Alexander Bershadsky

Katya Arnold
Christoph Ballestrem
Alex Chausovsky
Inna Grosheva
Miriam Lev Ran
Natalia Magid
Daniel Riveline
Michael Shtutman
Julia Zonis

Interplay between adhesion-dependent signaling and the cytoskeleton

Department of Molecular Cell Biology

Tel. 972 8 934 2884 **Fax.** 972 8 934 4125
E-mail: alexander.bershadsky@weizmann.ac.il

The major research interest of the laboratory is concentrated on the interplay between cytoskeleton dynamics, formation of adhesion structures, and signaling that control cell shape, motility and morphogenesis. The main directions of our work are summarized below.

Focal contacts as mechanosensors: dissection of the Rho-signaling pathway

The transition of cell-matrix adhesions from the initial punctate focal complexes into the mature elongated focal contacts, requires GTPase Rho activity. Activation of myosin II-driven contractility by a Rho target known as Rho-associated kinase, or ROCK, was shown to be essential for focal contact formation. To dissect the mechanism of Rho-dependent induction of focal contacts and to elucidate the role of cell contractility, we applied mechanical force to vinculin-containing dot-like adhesions at the cell edge using a micropipette (Fig. 1). Local centripetal pulling led to development of these structures into streak-like focal contacts, as revealed by the dynamics of green fluorescent protein (GFP)-tagged vinculin or paxillin. Inhibition of Rho activity by C3 transferase suppressed this force-induced focal contact formation. However, constitutively active mutants of another Rho target, the formin homology protein mDia1, were sufficient to restore force-induced focal contact formation in C3 transferase-treated cells. Force-induced formation of the focal contacts still occurred in cells subjected to myosin II and ROCK inhibition. Thus, as long as mDia1 is active, external tension force bypasses the requirement for ROCK-mediated myosin II contractility in the induction of focal contacts. Our experiments show that integrin-containing focal complexes behave as individual mechanosensors exhibiting directional assembly in response to local force.

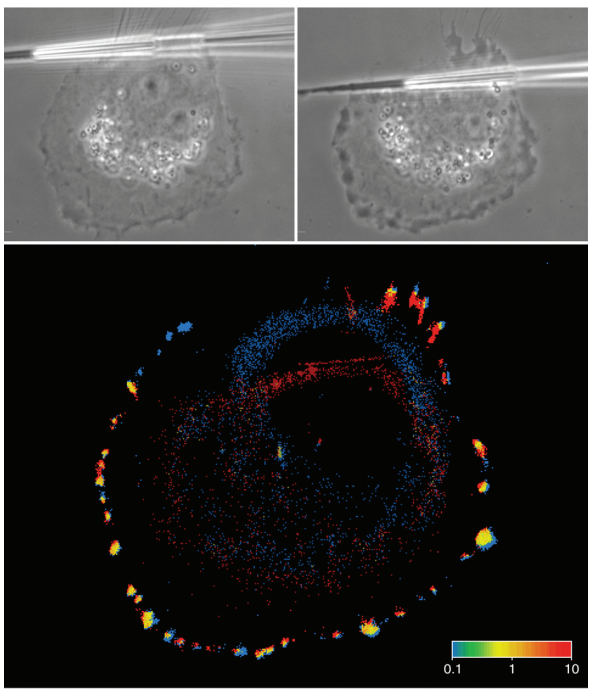

Fig. 1 *GFP-vinculin-transfected cell before (upper left) and after (right) pipette pulling. Lower image shows in spectrum scale, a ratio between intensity of GFP-vinculin fluorescence after pulling versus before pulling. Focal contacts grow upon pulling (red).*

Regulation of cell motility by cell-cell contact protein p120 catenin

Cadherins, the major receptors mediating cell-cell adhesion, associate in the cytoplasm with armadillo family proteins, including beta-and gamma-catenin and p120 catenin (p120ctn). We have shown that overexpression of p120ctn in fibroblasts and epithelial cells induces pronounced changes in cell shape, motility and adhesion to the extracellular matrix. p120ctn-transfected cells display increased filopodial/lamellipodial activity, decreased contractility (Fig. 2), and augmented migratory ability. These effects of p120ctn are mediated by small GTPases Rac and Cdc42. Direct assessment of the activity of these GTPases in cells overexpressing p120ctn revealed significant augmentation of the Rac and Cdc42 activity as compared to non-transfected control cells. Moreover, co-transfection of p120ctn with dominant-negative Rac and Cdc42 suppressed morphological effects of p120ctn. Confocal immunofluorescence visualization of the endogenous p120ctn in dense cultures showed that formation of cadherin-mediated cell-cell contacts is accompanied by sequestering of p120ctn to the junction regions. In sparse cultures p120ctn is distributed over the cytoplasm and enriched

in the lamellipodia and ruffles. Co-transfection with an excess of E-cadherin leads to sequestration of exogenous p120ctn to cell-cell junctions and abolishes p120ctn effects on cell morphology. Thus, p120ctn may couple the formation and disruption of cadherin-mediated contacts with regulation of cell motility by triggering pathway(s) affecting Rac and Cdc42 GTPases.

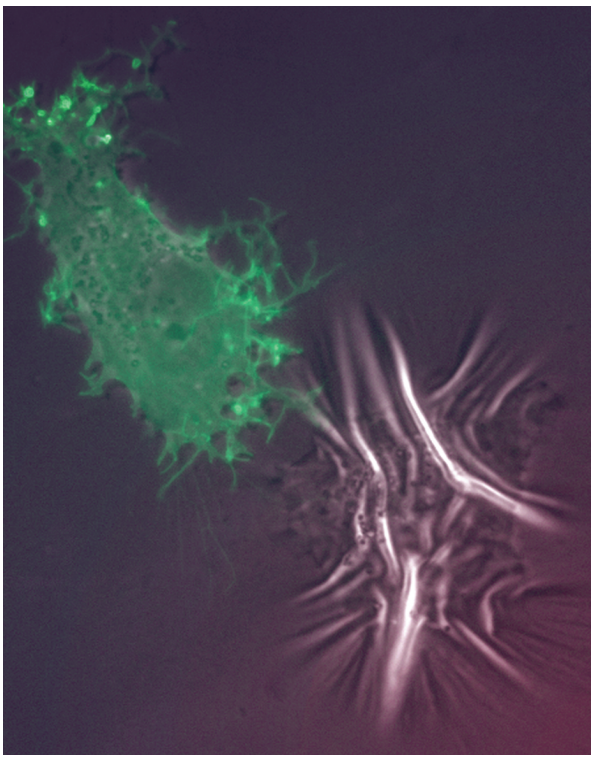

Fig. 2 *p120 catenin suppresses cell contractility. GFP-p120 catenin transfected cell (green) is unable to form wrinkles on the flexible substrate, while the neighboring non-transfected cell produces wrinkles.*

Cell-cell contact dependent regulation of microtubule dynamics

Epithelial polarization and neuronal outgrowth require the assembly of microtubule (MT) arrays not associated with centrosomes. Since these processes generally involve contact interactions mediated by cadherins, we investigated the potential role of cadherin signaling in the stabilization of non-centrosomal MTs. We analyzed MT organization and dynamics in cytoplasts prepared from CHO cells (which normally do not express cadherins) and transfectants of them stably expressing either N- or E-cadherin. Cadherin expression in centrosome-free cytoplasts increased MT polymer level and changed the behavior of MTs from treadmilling to dynamic instability. This effect was not a result of cadherin expression per se but depended on formation of cell-cell contacts. The effect of cell-cell contacts was mimicked by application of beads coated with stimulatory cadherin antibody and was suppressed by overexpression of the cytoplasmic cadherin tail. We suggest that cadherins initiate a signaling pathway, which alters MT organization by stabilizing MT ends.

Selected Publications

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Geiger, B., and Bershadsky, A. (2001) Assembly and mechanosensory function of focal contacts. Curr. Opin. Cell Biol. 13, 584-592.

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