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Review Article

Correlation between the interaction of the vinculin tail domain with lipid membranes, its phosphorylation and cell mechanical behaviour

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Abstract

Cell adhesion and cell–cell contacts are a pre-requisite for proper metabolism, protein synthesis and cell survival. Integrins are the transmembrane receptors that link the extracellular matrix via the FAC (focal adhesion complex) with the cytoskeleton. Vinculin is a pivotal FAC protein that has not only been implicated in regulating FAC formation and transmitting mechanical forces, but also in associating with membranous lipids in biological systems.

Keywords: cell mechanics; focal adhesions; lipid–membrane interaction; vinculin tail

Introduction

Cell adhesion, polarity, migration and the ability to invade are all possible because of two processes: the regulation of actin-filament dynamics and the production of specialized cell–extracellular-matrix adhesions (Pollard et al., 1991; Ridley et al., 2003). Both the regulation and production processes are coupled mechanically by large complexes of cytoplasmic proteins linking transmembrane receptors called integrins (Critchley, 2000). Current understanding of the integrin receptor function centres on the protein talin and other critical intracellular proteins which form plaques (complexes) under the cell membrane at adhesion sites (Giannone et al., 2003; Tadokoro et al., 2003). The binding of talin to the intracellular β-chains of the integrin activates the integrin receptors, giving them high affinity for the external substratum; *in vivo* this is the extracellular matrix (Calderwood et al., 2003; Tadokoro et al., 2003; Garcia-Alvarez et al., 2003). There is also an associated force coupling of the cell–matrix adhesion to the actin cytoskeleton (Alenghat et al., 2000; Goldmann and Ingber, 2002; Giannone et al., 2003; Jiang et al., 2003). This second process activates talin by force induction, exposing binding sites for the recruitment of the protein vinculin to the adhesion sites (Galbraith et al., 2002; Papagrigoriou et al., 2004; Vogel and Sheetz, 2006). Vinculin interacts with both talin and actin, and ensures that there is mechanical stability and a committed regulation (maturity) of the adhesions into a plaque containing a complex of proteins, often known as the FAC (focal adhesion complex) (Möhl et al., 2009). The control of vinculin levels, specifically the intracellular concentration of recruited functional vinculin, is essential for the efficient cell morphology. Many previous studies have demonstrated that vinculin plays a critical role in complexing cytoskeletal and cytoplasmic proteins and is vital for the correct control of cytoskeletal mechanics and the integrity of cell shape (Goldmann et al., 1995; Goldmann and Ezzell, 1996; Ezzell et al., 1997; Goldmann et al., 1998; Goldmann, 2002a, 2002b; Goldmann and Ingber, 2002).

Mutant or incorrectly functioning vinculin may be indicators of tumour behaviour and, in cell culture, overexpression of the wild-type protein has been reported to act as a regulator of tumour invasiveness (Rodríguez Fernández et al., 1992, 1993). Cells lacking vinculin are less adherent, less well spread, have fewer and smaller and less stable adhesions, and are more motile (Xu et al., 1998a, 1998b). In summary, vinculin overexpression reduces cell migration and conversely down-regulation of the protein enhances two-dimensional cell motility (Mierke et al., 2008).

Vinculin structure and function

Protein structure studies and crystals of fragments of vinculin reveal specific intermolecular hydrogen-bonding-binding sites for integral adhesion proteins. Many of the binding sites are well-characterized [summarized in Ziegler et al. (2006)]. The binding sites are cryptic and masked and must be exposed by breaking an intramolecular interaction between the N-terminal head domain and the C-terminal tail domain. Consequently, the protein exists in equilibrium between inactive (closed) and active (open) states. Active states are open conformations in which the head and tail have disengaged, exposing cryptic binding sites for partner proteins. A recent molecular modelling study has shown that exposure of the binding sites is allosteric and depends on a conformational change in the tertiary structure of vinculin, revealing different open and activated states (Chen et al., 2006). Analogous to the shape of a ball and chain, the head is ~95 kDa and the tail ~30 kDa in size respectively. The head domain interacts with talin and α-actinin, whereas the tail domain also contains binding sites which include actin and paxillin (Xu et al., 1998a; Turner, 2000). Both the head and the tail domains are composed of

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Abbreviations: FAC, focal adhesion complex; PIP_2, phosphatidylinositol 4,5-bisphosphate.

helical bundles. The tertiary structure of the vinculin tail domain is composed of a typical five-helical bundle adopting an anti-parallel topology, stabilized with intramolecular hydrogen bonds, but flexible enough to reveal its cryptic binding. The vinculin tail also binds to lipids. *In vitro* studies have shown that phospholipids, such as PIP_2 (phosphatidylinositol 4,5-bisphosphate), can activate (open) vinculin by interacting with the tail and separating it from the head domain (Weekes et al., 1996; Hüttelmaier et al., 1998).

Phospholipid-binding interactions of the vinculin tail

Site-directed mutagenesis studies showed that intermolecular interactions between the vinculin tail and lipids are controlled by two surfaces that expose basic (positively charged) residues called the 'collar' and the 'ladder' (Bakolitsa et al., 1999). The C-terminal (last) residues 1062–1066 (TPWYQ) are also directly implicated in lipid-membrane binding. In the crystal structure, these five hydrophobic residues form a hairpin (Bakolitsa et al., 2004). One hypothesis is that the C-terminal residues form an anchor for the core of a lipid membrane bilayer, such that it restricts the freedom of the entire vinculin protein and 'drags' the tail domain closer to the acidic lipid heads protruding from the surface of the membrane. Subsequent intermolecular hydrogen-bonding between the lipid heads and the basic residues of the vinculin tail could trigger the unfurling of the helical bundle, resulting in a tighter association of helices 2 and 3 with the acidic lipid heads (Johnson et al., 1998; Bakolitsa et al., 1999; 2004). Defective interaction between the vinculin tail and the phospholipids impairs cell spreading and motility directly (Chandrasekar et al., 2005; Ziegler et al., 2006). It is not yet clear whether these interactions *in vivo* are associated with membrane or soluble lipids in the cytosol. According to studies in my laboratory (Tempel et al., 1995; Scott et al., 2006), the entire 15 residues at the C-terminal region of the wild-type vinculin tail (1052–1066) can interact with lipids. These 15 residues are highly flexible in the crystal structure (Bakolitsa et al., 2004). Mutated vinculin lacking these 15 residues supresses and fails to restore normal spreading (Bakolitsa et al., 1999; Chandrasekar et al., 2005). The same mutant vinculin fails to associate with adhesions, but has a more fibrillar distribution in the cytosplasm (Saunders et al., 2006). The same mutant was also unable to interact with phosphatidylserine or PIP_2 artificial vesicles compared with a normal vinculin tail (Saunders et al., 2006).

Phospholipid-binding for focal adhesion turnover

PIP_2 is an example of a phospholipid that acts as a general regulator for actin dynamics and adhesion site turnover (assembly and disassembly). Integrin binding to talin, and many cytoskeleton proteins involved in cell adhesion are dependent on PIP_2. Furthermore, an imbalance in local PIP_2 levels can cause adhesion sites to lose their integrity (Weekes et al., 1996; Gilmore and Burridge, 1996; Hüttelmaier et al., 1998). Many studies have indicated that vinculin interacts with phospholipids, such as phosphatidylcholine, phosphatidylserine and PIP_2 (Johnson et al., 1998; Steimle et al., 1999; Niggli, 2001; Cohen et al., 2006). Chandrasekar et al. (2005) found that adhesion site dynamics and turnover, and consequently cell motility, were directly affected by the phospholipid-binding to the vinculin tail. Double mutations to the 'ladder' and 'collar' reduced adhesion turnover, causing a delay in cell spreading and motility. They found that the recruitment of vinculin during reinforcement (maturation) of adhesion sites does not depend on the capacity of vinculin to bind phospholipids such as PIP_2, but directly affects PIP_2-dependent disassembly of adhesions, consistent with the findings of Cohen et al. (2006) and Saunders et al. (2006). This work with PIP_2 raises the question of whether the vinculin tail interacts with soluble or membranous phospholipids, or perhaps both, during the turnover of adhesions. An investigation into the function of the C-terminal region is needed to answer this question. It is important to establish whether the C-terminal region simply anchors into phospholipid membranes or is employed to increase the affinity between the vinculin tail domain and the soluble phospholipid.

The influence of the C-terminal tail

Aspects of the function and the structure of the tail domain of vinculin and its C-terminal region at the atomic and cellular level, and by its phosphorylation, are under further investigation. A molecular dynamics study has looked at the behaviour of the C-terminal residues in different charged states under solvated conditions, approximating a range of cytosolic conditions. Because of the size of the entire vinculin tail, the simulations were kept computationally manageable by excluding the helical bundle. The last 21 residues were used, which included the six residues of the last helix followed by the 15 from the C-terminal (unstructured) region, the aim being to explore the conformational changes and identify energetically favourable geometries. These could be used either to act as putative anchors in a future lipid membrane model, or interact with conformations of the vinculin tail to ensure a binding site for soluble phospholipids (Diez et al., 2008).

Cellular and cytoskeletal responses were investigated by applying precisely controlled forces on the surfaces of cultured cells. The mechanical properties of populations of recruited integrin linkages were measured in response to applied forces. A magnetic tweezer method was developed to apply forces of up to 10 nN to super-paramagnetic beads attached to the extracellular surface of clustered adhesions (Alenghat et al., 2000; Mierke et al., 2008). Cell stiffness and creep measurements were performed on cultured cells by determining the strength between a given bead and the associated adhesions. In a recent study, cultured fibroblasts were transfected with either wild-type or mutant vinculin lacking the 15-residue C-terminal region (Diez et al., 2009). Differences in the bead-binding strengths of the two types of transfected cells were significant, based on the fraction of beads detaching at different applied forces. As an aside, intracellular fluorescence localization was used to confirm that the mutant vinculin was incorporated correctly at the focal adhesions (Diez et al., 2009).

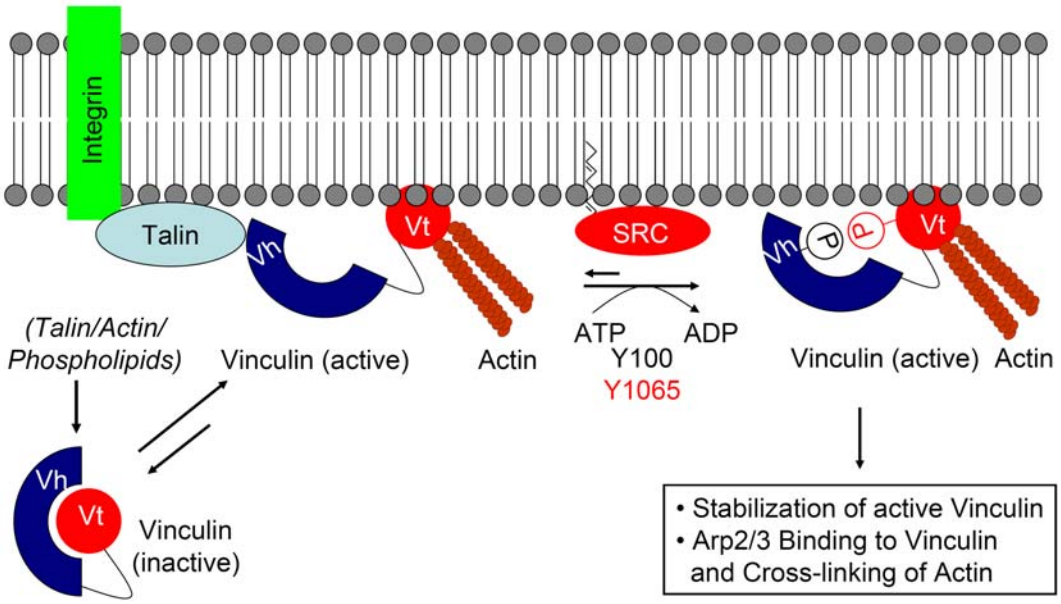

Figure 1 **Vinculin: a mechanism of action**
The model depicts the activation of vinculin by talin, actin or phospholipids, and the anchoring of vinculin tail to the lipid membrane. This process allows for *src*-dependent phosporylation on position Tyr^{100} and Tyr^{1065} of the vinculin molecule which is believed to stabilize the vinculin–lipid membrane complex and enables Arp2/3–vinculin interaction as well as further recruitment of actin. This connection strengthens the FAC, which facilitates the cell to generate transiently higher internal tension and strain energy for cell adhesion and migration.

The tyrosine phosphorylation site (Tyr^{1065}) on a vinculin tail were targeted by *c-src* kinase (Zhang et al., 2004). For instance, when its phosphorylation is prevented, interaction of vinculin with the Arp2/3 subunit p34Arc protein is inhibited, impairing cell spreading and migration (Möse et al., 2007). Furthermore, phospholipids stimulate the phosphorylation of vinculin by *src* kinase (Ito et al., 1982, 1983). In the presence of acidic phospholipids, the phosphorylated fraction of vinculin was elevated compared with vinculin in the absence of lipid vesicles (Ito et al., 1982). Furthermore, these authors described a higher degradation of the phosphorylated vinculin peptide by protease digestion assays in the presence of acidic phospholipids, which suggests that the interaction of vinculin with the lipid membrane induces a conformational shift, giving the *src* kinase the possibility of vinculin phosphorylation (Ito et al., 1983).

In summary, it is conceivable that the anchorage of vinculin to lipid membranes by its C-terminus alters its conformation, which in turn might enhance its activation and focal adhesion recruitment (Ito et al., 1983; Johnson and Craig, 1995; Gilmore and Burridge, 1996). Crystal structure data of vinculin show that the lipid anchor region is not buried inside the vinculin molecule when it is in a closed conformation (Borgon et al., 2004; Bakolitsa et al., 2004). Data from my laboratory confirm an interaction of the hydrophobic part of lipid membranes with the C-terminal peptide (Diez et al., 2008). A conformational switch may be important for *src*-dependent phosphorylation. Phosphorylation of Tyr^{1065} might therefore be essential for the binding of the p34 subunit of Arp2/3 to vinculin, and consequently for the reinforcement of the FAC and mechanical linkage to the actin cytoskeleton (Figure 1).

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