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NEWS AND VIEWS

Vinculin, talin and focal adhesions

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One of the most complicated connections between actin and the plasma membrane is the focal adhesion, a complex of proteins and lipids that forms at sites where cells attach to the extracellular matrix. A major protein component of focal adhesions is vinculin. As with other components of the focal adhesion complex, vinculin illustrates the apparent redundancy of proteins that mediate the connection of actin to the plasma membrane. During the past 5 years there have been several studies examining the role of vinculin in cell function. The purpose of this article is to discuss these findings and present an integrated model of vinculin's role in the cell.

Vinculin associates with talin and alpha-actinin via its N-terminal region (Burridge & Mangeat, 1984; Wachsstock *et al.*, 1987). It also self-associates to form head-to-tail dimers (Molony & Burridge, 1985; Johnson & Craig, 1994). There is further convincing evidence that vinculin contains an actin-binding domain (Menkel *et al.*, 1994; Johnson & Craig, 1995). Vinculin is also a ligand for the focal adhesion complex protein paxillin (Turner *et al.*, 1990). Furthermore, vinculin binds phospholipid bilayers non-covalently with an apparent two-step mechanism involving both electrostatic interactions with acidic head groups and insertion into the hydrophobic domain of lipid bilayers (Niggli & Burger, 1987). It has also been shown in *in vitro* lipid photolabelling studies that vinculin as well as talin directly inserts into the hydrophobic region of lipid bilayers (Goldmann *et al.*, 1992; Niggli *et al.*, 1994). In addition, vinculin interacts with phosphatidylinositol-4,5-bisphosphate (Fukami *et al.*, 1994). Therefore, vinculin binds the lipid bilayer directly through one of several mechanisms and also binds actin directly. Alternatively, vinculin may also bind to one of several proteins such as talin or alpha-actinin, which themselves may bind either directly to lipids or to the intracellular domain of integrin. These proteins or their complexes may then bind actin (Kaufmann *et al.*, 1992; Fritz *et al.*, 1993).

Chicken (82% cDNA; 95% protein), human (100% cDNA; 100% protein), and mouse (92% cDNA; 99% protein) vinculin have been cloned which show the indicated similarities between species (Price *et al.*, 1987; Weller *et al.*, 1990; Coll *et al.*, 1995). Both chicken and human vinculin contain 1066 amino acids. There is a single vinculin gene, but a difference in mRNA splicing (at amino acid 915) gives rise to the 150 kDa metavinculin variant in muscle cells. In nematode muscle, a vinculin homologue, identified by its amino acid sequence as similar to chicken vinculin, has been localized to dense plaques in the body wall muscle (Barstead & Waterston, 1989). The central region of vinculin contains three repeats of approximately 113 amino acids. The region N-terminal to the repeat region contains the talin binding domain. This domain is highly conserved. The C-terminal region to the repeats contains a proline-rich sequence thought to be important in separating the globular head of vinculin from its extended tail (Price *et al.*, 1989). The last 170 amino acids are thought to be important in the ability of vinculin to self-associate (Milam, 1985). The C-terminal of chicken vinculin has a pI of 9.7, compared with a pI of 5.9 for the intact protein (Coutu & Craig, 1988). This is thought to explain the ability of vinculin to interact directly with acidic phospholipids, and probably with the plasma membrane (Tempel *et al.*, 1995). Vinculin, as well as

integrin and paxillin, are all substrates for tyrosine phosphorylation by $pp60^{-src}$ (Sefton *et al.*, 1981; Maher *et al.*, 1985; Glenney & Zokas, 1989).

The role of vinculin *in vivo* has been studied in several species and cell lines. This is summarized in Table 1. Barstead and Waterston (1991) were the first to examine vinculins' function using a genetic screen designed to recover mutations in the vinculin gene of the nematode *Caenorhadbitis elegans*. Nematodes lacking vinculin had arrested development and disorganized muscle tissue indicating that vinculin is essential for normal muscle function. Rodriguez Fernandez and colleagues (1992) then showed that overexpression of vinculin in mouse 3T3 cells reduced cell locomotion. In another study, Rodriguez Fernandez and colleagues (1993) reduced vinculin expression in 3T3 cells using antisense constructs. The transfected cells exhibited a round phenotype with fewer vinculin-positive focal contacts, and displayed increased motility. Grover and colleagues (1987) treated mouse F9 embryonic carcinoma cells with the mutagen ethanemethylsulfonate to produce a adhesion-defective cell line, called 5.51. Samuels and colleagues (1993) found that 5.51 cells lacked vinculin and showed that transfection of chicken vinculin restored cell adhesion and normal actin organization. (Full restoration of cell spreading was not achieved probably because other genes were also mutated, for example, uvomorulin expression is reduced to 30–40% of normal in these cells; Adamson *et al.*, 1990). Goldmann and colleagues (1995) showed that 5.51 cells contained β 1-integrin, talin, and α-actinin that were localized in patches associated with the plasma membrane. They also observed that filopodia and lamellipodia in 5.51 cells were less stable in comparison to the wild-type cells. Varnum-Finney and Reichardt (1994) generated vinculin-deficient isolates of PC12 cell lines by transfection with vectors expressing antisense vinculin RNA. They showed that neurite outgrowth on laminin was reduced and that the formation of filopodia and lamellipodia was normal but less stable compared to PC12 control cells. Recently, Coll and colleagues (1995) created a F9 cell variant (called F9vin(−/−)) in which both copies of the vinculin gene were disrupted using homologous recombination. The loss of vinculin in these cells resulted in rounded morphology, decreased adhesion, and increased motility. Interestingly, the F9vin(−/−) cells formed focal adhesions which, based on fluorescence intensity measurements, contained larger amounts of talin, alpha-actinin, and paxillin (Volberg *et al.*, 1995).

All of these studies suggest that vinculin is important for cell attachment and spreading. The presence of focal adhesion complexes in both of the vinculin-deficient F9 cells lines (5.51 and F9vin(−/−)) suggest that there are other mechanisms for the formation of focal adhesions in the absence of vinculin. A likely candidate for a focal adhesion complex protein to be involved in an alternative linkage is talin (Isenberg & Goldmann, 1992). The differences in the ability of vinculin and talin to self-associate and interact with other cytoskeletal proteins might be the key. For example, both talin and vinculin form dimers (Goldmann *et al.*, 1994; McLachlan *et al.*, 1994; Johnson & Craig, 1995). Nuckolls and colleagues (1992) microinjected antibodies against talin into fibroblasts which inhibited spreading and migration, and disrupted recently formed focal adhesions and stress fibres. The binding

Table 1. Modification of vinculin in various cell lines and the phenotypical expression.

Organism/cell type	*Mechanism of disruption*	*Phenotype*
Nematodes *Caenorhadbitis elegans* (Barstead & Waterston, 1991)	Vinculin knockout	Elongation disrupted; disorganized muscle
BALB/c 3T3 cells (Rodriguez Fernandez *et al.*, 1992)	Vinculin overexpression	Altered dynamic properties
BALB/c 3T3 cells (Rodriguez Fernandez *et al.*, 1993)	Vinculin antisense transfection	Round with smaller and fewer plaques
Embryonal carcinoma cells F9 (5.51) and	Vinculin chemically mutagenized	Round, no lamellipodia and actin stress fibres
Embryonal carcinoma cells F9 (5.51 vin3+4) (Samuels *et al.*, 1993; Goldmann *et al.*, 1995)	Exogenic vinculin gene expression	Exhibit actin stress fibres, filopodia, and lammelipodia
PC 12 neurite cells (Varnum-Finney & Reichardt 1994)	Vinculin antisense transfection	Exhibits less stable filopodia and lamellipodia
Embryonal carcinoma cells F9 (γ229)	F9 vinculin knockout	Round, locomotion
(Coll *et al.*, 1995; Volberg *et al.*, 1995)	F9 vinculin (−/−)	Shape, adhesion, locomotion; increased talin, paxillin, at focal adhesion
Embryonic stem cells (Coll *et al.*, 1995)	ES vinculin (−/−)	Shape, adhesion

of talin to integrins may be under the control of phosphorylation caused for example by interleukin-1-β (Qwarnstrom *et al.*, 1991), and talin's function as a linker protein can be reversed by the calcium-dependent protease calpain (Turner *et al.*, 1989). Originally, it was thought that talin was linked to actin filaments through other proteins such as vinculin and α-actinin, but talin has also been shown to bind directly to actin (Goldmann & Isenberg, 1991). There are therefore two independent links of actin filaments to integrins via talin and vinculin, and both links are found in the same focal adhesion sites. In addition, vinculin binds to α-actinin, an actin cross-linking protein, while talin does not. Finally, the identification of sites on talin that binds vinculin suggests that dimers of talin may themselves be linked by vinculin (Gilmore *et al.*, 1993). Therefore, our interpretation is that talin supports linear membrane extension (i.e. filopodia) driven by actin polymerization. Vinculin, with its greater linking (through α-actinin and talin) or force-transducing properties, would support the broader, more stable cytoplasmic extensions of lamellipodia. In Fig. 1 we present a model showing how vinculin and talin may interact with actin and the plasma membrane in the focal adhesion complex. Since vinculin and talin are known components of the pathways that link the cytoskeleton to the cell membrane and regulate cell motility, the deletion or functional mutation of both proteins should prevent the cell from adhering and forming focal contacts.

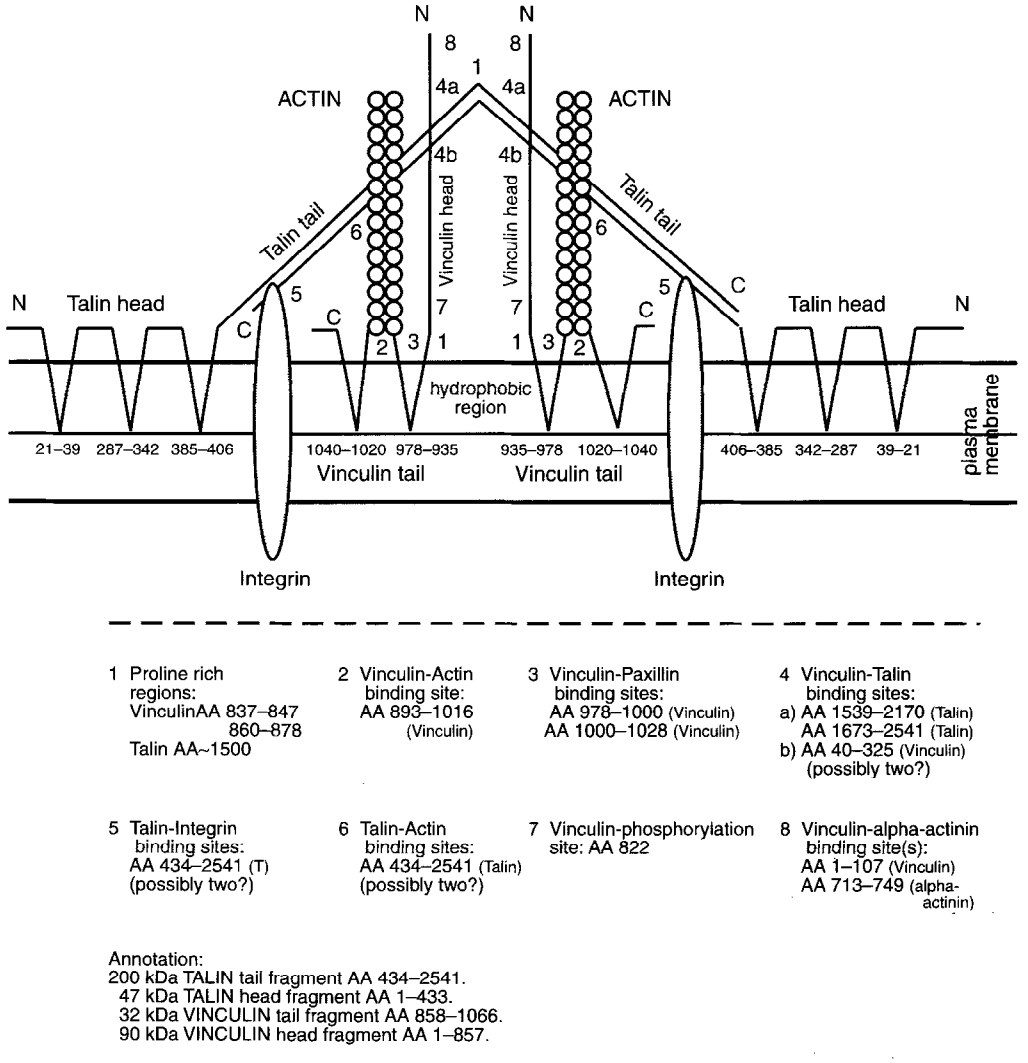

Fig. 1. Model of vinculin and talin interactions at the plasma membrane. We present a detailed model of vinculin and talin which is based on recent experimental and theoretical data. According to Tempel and colleagues (1995) two regions in the vinculin molecule (amino acids 978–935 and 1020–1040) and three regions in the talin molecule (amino acid 21–39, 287–342, and 385–406) are good candidates for lipid interactions. It is, therefore, likely that (vinculin-talin)-lipid and (vinculin-talin)-actin interactions are the crucial activities needed for cell spreading and focal contacts, and that the loss of vinculin or talin may be compensated by one or more proteins.

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