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March
2014

this issue

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Integrin-mediated redox control of β -actin: PDI's Emergence

Cell migration is an integral part of several biological processes including angiogenesis, wound healing, and immune surveillance¹. Integrins are $\alpha\beta$ heterodimeric transmembrane receptors that link a cell's dynamic interactions with the extracellular matrix (ECM) to the cytoskeletal rearrangements that are necessary to promote cell motility (Fig. 1). The ECM proteins that are engaged by integrins include fibronectin, collagen, and laminin; multiple integrins exist with varying affinities for each ECM protein (e.g., nine different integrin isoforms can bind fibronectin)². Consequently, cell adhesion dynamics and motility are influenced by the repertoire of integrins employed by a cell², and changes in the profile of expressed integrins in various cancers have been linked to increased invasive behavior and metastasis^{3,4}.

The transduction of integrin receptor signals to the actin cytoskeleton is a complex and multifaceted process (Fig. 1). Integrin receptors cluster upon engaging the ECM and transmit signals to actin through multiple means, including bridging proteins (e.g., talin, vinculin and α -actinin), the recruitment of the Arp2/3 complex, and activation of kinases (e.g., FAK, ILK, etc.) and small G-proteins (e.g., Rho GTPases)¹. Integrin engagement also leads to the production of reactive oxygen species (ROS) by varied means (e.g., NADPH oxidase, 5-lipoxygenase, and mitochondria⁵), which can result in actin oxidation. Of the six conserved cysteine residues that are present in β -actin, only Cys374 is both redox-sensitive and accessible in both the globular (monomeric) and filamentous (polymeric) forms of actin (i.e., G-actin and F-actin, respectively)⁶⁻⁸. Oxidation of β -actin is necessary for cell spreading in response to integrin engagement with fibronectin and has been shown to occur via the formation of a mixed disulfide bond between Cys374 and glutathione in a reversible process known as S-glutathionylation⁹. This modification actually serves a dual purpose in that it protects actin from irreversible oxidation and it provides a signal for downstream events. One of the important consequences of β -actin S-glutathionylation is the disassembly of the actomyosin complex (i.e., F-actin and myosin), which is a necessary step for successful cell adhesion and migration⁹ (Fig. 1).

The reversal of S-glutathionylation can occur in both an enzyme-dependent and independent manner^{7,10}. The primary enzyme

responsible for this process is thought to be glutaredoxin, although thioredoxin, sulfiredoxin, and protein disulfide isomerase (PDI) can also perform this function^{7,11}. PDI has a canonical role in the formation of correct disulfide bonds in nascent proteins in the endoplasmic reticulum (ER)¹²; however, PDI also functions extracellularly in platelet aggregation^{13,14} and has been shown to be released from the ER in a signal-dependent manner¹⁵. Recent evidence suggests that PDI has a major role in the regulation of β -actin oxidation in response to α IIb β 3 integrin engagement by fibronectin¹⁶. In this study by Sobierajska *et al*¹⁶, PDI was shown to form a mixed disulfide bond with β -actin Cys374 under conditions of integrin activation (Fig. 1). Consistent with this role for PDI residing outside of the ER, immunofluorescence experiments demonstrated that PDI and β -actin colocalize in membrane protrusions (i.e., lamellipodia) associated with cell spreading. The physiological relevance of the

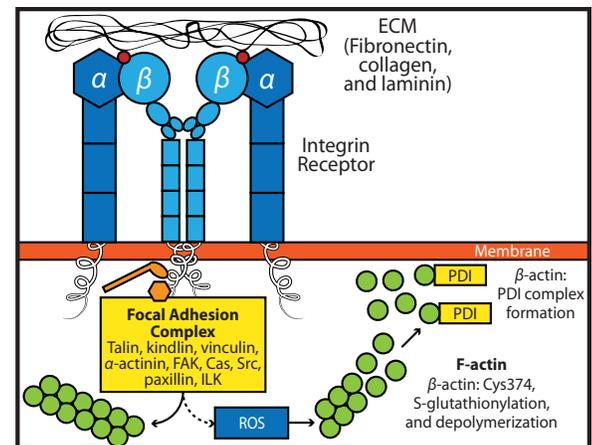


Fig. 1: Impact of integrin receptor interactions with the ECM on actin dynamics. The engagement of the integrin receptor with ECM proteins (e.g., fibronectin, collagen, or laminin) leads to receptor clustering, focal adhesion complex formation and the stimulation of local actin polymerization. In contrast, downstream signaling leads to the production of ROS that promotes β -actin Cys374 oxidation, resulting in S-glutathionylation of this residue and the association of β -actin with PDI, a step that is essential for cell motility.



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Actin Protein PRODUCTS

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β -actin:PDI complex was also demonstrated using antisense RNA to PDI, where the downregulation of PDI expression resulted in impaired cell adhesion and spreading on fibronectin.

The finding that PDI has an important role in regulating the oxidation of β -actin in response to integrin signaling suggests it may be important in other signal transduction pathways where β -actin is S-glutathionylated. Furthermore, it will be fascinating to see if further research in this area reveals a broader role for PDI in regulating other S-glutathionylated proteins, and whether PDI-linked proteins will show promise as biomarkers of disease.

At Cytoskeleton, we offer a variety of actin-based tools to help the researcher study redox regulation of β -actin. Products include purified β -actin (non-muscle; unlabeled and rhodamine-labeled) actin polymerization assay kits, G-/F-actin *in vivo* assay kits, assorted actin binding proteins (e.g., α -actinin, Arp2/3 complex), and antibodies. In addition, we offer custom-produced proteins and assays along with compound screening. For more information on these products and services or if you have questions about redox regulation of actin, please send an email to Cytoskeleton's Technical Support Team (tservice@cytoskeleton.com.)

Actin Protein Products

Actin Binding Proteins	Source	Purity	Cat. #	Amount
α -Actinin Protein	Rabbit skeletal muscle	>90%	AT01-A AT01-C	2 x 50 μ g 10 x 50 μ g
Arp2/3 Protein Complex	Bovine brain	>90%	RP01-A	2 x 50 μ g
Arp2/3 Protein Complex	Porcine brain	>90%	RP01P-A RP01P-B	2 x 50 μ g 6 x 50 μ g
Cofilin Protein	Recombinant human cofilin 1	95%	CF01-A CF01-C	1 x 100 μ g 4 x 100 μ g
Gelsolin Protein	Recombinant human, plasma isoform	>95%	HPG6-A HPG6-B	4 x 20 μ g 20 x 20 μ g
Myosin Cardiac Protein	Bovine cardiac muscle	95%	MY03-A MY03-B	5 x 1 mg 10 x 1 mg
Myosin: Heavy Meromyosin Protein	Chymotrypsin digest of rabbit skeletal muscle myosin II	70%	MH01-A	4 x 50 μ g
Myosin II Protein	Rabbit skeletal muscle	95%	MY02-A MY02-B	5 x 1 mg 20 x 1 mg
Profilin Protein	Recombinant human profilin 1	>95%	PR01-A	1 x 50 μ g
WASP protein VCA Domain: GST tagged Binds & activates Arp2/3	Recombinant human	>95%	VCG03-A	1 x 500 μ g

Actin Proteins	Source	Purity	Cat. #	Amount
Rhodamine Actin Protein (labeled)	Human platelet, non-muscle	>99%	APHR-A APHR-C	4 x 10 μ g 20 x 10 μ g
Actin Protein (unlabeled)	Human platelet, non-muscle	>99%	APHL99-A APHL99-C APHL99-E	2 x 250 μ g 1 x 1 mg 5 x 1 mg

Antibodies	Antigen	Grade	Cat. #	Amount
Actin Antibody (rabbit)	C-terminal of actin	Affinity Purified	AAN01-A AAN01-B	1 x 100 μ g 3 x 100 μ g
Cofilin Antibody (rabbit)	N-terminal of human cofilin1	Affinity Purified	ACFL02-A ACFL02-B	1 x 50 μ g 3 x 50 μ g
Profilin Antibody (rabbit)	Purified human profilin	Affinity Purified	APUF01-A	1 x 50 μ g

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Actin Protein Kits

Product	Cat. #	Amount
Actin Binding Protein Spin-Down Assay Biochem Kit™ Muscle	BK001	30-100 assays
Actin Polymerization Biochem Kit™	BK003	30-100 assays
Actin Binding Protein Spin-Down Assay Biochem Kit™ Non-muscle	BK013	30-100 assays
G-actin/F-actin <i>In Vivo</i> Biochem Kit™	BK037	30-100 assays