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NEWS AND VIEWS

Actin-membrane coupling: a role for talin

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The principle of apparent redundancy which governs cellular organization and motility, as reflected by the variety of actin-binding proteins and diverse cytoplasmic motors, probably also holds true for mechanisms responsible for coupling cytoskeletal proteins to membranes (Isenberg, 1991).

When we focus on talin, a new candidate for nucleating and linking actin filaments to plasma membranes, one should be aware that we have selected only one of several possible mechanisms of interest.

By naming a new protein found in adhesion plaques and ruffling membranes 'talin' (derived from the latin word talus = ankle), Burridge and Connell (1983a,b) were keen to envisage a possible function for this protein: namely to establish a link between the cell skeleton and adhesion zones.

Efforts directed towards talin in the 1980s concerning its localization and interaction with other proteins have been reviewed by Burridge *et al.* (1988) and Beckerle and Yeh (1990). Talin has mostly been isolated from platelets or chicken gizzard. The high molecular weight protein folds into a rod-like molecule with the tendency to form protein dimers (Molony *et al.*, 1987). Its apparent molecular weight on SDS-gels is 225–235 kDa; however, the actual molecular weight deduced from the primary sequence (Rees *et al.*, 1990) is 269,85 kDa. A relevant function for talin in establishing a transmembrane linkage became obvious when the interaction with two prominent adhesion plaque proteins; vinculin (Burridge & Mangeat, 1984) and integrin (Horwitz *et al.*, 1986) could be demonstrated *in vitro*. Since vinculin is now accepted as an actin-binding protein (Isenberg *et al.*, 1982; Ruhnau & Wegner, 1988; Westmeyer *et al.*, 1990), a hypothetical linker cascade could involve the proteins integrin–talin–vinculin–actin (from the outside to the inside) or alternatively integrin–α-actinin–actin (Pavalko *et al.*, 1991).

A more direct way of interaction in favour of the previously suggested multi-protein links (c.f. Burridge *et al.*, 1990) becomes more attractive, since it was recently shown that talin can directly bind to actin (Muguruma *et al.*, 1990, 1992; Goldmann & Isenberg, 1991; Kaufmann *et al.*, 1991, 1992; Goldmann *et al.*, 1992).

Once the amino acid sequence of talin was determined (Rees *et al.*, 1990) some first predictions concerning its structural domains could be made (Rees *et al.*, 1990; Turner & Burridge, 1991; Critchley *et al.*, 1991). Talin can be cleaved into a 47 kDa and a 190 kDa fragment by the endogenous calpain II protease (Fox *et al.*, 1985; Beckerle *et al.*, 1987). The 47 kDa subdomain carries the N-terminal containing regions of sequence homologous with the membrane binding regions of band 4.1 and ezrin. It was therefore speculated that the N-terminal of talin contains the membrane binding site. However, both the 47 kDa and the 190 kDa talin subunits redistribute into focal contacts after microinjection (Nuckolls *et al.*, 1990). Since lipid and protein interactions may be involved in this reassembly and since these interactions for both subdomains have not been fully investigated individually, the actual membrane-binding domain awaits its precise characterization.

Defining protein-binding domains on the basis of linear sequence may not be sufficient to explain the whole binding process, which involves protein folding. This may become relevant by looking at the talin–integrin interaction. *In vitro* binding assays suggest that the integrin binding site is localized on the 190 kDa fragment (Horwitz *et al.*, 1986), though an interaction of integrin with the N-terminal membrane binding domain seems more plausible. We have attempted to resolve this in our current model (see Fig. 1). Using talin fusion proteins, Critchley *et al.* (1991) were able to map at least one binding site for vinculin within the last 500 amino acid residues of the 190 kDa carboxy-terminal end of the talin sequence. Vinculin, on the other hand, was shown to expose one talin binding site along the first 258 residues of the N-terminal 90 kDa globular head fragment (Jones *et al.*, 1989; Critchley *et al.*, 1991). This interaction is also included in our model (Fig. 1). Following the arguments of Muguruma *et al.* (1990), it is tempting to speculate that the actin binding site is localized within the 190 kDa carboxy-terminal talin fragment (c.f. Fig. 1).

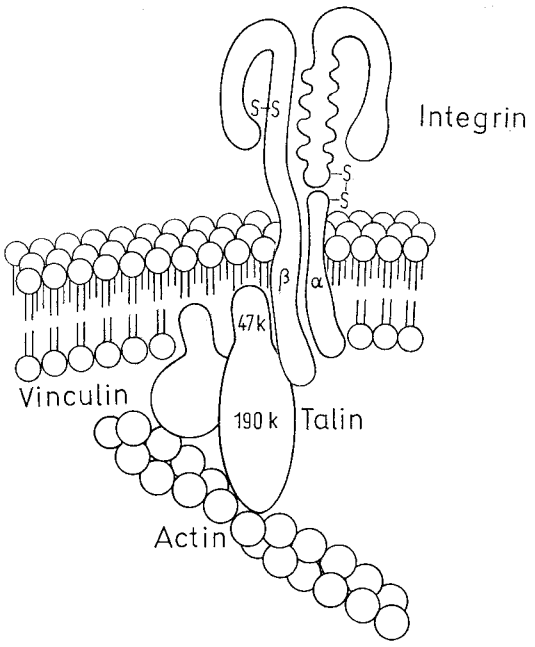

Fig. 1. Talin interactions at the plasma membrane: a model deduced from experimental data. Talin, like vinculin, interacts with membrane lipids and partially inserts into the hydrophobic part of the lipid bilayer. Extracellular matrix junctions may occur through integrin binding, involving the 190 kDa subunit. In addition, this subunit on the cytoplasmic site may bind to vinculin (an actin-binding protein) and actin, facilitating nucleation and actin filament assembly.

Since it was shown that talin preferentially interacts with negatively charged phospholipids (Heise *et al.*, 1991), it is of interest to establish whether the highly charged carboxy-terminal segment, which is shared among the three membrane binding proteins (talin, band 4.1 and ezrin), is of relevance.

Talin becomes phosphorylated on serine and threonine residues by protein kinase C (Lichtfield & Ball, 1986; Beckerle, 1990) and on tyrosine residues in Rous-sarcoma-virus-transformed cells by the tyrosine kinase $p60^{v\text{-}src}$ (Pasquale *et al.*, 1986). The precise phosphorylation sites have not yet been mapped. There are indications (Burn *et al.*, 1988) that phosphorylation of talin determines its transmembrane linkage with integrins.

The unique properties of talin may allow it to act as a key protein during protrusion events in leading lamellae of moving cells. Talin is a true nucleating protein for actin polymerization (Kaufmann *et al.*, 1991; Goldmann *et al.*, 1992): it binds to G-actin, it overcomes the rate limiting step in actin assembly by facilitating actin nuclei formation and enhances actin polymerization by favouring an increase of filament number concentration over filament length. Talin, although it nucleates actin filament growth, does not restrict assembly of actin monomers at either end since it is not a capping protein.

In this respect talin exactly matches the requirements which have been predicted to be essential for pseudopod formation during cell movement (Stossel, 1989; Condeelis, 1992). Indeed, talin was found "to be concentrated without exception at the tip of each motile F-actin 'rib', the earliest precursor structure of actin bundles in the extreme edge of a ruffling membrane" (Izzard, 1988; DePasquale & Izzard, 1991).

The long existing hypothesis of vectorial force production by the unidirectional polymerization of actin (Isenberg *et al.*, 1978) is supported by the morphology showing a single polarity of actin filaments in this region of cells (Small *et al.*, 1978) and the observation that barbed-end growth is favoured over pointed-end growth due to the difference in critical concentrations at each end (Wegner & Isenberg, 1983).

The elegant work of Theriot and Mitchison (1991) shows that actin polymerization directly correlates with the advancement of lamellipodia supporting this long-standing view. Though different, but in a sense equivalent, Tilney *et al.* (1992a,b) have recently described how actin polymerization could provide a driving force for intracellular movement of the bacterium *Listeria* once filaments become nucleated at the membrane surface. These findings support the notion that actin alone may be the driving force as soon as it becomes nucleated at the membrane interface (c.f. Heath & Holifield, 1991a,b; Rinnerthaler *et al.*, 1991).

De novo formation of actin nuclei at membranes (rather than uncapping of pre-existing actin filament ends) seems to occur in *Dictyostelium* (Shariff & Luna, 1992). In the search for some magic nucleation factors, talin may prove to be adequate, i.e. it does the right thing in the right place. So, why not consider talin as a key protein in mediating actin assembly at cell membranes?

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