Cytoskeleton-adhesion crosstalk: molecular and biophysical aspects

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Focal adhesions as mechanosensors: a physical mechanism

Cells adhere to the extracellular matrix (ECM) via transmembrane integrin family receptors, which together with numerous associated "plaque proteins" form focal adhesions (FAs) linking the ECM to the actin cytoskeleton. FAs are both adhesion and signal transduction organelles, informing cells about the state of the ECM. We have shown that FAs function as touch receptors responding to the mechanical characteristics of the microenvironment (Bershadsky et al., 2003).

In our theoretical studies (in collaboration with group of Prof. M. Kozlov, TAU), we demonstrated that the major features of the FA mechanosensitive behavior can be explained by thermodynamic principles, which govern self-assembly of molecules into an aggregate subjected to pulling force. Elastic stresses generated within a protein complex decrease the chemical potential of the aggregated molecules relative to the pool of non-assembled molecules . This means that self-assembly of proteins is favored when pulling forces act on the aggregate and disfavored when these forces are relaxed (Fig.1). Considering various types of linkage between the aggregate and the substrate, we predicted different modes of FA assembly and disassembly and showed that the suggested model accounts for the major types of FA behavior observed experimentally (Shemesh et al., 2005a). Thus, this model based on very general assumption allows to portray qualitatively the FA mechanosensitive behavior. A hierarchy of diverse signaling circuits shown to be involved in the focal adhesion mechanosensitivity could be build up as a superstructure onto this basic mechanism.

A mechanism of force-driven focal adhesion assembly can be based on the formin function

Signal from small G-protein RhoA is required for the formation of mature FAs and the associated actin filament bundles (stress fibers). Our studies showed that formin homology protein Dia1 is a downstream target of Rho that mediates force-induced FAs formation (reviewed in Bershadsky et al., 2003). More recent experiments with Dia1 knockdown by siRNA (L. Carramusa et al.) confirmed that Dia1 is necessary for the transformation of initial focal complexes into FA and/or for further elongation of FA (Fig. 2).

Analysis of formin interaction with actin indicates to a possible direct involvement of formins in the functioning of cellular mechanosensory units. Specifically, theoretical consideration predicted a novel phenomenon, the force-driven polymerization of actin mediated by proteins of the formin family (Kozlov and Bershadsky, 2004). Formins localize to the barbed ends of actin filaments, but, in contrast to the regular capping proteins, allow for actin polymerization in the barbed direction (the so-called "leaky" or "processive" capping mechanism). We proposed that the mechanism of leaky capping is based on the elasticity of the formin dimer (Kozlov and Bershadsky, 2004) or, more precisely, elasticity of the formin dimer/barbed end complex (Kozlov and Bershadsky, 2004; Shemesh et al., 2005b). The phenomenon of force-driven actin polymerization is a direct consequence of the phenomenon of leaky capping of actin filaments by formins. We showed that if a pulling force is applied to the formin capping the filament end, the elastic mechanism drives filament growth. Specifically, a moderate pulling force of ~3.5 pN (which can be developed by a single myosin molecule) reduces the critical concentration by an order of magnitude (Kozlov and Bershadsky, 2004).

Cross-talk between focal adhesions and microtubules. A novel role of Dia1 formin

We have shown that Dia1 activation promotes microtubule interactions with FAs (Ballestrem et al., 2004). Thus, Dia1 plays a dual role in the regulation of FAs. First, it is necessary for the FA growth that is induced by force. Second, Dia1 promotes microtubule targeting to FAs, which locally inhibit myosin II-driven contractility and facilitates FA

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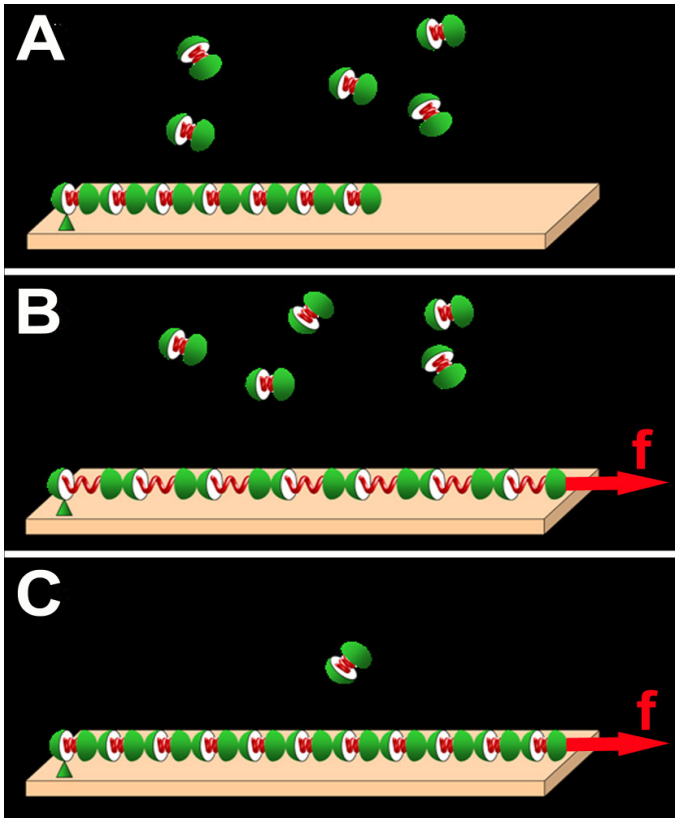

Fig. 1 *Schematic representation of self-assembly upon pulling force. (A)The protein aggregate and the free proteins in the surrounding medium. (B) Application of the pulling force results in the aggregate stretching and the related accumulation of the elastic stresses within the aggregate. (C) Insertion of new proteins into the aggregate results in stress relaxation. From Shemesh et al (2005a).*

turnover (Ballestrem et al., 2004). Dia1 dramatically suppresses microtubule plus end growth, in an actin dependent manner (Bershadsky et al., 2006). In our recent studies we have found that alpha-tubulin deacetylase, HDAC6, affects microtubule dynamics and cooperates with Dia1 in its effect on microtubule growth. These and other results (Bershadsky et al., 2006) suggest that mDia1 and HDAC6 are constituents of a molecular complex responsible for a dynamic link between growing ends of microtubules and microfilaments. Taken together, our data show that Dia1 coordinates the activities of two major cytoskeletal systems, actin and microtubules, in the process of formation and turnover of FAs.

Cadherin-mediated cell-cell adherens junctions

Cells adhere to each other via homophilic interactions between surface adhesion molecules, among which, the transmembrane receptors of cadherin family play a major role. Cadherin-mediated contacts, similarly to integrin-mediated cell-matrix adhesions, trigger the reorganization of the actin cytoskeleton (Bershadsky, 2004). We established that Dia1 formin is required for the integrity of cadherin-mediated cell-cell junctions, and localizes to the junctions via its N-terminal domain (L.Carramusa et al.). Another cadherin's partner, known as p120 catenin (p120), was shown to be an important mediator in the formation of dynamic actin networks at the cell-cell junctions and in other cell compartments. Down regulation of p120, by siRNA, impaires formation of cell-cell junctions and decreases cell spreading on the cadherin-coated substrates (Gavard et al., 2004). We showed recently that down regulation of p120 in epithelial cells leads also to a striking decrease in lamellipodial persistence and in focal adhesion formation. Both in cell-cell junctions and in other dynamic actin structures, p120

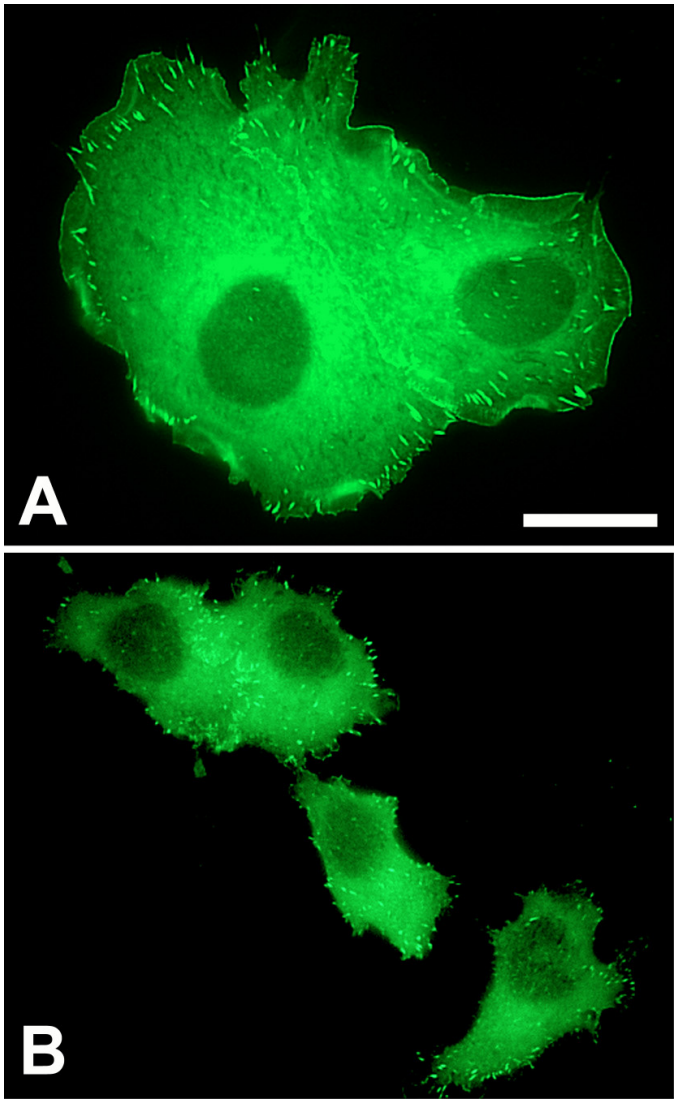

Fig. 2 *Dia1 formin is required for the focal adhesion maturation. Control MCF-7 cells (A) and MCF-7 cells transfected with a vector encoding Dia1 siRNA (B) are both transfected with GFP-VASP to visualize focal adhesions and lamellipodia. Note that Dia1 knockdown prevents formation of mature elongated focal adhesions and reduces lamellipodial activity. Scale bar: 20 μm*

colocalizes with actin-nucleating Arp2/3 complex and with the Arp2/3-activating protein, cortactin. Moreover, we presented evidence of direct interaction between cortactin and p120 (Boguslavsky et al., 2006). Arp2/3 complex and cortactin play an important role in the actin-polymerization-driven lamellipodia extension and in the maintenance of cell-cell junctions (reviewed in Bershadsky, 2004). p120 depletion led to dramatic loss of cortactin and Arp2/3 from cell-cell junctions and from the cell leading edge. We propose a common mechanism underlying p120 functions in both cell-cell junctions and lamellipodia, based on the involvement of p120 in the local regulation of actin polymerization through the modulation of the cortactin-Arp2/3 complex localization.

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