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Commentary

Mechanotransduction and focal adhesions

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Abstract

Cellular FAs (focal adhesions) respond to internal and external mechanical stresses which make them prime candidates for mechanotransduction. Recent observations showed that the FA proteins including vinculin, FAK (FA kinase) and p130Cas are crucial for the ability of cells to transmit forces and to generate cytoskeletal tension. When mechanically stimulated, cells respond by modulating the spreading area, remodel the actin cytoskeleton, activate actomyosin interactions, recruit integrins and reinforce FAs and cytoskeletal structures. These complex cellular responses are orchestrated such that mechanical stresses within the FA complex remained within a narrow range.

Keywords: cytoskeleton; focal adhesions; integrins; mechanotransduction

1. Introduction

Cell adhesion and cell–cell contacts critically influence cell metabolism, protein synthesis, cell survival, cytoskeletal architecture and consequently cell mechanical properties such as migration, spreading and contraction (Goldmann, 2002a, 2002b; Klemm et al., 2006; Grashoff et al., 2010; Hoffman et al., 2011). An important group of adhesive transmembrane receptors that mechanically link the ECM (extracellular matrix) with the internal cytoskeleton are integrins (Hynes, 2002). Integrins are intimately connected with the FAs (focal adhesions) which consist of proteins, including vinculin, p130Cas and FAK (FA kinase; Alenghat and Ingber, 2002). The formation of FAs is greatly augmented either through externally applied tension to the cell or internally through myosin II-driven cell contractility. FAs sense mechanical stresses and function regardless of the intracellular versus extracellular origin (Balaban et al., 2001; Geiger and Bershadsky, 2002; Bershadsky et al., 2003, 2006).

2. Mechanotransduction

Seminal observations by Don Ingber and others (Wang et al., 1993; Ezzell et al., 1997; Goldmann et al., 1998; Dey et al., 2011), using a magnetic twisting device to transfer forces directly from integrins to the local cytoskeleton suggests that mechanical deformation of one or more adhesion plaque proteins is the proximal step in an intracellular signalling cascade that leads to global cytoskeletal rearrangements and mechanotransduction at multiple, distant sites within the cell. Phosphorylation of FA proteins assists in the recruitment and binding of FA proteins by regulating protein–protein interactions in proteins that contain the SH2 (Src homology 2) domain, including paxillin, FAK and p130Cas, whereas Src kinase activation and interaction with vinculin initiates tyrosine phosphorylation of paxillin and p130Cas that leads to FA turnover and cell migration (Brabek et al., 2004; Subauste et al., 2004).

Exactly which protein within FAs acts as a mechanosensor/coupler/regulator or transmitter, however, is currently debated and numerous candidates have been proposed. In the following, I focus on three proteins that are located within the FAs and presently regarded as principal mechanotransducer of adherent cells.

3. FAs

It was shown that in mechanically stimulated fibroblasts and myocytes, FAK and paxillin were mainly affected (Wang et al., 2001; Sawada and Sheetz, 2002; Torsoni et al., 2003). These proteins are recruited to FAs and not to the actin cytoskeleton, which indicates that mechanical distortion of FAs itself is at the origin of mechanical signalling. Roovers and Assoian (2003) demonstrated that the effect of integrin clustering (in response to force generation) on FAs is required for sustained FAK and MAPK (mitogen-activated protein kinase) activation. Artificially clustering of integrins with antibodies relieved the requirement for actomyosin-dependent tension, which strongly suggests that FAs are the key site of mechanosensing. Experiments carried out on matrices that have different regions of elasticity, cells migrated towards areas of higher substrate rigidity. This effect required the expression of FAK, suggesting that this molecule is involved in sensing forces (Lo et al., 2000). Interestingly, FAK phosphorylation on Tyr^{397}, is sensitive to the tethering of integrins to a rigid substratum, whereas integrin clustering alone regulates phosphorylation on other sites of FAK (Shi and Boettiger, 2003). Stretching of the actin cytoskeleton also increased the binding of FAK to paxillin and p130Cas (Sawada et al., 2006). It appears that FAK is a key component of the mechanosensing apparatus and both FAK and its interacting partners seem candidates for mechanotransduction (Figure 1). In all, the information presented

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Abbreviations: ECM, extracellular matrix; ERK1/2, extracellular-signal-regulated kinase 1/2; FA, focal adhesion; FAK, FA kinase; MAPK, mitogen-activated protein kinase; SH2, Src homology 2.

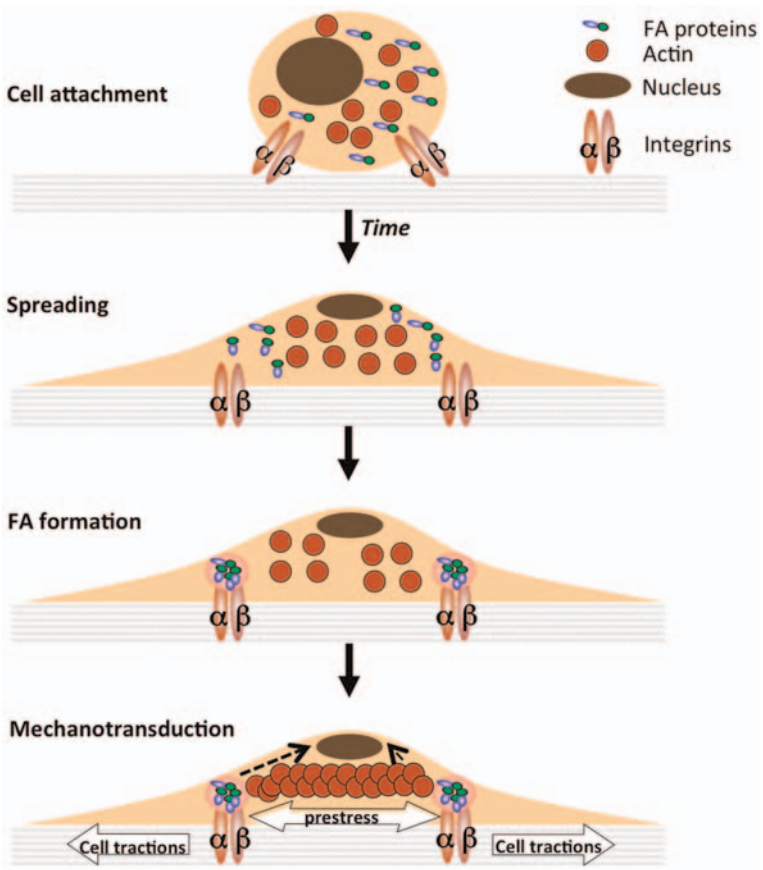

Figure 1 Upon cell attachment via integrins to the ECM, cells start to spread, form FAs, and connect to the actin cytoskeleton to generate internal prestress and cellular tractions and transmit signals to the nucleus

from various research groups suggests a possible mechanism(s) for mechanotransduction within FAs: (i) when force is applied to integrins, which results in clustering because of increased actomyosin recruitment and cytoskeletal assembly, this could contribute to increased receptor density and integrin signalling and (ii) that tension alters the conformation of FAs to induce new binding interactions or direct modulation of enzymatic activity. Conformational changes resulted in activation of tyrosine kinases such as FAK to mediate mechanotransduction. Wang et al. (2001) and Lo et al. (2000) reported that FAK-null and FAK-Y397F expressing fibroblasts failed to reorient in response to mechanical force and to form prominent FAs compared with FAK expressing cells; and Yamamoto et al. (2001) showed MAPK activation and gene expression in neonatal cardiac myocytes after applying mechanical strain. Fundamental questions about mechanotransduction within integrin-mediated adhesions, however, remain unanswered. Elucidating the detailed mechanism by which forces are converted to chemical signals and cytoskeletal rearrangements is the endeavour of many laboratories, including ours.

Vinculin levels within FAs were reported to correlate linearly with traction forces (Balaban et al., 2001). Using beads coated with fibronectin in laser trapping studies showed that the recruitment of vinculin was required for the development of tension between the bead and the cell. This was associated with FAs strengthening or reinforcements (Galbraith et al., 2002). The loss of cytoskeletal tension resulted in a rapid dissociation of vinculin from FAs suggesting that force is required for both FAs development and maintenance (Rottner et al., 1999; Möhl et al., 2009). Vinculin in FAs must therefore be regarded as mechanical coupling and regulating but not as mechanosensing protein (Goldmann et al., 1995; Ezzell et al., 1997). Other reports showed that the interaction between FAK and paxillin, and its activation are also critical for intracellular signalling (Ostergaard et al., 1998; Turner, 1998, 2000; Turner et al., 1999). Subauste et al. (2004) described a signalling pathway whereby vinculin controls FAK–paxillin interaction and alters ERK1/2 (extracellular-signal-regulated kinase 1/2) activity that includes p130Cas and Crk-II, by regulating cell survival and motility. We recently showed that vinculin acts as a regulator for contractile force generation, and that vinculin transfection restores the contractility of vinculin-deficient F9 mouse embryonic carcinoma cells and MEFs (mouse embryonic fibroblasts; Mierke et al., 2008, 2010; Diez et al., 2009; Fabry et al., 2011).

Mechanoreception, transduction and force sensing is a crucial function for p130Cas (Tamada et al., 2004; Yoshigi et al.,

2005). As a mechanosensor, p130Cas responds to the external stress by phosphorylation, which initiates the downstream pathways through MAPK cascade (Sawada and Sheetz, 2002). Basal- or stress-mediated phosphorylation of p130Cas, requires its binding partners, such as FAK and SFK (Src family kinase) (Sawada et al., 2006). p130Cas was reported as an actin filament assembling protein (Honda et al., 1999), though no direct interaction with actin has been reported to date. Detection of p130Cas in FAs of FAK null cells (Nakamoto et al., 1997) indicates the presence of other SH3 domain binding partners, which may connect the gap between p130Cas and actin fibres. Reports of co-localized p130Cas and vinculin (Nakamoto et al., 1997) triggered a study to examine the interaction between them (Dey et al., 2011). Vinculin reacts with talin or other neck-binding proteins (Cohen et al., 2005), and in turn recruits paxillin to enhance integrin clustering (Humphries et al., 2007). From a more recent study by Janostiak et al. (2011), the position Tyr^{12} of p130Cas SH3 domain was found to be extremely influential for FAK binding and successive maturation of FA complexes. We also studied the effect of the Tyr^{12} position on vinculin binding, and its influence over the subsequent stretch-mediated mechanotransduction pathway, through p130Cas phosphorylation. We found that vinculin indeed interacts with p130Cas and influences its basal level or stretch-mediated phosphorylation upon mechanical stress (unpublished observation by Radoslav Janostiak, Jan Brabek, Daniel Rosel, University of Prague and Wolfgang H Goldmann, University of Erlangen). The impact of vinculin on subsequent ERK1/2 phosphorylation was found to be significant and comparable to FAK. Phospho-mimicking and non-phosphorylatible mutations were observed to control vinculin binding *in vivo* and successive phosphorylation of p130Cas and ERK1/2. As a FA and mechanosensing protein, p130Cas is involved in integrin-mediated adhesion and cell migration through multiple signalling pathways (Tikhmyanova et al., 2010). Complete absence of FAK, does not abolish the FA targeting of p130Cas fully (Meenderink et al., 2010), which makes the existence of some other binding partner for SH3 domains very obvious. As an FA protein, p130Cas has itself no actin-binding domain, but influences the actin stress fibre formation (Honda et al., 1999) possibly through one of its binding partners. In some previous study, vinculin was reported to co-localize with p130Cas (Nakamoto et al., 1997). Presently, we analyse the interaction between vinculin and p130Cas, as vinculin might be the 'other' binding partner for p130Cas, through its proline-rich neck region (Winkler et al., 1996; Critchley, 2000).

Future research should advance our understanding on how the cytoskeleton of cells deforms and transmits stresses via FA sites. To test that force transmission through the cytoskeleton is mechanically highly heterogeneous (Goldmann, 2012), and that the FA proteins are important mechanotransducer for a variety of fundamental cell functions including cell division, motility and differentiation, this research will have wide implications in medicine and biology that go well beyond the immediate issues of cellular function.

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