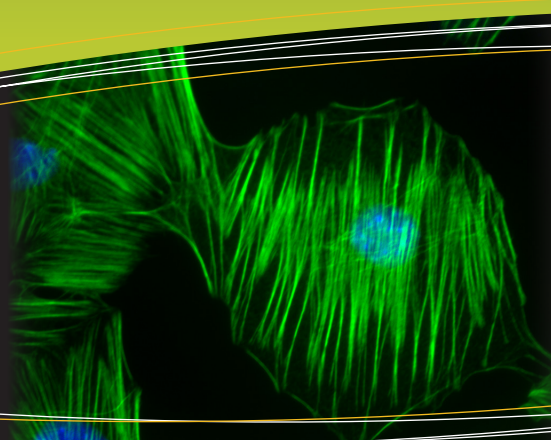




Helping advance science,
one protein at a time.

SEPT 2011



this issue

Small G-protein Activation In Cells

New Direct Modulators

G-switch™ Products

Small G-protein activation in cells: where do you start?

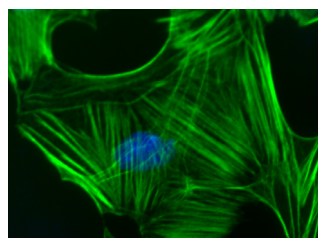
Aberrant regulation of cellular Rho family small GTPase activity has been linked to various disease states (e.g., cancer and cardiovascular, pulmonary and neurodegenerative diseases), and several tools currently exist for the study of Rho family small G-proteins in cells, but each have their limitations.

Growth factors and other physiological signaling molecules that act through membrane-bound receptors (e.g., EGF, PDGF, lysophosphatidic acid (LPA), bradykinin etc.) offer a means of indirectly activating endogenous Rho family small G-proteins in their native setting. These reagents may offer the most physiological approach to activating Rho GTPases, but they introduce other variables that can impact data interpretation. Rho GTPases are not the only proteins that are activated in response to these stimuli. The receptors for each of these molecules also activate other signal transduction proteins in the cell (e.g., Ras, PI3 kinase, phospholipase C, STAT etc.).

For studies of RhoA and its isoforms, it is possible to limit pleiotropic signaling by using the cell permeable small molecule calpeptin. Calpeptin is thought to indirectly activate endogenous Rho isoforms by inhibiting the tyrosine phosphatase (SHP-2) that functions as an upstream negative regulator of RhoA. Calpeptin is more selective than the physiological signaling molecules described above, which makes it a useful tool when indirect activation of Rho GTPase isoforms is desired.

DNA transfection-based tools allow for the expression of exogenous constitutively-active or dominant-negative small G-proteins and offer a way to have selective activation of the small G-protein of interest. The interpretation of data from these experiments can unfortunately be complicated by the fact that supraphysiological levels of the small GTPase are produced in the cell by this approach, which can titrate out cellular factors and impact other cellular pathways.

Cytoskeleton, Inc. has developed the new G-switch™ small G-protein activators that offer the selectivity of DNA transfection approaches and eliminate the issues related to protein overexpression. These new cell permeable activators create constitutively active endogenous Rho family small GTPases in the cell. These cell permeable activators are based



Actin cytoskeleton morphological changes induced by Rho/Rac/Cdc42 Activator 1 (Cat. # CN04) treatment of Swiss 3T3 cells. Cells were fixed, stained with Acti-stain™ 488 phalloidin (Cat. # PHDG1), and visualized by fluorescence microscopy.

on the bacterial CNF toxins that directly modify Rho family G-proteins (Gln-63 of RhoA, Gln-61 of Rac1 or Cdc42). Deamidation of Gln-63/61 by these activators converts the glutamine to glutamate, resulting in a small G-protein that is constitutively active and cannot be inactivated by cellular GTPase

activating proteins (GAPs). Consequently, data interpretation becomes much clearer since any observed cellular response can be directly linked to the activation of endogenous small G-protein(s) and is not the result of pleiotropic signaling.

Publication Spotlight

Anderson et al. 2011. The L6 domain tetraspanin Tm4sf4 regulates endocrine pancreas differentiation and directed cell migration. *Development*. **138**, 3213-3224.

Aguilar et al. 2011. Phos-Tag-Based Analysis of Myosin Regulatory Light Chain Phosphorylation in Human Uterine Myocytes. *PLoS ONE*. **6**, e20903

Schoenwaelder et al. 2000. The protein tyrosine phosphatase Shp-2 regulates RhoA activity. *Current Biology*. **10**. 1523-1526

Upcoming Meetings

- Neuroscience Nov 12-16
- AACR-NCI-EORTC Nov 12-16
- ASCB Dec 3-7

Cytoskeleton Products

- Actin Proteins
- Antibodies
- Activation Assays
- ELISA Kits
- G-LISA® Kits
- Pull-down Assays
- Motor Proteins
- Small-G-Proteins
- Tubulin Proteins

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Helping advance science,
one protein at a time.

G-switch™ PRODUCTS

Rapid results

Modulates Rho in multiple species and cell types

Direct modulation

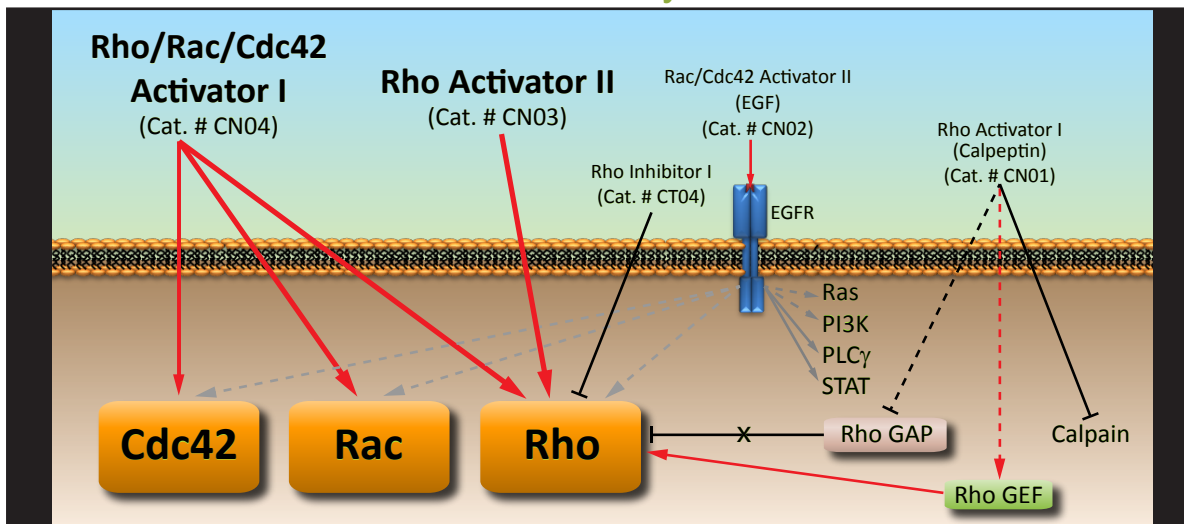
Visit cytoskeleton.com for more information on our G-switch™ activators and inhibitors.

Introducing G-switch™ Activators and Inhibitors

The G-switch™ line of small G-protein tools has been developed with an emphasis on creating highly potent reagents that target endogenous Rho family proteins and pathways. In contrast to methods that rely on over-expression or knockdown of target proteins (e.g., DNA transfection of dominant-negative or constitutively-active Rho mutants, RNAi knockdown), G-switch™ reagents act rapidly on the endogenous target protein (in minutes

to hours, depending on the product), thereby optimizing the chance of generating a more physiologically relevant response. The G-switch™ product line includes reagents that directly and indirectly modulate Rho family signal transduction, thereby offering a wide range of mechanistic tools to study these critical cellular functions. See Cytoskeleton's web site for the latest G-switch™ information.

Rho Activator and Inhibitor Pathways



G-switch™ Products

G-Protein Modulators		Rho Family Protein Modulation	Cell Entry Mechanism	Cat. #	Amount
Direct Modulators	Rho Activator II Deamidation of Rho Gln-63	Direct	Cell permeable	CN03-A CN03-B	1 x 20 µg 5 x 20 µg
	Rho Inhibitor I ADP ribosylation of Rho Asn-41	Direct	Cell permeable	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	Direct	Cell permeable	CN04-A CN04-B	1 x 20 µg 5 x 20 µg
Indirect Modulators	Rho Activator I SHP-2 phosphatase mediated Rho activation	Indirect	Cell permeable	CN01-A CN01-B	5 x 10 units 20 x 10 units
	Rac/Cdc42 Activator II EGF receptor mediated Rac/Cdc42 activation	Indirect	Receptor mediated	CN02-A CN02-B	5 x 10 units 20 x 10 units

