Mechanics and dynamics of cell adhesion: focus on the cytoskeleton

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Molecular complexes involved in cell adhesion to other cells or to the extracellular matrix (Fig. 1) are not just passive links between the external milieu and the intracellular cytoskeletal machinery, but rather serve as signal-transduction organelles informing the cell about biochemical and mechanical characteristics of their microenvironment. In particular, we have previously shown that integrin mediated cell-matrix contacts, known as focal adhesions, function as mechanosensors: they grow in response to mechanical force and shrink upon force relaxation (Bershadsky et al, 2003). A growing body of evidence has demonstrated that cadherin-mediated cell-cell adherens junctions could respond to mechanical forces in a similar manner. The major mechanisms responsible for these unique features of the adhesion complexes are based on specific interactions of adhesion receptors with the cytoskeleton via a system of structural and signaling components working together as a dynamic force-sensing device. Here, we examined and analyzed function of several basic structural and regulatory elements involved in the crosstalk between the adhesion complexes and the cytoskeleton.

1. Role of formin family proteins in the regulation of cell adhesion and actin-microtubule crosstalk

In search of a possible mechanism for adhesion-dependent mechanosensitivity, our group has studied formin family proteins characterized by the presence of highly conserved formin-homology 1 and 2 (FH1 and FH2) domains. Formins are known to be potent stimulators of actin filament nucleation and elongation. Our theoretical studies predicted that formin-mediated actin polymerization could be significantly enhanced by the application of a piconewton pulling force to the formin-capped filament end (Kozlov and Bershadsky, 2004; Shemesh et al, 2005).

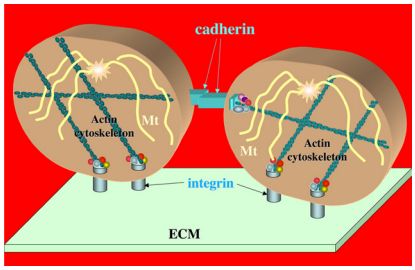

Fig. 1 *A scheme depicting interplay between cytoskeleton and adhesion. Both integrin-mediated cell-matrix adhesions and cadherin-mediated celll-cell adhesions are dynamically linked to actin cytoskeleton and reciprocally controlled by microtubules.*

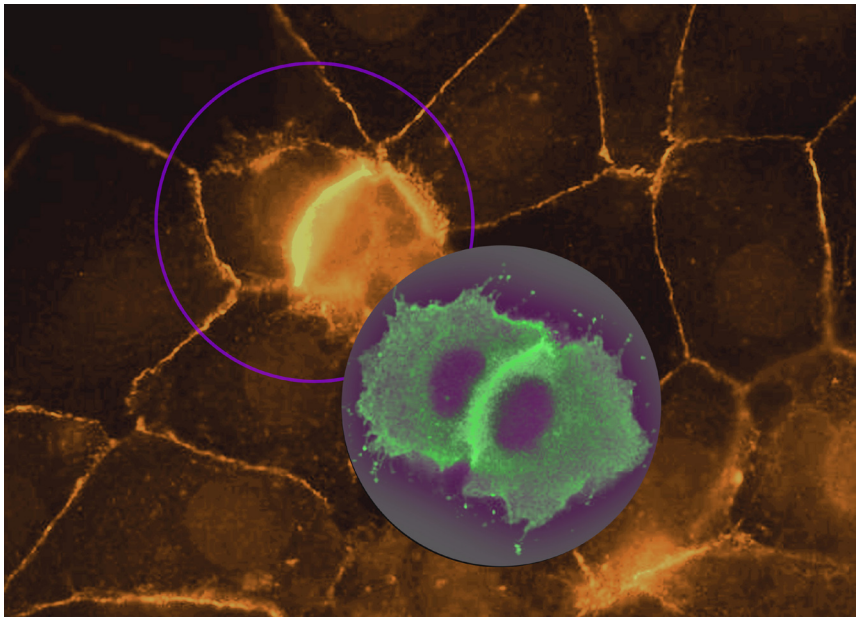

Fig. 2 *Mammalian formin Dia1 activated by RhoA localizes to cell-cell adherens junctions and strengthens them. Adherens junctions in the monolayer of MCF7 cells are visualized by immunofluorescence staining of β-catenin (orange). Two cells, on the upper left (encircled), demonstrate an exaggerated junction with very high β-catenin levels. These cells, as shown in the inset, were transfected with a GFP fusion construct of Dia1 (green) along with a constitutively active RhoA mutant, RhoA-V14. GFP-Dia1 localizes to adherens junctions and to the tips of filopodia produced along the periphery of the transfected cells. See article by L. Carramusa et al. (2007)*

1.1. Mammalian diaphanous-related formin Dia1 controls the organization of E-cadherin-mediated cell-cell junctions

It is well established that both formation and maintenance of cell-cell junctions in epithelial cells depend on the actin cytoskeleton. Formation of cadherin-mediated cell-cell junctions (adherens junctions) is accompanied by profound changes in the actin cytoskeleton and accumulation of polymerized actin at the contact area (Bershadsky, 2004; Erez et al, 2005). Inhibition of actin assembly by treatment with pharmacological reagents such as latrunculin or cytochalasin prevents the formation and strengthening of cadherin-mediated adhesions in addition to disrupting those already in existence. However, the molecular mechanisms underlying the crosstalk between cadherin signaling and the actin cytoskeleton are not fully understood. In particular, the specific role of proteins regulating actin polymerization in the formation and maintenance of the adhesion contacts has yet to be elucidated.

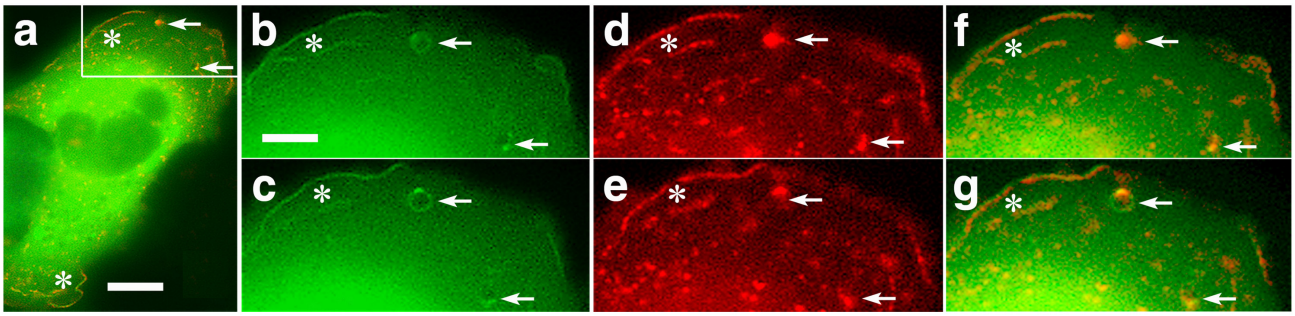

Fig. 3 *Association of p120 catenin with cortactin, an activator of actin polymerization. Colocalization of p120 and cortactin at the cell leading edge, ruffles, and vesicle-associated halos in B16 F1 cells. Cells were cotransfected with GFP-p120 and RFP-cortactin and filmed using double color fluorescence microscopy. A low-magnification merged image is shown in a, and enlarged images of the cell part shown in this photograph inside the rectangular frame are shown in (b–g). GFP-p120 fluorescence is shown in green (b and c), RFP-cortactin fluorescence is shown in red (d and e), and merged images are presented in (f) and (g). The time interval between images (b,d,f) and (c,e,g), respectively, is 30 sec. Arrows indicate halos associated with moving endocytotic vesicles. Asterisks indicate localization of the ruffles. (Scale bars: a, 10μm; b–g, 4.5μm.). See article by S. Boguslavsky et al. (2007)*

The basic unit of the adherens junction is a cadherin/catenin complex, which consists of transmembrane adhesion receptors known as classic cadherins. These interact in a homophilic manner with the cadherins of neighbouring cells. The cytoplasmic domains of classic cadherins interact directly with armadillo family proteins: p120 catenin - via the juxtamembrane domain, and beta-catenin or plakoglobin - via the C-terminal domain. Both beta-catenin and plakoglobin can bind to alpha-catenin, which is another essential element of the cadherin/catenin complex (for review see Erez et al 2005). Monomeric alpha-catenin interacts with beta-catenin or plakoglobin, while homodimers of alpha-catenin can bind actin filaments and regulate their polymerization and cross-linking. p120 catenin is another protein functioning at the interface between cadherin and actin, and this regulates Rho GTPases and (as will be discussed in more detail below) interacts with the actin-binding protein cortactin (Boguslavsky et al., 2007).

Regulators of actin nucleation appear to be critical for the control of cadherin-mediated cell-cell adhesion. For example, the Arp2/3 complex, which promotes the formation of a branched actin filament network, was shown to localize to adherens junctions and physically associate with the cadherin/catenin complex. Moreover, activator of Arp2/3 function, cortactin, demonstrate similar localization. Knockdowns of these components induce weakening of adherens junctions exemplified by the reduced density of cadherin and associated proteins. Another group of molecules controlling actin polymerization comprises the formin family proteins. Formin-1, which was historically the first representative of this group of proteins is now found to be a partner for alpha-catenin. Formin-1 localizes to cadherin-mediated cell-cell junctions in an alpha-catenin dependent manner; its association with the cadherin-catenin complex is necessary for the formation and maintenance of adherens junctions in keratinocytes (see for a review Bershadsky, 2004).

The potential role in cadherin-mediated adhesion of Diaphanous related formins is of particular interest, since their activity is controlled by small G-proteins of the Rho family. In several cell types, Rho, Rac and Cdc42 were shown to be essential for, and activated by cadherin-mediated cell-cell adhesion (Erez et al., 2005). While the roles of Cdc42 and especially Rac can be at least partially related to the regulation of Arp2/3-mediated actin polymerization, the mechanism of apparent Rho-dependence of the adherens junctions is still poorly understood. Diaphanous related formin 1 (Dia1) is one of the Rho targets; RhoA binding triggers the transition of Dia1 from a closed inactive to an open active conformation.

In our study (Carramusa et al., 2007), an RNAi mediated silencing approach was used to demonstrate that Dia1 is required for the adherens junction integrity. Moreover, we show that, upon activation by RhoA, Dia1 localizes to adherens junctions, which leads to their dramatic strengthening and enlargement. In particular, we demonstrated that shRNA-mediated downregulation of Dia1 in MCF7 epithelial cells disrupts adherens junctions, as manifested by significantly decreased localization of E-cadherin and associated proteins to cell-cell contacts. Expression of mouse Dia1, which is insensitive to the human Dia1-specific shRNA, rescued the junctional integrity.

Co-expression of GFP-tagged Dia1 and a constitutively active RhoA mutant, RhoA-V14, resulted in localization of the exogenous GFP-Dia1 to the cell-cell junctions. This localization was accompanied by a strong increase in the width of the adhesion zone and augmentation of the actin, E-cadherin, and beta-catenin content in the junctions (fig. 2).

Adherens junction enhancement induced by Dia1 and active RhoA did not require microtubules, but depended on myosin II activity. Inhibition of myosin II activity abolished the Dia1-mediated reinforcement of cell-cell junctions and instead induced the formation of numerous actin-rich filopodia at the contact zone.

Thus, our data show that the

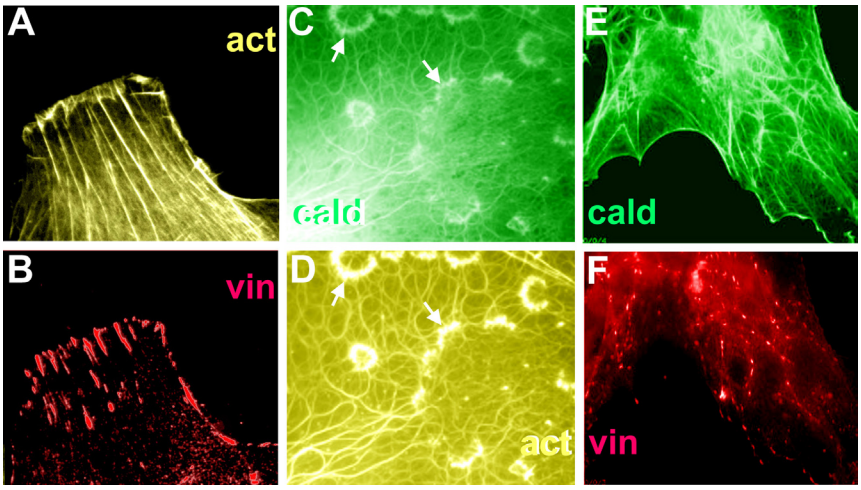

Fig. 4 *Organization of the actin cytoskeleton and focal adhesions in cultured HTM cells and effect of over-expression of caldesmon on these structures. (A) Phalloidin staining reveals filamentous actin (act) shown in yellow. In control cells, actin forms straight bundles (stress fibers) and peripheral lamellipodia. (B) Vinculin (vin) antibody staining of the same cell (shown in red) visualizes numerous elongated focal adhesions localized to the ends of the stress fibers, as well as few small dot-like focal complexes associated with lamellipodia at the cell edges. (C-F) Examples of HTM cells transduced with adenovirus encoding GFP-caldesmon. Distributions of GFP-caldesmon (cald) are shown in (C) and (E) in green; (D) distribution of actin in the cell (C) shown in yellow; (F) distribution of vinculin in the cell (E) shown in red. Caldesmon localized to the actin-containing structures dramatically alters their organization in the cell. The stress fibers disappeared and are substituted by a network of curved actin fibers (C,D,E). In (C,D), numerous actin- and caldesmon-containing circular or semi-circular "waves" are clearly seen (arrows). Staining caldesmon-over-expressing cells with antibody to vinculin (F) does not reveal mature elongated focal adhesions typical for control cells (compare B with F). Small, residual patches are sometimes seen in association with remaining actin fibers (F). See article by Grosheva et al (2006).*

formin protein Dia1 localizes to and controls cadherin-mediated junctions in a RhoA-dependent manner and is an indispensable component of the cell-cell junctions in epithelial cells. The proper function of Dia1 at adherens junctions requires myosin-II activity and may depend on myosin-II driven tension forces.

1.2. Dia1 formin as a possible link between microtubules and actin cytoskeleton

Coordinated activity of the actin cytoskeleton and microtubules is critically important for a variety of basic cellular functions, including cell motility, adhesion, and polarization. We present evidence of formin involvement in the actin-microtubule crosstalk. We have shown that constitutively active Dia1 triggers a decrease in microtubule growth and shrinking velocities; this decrease is not accompanied by any changes in probabilities of microtubule "catastrophe" and "rescue" events. Consistent with these results, knockdown of Dia1 leads to an increase in microtubule growth velocity. The effect of Dia1 on microtubule dynamics was actin-dependent and could be abolished by the inhibitors of actin polymerization, cytochalasin D or latrunculin B. We hypothesize that Dia1 and, perhaps, other formins may create dynamic connections between microtubule ends and actin filaments (Bershadsky et al, 2006a). The possible mechanisms of these effects based on formin interactions with proteins regulating microtubule functions, such as dynactin complex and the alpha-tubulin deacetylase HDAC6 are now under investigation.

2. p120 catenin regulates lamellipodial dynamics and cell adhesion in cooperation with cortactin

Catenin p120 (also known as p120ctn and p120cas) is an armadillo family protein that binds to the juxtamembrane domain of classical cadherins. Knockout or knockdown of p120, as well as experimental interventions blocking its binding to cadherins, leads, in many cellular systems, to perturbation of adherens junction formation/maintenance and impeding cell spreading on the cadherin-coated substrates. In addition to its role in cell–cell junctions, p120 appears to have other functions. Possible involvement of p120 in the regulation of cell migration is especially intriguing because the increased migratory and invasive ability of several types of tumor cells correlates with increased cytoplasmic levels of p120. We have shown (Boguslavsky et al, 2007) that in culture, overexpression of p120 enhances cell motility, while knockdown of p120 reduces it. To elucidate the mechanisms of p120 involvement in motility regulation, we characterized the subcellular localization of p120 in cells and analyze its roles in different processes associated with cell motility and matrix adhesion by using RNAi-mediated gene silencing.

We show that p120 is concentrated at major sites of actin polymerization such as membrane ruffles, lamellipodia, and actin halos associated with endocytotic vesicles. In all these sites, p120 colocalizes with the actin- and Arp2/3 complex-binding protein cortactin (fig. 3). We present evidence for direct interaction between cortactin and p120 and involvement of the latter in the proper localization of cortactin and Arp3 to the ruffles and lamellipodia. Down-regulation of p120 not only destabilizes adherens junctions, but also interferes with persistent lamellipodia extension and formation of focal adhesions. We propose that p120, in cooperation with cortactin, is involved in the regulation of cell leading edge dynamics (Boguslavsky, 2007).

3. Cell adhesion and the actin cytoskeleton of cultured human trabecular meshwork cells:

modulation by caldesmon

Caldesmon is a multifunctional ubiquitous regulator of the actin cytoskeleton, which can affect both actomyosin contractility and actin polymerization. Our previous studies showed that caldesmon over-expression in cultured fibroblasts produces effects that resemble those of chemical inhibitors of cellular contractility. Since these inhibitors (H-7, Y-27632, etc.) have been shown to lower intraocular pressure and increase outflow facility from the anterior chamber of the eye, we proposed that caldesmon might be used for gene therapy of glaucoma. In our study (Grosheva et al 2006) we examined the effects of expression of adenovirus-delivered rat non-muscle caldesmon fused with green fluorescent protein (AdCaldGFP) on the actin cytoskeleton and matrix adhesions in cultured human trabecular meshwork (HTM) cells.

Cultured HTM cells demonstrate a well-developed actin cytoskeleton, comprising mainly arrays of parallel actomyosin bundles (stress fibers). Lamellipodial protrusions containing dense actin networks are also observed (fig. 4). Cell-matrix adhesions are dominated by focal adhesions (FAs) associated with the ends of the stress fibers and focal complexes in lamellipodia (fig. 4). Treatment of HTM cells with AdCaldGFP resulted in dose-dependent morphological changes within 24–48 hr post-infection (fig. 4). About 40–50% of the population of caldesmon-expressing cells demonstrated high levels of GFP–caldesmon expression and severe changes in the actin cytoskeleton, manifested by the disappearance of stress fibers and the formation of curved actin- and myosin-containing bundles. These bundles formed a dynamic network consisting of pulsating loops filling the entire cytoplasm. Another type of novel actin structures induced by caldesmon over-expression were highly dynamic circular waves that propagated over the affected cells. In cells with disrupted stress fibers, vinculin-containing FAs had also essentially vanished. However, punctate focal complexes were still prominent throughout the lamellipodia of these cells (Fig. 4). Thus, our study shows (Grosheva et al, 2006) that caldesmon overexpression induces unique reorganization of the actin cytoskeleton in affected cells, accompanied by disruption of focal cell–matrix adhesions. Inducing such changes in HTM cells in glaucomatous eyes in vivo produced a therapeutically useful increase in outflow facility (Gabelt et al, 2006; Borras et al, 2007).

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