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Short review

Vinculin, an adapter protein in control of cell adhesion signalling

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ABSTRACT

Vinculin, discovered in 1979 (Geiger, 1979), is an adapter protein with binding sites for more than 15 proteins. Biochemical and structural analyses have contributed to detailed knowledge about potential binding partners and the understanding of how their binding may be regulated. Despite all this information the molecular basis of how vinculin acts in cells and controls a wide variety of signals remains elusive. This review aims to highlight recent discoveries with an emphasis on how vinculin is involved in the coordination of a network of signals.

Introduction

Vinculin is a 117 kDa protein, which localises to integrin-mediated cell–matrix adhesions and cadherin-mediated cell–cell junctions. In *C. elegans* vinculin deficiency leads to the loss of muscular activity and lethality at an early larval stage (Barstead and Waterston, 1989). Mouse embryos deficient in vinculin are small and die at day E10.5 with major defects in brain and heart development (Xu et al., 1998a). Embryos at E9.5 are about a third smaller than normal embryos and their mutant tissue seems more fragile suggesting that vinculin plays a role in strengthening cell attachments to its environment. Mouse embryonic fibroblasts isolated from vinculin knock-out animals at E9.5 spread less, have smaller focal adhesions (FA) and show decreased adhesion strength to fibronectin, laminin, vitronectin and collagen, but they migrate faster than their wild-type counterparts (Xu et al., 1998a). Earlier studies using vinculin-null F9 embryonic carcinoma cells made similar observations (Coll et al., 1995). Re-expression of vinculin rescued these defects in vinculin-null cells (Coll et al., 1995; Xu et al., 1998b; Saunders et al., 2006). The phenotypic changes of reduced cell adhesion and an increase in cell motility associated with the loss of vinculin is thought to drive the formation of tumour metastases. Other studies showed that expression of vinculin in tumour cell lines with diminished levels of the endogenous protein suppressed their tumorigenic ability and increased adhesion strength (Rodríguez Fernández et al., 1992; Lifschitz-Mercer et al., 1997). However it remains to be established which signals lead to the enhanced tumourigenicity of vinculin-deficient cells.

During cell migration small dot-like adhesion sites (focal complexes; FX) form at the leading edge of a cell, which then mature into streak-shaped FA. In the lamellum, an area in front of the nucleus, adhesions start to disassemble (Fig. 1a). For controlled cell motility, the formation and disassembly of adhesion sites needs to be coordinated. However, it should be noted that FA do not only form during continuous cell migration, they can also be constantly formed in the cell periphery of stationary cells mostly at sites of local protrusions and retractions (Smilenov et al., 1999; Ballestrem et al., 2001; our observations). Although many of those FA, especially in less migratory cell types such as fibroblasts or epithelial cells, seem relatively stable over time, there is an astonishing level of mobility of proteins therein (Lele et al., 2008). Many FA proteins, including vinculin, can cycle in and out of these FA. The rate of vinculin cycling is dependent on its activity status (Fig. 1b), which in turn can affect the cycling rate of other FA proteins (Cohen et al., 2006; Humphries et al., 2007). Although, it is known that vinculin is recruited to FA very early during their development, little is known about the molecular basis of how these events are controlled. Similarly, even though it is known that the activation status of vinculin controls its mobility, we are far from understanding how this can control key signals in FA.

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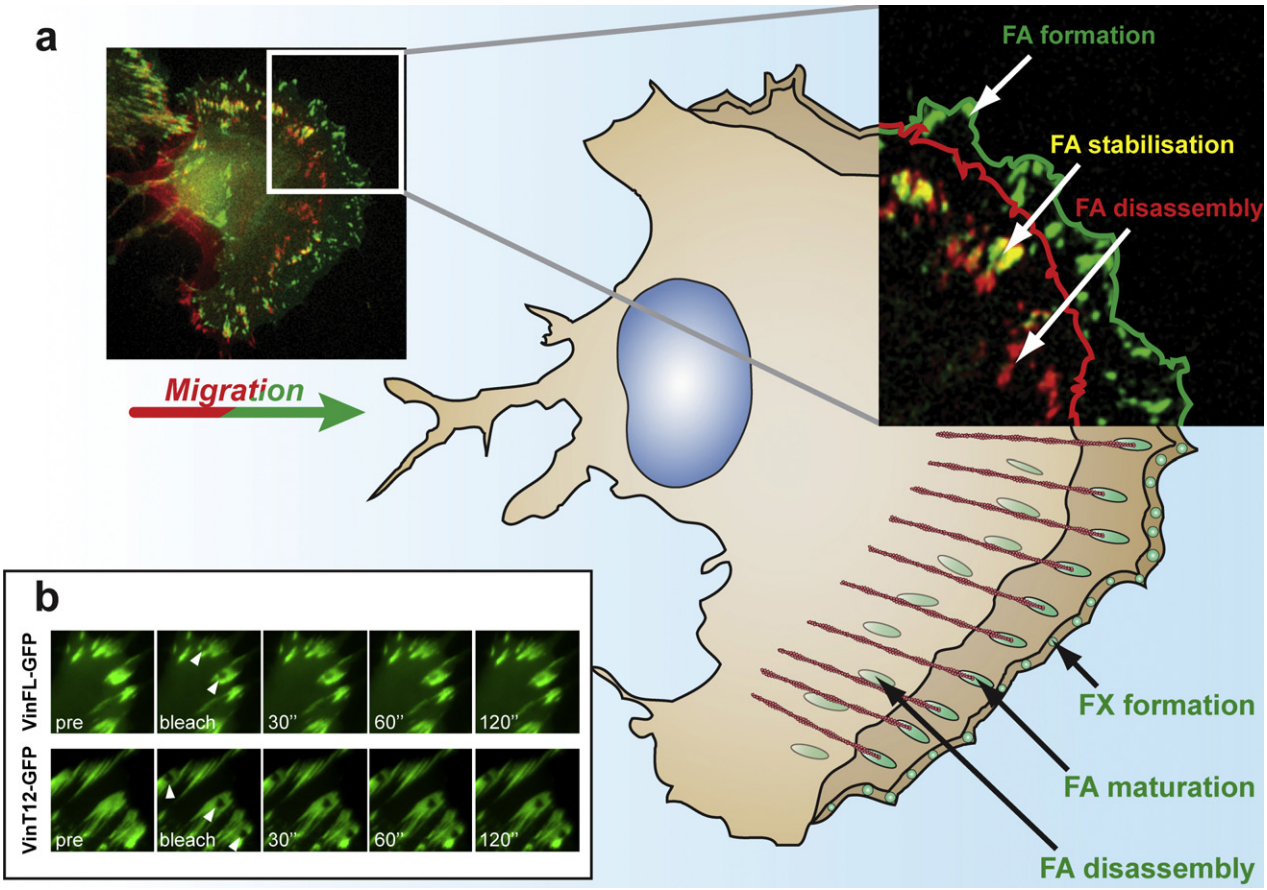

Fig. 1. (a) Schematic representation originating from a migrating B16 melanoma cell expressing β3-integrin – GFP showing areas of FA formation and disassembly. To outline these areas, two images of time-lapse recordings taken at 5 min intervals were labeled in green and red respectively and then superimposed. Newly formed FA are labeled in green; structures that remained at the same place during the 5 min interval are seen in yellow; FA that have disassembled during cell motility in the later time point are labeled in red. Note that cell–matrix contacts start forming at the protrusive edge of the cells (focal complexes; FX), they then increase in size to mature into streak-shaped focal adhesions (FA), and then disassemble in an area before the nucleus. (b) Fluorescence Recovery After Photobleaching (FRAP) of vinculin-GFP fusion constructs in vinculin-deficient mouse embryonic fibroblasts (MEF). The top row shows the recovery of wild-type vinculin-GFP, the lower row the recovery of constitutively active vinculinT12-GFP in pre-bleached FA areas (see arrowheads). Note that the turnover of wild-type vinculin in FA is dramatically faster than the constitutively active vinculin T12 mutant.

Activation of vinculin

In 2005, Chen et al. were able to demonstrate that vinculin undergoes conformational changes when localising to FA (Chen et al., 2005). Using intramolecular Foerster Resonance Energy Transfer (FRET) they showed that only the active extended form of vinculin localises to focal adhesions whereas the folded inactive form resides in the cytoplasm. Evidence for such events was first obtained using biochemical and structural analyse of vinculin (Johnson and Craig, 1994) which revealed that vinculin is composed of head, neck and tail domains (Fig. 2) with the interaction between head and tail domains masking the binding sites for vinculin-binding partners (Ziegler et al., 2006). It is thought that, *in cellulo*, vinculin exists in an equilibrium between active and inactive states and it can be stabilised in the active form by interactions with a subset of binding partners. Several models for the activation of vinculin have been proposed all of which lead to its continued localisation to FA and the full unmasking of all binding sites. Again there is biochemical evidence from *in vitro* studies that talin or α-actinin, either as a single binding component (Izard et al., 2004; Izard and Vonrhein, 2004; Bois et al., 2006), or together with PIP2 (phosphatidylinositol-4,5-bisphosphate) or actin in what can be described as the combinatorial model (Bakolitsa et al., 2004; Chen et al., 2006), is able to disrupt the head–tail interaction and activate vinculin (Gilmore and Burridge, 1996; Izard et al., 2004; Bois et al., 2006; Janssen et al., 2006). Current evidence favours the combinatorial model since the tight intramolecular binding between the vinculin tail and head appears too strong for a single ligand to overcome (Janssen et al., 2006; Ziegler et al., 2006).

From studies in cell culture it seems likely that talin, rather than α-actinin, is the major protein involved in the activation of vinculin, since talin rather than α-actinin, co-localises with vinculin in FX (Zaidel-Bar et al., 2003). More importantly, the presence of talin is a prerequisite for vinculin localisation to FX (Zhang et al., 2008) and to FA (our unpublished observations). Whether PIP2 (Gilmore and Burridge, 1996; Weekes et al., 1996; Hüttelmaier et al., 1998) or actin (Chen et al., 2006) is the preferred partner for talin is yet unclear, but as the binding sites for actin and PIP2 overlap making it unlikely that both bind simultaneously (Steimle et al., 1999). A recent study has shown that vinculin mutants deficient in PIP2 binding readily localise to FA but inhibit FA turnover and subsequently decrease cell motility (Chandrasekar et al., 2005). The authors propose therefore that PIP2, rather than being involved in the activation of vinculin, regulates its release from FA plaque. One possibility is that PIP2 can compete and replace F-actin binding to vinculin (Chandrasekar et al., 2005). The precise role of actin in the activation process is also unclear and a number of questions arise. Would an initial contact with actin be sufficient to activate vinculin or are forces induced by the actomyosin machinery of the cell required for full activation? Evidence for the latter is that