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COMMENTARY

Vinculin-p130Cas interaction is critical for focal adhesion dynamics and mechano-transduction

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Abstract

Adherent cells, when mechanically stressed, show a wide range of responses including large-scale changes in their mechanical behaviour and gene expression pattern. This is in part facilitated by activating the focal adhesion (FA) protein p130Cas through force-induced conformational changes that lead to the phosphorylation by *src* family kinases. Janostiak et al. [Janostiak et al. Cell Mol Life Sci (2013) DOI 10.1007/s00018-013-1450-x] have reported that the phosphorylation site Y12 on the SH3 domain of p130Cas modulates the binding with vinculin, a prominent mechano-coupling protein in FAs. Tension changes in FAs (due to the anchorage of the SH3 domain and C-terminal) bring about an extension of the substrate domain of p130Cas by unmasking the phosphorylation sites. These observations demonstrate that vinculin is an important modulator of the p130Cas-mediated mechano-transduction pathway in cells. The central aim should be now to test that vinculin is critical for p130Cas incorporation into the focal adhesion complex and for transmitting forces to the p130Cas molecule.

Keywords: cell mechanics; focal adhesions; p130Cas; vinculin

Integrin-associated focal adhesions (FAs) are the main cellular structure for cell adhesion. They consist of several hundred different proteins (Zaidel-Bar et al., 2007) that together, critically influence a large number of integrin-mediated cell signalling events such as cell survival and proliferation, contraction, migration and differentiation. By far the most important factor that determines integrin-mediated cell signalling is the mechanical environment of the cell, namely its adhesiveness, stiffness, topology and strain fluctuations. Consequently, an understanding of the molecular processes that enable cells to sense their mechanical environment is of great interest (Goldmann, 2002, 2012a, b; Goldmann et al., 2013).

One of the most prominently discussed mechano-sensing molecules is p130Cas. Originally described as a *crk*-associated substrate, p130Cas is a member of the FA scaffold protein family (Nakamoto et al., 1997; Honda et al., 1999; Defilippi et al., 2006; Thompson et al., 2009). p130Cas is a multi-domain protein (Nasertorabi et al., 2004) that interacts with focal adhesion kinase (FAK) (Polte and Hanks, 1995; Harte et al., 1996), Pyk2 (Birge et al., 2009) and several other proteins, including FRNK, RapGEF1, Aurora kinase A, PI3K, NMP4, NCK1 and SHIP2 and NSP (Chen et al., 1995; Liu et al., 1996; Pratt et al., 2005; Roselli et al., 2010; Mace et al., 2011), that has been reviewed by Cabodi et al. (2010) (Figure 1).

The current working model of how extracellular and intracellular (contractile) mechanical stimuli are thought to be transmitted to p130Cas is that: (i) forces are sent out from two 'handles' of p130Cas that lead to protein stretching, and (ii) stretching of p130Cas opens up cryptic binding sites on the substrate binding domain (SBD) to enable the docking and activation of non-receptor tyrosine kinases of the *src* and *crk* family (Parsons and Parsons, 1997; Abram and Courtneidge, 2000). This is followed by the successive phosphorylation of the substrate domain (SD) of p130Cas (Polte and Hanks, 1995; Sawada et al., 2006), which in turn activates downstream signalling, including the mitogen activated protein (MAP) kinase cascade (Goldberg et al., 2003), activation of small GTPase proteins (Sawada et al., 2001, 2006; Sawada and Sheetz, 2002), and tyrosine phosphorylation of several other adhesion proteins (Giannone and Sheetz, 2006). In agreement with this working model, p130Cas is in a

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Abbreviations: ECM, extracellular matrix; FAs, focal adhesions

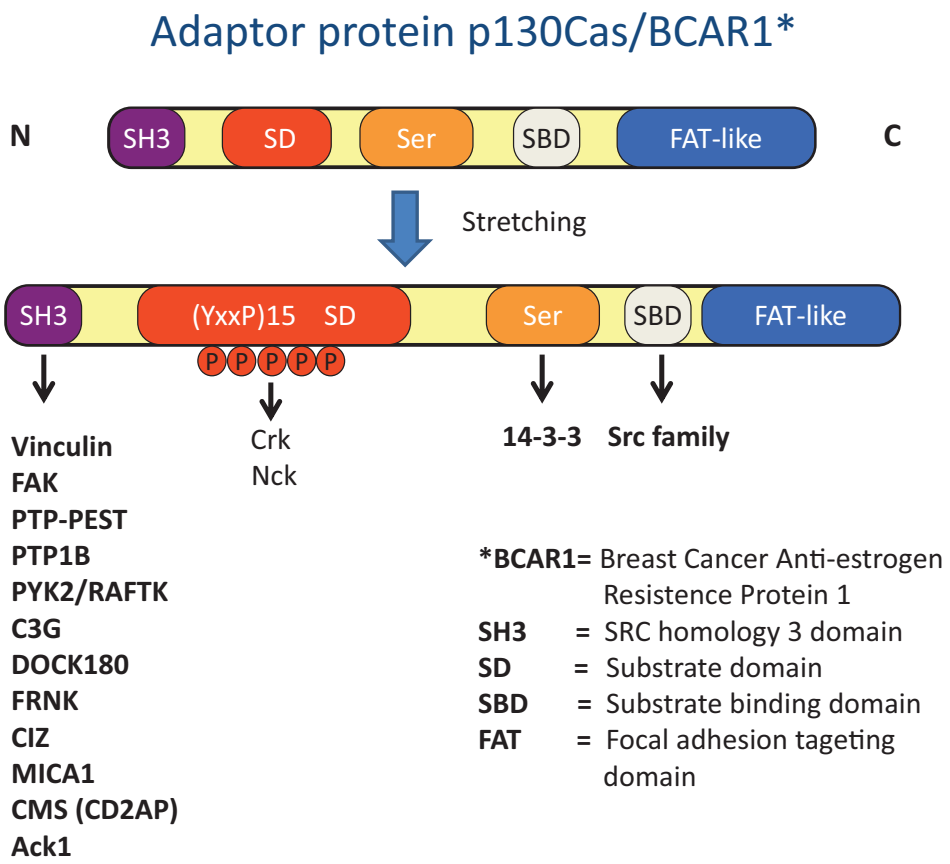

Figure 1 A modified schematic representation of the adaptor protein p130Cas which consists of a well-known FAT-domain, substrate binding domain, a 14-3-3 protein and tyrosine binding domain as well as a SH3 domain. The role of the SH3 domain of p130CAS as a docking molecule, which is involved in numerous protein–protein interactions, is well established (Cabodi et al., 2010). Stretching the molecule allows protein binding and opens up phosphorylation sites.

phosphorylated state in highly invasive cells (Cowell et al., 2006; Schuh et al., 2010). Moreover, cells transformed with *v-src* and *v-crk* have increased p130Cas phosphorylation and invasiveness in a 3-D culture system (Brabek et al., 2004, 2005).

There are conceptual problems, however, with this working model. For p130Cas to be stretched, the mechanical forces need to be transmitted to the p130Cas molecule on two distant sites, namely via FAK, Pyk2 and other proteins on the SH3-domain near the N-terminus 'first handle', and via other as yet unspecified FA proteins that bind to the focal adhesion targeting region (FAT) of p130Cas near the C-terminus 'second handle'. Whether FAK, Pyk2 and other proteins can act as a mechano-coupling and force-transmitting protein, however, remains unknown. Similarly, the list of plausible candidates for the other mechano-coupler near the C-terminus has not been narrowed down by clear experimental evidence.

A possible candidate for the missing p130Cas binding partner proposed by Janostiak et al. (2011) is vinculin. This idea is supported by reports of co-localisation of p130Cas and vinculin (Nakamoto et al., 1997). Vinculin, as a dominant and abundant FA protein (Burridge and Feramisco, 1980; Eimer et al., 1993) binds to talin, alpha-actinin, actin and several other neck binding proteins (Burridge and Feramisco, 1980). It recruits paxillin to enhance integrin clustering (Humphries et al., 2007) and is a major mechano-coupling/regulating protein within the FA complex (Goldmann et al., 1995, 1998; Goldmann and Ezzell, 1996; Ezzell et al., 1997).

Proof of p130Cas-vinculin binding has now come from Janostiak et al. (2013) introducing point mutations on the SH3 domain of p130Cas at position 12 and vinculin's neck region at position 861-4. Changing wildtype p130Cas 12Y to 12F or 12E in mouse embryonic fibroblasts (MEFs) and studying the location of these mutant proteins by fluorescence imaging using antibodies for p130Cas variants and vinculin, they have shown that the wildtype and 12F mutant co-localise in FAs, whereas the 12E variant does not. To test whether vinculin binding is necessary for mechanical activation of p130Cas, Janostiak et al. (2013) cultured MEFs on a flexible PDMS substrate and exposed the cells to stretch by a cell stretcher. There was no increase in

phosphorylation of p130Cas at position Y410 and ERK1/2 in Vin−/− and FAK−/− cells, whereas in wildtype cells p130Cas(Y410) and pERK1/2 phosphorylation was increased compared to unstretched conditions. Since binding of p130Cas to FAK or vinculin is required for localisation of p130Cas at the FA sites, they hypothesised that the stretch-induced phosphorylation of p130Cas at Y410 also requires proper localisation of p130Cas in FAs. They could demonstrate that the constitutively phospho-mimicking (i.e. vinculin binding deficient) 12E p130Cas mutant showed no detectible activation. In contrast, the non-phosphorylatible Y12F p130Cas mutant (with strong vinculin and FAK binding) increased stretch activation.

Janostiak et al. (2013) speculated that cells with impaired p130Cas-mediated mechano-chemical signalling may show reduced FA reinforcement, and consequently reduced stiffness and increased cytoskeletal fluidity. To test this hypothesis, they determined how cells deform under external force using magnetic tweezers. The cell stiffness was lower in the phospho-mimicking (Y12E) p130Cas mutants where it was poorly associated with FAs, and the cell fluidity was highest. The lower stiffness suggests that these cells have a lower contractile pre-stress, as confirmed by traction microscopy.

To ensure that lower traction forces of the phospho-mimicking mutants are not caused by diminished adhesion strength, they ramped up the force of the magnetic tweezers until the integrin-bound beads detached from the cells. Repeating this for hundreds of cells gives a probability that the adhesions break at a given force, and thus is a quantitative measure of adhesion strength. The bead detachment (i.e. binding strength) probabilities are not markedly different between the wildtype and p130Cas mutant cells. Therefore, the reduced traction forces that they observed in the phospho-mimicking 12E mutants are not caused by poor adhesion, but are probably due to diminished contractile activation.

In summary, data from Janostiak et al. (2013) confirm that: (i) p130Cas interacts with vinculin in a FAK-independent manner, (ii) vinculin is necessary for stretch-activation of p130Cas and ERK1/2 phosphorylation, (iii) binding to vinculin is regulated by p130Cas phosphorylation on position 12, and (iv) the Y12E (phospho-mimicking) mutant prevents p130Cas stretch-activation, increases FA turnover, decreases FA size but not adhesion strength, increases cell migration and cell fluidity, and reduces cell stiffness and tractions. These observations show that vinculin is probably the 'first handle' and an important modulator of the p130Cas-mediated mechano-transduction pathway in cells.

Future work has to address the 'second handle' at the C-terminal end through which p130Cas mechanically couples to partner proteins (the 'first handle' at the N-terminal end of p130Cas being the SH3-domain). Sawada et al. (2006) suggest that in order for the p130Cas molecule to open up, that is to act as a mechano-sensor, the SBD must be targeted to FAs. However, other studies contradict this assumption (Harte et al., 2000; Donato et al., 2010). Donato et al. (2010) showed that the C-terminal homology (CCH) domain is necessary for proper targeting of p130Cas to FAs. Their results suggest that the C-terminal CCH-region is also the 'second handle' for coupling forces to p130Cas to ensure its mechano-sensing function.

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