is inhibited by PIP-2, is distinguishable from the Ca^{++}-sensitive actin-binding domain. By analyzing proteolytic peptides in respect to their function (Yin et al., 1988) and by deletional mutagenesis, using COS cells and *Escherichia coli* to produce truncated plasma gelsolin after DNA transfection (Kwiatkowski et al., 1989; Way et al., 1989), the following model for the domain structure has emerged. Gelsolin potentially has three actin-binding sites (Yin et al., 1988; Bryan, 1988; Way et al., 1989). Ca^{++}-regulation of the intact gelsolin molecule as well as Ca^{++}-sensitive actin binding involved in nucleation, occurs in the C-terminal half which itself undergoes a Ca^{++}-induced conformational

change. The two other actin-binding sites are located in the NH_2-terminal half of the molecule. CT 17 N (residues 1–149) contains a high-affinity binding site for actin monomers and filament ends, whereas CT 28 N (residues 150–406) contains the only PIP-2 inhibited binding site for actin molecules arranged in a filament (Kwiatkowski et al., 1986; Bryan, 1988; Kwiatkowski et al., 1989). Although severing by the NH_2-terminal half-fragment is inhibited by PIP-2 (Janmey et al., 1987), the PIP-2 binding fragment CT 28 N by itself does not have severing activity (Yin et al., 1988). Deletion of 79% from the C-terminal end yields a 160-amino-acid fragment (PG 160) that severs and is PIP-2 regulated. Hence by exclusion, the PIP-2 binding site must reside in a small sequence of about 11 amino acids (residues 150–160) (Bryan, 1988; Yin et al., 1988; Kwiatkowski et al., 1989). The recent published structure of gelsolin–segment-1-actin complex at 2.5 angstroms resolution (McLaughlin et al., 1993) supports this conclusion. This region contains the predicted hydrophobic sequence (Janmey and Stossel, 1987) capable of interacting with the acyl chains of the phospholipid as well as certain basic residues which have been suggested to bind to the negatively charged phosphate groups on PIP-2 (Kwiatkowski et al., 1989).

Like profilin, gelsolin binds to clusters of PIP-2 molecules (Janmey and Stossel, 1987). Studies by the same authors (1989) suggest that the physical state of phosphatidylinositolphosphates within the membrane is important for its inhibitory effects; even at low concentrations in mixed lipid vesicles (e.g., PC containing vesicles) PIP-2 fully inhibits gelsolin, provided that diffusion-limited clustering can occur and that the exposure of hydrophilic phosphatidylphosphoinisitol headgroups is facilitated (cf. talin–lipid interaction above). The latter can efficiently be hindered (i) by trapping into multilamellar lipid sheets, (ii) by the formation of hexagonally packed quasi-crystals of PIPs induced by divalent cations or neomycin and (iii) by specific high-affinity binding to other PIP-2 binding proteins, such as profilin. Quasi-elastic light scattering data (Janmey and Stossel, 1989) also suggest that PI incorporation directly influences the PC diffusion constant and hence affects the phospholipid arrangement and the overall membrane structure. It should, therefore, be intriguing to investigate the long-range effect of PIP-2 within the membrane on proteins that do not bind directly to phosphoinositolphosphates but interact in a hydrophobic or amphiphilic way with other lipid bilayer components.

C. Severin, Fragmin, Villin, Cofilin

Severin (40 kDa), fragmin (42 kDa) and villin (95 kDa) are F-actin fragmenting proteins with extensive sequence homologies to gelsolin (André et al., 1988). In structure and function severin is regarded as a prototype of gelsolin (Yin et al., 1990) since both proteins stem from an ancestral gene, from which gelsolin has been derived by duplication. Regulation by phosphoinositols *in vitro* is common to all three proteins. Severin has at least two PIP-2 binding sites since the activity of the two nonoverlapping severin fragments (domain 1 and 2+3) are strongly inhib-

ited by PIP-2 (Eichinger and Schleicher, 1992). gCap39, which has 49% sequence homology with gelsolin and is not inhibited by PC, PE, PS, PI and IP_3 (Yu et al., 1990). In contrast Eichinger and Schleicher (1992) reported that PC and PC/PE vesicles as well as PC and PE/PS vesicles had no or only slightly an inhibitory effect on severing activity; however, vesicles composed of PC and PS surprisingly do have inhibitory effects! The same authors also reported a pH-dependence for severin and lipid interactions. Hence, the severin activity *in vivo* could be modulated by PIP-2, pH and membrane lipid composition.

The brush border protein villin has been reported to partition into the hydrophobic (Glenney and Glenney, 1984) and hydrophilic phases (Conzelman and Mooseker, 1986), depending on whether detergents are included in the isolation protocol. Cap-100 (Hofmann et al., 1992) is highly homologous to villin and appears to be a premature villin-type protein (protovillin) (Hofmann et at., 1993). Since Cap-100 binds to PIP-2, also villin might be regulated by phosphoinositolphosphates. Cofilin is also inhibited by PIP-2 (Yonezawa et al., 1990).

VIII. THE F-ACTIN CROSS-LINKING PROTEINS: SPECTRIN AND FILAMIN

The role of spectrin in red cell cytoskeleton–membrane interactions is reviewed in this Volume by Niggli. Evidence for electrostatic coupling of spectrin to charged phospholipids was obtained by analyzing the penetration into lipid monolayers and by evaluating the shifts of phase transitions and phase boundaries of lipid mixtures in the presence of spectrin (Mombers et al., 1980; Maksymiw et al., 1987). The selective binding of spectrin to negatively charged phospholipids (PS and PG) results from locally clustered positive charges along the folded spectrin polypeptide chain, although the whole protein has a net negative charge. Hydrophobic interactions with PC and PE are probable because hydrophobic ligands have been shown to act as strong quenchers for intrinsic protein fluorescence (Isenberg et al., 1981). *In situ* labeling of erythrocyte spectrin was achieved by application of the hydrophobic label phenylisothiocyanate (Sikorski and Kuczek, 1985). Interaction with lipid bilayers is facilitated by covalently bound palmitate; however, only a small fraction of spectrin was shown to be ^{3}H-palmitoylated (Mariani et al., 1993).

Filamin is a high molecular weight (250 kDA) actin-binding protein, the analogue of which in nonmuscle cells is known as actin binding protein (ABP) with a corresponding molecular weight of 280 kDa. The molecular design and function of filamin are summarized elsewhere (Hartwig and Kwiatkowsky, 1991; Small et al., 1992). In smooth muscle where filamin is an abundant protein, it is arranged in an alternative pattern with vinculin and dystrophin in regions closely aligned beneath the plasma membrane. Initial indications for filamin–lipid interactions were obtained by the work of Furuhashi et al. (1992). These authors reported inhibition of filamin–actin interaction and consequently an inhibition of gelation

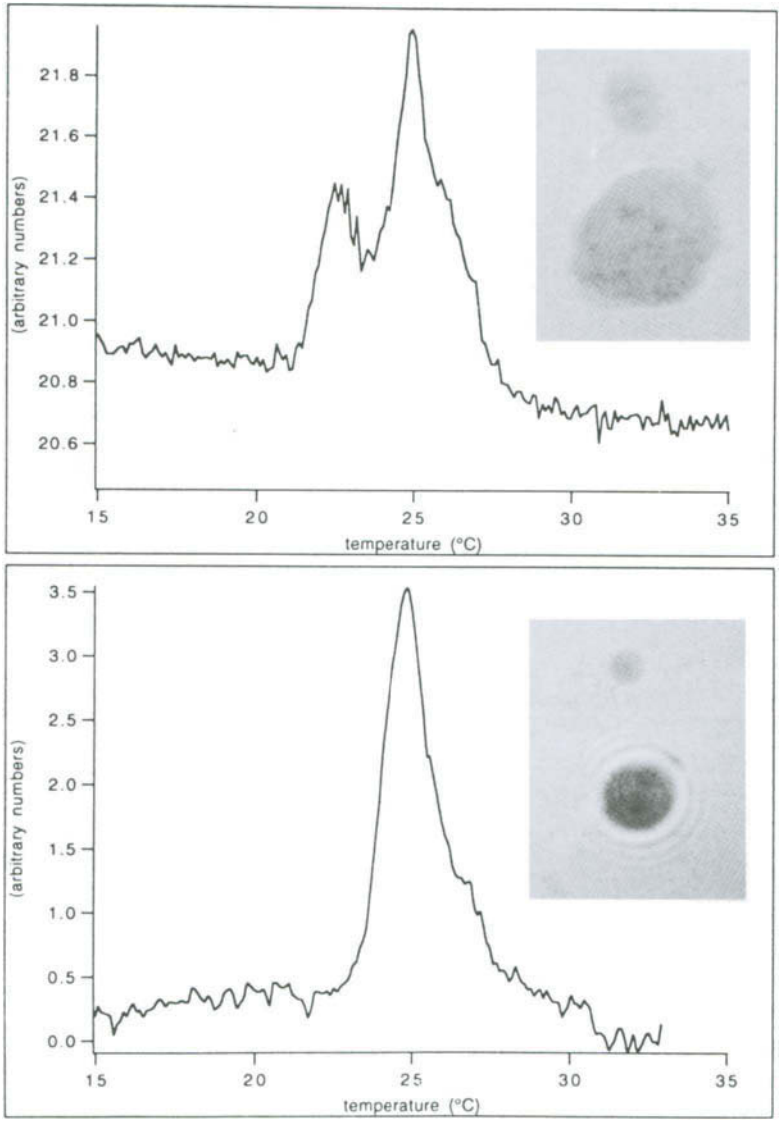

Figure 9. Differential scanning calorimetry (DSC). Top picture: DMPG/DMPC (1:1) in the absence of filamin. Bottom picture: DMPG/DMPC (1:1) in the presence of filamin. Inset: Corresponding phase contrast microscopy of vesicles (-/+) filamin. Taken from Goldmann et al., 1993, with permission.

upon incubation with phosphoinositols in the order PIP-2 > PIP > PI. Three mol PIP-2/mol filamin were sufficient to achieve complete inhibition of filamin cross-linking, which compares to the inhibition of capping proteins (see above). In addition, there is sound evidence from work in this laboratory that filamin not only binds to phosphoinositols in solution but also interacts directly with lipid membranes (Goldmann et al., 1993; Tempel et al., 1994). DSC measurements in combination with hydrophobic labeling and film balance studies show insertion of filamin into the lipid bilayer when reconstituted into vesicles and lipid monolayers. This lipid–filamin interaction is highly charge dependent as hydrophobic interactions diminish with rising salt concentrations. Filamin incubation with lipid vesicles (DMPG/DMPC, 1:1) leads to the formation of condensed rounded-up vesicles with a smooth surface when viewed under the light microscope. This observation agrees with similar effects described for PC and PE vesicles loading from the inside with actin–filamin mixtures (Cortese et al., 1989). The possibility that actin-binding proteins influence shape and deformability of lipid layers is significant for cellular shape changes and pseudopod formation.

IX. ACTIN-SEQUESTERING PROTEINS

A. Profilin

Some of the pioneer work concerning actin-binding proteins/lipid interactions was performed on profilin, an actin monomer sequestering protein (cf. Pollard and Cooper, 1986).

Profilin inhibits actin polymerization by binding to actin monomers and to a lesser extent to actin filament ends (Pollard and Cooper, 1986). Profilin and non-muscle actin monomers form a high affinity complex *in vitro* and *in vivo* (K_d ~10-400 nM). Lassing and Lindberg (1985) were the first to demonstrate that this high affinity complex can be dissociated by phospholipids. After preincubation with various lipids at low salt concentration, the effectiveness in dissociating the profilin-actin complex *in vitro* decreased in the following order of phospholipids: PIP-2 > PA > PS > PI > PG. Under physiological ionic conditions (80 mM KCl and Ca^{++}-concentrations below 10^{-5} M) only PIP-2 effectively dissociates the profil-actin complex. PIP, when reconstituted in lipid bilayers is much less active and PI, as well as the cationic phospholipids (PC and PE), is inactive (Lassing and Lindberg, 1988a; Goldschmidt-Clermont et al., 1990). From these results it was suggested that the onset of actin polymerization which is frequently observed to occur upon cell stimulation could be accounted for by increased production of PIP-2, a subsequent binding to the profilin-actin complex at the membrane interface and the liberation of G-actin (Lassing and Lindberg, 1988b).

More recently, the binding of profilin to PIP-2 has been studied in greater detail (Goldschmidt-Clermont et al., 1990). Large unilamellar vesicles (LUVETs) (Mayer

et al., 1986) were used to determine the binding stoichiometry of profilin to PIP-2, which by nuclear magnetic resonance (Van Paridon et al., 1986) has been shown to partition between the two leaflets of such vesicles. According to this report (Goldschmidt-Clermont et al., 1990) profilin binds to reconstituted PIP-2 with a submicromolar affinity ($K_d < 0.1$ μM in pure PIP-2 micelles) and a stoichiometry of 1 : 7 or with a molar ratio of 1 profilin per 5 PIP-2 molecules and a $K_d < 1.0$ μM in vesicles containing PIP-2 and PC in a molar ratio of 1 : 5. Profilin probably does not interact hydrophobically with the acyl chains of the inositol glycerol backbone (Goldschmidt-Clermont et al., 1991) but interacts by a cluster of basic residues close to its COOH-terminal (Goldschmidt-Clermont et al., 1990). Since the actin-binding site is also localized within this sequence region, PIP-2 and actin can compete for profilin binding. However, with equal concentrations of monomeric actin and PIP-2 (at least in platelets; 140–240 mM) and an affinity, which is up to 10-fold higher for profilin than for actin, a large amount of the membrane integrated PIP-2 is probably bound to profilin (Goldschmidt-Clermont et al., 1991). In support of this notion is the demonstration using electronmicroscopy and immunolocalization that in platelets the membrane association of profilin reversibly increases upon activation (Hartwig et al., 1989). Most interestingly, the profilin-PIP-2 interaction is not only involved in actin regulation but also interferes with the cytosolic phospholipase C-catalyzed PIP-2 hydrolysis (Goldschmidt-Clermont et al., 1990) which normally leads to the generation of inositoltriphosphate (IP3) and diacylglycerol, a potent activator of protein kinase C. Profilin bound to PIP-2 is a negative regulator of phospholipase C (PLC) activity. Since the cycle of inositol turnover has to be complete there must also exist a mechanism which overcomes this inhibitory effect of profilin upon phospholipase C. Goldschmidt-Clermont et al. (1991) have found that PLC-γ, when phosphorylated by the EGF receptor can effectively compete with profilin for PIP-2 binding and thereby switches the system on. Hence, PIP-2 hydrolysis, dissociation of the profilin-PIP-2 complex and profilin-actin complex formation could be the reversal steps following cellular stimulation.

X. MOLECULAR MOTOR PROTEINS

A. The Myosin I Family

Myosin I, which was first identified in Acanthamoeba (Pollard and Korn, 1973), represents a new class of mechanoenzymes necessary for actin-based motility (Korn and Hammer, 1988, 1990; Adams and Pollard, 1989a). Myosin I isolated from Acanthamoeba (Adams and Pollard, 1989b; Miyata et al., 1989; Doberstein and Pollard, 1992; cf. Pollard et al., 1991) and brush border membranes (Hayden et al., 1990) has been shown to interact with membrane lipids. Three independent groups have reported that myosin I binds to salt-treated 'stripped' plasma membranes devoid of actin and myosin as well as to pure, negatively charged pho-

pholipid vesicles. In all cases myosin I follows saturation kinetics with an overall binding capacity which exceeds that of actin several fold. Apparent dissociation constants were found to range between 0.3–0.5 $\times$ 10^{-7} M for the binding of Acanthamoeba myosin I to KI stripped plasma membranes (Miyata et al., 1989) and 1.4–3.0 $\times$ 10^{-7} M for the binding of myosin I to NaOH stripped membranes and to phospholipid vesicles with selected lipid compositions (Adams and Pollard, 1989b; Hayden et al., 1990; this reflects the higher affinity of myosin I for lipids than for pure F-actin. No evidence was presented that myosin I binds to neutral phospholipids such as phosphatidylcholine (PC). Instead, myosin I associates with liposomes containing phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylinositol (PI) or PIP-2 or mixtures of these anionic lipids with neutral ones at an estimated ratio of 4–5 pmol protein / nmol phospholipid; this is similar to that of other protein-lipid interactions.

Since a high ionic strength is required to solubilize myosin I from membranes (Miyata et al.,1989), it has been questioned if this salt treatment could lead to an artificial exposure of basic sequences which could in turn favor an electrostatic interaction of myosin I with membranes. However, a major fraction of the myosin I, including its associated kinase, is also linked to membranes *in situ* (Kulesza-Lipka et al.,1991; Baines et al., 1992). Interestingly, the kinase, which itself is activated by a phospholipid enhanced autophosphorylation *in vitro* (Brzeska et al., 1990, 1992), is no longer activated by phosphorylation when operating in a membrane-bound form, whereas lipids still stimulate myosin I phosphorylation through this kinase (Kulesza-Lipka et al., 1993). This example convincingly demonstrates that (i) the myosin-I-lipid interaction is not merely electrostatic and (ii) specific protein–lipid interactions exist and these may differ, depending on whether the purified components are mixed in solution, reconstituted into lipid-bilayers or react as constituents of purified plasma membranes.

XI. OTHERS

In the nervous system the vesicle specific phosphoprotein synapsin I is a lipid-and-actin binding protein. Synapsin I acts as a phophorylation-dependent, actin nucleating protein *in vitro* (Bähler and Greengard, 1987; Petrucci and Morrow, 1987; Valtorta et al., 1992; Benfenati et al., 1992). The proline-rich hydrophobic head region of the molecule inserts into the hydrophobic core of lipid membranes and simultaneously reacts with acidic phospholipids (Benfenati et al., 1989 a,b; Südhof et al., 1989; Benfenati, personal communication).

Hydrophobic and electrostatic interactions with phospholipids (PS) have also been measured for caldesmon (Vorotnikov and Gusev, 1990; Czurylo et al., 1993), an actin-binding regulatory protein in smooth muscle contraction. The near future will show whether the cytoskeleton and membrane associated members of the

rho-family (small GTP-binding proteins) and the connected activating proteins (e.g. GAP) interfere with membrane lipids (Ridley & Hall, 1992).

Finally, enzymes like 5′-nucleotidase and proteins from the erythrocyte membrane skeleton such as protein 4.1 appear to interact with both actin and membrane components (see Niggli, this volume and previous reviews: Isenberg, 1991; Luna & Hitt, 1992).

XII. CONCLUSIONS

It is surprising how many of the known actin-binding proteins can bind to lipids (see Table I). It also appears likely that some of these actin-binding proteins whose lipid-binding properties have not been investigated the ezrin-radixin-moesin family, ERM; Algrain et al., 1993, zyxin; Sadler et al., 1992, or the membrane associated protein CD43; Yonemura et al., 1993, may have to lipid-binding properties.

Table 1. Actin and Lipid Binding Proteins

Protein	*Molecular weight (kDa)*	*Origin*	*Lipid*
Talin	269	vertebrates	PS;PG;PC
Vinculin	116	vertebrates	PS;PG;PI;PA
α-Actinin	200	higher and lower organisms	PA;DG;PIP-2
MARCKS	31 (70)	vertebrates	PS;PC
Ponticulin	17	*Dictyostelium*	DAG
Cap 32/34	32/34	*Acanthamoeba, Dictyostelium,* brain, skeletal muscle etc.	PIs
Cap 100	100	*Dictyostelium*	PIP-2
gCap 39	39	macrophages	PIP-2
Gelsolin	90	vertebrate cells	PIP-2;PIP
Severin (Fragmin)	42	lower eukaryotic cells	PIP-2;PIP;PC/PS
Villin	95	vertebrates	PIs;acidic phospholipids
Cofilin	15–20	vertebrates	PIs
Spectrin	240 (2x)	erythrocytes	PS;PG;PE
Filamin	250–270	smooth muscle	PS;PG
Profilin	12–15	higher and lower organisms	PIP-2;PIP
Myosin I	110–140	higher and lower organisms	PG;PS;PI;PIP-2
Protein 4.1	78	erythrocytes	PS;PIP-2
Synapsin	80/86	brain	acidic phospholipids
Caldesmon	70–80	smooth muscle, non-muscle cells	PS
5′-Nucleotidase	71	eurkaryotic cells	GPI

Note: *Abbreviations*: PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PA, palmitic acid; DAG, diacylglycerol; PI, phosphatidylinositol; PIP-2, phosphatidylinositol-4,5-biphosphate; PPI, phosphatidylphosphoinositol; IP3, inositoltriphosphate; GPI, glycosylphosphoinositol.

Claims have been made that most of the lipid binding capacity is artefactual and of no biological relevance. As we have tried to point out, lipid interactions, however, are not as trivial as they appear at first glance. Clearly, investigation of lipid-binding in solution is of limited interest since one has to expect that a charged protein with hydrophobic pockets will readily interact with polarized lipid molecules.

On the other hand, one has to realize that a group of actin-binding proteins primarily binds phosphoinositolphosphates and is regulated by these lipids in its functions. Hence, there exists *specificity*. We have pointed out the possibility that lipid interactions can be modulated by the presence of additional proteins. This stresses the factor of *competition* and *selective binding*. We know that even a reconstituted bilayer system may not be sufficient enough to mirror the *in vivo* situation in biological membranes. Many other factors including physical factors such as diffusion rates, curvature, lateral pressure and microviscosities will certainly be important in determining the biological role of actin-binding protein/lipid interactions inside a cell.

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