

The Small GTP-Binding Protein rho Regulates the Assembly of Focal Adhesions & Actin Stress Fibers in Response to Growth Factors

Anne J. Ridley & Alan Hall
1992. Cell

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(<http://www.siena.edu/biology/faculty/cellbiology/actin.jpg>)

Background

- Rearrangement of cytoskeleton important for biological processes: cell shape & locomotion
- Rho family, monomeric GTPases (5 subfamilies): Rac-, **Rho-**, & Cdc42-like
- Prominent, observable structures associated with rearrangement (fibroblast motility): *membrane ruffles & lamellipodia extensions (Rac-like)*, *stress fibers & focal adhesions (Rho-like)*
- 1992: regulation mechanism of cytoskeleton rearrangement unknown (stress fibers, focal adhesions, etc.)

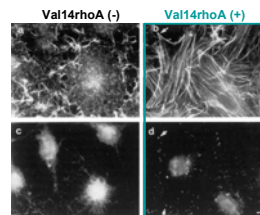


Introduction:

- rho family proteins are monomeric GTPases in ras superfamily (oncogenesis)
- C3 transferase ADP-ribosylates rho proteins (inhibits GEFs) & introduction of C3 into cells causes loss of stress fibers
- Microinjection of cells with recombinant rho proteins causes *stress fiber formation*

Hypothesis: rho proteins are involved in regulating the organization of polymerized actin

Are focal adhesions associated with ends of rho-induced actin stress fibers?



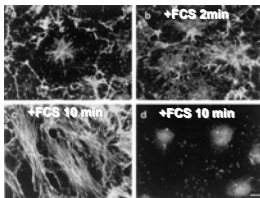
Actin & Vinculin distribution in serum-starved 3T3 cells (FITC-phalloidin & anti-vinculin Ab)

- A. punctate, few fibers
- B. **abundant stress fibers**
- C. vinculin in cytoplasm, rarely associated with fibers
- D. **vinculin @ ends of fibers marking focal adhesions**

- rho-induced stress fiber formation are associated with focal adhesions (arrows)
- Rapid formation (began w/in 10 min) suggestive of pathway

Is rho part of a signaling pathway leading to stress fiber/ focal adhesion formation?

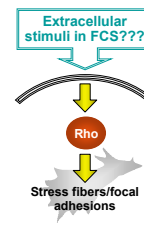
Serum-starved 3T3 cells with or without the addition of 0.2% FCS:



- A. punctate actin in serum-starved 3T3s
- B. stress fiber formation @ 2 min
- C. 10 min peak density of stress fibers
- D. vinculin @ ends of fibers marking focal adhesions (1st detectable @ 2 min & peak @ 10 min)

- Serum stimulates stress fiber/focal adhesion formation similarly to rho
- Suggests serum may have factor acting upstream of rho

Is a factor in FCS activating a rho-dependant signaling pathway?



Added peptide factors back to starved cells (6 present in serum):

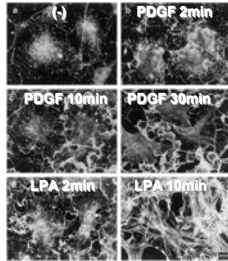
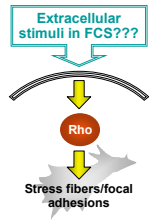
Table 1. Changes in the Actin Cytoskeleton of Serum-Starved Swiss 3T3 Cells Induced by Growth Factors

Addition	Stress Fibers 5 Min	Stress Fibers 30 Min	Ruffling 10 Min
FCS (0.2%)	++++	++++	+
LPA (20 ng/ml)	++++	++++	--
Bombesin (10 nM)	++	++	++
PDGF (3 ng/ml)	--	++	+++
EGF (10 ng/ml)	--	++	+++
Insulin (1 g/ml)	--	+	++
Thrombin (40 ng/ml)	--	+	+

(*s indicative of stress fiber relative density)

- PDGF etc. different rate/level formation from FCS
- Bombesin only factor that ~mimics FCS-mediated stress fiber/focal adhesion formation (antagonist blocked formation)

Is a factor in FCS activating a rho-dependant signaling pathway?



- PDGF: ruffles @ 2 min; peak @ 5-10 min; some fibers by 30 min (<D)
- LPA: rapidly induces stress fibers
- Tested factors in combos with no effect: *not a peptide growth factor*

Is FCS activity due to a phospholipid?

Fig 4: Fractionated FCS & tested each fraction for activity

- Activity corresponds to fraction co-migrating with 69kDa BSA (some lipids bound to serum albumin in plasma) & trypsin/heat R

Table 2: Fractionated FCS & tested each fraction for activity

- Fraction 27 from active FCS; LPA & PA mixed with BSA, fractionated, & treated with phospholipases B (breaks both FA-glycerol ester bonds) or A2 (converts phospholipids to lysophospholipids)

- LPA the ligand!

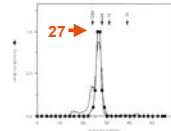


Table 2. Stress Fiber Induction by Phospholipids and an Active Serum Fraction after Treatment with Phospholipases

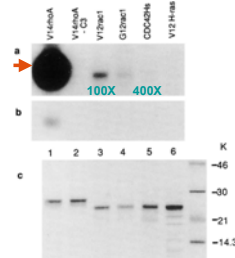
	No Addition	Phospholipase B	Phospholipase A2
No addition	---	---	---
Fraction 27 (50 u/ml)	+++	+	+++
Fraction 27 (10 u/ml)	+	---	++
LPA (200 ng/ml)	+++	---	+++
LPA (20 ng/ml)	++	---	++
PA (200 ng/ml)	---	---	+++
PA (20 ng/ml)	---	---	+
Bombesin (10 nM)	++	++	ND

Does endogenous rho stimulate stress fiber formation?

Used in vitro assay to see if C3 ribosylation rho-specific:

- A/B. PAGE gels @ different exposures: proteins incubated w/ C3 transferase & P32-NAD⁺ (ribosylation substrate)

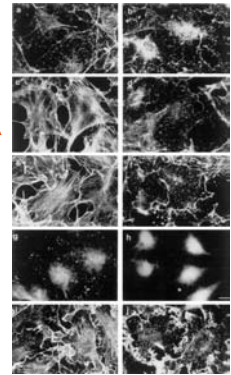
- C. coomassie stain of A/B



Does endogenous rho stimulate stress fiber formation?

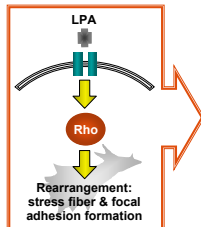
Starved cells injected & stimulated:

- (a) not injected; (b) C3 160ug/ml
- (c) C3 80ug/ml+LPA; (d) C3 160ug/ml + LPA
- (e) buffer + LPA; (f) ribosylated rhoA + LPA
- (g) LPA (Vinculin); (h) C3 160ug/ml + LPA (Vinculin)
- (i) PDGF; (j) C3 160ug/ml + PDGF

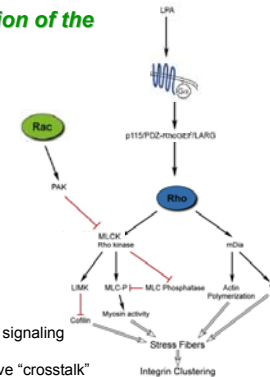


- C3 & dominant-negative, ribosylated rhoA inhibit stress fibers & focal adhesions

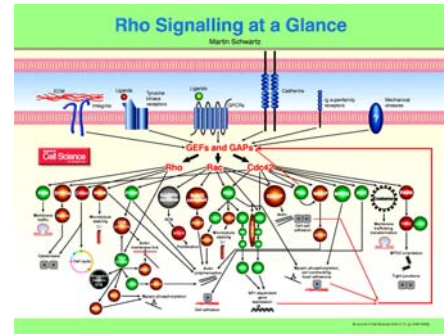
Conclusions & evolution of the model



- Papers identified Rho/Rac as key signaling molecules
- Showed signaling pathways involve "crosstalk"



Rho-like family: major switches in many pathways



Schwartz, M. J Cell Sci 2004;117:5457-5458