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Cross Talk Between Cell Adhesion, Microtubules and The Actin Cytoskeleton

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The research interests of Hmy laboratory are concentrated on the mechanisms that control cell shape, motility and polarity. These processes depend on coordinated remodeling of the cytoskeleton and adhesion structures. Rho family G-proteins are molecular switches that trigger cytoskeletal reorganizations in response to external signals, including the signals from adhesion receptors. Modulators and effectors of the Rho family proteins are the major factors determining cell morphology and locomotory behavior. We discuss below novel functions of two prominent players in this signaling network: the formin homology protein, mDia1, and the armadillo family protein, p120 catenin.

Coordinate regulation of focal adhesion assembly and microtubule dynamics by formin homology protein mDia1

The formin homology protein, mDia1, is a major target of Rho and is responsible, together with the Rho-associated kinase (ROCK), for the formation of focal adhesions (FAs). Focal adhesions are dynamic molecular complexes associated with integrin-family transmembrane receptors connecting the actin cytoskeleton with the extracellular matrix (Fig. 1). Cell motility depends on both assembly and disassembly of the FAs. In the pathway controlling FAs assembly, ROCK regulates myosin II-driven contractility, while mDia1 is implicated in actin polymerization. At the same time, FA turnover depends on another class of cytoskeletal elements, microtubules (MTs). We found that a constitutively active form of mDia1 (mDia1ΔN3) affects three aspects of MT dynamics. In cells expressing mDia1ΔN3, (1) the growth rate at the MT plus-ends in the cell body decreased by half, (2) the rates of MT plus-end growth and shortening at the cell periphery also decreased, while the frequency of catastrophes and rescue events remained unchanged, and (3) mDia1ΔN3 expression in cytoplasts lacking a centrosome stabilized free MT minus-ends. The changes in MT behavior in the mDia1ΔN3-expressing cells increased MT targeting toward FAs (Fig. 2). Thus, mDia1-dependent alterations in MT dynamics augment the MT-mediated negative regulation of FAs. This function of mDia1 may create a negative feedback loop controlling FA growth.

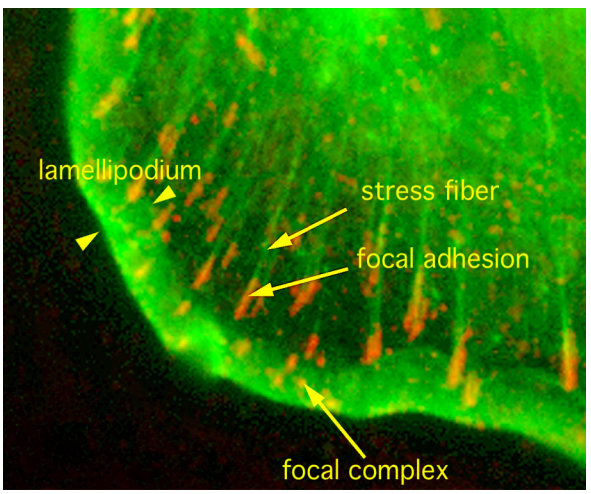

Fig. 1 Association of focal adhesions with the actin cytoskeleton. *A portion of the leading lamella of T47D cell stimulated by neuregulin is shown. Actin is visualized by phalloidin staining (green), and adhesion structures - with anti-phosphotyrosine antibodies (red). The margins of the lamellipodium contain a dense actin network, marked by arrowheads. Here nascent matrix adhesions (focal complexes) are formed.*

Association of p120 catenin with dynamic actin structures and its involvement in the control of actin polymerization-driven motility

The armadillo family protein p120 catenin (p120ctn) binds juxtamembrane domains of classical cadherins and localizes to cell-cell junctions. Upon

overexpression, it enhances the activity of the Rho family GTPases Rac and Cdc42 and augments cell motility. Recently we found p120 in the actin-enriched structures: lamellipodia, ruffles, and 'tails' associated with moving endocytotic vesicles. Co-immunoprecipitation revealed the association of p120 with another component of these structures, cortactin. Downregulation of p120 by siRNA not only destabilized cadherin junctions, but also disorganized lamellipodial activity, resulting in inhibition of cell spreading and migration. Finally, intracellular vesicle velocity increased upon p120 overexpression and decreased upon its downregulation. Thus, in addition to its function at cell-cell junctions, p120 appears to be a potent regulator of actin polymerization-driven cell motility. We propose that a common mechanism underlying all these p120 activities is based on its involvement in local Rac-, Cdc-42-, and cortactin-dependent regulation of actin polymerization. Thus, p120 may couple the formation and disruption of cadherin-mediated contacts with the regulation of cell motility.

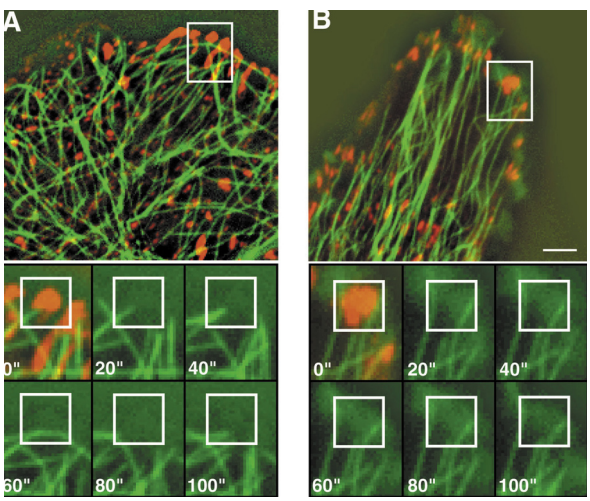

Fig. 2 mDia1 alters the mode of microtubule interactions with focal adhesions.

B16F1 cells expressing tubulin-GFP (green) were super-transfected with a marker for focal adhesions RFP-zyxin (red) alone (A) or together with constitutively active mDia1(B). Sequences of microtubule images presented in the lower part of the figure show that microtubules in cells mDia1 expressing activated remain in proximity of focal adhesions for longer times than highly dynamic microtubules in control cells.

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