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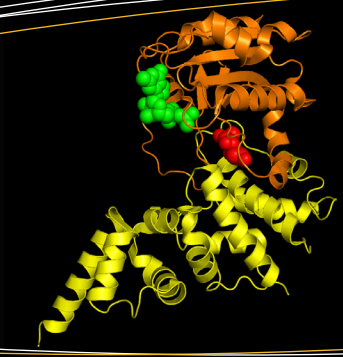
CYTOSKELETON NEWS

NEWS FROM CYTOSKELETON INC.

APR 2012



this issue

Upcoming Meetings

American Association for Cancer Research (AACR)
March 31st - April 4th, 2012
Booth # 4207

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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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Trafficking: Arfs and the Cdc42/Rac connection

The mammalian ADP-ribosylation factor (Arf) subfamily of Ras-related small G-proteins were originally named for their ability to stimulate cholera toxin mediated ADP-ribosylation of the $G_{s\alpha}$ subunit utilized by many GPCRs[1]. The Arf GTPases have been grouped into three classes based on their size and amino acid similarity[2]: class I (Arf1 and Arf3), class II (Arf4 and Arf5), and class III (Arf6). Within this subfamily, Arf1 and Arf6 are the two most widely studied GTPases and they play distinct but similar roles in protein and membrane vesicle trafficking in the cell[3]. Arf1 has historically been viewed as having a primary role in the development and maturation of coated vesicles that transport proteins from the endoplasmic reticulum to the Golgi apparatus, but recent data have also implicated a role for Arf1 in the transport of proteins from the Golgi to the plasma membrane[4]. Arf6 is localized at the plasma membrane and endocytic compartments and plays a significant role in endocytic membrane trafficking and localized actin dynamics, the latter involving cross-talk with Rho family GTPases[3].

Like other small G-proteins, the activation of Arf GTPases is accomplished through the exchange of bound GDP for GTP mediated by guanine-nucleotide exchange factors (GEFs). Several GEFs have been identified for the Arf small G-proteins and each GEF harbors a 200 amino acid Sec7 domain that has been shown to be sufficient for the GEF activity[5]. These GEFs vary greatly in overall size and have classically been categorized by their sensitivity or resistance to inhibition by the fungal antibiotic Brefeldin A (BFA), and more recently through phylogenetic analysis[6,7]. Arf1 is known to be activated by GEFs that fall into both categories with respect to BFA sensitivity, whereas Arf6 appears to be exclusively activated by BFA resistant GEFs[3]. One BFA resistant GEF that is able to activate both Arf1 and Arf6 in biochemical assays is ARNO (a.k.a. cytohesin-2)[8]. Although ARNO exhibits a substrate preference for Arf1, it does activate Arf6 when exogenously expressed in cells[9-11]. Moreover, the activated GTP-bound Arf6 can recruit ARNO to the plasma membrane, leading to subsequent Arf1 activation[11].

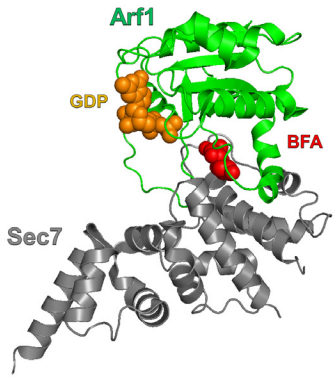

Figure Legend: The structure of the Arf1-Sec7 complex with bound GDP and BFA (PDB ID: 1RE0). Adapted from ref. 16.

Although Arf1 and Arf6 reside primarily in different subcellular locations, they accomplish similar tasks when activated. Both Arf1 and Arf6 are known to activate lipid modifying enzymes that can alter local membrane environments[3]. Changes in the actin cytoskeleton also occur in the vicinity of these membrane micro-environments through Arf1 and Arf6 mediated activation of the Rho family GTPases Cdc42 and Rac, respectively[12]. Golgi-associated active Arf1 recruits Cdc42 to coat protein complex I (COPI) on developing Golgi vesicles where it activates N-WASP, leading to the activation of the Arp2/3 complex and actin

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Continued from Page 1

polymerization[13]. The resulting changes in the local actin cytoskeleton are thought to facilitate Golgi vesicle maturation. At the plasma membrane, Arf6 utilizes Rac to modulate the local actin cytoskeleton in a variety of processes that include phagocytosis and cell adhesion, migration, and invasion[3]. Rac activation via Arf6 is mediated primarily through the recruitment of the Rac GEFs Dock180 (Dock180-ELMO complex), Kalirin5, and Trio[14,15].

Coming soon from Cytoskeleton will be a variety of reagents that can be used for the study of Arf1 and Arf6 and their associated pathways. These product offerings will include activation assays in pull-down format for Arf1 and Arf6, as well as purified recombinant Arf1, Arf6, and the ARNO GEF domain. To study the effect of Arf activation on Rho family GTPases, we also offer activation assays in pull-down and G-LISA® format for Cdc42 (Cat# BK034 and BK127, respectively) and Rac1 (Cat# BK035 and BK128, respectively).

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Arf Protein Research Tools

Proteins	Purity	Cat. #	Amount
Cdc42 His Protein, constitutively-active (Q61L)	>70%	C6101-A C6101-C	1 x 10 µg 4 x 10 µg
Cdc42 GST Protein, dominant-negative (T17N)	>90%	C17G01-A C17G01-C	1 x 25 µg 4 x 25 µg
Cdc42 GST Protein, wild-type	>90%	CDG01-C	8 x 25 µg
Cdc42 His Protein, wild-type	>90%	CD01-A CD01-C CD01-XL	1 x 100 µg 3 x 100 µg 1 x 1 mg
Rac1 His Protein, constitutively-active (Q61L)	>90%	R6101-A R6101-C	1 x 10 µg 4 x 10 µg
Rac1 GST Protein, dominant-negative (T17N)	>90%	R17G01-A R17G01-C	1 x 25 µg 4 x 25 µg
Rac1 GST Protein, wild-type	>90%	RCG01-C	8 x 25 µg
Rac1 His Protein, wild-type	>90%	RC01-A RC01-C RC01-XL	1 x 100 µg 3 x 100 µg 1 x 1 mg
Rac2 His Protein, wild-type	>90%	RC02-A RC02-B	1 x 100 µg 3 x 100 µg

Activation Assays	Cat. #	Amount
Rac1,2,3 G-LISA® Activation Assay, colorimetric	BK125	96 assays
Rac1 G-LISA® Activation Assay, colorimetric	BK128	96 assays
Rac1 Activation Assay Biochem Kit™, Pull-down format	BK035	50 assays
Cdc42 G-LISA® Activation Assay, colorimetric	BK127	96 assays
Cdc42 Activation Assay Biochem Kit™, Pull-down format	BK034	50 assays

Small G-protein Antibodies	Host	Type	Species Reactivity	Cat. #	Amount
Cdc42 Specific Antibody Human Cdc42 Peptide (a.a.123-141)	Mouse	mAb	Hu, Ms, Rt, other extracts	ACD03-A ACD03-B	1 x 100 µg 3 x 100 µg
Rac1 Specific Antibody Human C-terminal Peptide	Mouse	mAb	Hu, Ms, Rt, other extracts	ARC03-A ARC03-B	2 x 50 µg 6 x 50 µg

Phalloidin	Excitation	Emission	Signal stability * ($T_{1/2}$ in secs)	Cat. #	Amount
Acti-stain™ 488 phalloidin	480 nm	535 nm	57	PHDG1-A	300 Slides
Acti-stain™ 535 phalloidin (Rhodamine Phalloidin)	535 nm	585 nm	27	PHDR1	300 Slides
Acti-stain™ 555 phalloidin	535 nm	585 nm	46	PHDH1-A	300 Slides
Acti-stain™ 670 phalloidin	640 nm	670 nm	8	PHDN1-A	300 Slides

* Stability measured without antifade. For comparison, fluorescein phalloidin has a T1/2 of 6 secs.
** One slide equals enough phalloidin to stain a 25 mm^2 coverslip

New Arf Products Coming Soon!

- Arf1 protein
- Arf1 Activation Assay (Pull-down format)
- Arf6 protein
- Arf6 Activation Assay (Pull-down format)
- ARNO protein