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JUNE 2012

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Contact Us

P: 1 (303) 322.2254
F: 1 (303) 322.2257
E: cserve@cytoskeleton.com
W: cytoskeleton.com

Distributors

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Fibronectin Regulation of Cell Adhesion and Fibrillogenesis

Cellular fibronectin (FN) is a dimeric glycoprotein secreted by cells in soluble, nonfunctional form. After attaching to a cell surface, FN polymerizes into insoluble fibrils that are a ubiquitous, functional component of the vertebrate extracellular matrix (ECM) (1,2). FN subunit sizes range from 230-270 kDa, largely due to differential splicing (3). Fibronectins are complex multi-domain proteins with binding sites for many partners including, integrins (4), syndecan-4 (5), gelatin/collagen (6), growth factors (7), heparin (8), and fibronectin (3). This newsletter focuses on the role of fibronectins in cell adhesion and assembly of the insoluble fibronectin matrix (fibrillogenesis) (Figure 1). Both of these processes are regulated by the Rho family of small G-proteins.

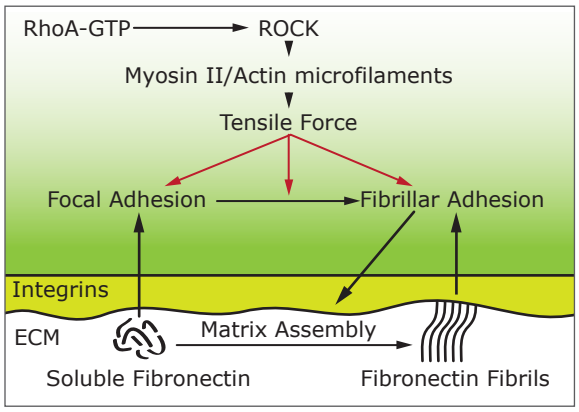

Figure 1: Schematic representation of signaling events involved in fibronectin-mediated cell adhesion and fibrillogenesis.

Fibronectin and Cell Adhesion

Most adherent cell types in 2D cultures attach to soluble fibronectin through interactions with integrin transmembrane receptors (9). The attachment of cells to fibronectin results in cell spreading and early fibronectin-driven integrin clustering. Enhanced integrin clustering subsequently occurs via contractile forces within the cell through activation of the small GTPase RhoA (10). RhoA activation leads to downstream activation of Rho kinase (ROCK), which in turn, activates the ATPase (force generating) and cross-linking functions of the actin motor protein myosin II (11). Cross-linking of actin filaments by myosin results in the formation of actin stress fibers. Motor activity on stress fibers results in a tensile force that causes enhanced integrin clustering and recruits actin and actin binding proteins, as well as signaling, structural and transmembrane proteins, to the clusters. As a result of this clustering and protein recruitment, focal adhesion (FA) complexes form that mediate motility and adhesion of cells onto the soluble fibronectin substrate (4) (Figure 1).

Fibronectin Matrix Assembly (Fibrillogenesis)

The fibronectin matrix assembly nucleates at focal adhesions shortly after cell attachment to the fibronectin substrate (12). The key fibronectin domains involved in polymerization have now been defined and are described in detail in a recent paper (3). Matrix assembly requires RhoA driven cell contractility to induce conformational changes that expose fibronectin binding sites required for FN-FN interactions and early fibrillogenesis (13). Other signaling pathways operating through focal adhesions have been shown to be essential for fibrillogenesis, including activated focal adhesion kinase (FAK) (14). The final step in matrix assembly involves maturation from deoxycholate (DOC) detergent soluble fibronectin filaments to a DOC insoluble matrix. This process is achieved through continuous deposition of FN dimers onto fibrils (15) (Figure 1). The dynamics of fibrillogenesis can easily be tracked with the use of fluorescently tagged fibronectins (13, see page 2 for products).

Trends and Future Directions

Over the last decade, it has become clear that the ECM environment is not simply a cellular support medium, but rather a highly complex structure that continuously sends and receives behavioral cues to and from cells, respectively, to regulate survival, proliferation, differentiation, shape, polarity, and motility (7). The trend to move away from 2D cultures in

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favor of more physiological 3D structures is well under way in the research community and there are now many commercial sources of 3D ECMs (16). Other exciting emerging trends include efforts to apply 3D technology to drug development and even to personalized medicine (16). The understanding that guiding stem cell fate and tissue regeneration will likely require a better characterization and replication of ECM compositions is also a driving force in fibronectin research (17).

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Fibronectin Research Tools

ECM Proteins

Introducing a range of fluorescently labeled and biotinylated fibronectins and laminins.

Fluorescent ECM	Source	Purity	Cat. #	Amount
Fibronectin Red fluorescent, rhodamine	Bovine serum	>80%	FNR01-A FNR01-B	5 x 20 µg 20 x 20 µg
Fibronectin Green fluorescent, HiLyte488™	Bovine serum	>80%	FNR02-A FNR02-B	5 x 20 µg 20 x 20 µg
Fibronectin Biotinylated	Bovine serum	>80%	FNR03-A FNR03-B	5 x 20 µg 20 x 20 µg
Laminin Red fluorescent, rhodamine	Engelbreth-Holm-Swarm mouse tumor	>90%	LMN01-A LMN02-B	5 x 20 µg 20 x 20 µg
Laminin Green fluorescent, HiLyte488™	Engelbreth-Holm-Swarm mouse tumor	>90%	LMN02-A LMN02-B	5 x 20 µg 20 x 20 µg
Laminin Biotinylated	Engelbreth-Holm-Swarm mouse tumor	>90%	LMN03-A LMN03-B	5 x 20 µg 20 x 20 µg

Small G-protein Activation Assays

Cytoskeleton's G-LISA offers a fast and sensitive way of performing small G-protein Activation Assays.

Small G-protein Activation Assays	Method	Cat. #	Amount
RhoA G-LISA® Activation Assay, colorimetric	G-LISA®	BK124	96 assays
RhoA G-LISA® Activation Assay, luminescence	G-LISA®	BK121	96 assays
RhoA Pull-down Activation Assay Biochem Kit™	Pull-down	BK036	80 assays
Rac1,2,3 G-LISA® Activation Assay, colorimetric	G-LISA®	BK125	96 assays
Rac1 G-LISA® Activation Assay, colorimetric	G-LISA®	BK128	96 assays
Rac1 G-LISA® Activation Assay, luminescence	G-LISA®	BK126	96 assays
Rac1 Pull-down Activation Assay Biochem Kit™	Pull-down	BK035	50 assays
Cdc42 G-LISA® Activation Assay, colorimetric	G-LISA®	BK127	96 assays
Cdc42 Pull-down Activation Assay Biochem Kit™	Pull-down	BK034	50 assays
RalA G-LISA® Activation Assay, colorimetric	G-LISA®	BK129	96 assays
RalA Pull-down Activation Assay Biochem Kit™	Pull-down	BK040	50 assays
Ras G-LISA® Activation Assay, colorimetric	G-LISA®	BK131	96 assays
Ras Pull-down Activation Assay Biochem Kit™	Pull-down	BK008	50 assays

G-switch™ Activators and Inhibitors

The most defined way of controlling endogenous Rho protein activity.

G-protein Modulator	Cell Entry Mechanism	Protein Modulation	Cat. #	Amount
Rho Activator II Deamidation of Rho Gln-63	Cell permeable	Direct	CN03-A CN03-B	3 x 20 µg 9 x 20 µg
Rho Inhibitor I ADP ribosylation of Rho Asn-41	Cell permeable	Direct	CT04-A CT04-B	1 x 20 µg 5 x 20 µg
Rho/Rac/Cdc42 Activator I Deamidation of Rho Gln-63 & Rac/Cdc42 Gln-61	Cell permeable	Direct	CN04-A CN04-B	3 x 20 µg 9 x 20 µg
Rho Pathway Inhibitor I Rho kinase (ROCK) inhibitor Y-27632	Cell permeable	Direct	CN06-A CN06-B	5 x 10 units 20 x 10 units
Rho Activator I SHP-2 phosphatase-mediated Rho activation	Cell permeable	Indirect	CN01-A CN01-B	5 x 10 units 20 x 10 units
Rac/Cdc42 Activator II EGF receptor-mediated Rac/Cdc42 activation	Receptor mediated	Indirect	CN02-A CN02-B	5 x 10 units 20 x 10 units