

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

Wolfgang H. Goldmann¹

Center for Medical Physics and Technology, Biophysics Group, Friedrich-Alexander-University of Erlangen-Nuremberg, Henkestrasse 91, 91052 Erlangen, Germany

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

¹email wgoldmann@biomed.uni-erlangen.de

Abbreviations: FAC, focal adhesion complex; PIP₂, 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 PIP₂ (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 suppresses 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 cytoplasm (Saunders et al., 2006). The same mutant was also unable to interact with phosphatidylserine or PIP₂ artificial vesicles compared with a normal vinculin tail (Saunders et al., 2006).

Phospholipid-binding for focal adhesion turnover

PIP₂ 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 PIP₂. Furthermore, an imbalance in local PIP₂ 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 PIP₂ (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 PIP₂, but directly affects PIP₂-dependent disassembly of adhesions, consistent with the findings of Cohen et al. (2006) and Saunders et al. (2006). This work with PIP₂ 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).

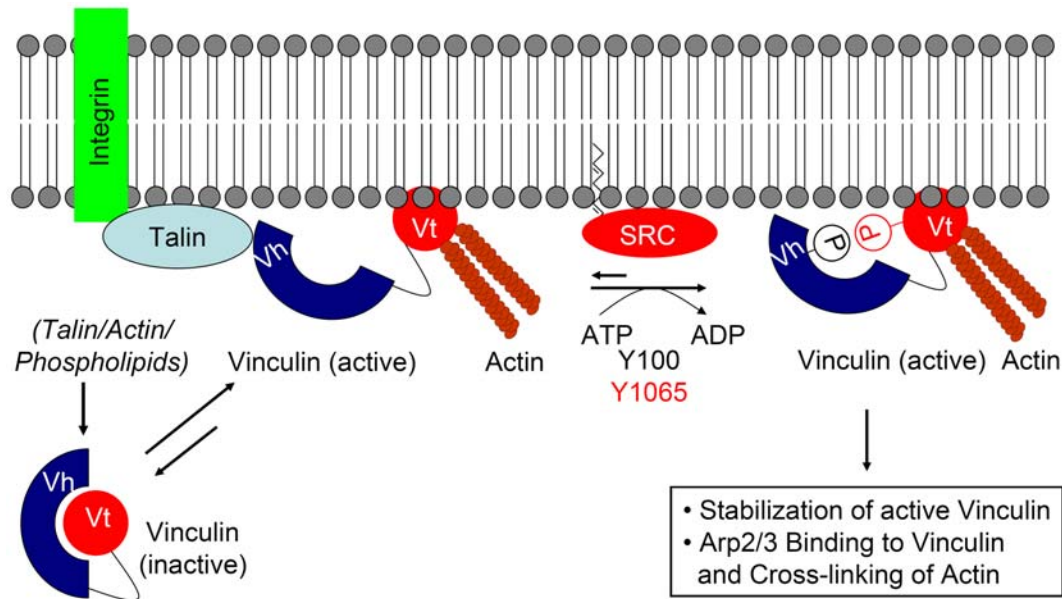


Figure 1 Vinculin: a mechanism of action

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 phosphorylation on position Tyr¹⁰⁰ and Tyr¹⁰⁶⁵ 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 (Tyr¹⁰⁶⁵) 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 Tyr¹⁰⁶⁵ 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).

Acknowledgements

I thank Dr Gerold Diez, Dr Wolfgang H. Ziegler, Dr Ben Fabry and Dr Bernd Hoffmann for excellent discussions, and thank Dr James Smith and Liz Nicholson (MA) for comments on the manuscript prior to acceptance.

Funding

The work in the author's laboratory is funded by the Deutsche Forschungsgemeinschaft; DAAD (Deutscher Akademischer Austausch Dienst); BaCaTec (Bavaria California Technology Center); and BFHZ (Bayerisch-Französisches Hochschulzentrum).

References

- Alenghat FJ, Fabry B, Tsai KY, Goldmann WH, Ingber DE. Analysis of cell mechanics in single vinculin-deficient cells using a magnetic tweezer. *Biochem Biophys Res Commun* 2000;277:93–9.
- Bakolitsa C, de Pereda JM, Bagshaw CR, Critchley DR, Liddington RC. Crystal structure of the vinculin tail suggests a pathway for activation. *Cell* 1999;99:603–13.
- Bakolitsa C, Cohen DM, Bankston LA, Bobkov AA, Cadwell GW, Jennings L et al. Structural basis for vinculin activation at sites of cell adhesion. *Nature* 2004;430:583–6.
- Borgon RA, Vonrhein C, Bricogne C, Bois PR, Izard T. Crystal structure of human vinculin. *Structure (Camb)* 2004;12:1189–97.
- Calderwood DA, Fujioka Y, de Pereda JM, Garcia-Alvarez B, Nakamoto T, Margolis B et al. Integrin β cytoplasmic domain interactions with phosphotyrosine-binding domains: a structural prototype for

- diversity in integrin signaling. *Proc Natl Acad Sci USA* 2003;100:2272–7.
- Chandrasekar I, Stradal TE, Holt MR, Entschladen F, Jockusch BM, Ziegler WH. Vinculin acts as a sensor in lipid regulation of adhesion-site turnover. *J Cell Sci* 2005;118:1461–72.
- Chen H, Choudhury DM, Craig SW. Coincidence of actin filaments and talin is required to activate vinculin. *J Biol Chem* 2006;281:40389–98.
- Cohen DM, Kutscher B, Chen H, Murphy DB, Craig SW. A conformational switch in vinculin drives formation and dynamics of a talin–vinculin complex at focal adhesions. *J Biol Chem* 2006;281:16006–15.
- Critchley DR. Focal adhesions: the cytoskeletal connection. *Curr Opin Cell Biol* 2000;12:133–9.
- Diez G, List F, Smith J, Ziegler WH, Goldmann WH. Direct evidence of vinculin tail-lipid membrane interaction in β -sheet conformation. *Biochem Biophys Res Commun* 2008;373:69–73.
- Diez G, Kollmannsberger P, Mierke CT, Koch TM, Vali H, Fabry B et al. Anchorage of vinculin to lipid membranes influences cell mechanical properties. *Biophys J* 2009;97:3105–12.
- Ezzell RM, Goldmann WH, Wang N, Parasharama N, Ingber DE. Vinculin promotes cell spreading by mechanically coupling integrins to the cytoskeleton. *Exp Cell Res* 1997;231:14–26.
- Galbraith CG, Yamada KM, Sheetz MP. The relationship between force and focal complex development. *J Cell Biol* 2002;159:695–705.
- Garcia-Alvarez B, de Pereda JM, Calderwood DA, Ulmer TS, Critchley DR, Campbell ID et al. Structural determinants of integrin recognition by talin. *Mol Cell* 2003;11:49–58.
- Giannone G, Jiang G, Sutton DH, Critchley DR, Sheetz MP. Talin1 is critical for force-dependent reinforcement of initial integrin-cytoskeleton bonds but not tyrosine kinase activation. *J Cell Biol* 2003;163:409–19.
- Gilmore AP, Burridge K. Regulation of vinculin binding to talin and actin by phosphatidylinositol-4-5-bisphosphate. *Nature* 1996;381:531–5.
- Goldmann WH. The coupling of vinculin to the cytoskeleton is not essential for mechano-chemical signaling in F9 cells. *Cell Biol Int* 2002a;26:279–86.
- Goldmann WH. Mechanical aspects of cell shape regulation and signaling. *Cell Biol Int* 2002b;26:313–17.
- Goldmann WH, Ingber DE. Intact vinculin protein is required for control of cell shape, cell mechanics, and rac-dependent lamellipodia formation. *Biochem Biophys Res Commun* 2002;290:749–55.
- Goldmann WH, Schindl M, Cardozo TJ, Ezzell RM. Motility of vinculin-deficient F9 embryonic carcinoma cells analyzed by video, laser confocal, and reflection interference contrast microscopy. *Exp Cell Res* 1995;221:311–19.
- Goldmann WH, Ezzell RM. Viscoelasticity in wild-type and vinculin-deficient (5.1) mouse F9 embryonic carcinoma cells examined by atomic force microscopy and rheology. *Exp Cell Res* 1996;226:234–7.
- Goldmann WH, Galneder R, Ludwig M, Xu W, Adamson ED, Wang N et al. Differences in elasticity of vinculin-deficient F9 cells measured by magnetometry and atomic force microscopy. *Exp Cell Res* 1998;239:235–42.
- Hüttelmaier S, Mayboroda O, Harbeck B, Jarchau T, Jockusch BM, Rüdiger M. The interaction of the cell-contact proteins VASP and vinculin is regulated by phosphatidylinositol-4,5-bisphosphate. *Curr Biol* 1998;8:479–88.
- Ito S, Richert N, Pastan I. Phospholipids stimulate phosphorylation of vinculin by the tyrosine-specific protein kinase of Rous sarcoma virus. *Proc Natl Acad Sci USA* 1982;79:4628–31.
- Ito S, Werth DK, Richert ND, Pastan I. Vinculin phosphorylation by the src kinase. Interaction of vinculin with phospholipid vesicles. *J Biol Chem* 1983;258:14626–31.
- Jiang G, Giannone G, Critchley DR, Fukumoto E, Sheetz MP. Two-piconewton slip bond between fibronectin and the cytoskeleton depends on talin. *Nature* 2003;424:334–7.
- Johnson RP, Craig SW. The carboxy-terminal tail domain of vinculin contains a cryptic binding site for acidic phospholipids. *Biochem Biophys Res Commun* 1995;210:159–64.
- Johnson RP, Niggli V, Durrer P, Craig SW. A conserved motif in the tail domain of vinculin mediates association with and insertion into acidic phospholipid bilayers. *Biochemistry* 1998;37:10211–22.
- Mierke CT, Kollmannsberger P, Paranhos-Zitterbart D, Smith J, Fabry B, Goldmann WH. Mechano-coupling and regulation of contractility by the vinculin tail domain. *Biophys J* 2008;94:661–70.
- Möhl C, Kirchgessner N, Schäfer C, Küpper K, Born S, Diez G et al. Becoming stable and strong: the interplay between vinculin exchange dynamics and adhesion strength during adhesion site maturation. *Cell Motil Cytoskeleton* 2009;66:350–64.
- Möse S, Selbach M, Brinkmann V, Karlas A, Haimovich B, Backert S et al. The *Helicobacter pylori* CagA protein disrupts matrix adhesion of gastric epithelial cells by dephosphorylation of vinculin. *Cell Microbiol* 2007;9:1148–61.
- Niggli V. Structural properties of lipid-binding sites in cytoskeletal proteins. *Trends Biochem Sci* 2001;26:604–11.
- Papagrigoriou E, Gingras AR, Barsukov IL, Bate N, Fillingham IJ, Patel B et al. Activation of a vinculin-binding site in the talin rod involves rearrangement of a five-helix bundle. *EMBO J* 2004;23:2942–51.
- Pollard TD, Doberstein SK, Zot HG. Myosin-I. *Annu Rev Physiol* 1991;53:653–81.
- Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G et al. Cell migration: integrating signals from front to back. *Science* 2003;302:1704–9.
- Rodríguez Fernández JL, Geiger B, Salomon D, Sabanay I, Zöller M, Ben-Ze'ev A. Suppression of tumorigenicity in transformed cells after transfection with vinculin cDNA. *J Cell Biol* 1992;119:427–38.
- Rodríguez Fernández JL, Geiger B, Salomon D, Ben-Ze'ev A. Suppression of vinculin expression by antisense transfection confers changes in cell morphology, motility, and anchorage-dependent growth of 3T3 cells. *J Cell Biol* 1993;122:1285–94.
- Saunders RM, Holt MR, Jennings L, Sutton DH, Barsukov IL, Bobkov A et al. Role of vinculin in regulating focal adhesion turnover. *Eur J Cell Biol* 2006;85:487–500.
- Scott DL, Diez G, Goldmann WH. Protein–lipid interactions: correlation of a predictive algorithm for lipid-binding sites with three-dimensional structural data. *Theor Biol Med Model* 2006;3:17.
- Steimle PA, Hoffert JD, Adey NB, Craig SW. Polyphosphoinositides inhibit the interaction of vinculin with actin filaments. *J Biol Chem* 1999;274:18414–20.
- Tadokoro S, Shattil SJ, Eto K, Tai V, Liddington RC, de Pereda JM et al. Talin binding to integrin β tails: a final common step in integrin activation. *Science* 2003;302:103–6.
- Tempel M, Goldmann WH, Isenberg G, Sackmann E. Interaction of the 47-kDa talin fragment and the 32-kDa vinculin fragment with acidic phospholipids: a computer analysis. *Biophys J* 1995;95:228–41.
- Turner CE. Paxillin, and focal adhesion signalling. *Nat Cell Biol* 2000;2:E231–6.
- Vogel V, Sheetz M. Local force and geometry sensing regulate cell functions. *Nat Rev Mol Cell Biol*. 2006;7:265–75.
- Weekes J, Barry ST, Critchley DR. Acidic phospholipids inhibit the intramolecular association between the N- and C-terminal regions of vinculin, exposing actin-binding and protein kinase C phosphorylation sites. *Biochem J* 1996;314:827–32.
- Xu W, Coll JL, Adamson ED. Rescue of the mutant phenotype by reexpression of full-length vinculin in null F9 cells; effects on cell locomotion by domain deleted vinculin. *J Cell Sci* 1998a;111:1535–44.
- Xu W, Baribault H, Adamson ED. Vinculin knockout results in heart and brain defects during embryonic development. *Development* 1998b;125:327–37.
- Zhang X, Izaguirre G, Lin SY, Lee HY, Schäfer E, Haimovich B. The phosphorylation of vinculin on tyrosine residues 100 and 1065, mediated by SRC kinases, affects cell spreading. *Mol Biol Cell* 2004;15:4234–47.
- Ziegler WH, Liddington RC, Critchley DR. The structure and regulation of vinculin. *Trends Cell Biol* 2006;16:453–60.

Received 2 February 2010/accepted 4 February 2010

Published on the Internet 8 March 2010, doi 10.1042/CBI20100085