Mathematical Cell Biology Graduate Summer Course

University of British Columbia, May 1-31, 2012

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Small GTPases and cell polarization: Rock'N Roll with Rac and Rho (and Cdc42)

www.math.ubc.ca/~keshet/MCB2012/

Big questions

- How does a cell know when to divide?
- How does it coordinate the process of division ("cytokinesis")?

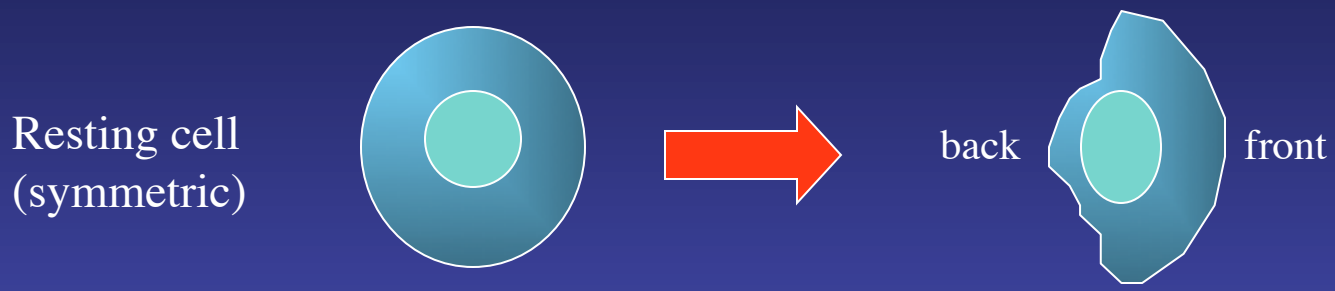
- How do cells move? What guides them?
- How do cells sense "directional cues"?

- How does a multi-cellular organism get its form/shape? ("morphogenesis")

Subsidiary question

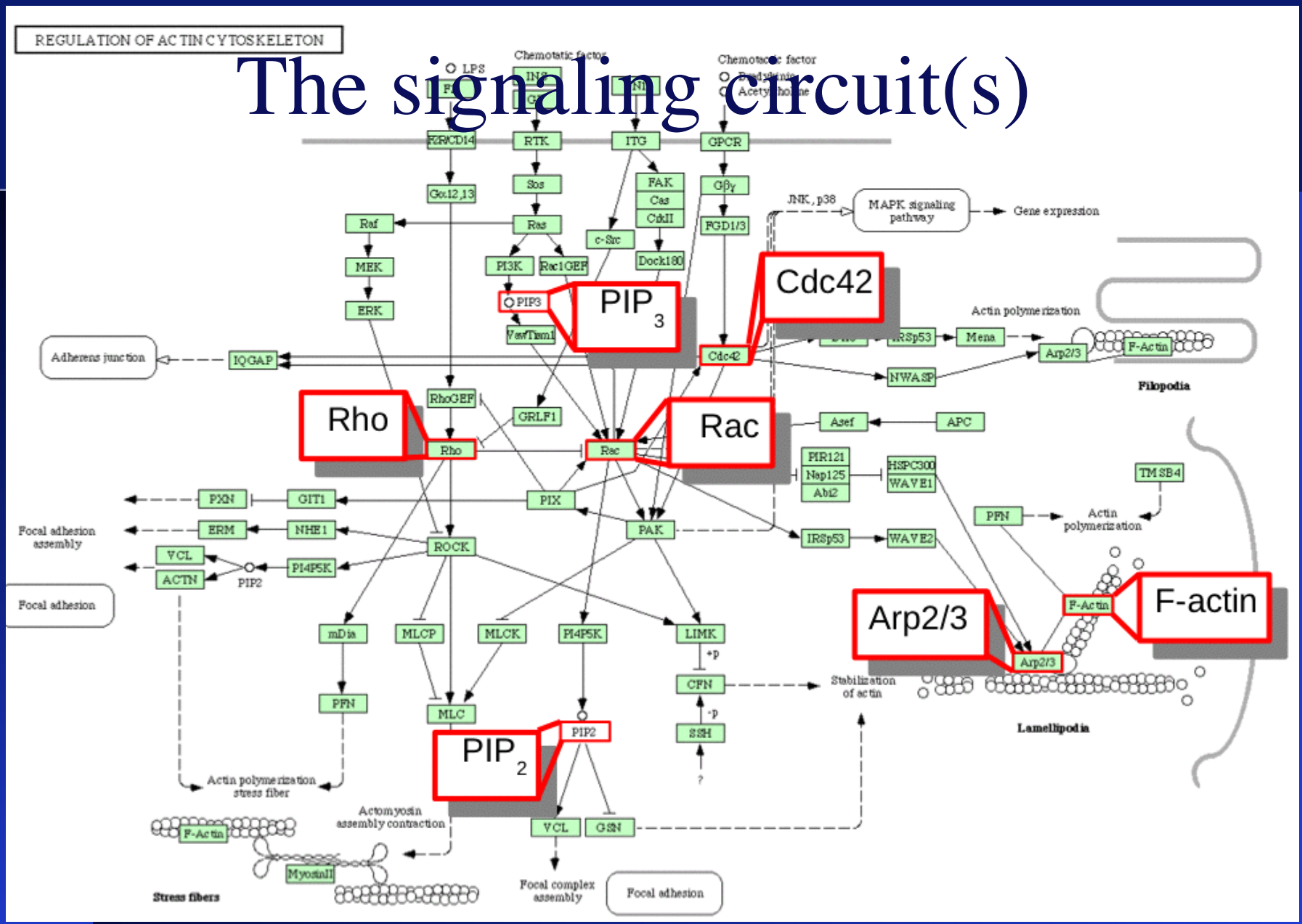
- How do cells polarize?
- How do cells sense “directional cues”?

Polarization (morphological)



Cell senses shallow gradient (or other stimuli) to decide which way to go

The signaling circuit(s)

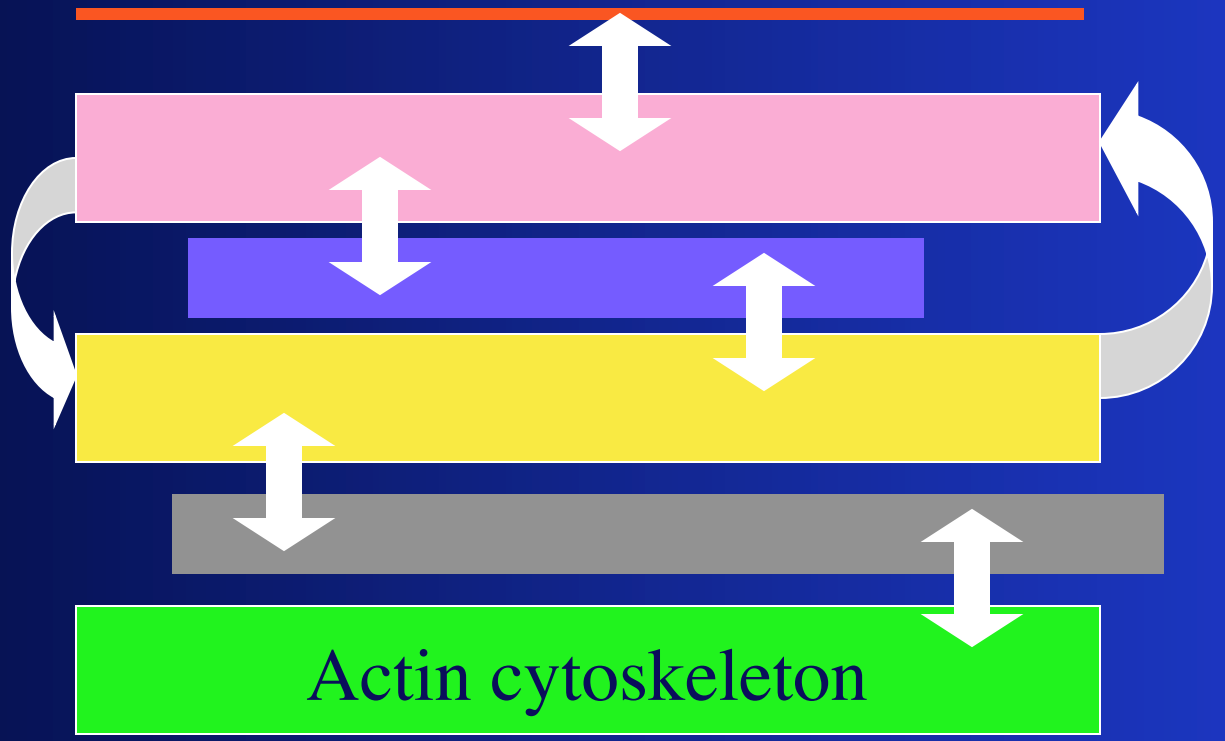


Signaling to actin (KEGG): www.genome.ad.jp/kegg highlights credit: A T Dawes

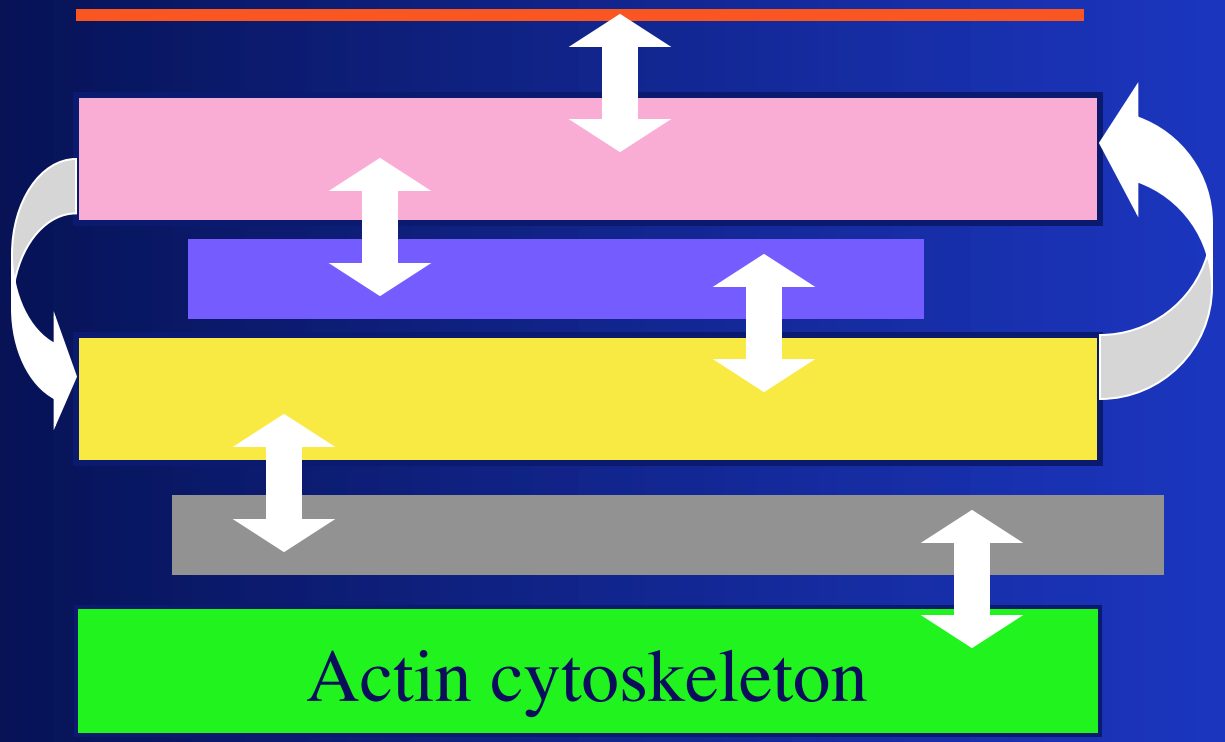
Abstraction:

"Layers" (or modules) of related proteins

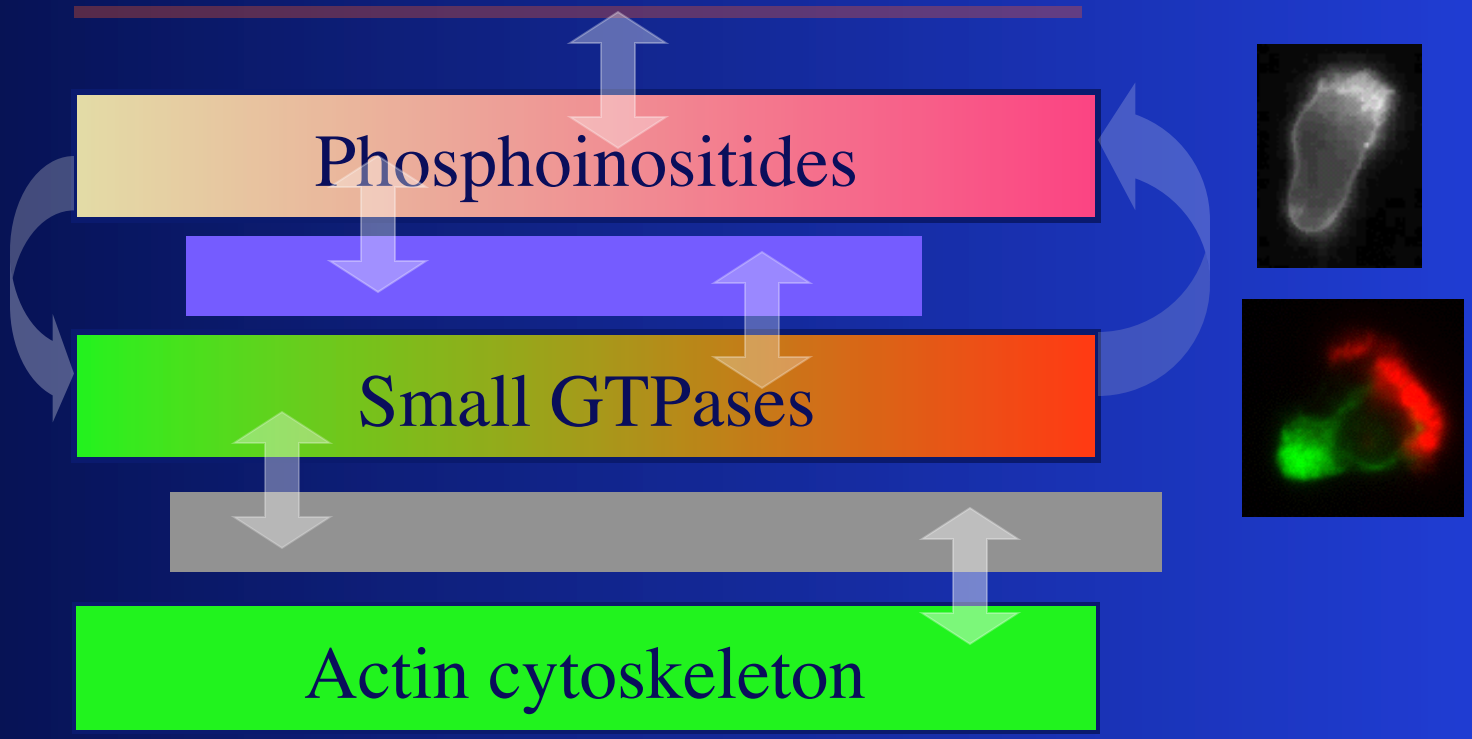
Many interconnections



Focus on a subset



Particularly those that are involved in cell polarization



Study each layer on its own

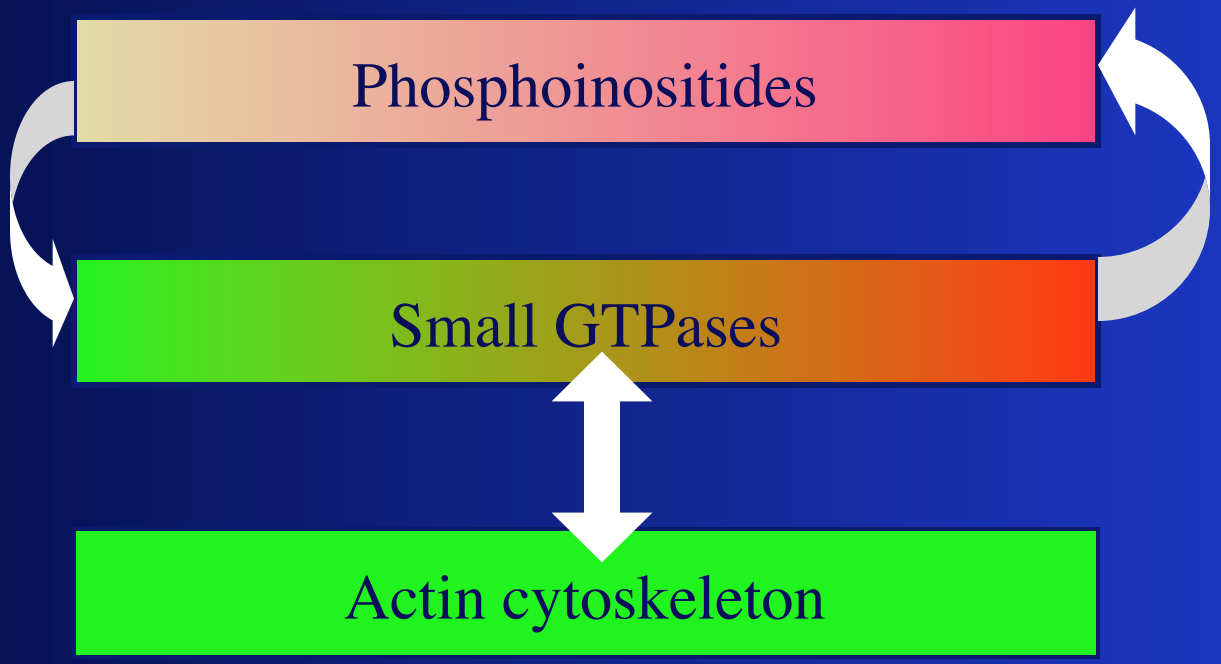
Determine its role in polarization

Phosphoinositides

Small GTPases

Actin cytoskeleton

Then connect up to other layers and to the actin cytoskeleton

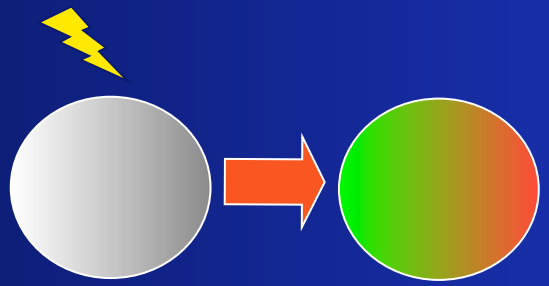


Step 1: Small GTPases

Detail:

Hypothesis:

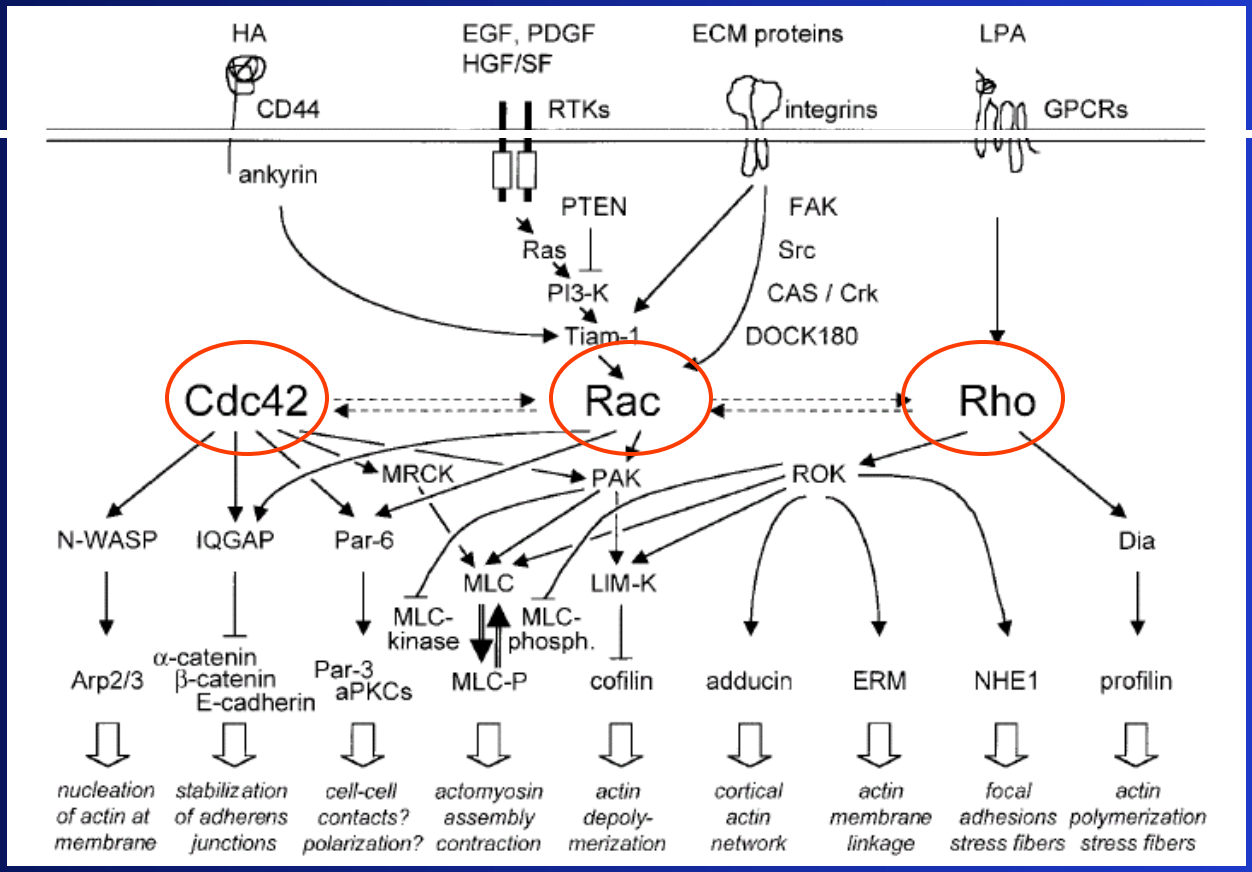
This module has inherent ability to polarize



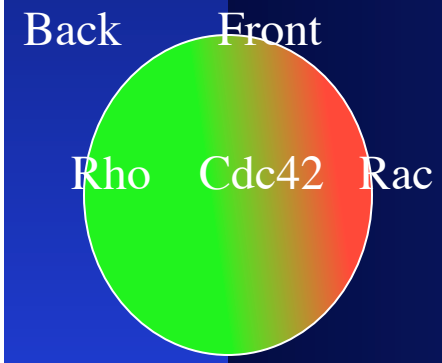
Step 1: Small GTPases

Jilkine A, Maree AFM, LEK (2007) Bull Math Biol

Rho GTPases (Cdc42, Rac, Rho)



Schmitz et al (2000) Expt Cell Res 261:1-12

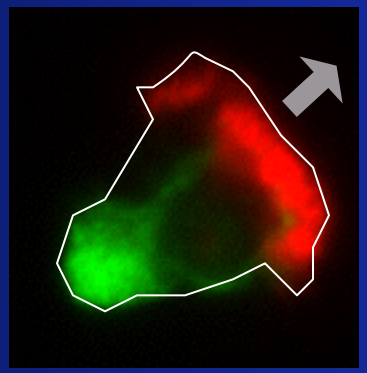


Polarization (biochemical)

Rear

Front

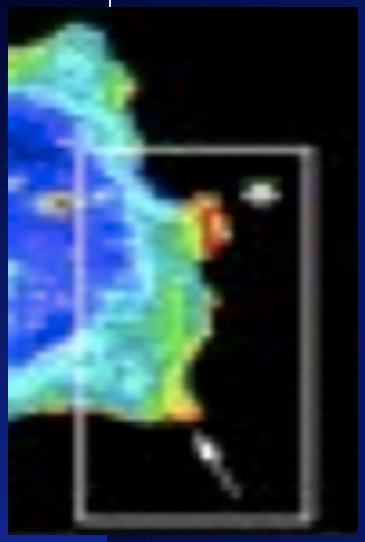
Timescale:
seconds to
minutes



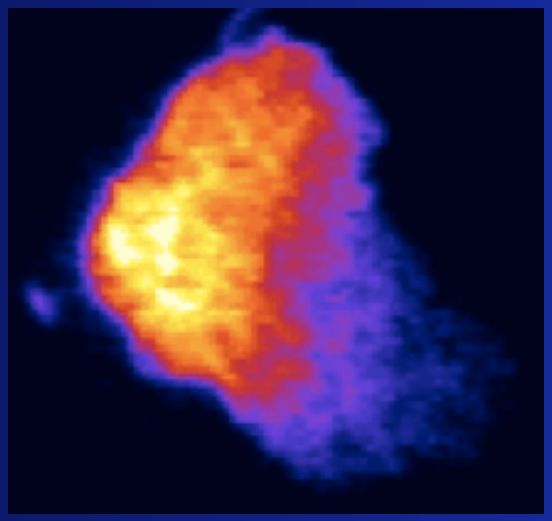
Henry Bourne's Lab

RhoA actin

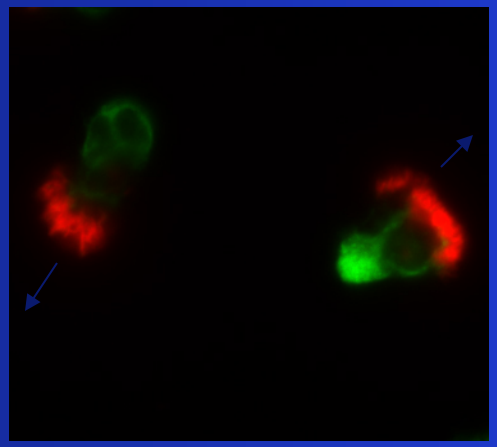
GTPases polarize rapidly in the cell



Cdc42 (red) in front
Nabant et al (2004) Science



Rac in front, neutrophil
Weiner et al (2007) PLoS Biology 5

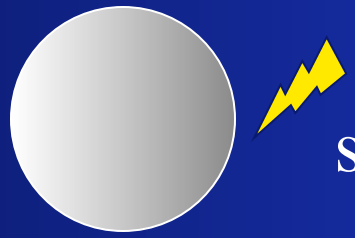


Rho (green) in back
actin (red) in front (neutrophil)
Bourne lab
http://www.cmpharm.ucsf.edu/bourne/

Fig panels credit: Jilkine

Sequence of events:

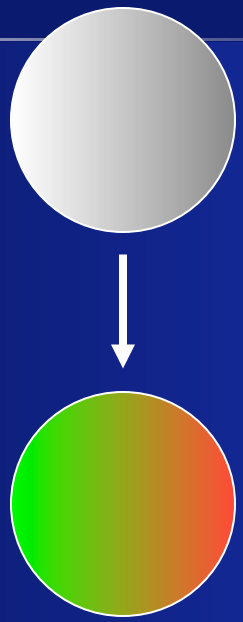
Resting cell



stimulus

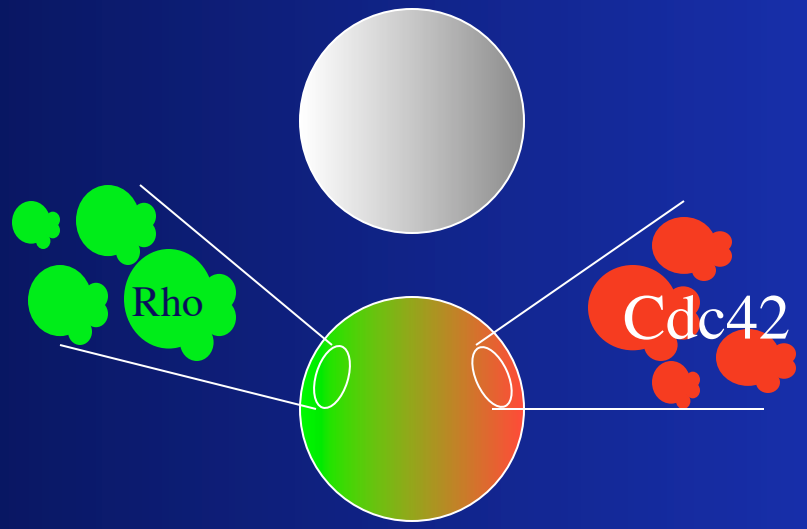
Rapid polarization

Resting cell



Polarized cell

Front vs Back

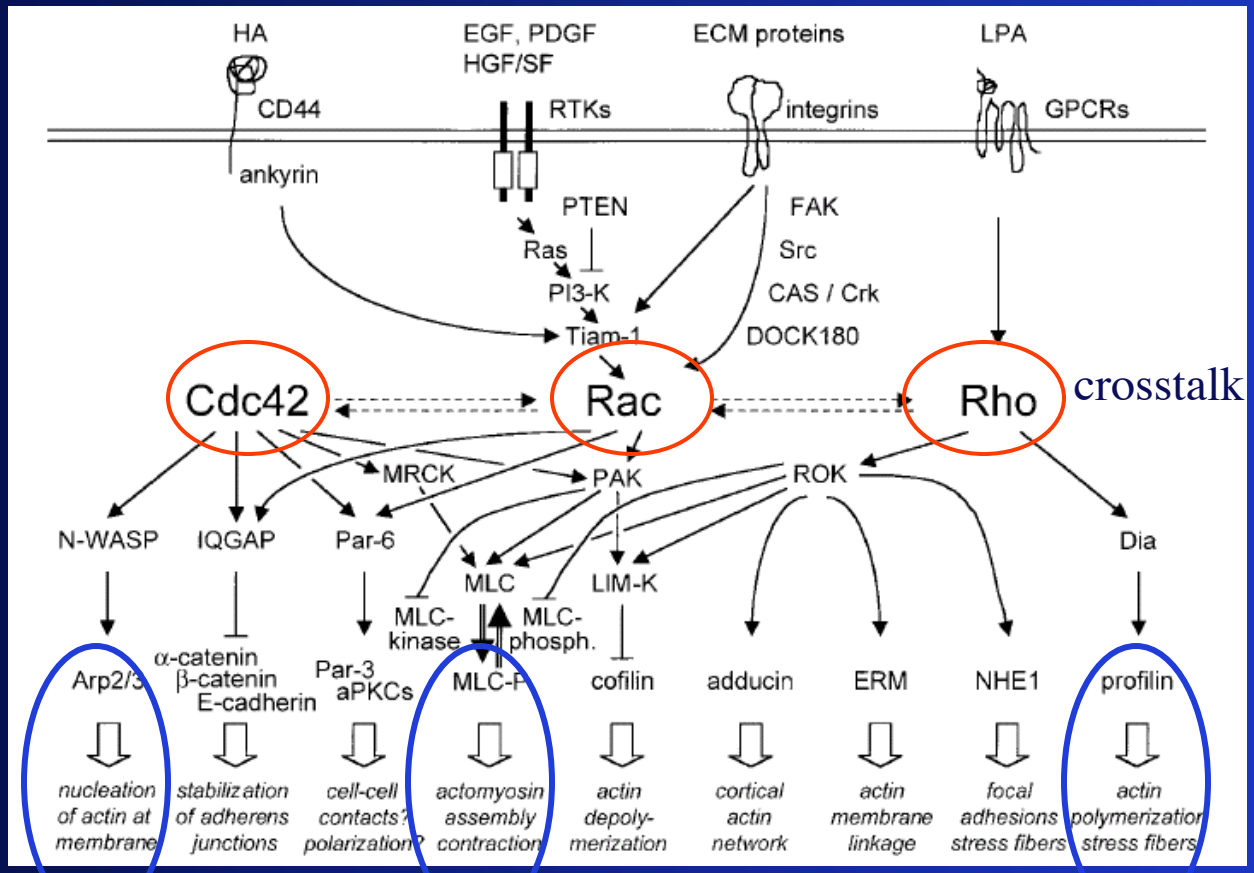


"Chemical polarization"

Rho in the back

Cdc42 in the front

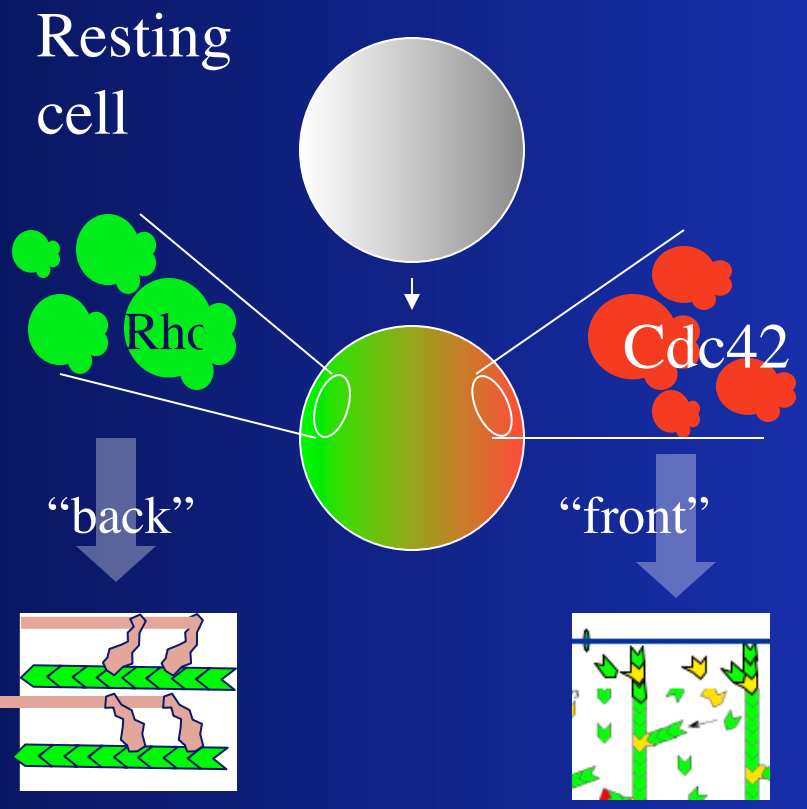
Rho GTPases (Cdc42, Rac, Rho)



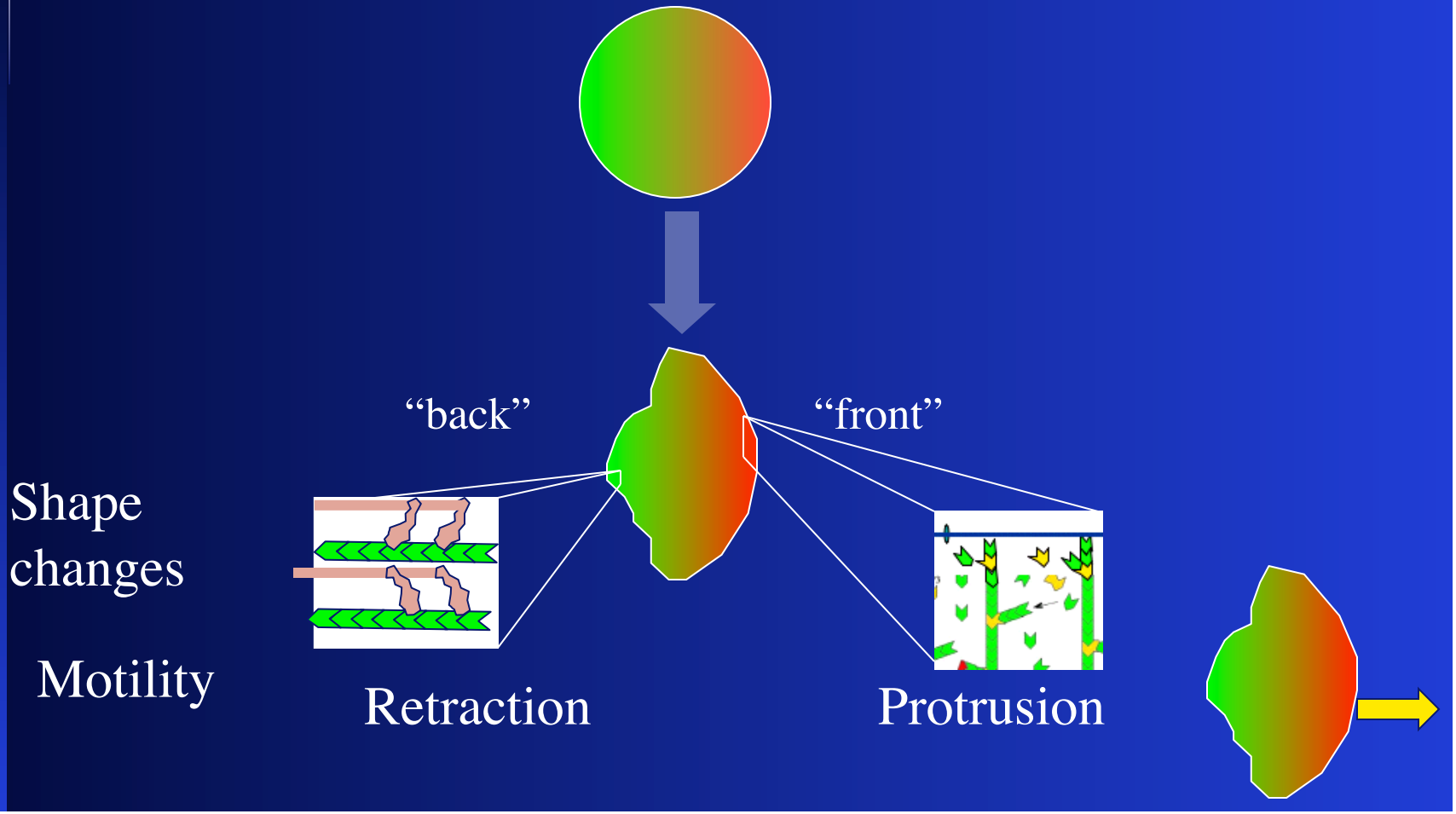
Schmitz et al (2000) Expt Cell Res 261:1-12

These affect actin and myosin

Chemical polarization

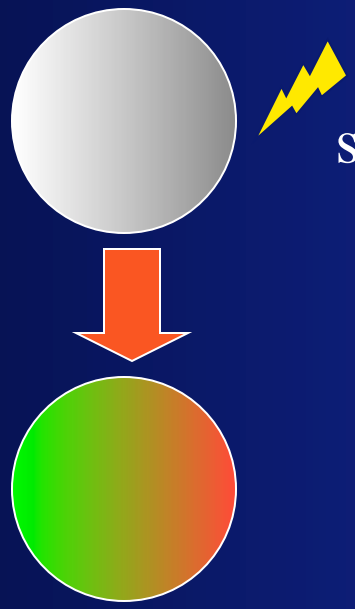


Leading to cell shape changes and motion



What we want to explain

Resting cell

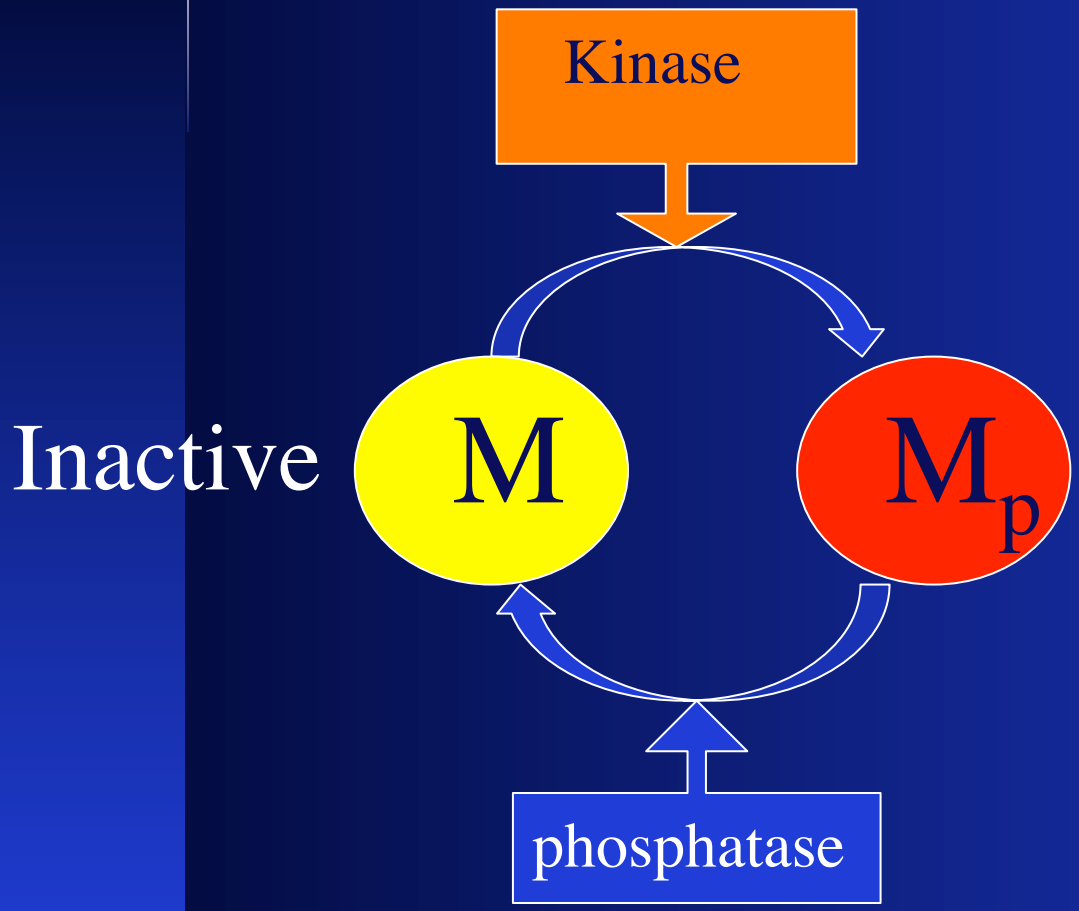


Chemical polarization

- How does the cell polarize?

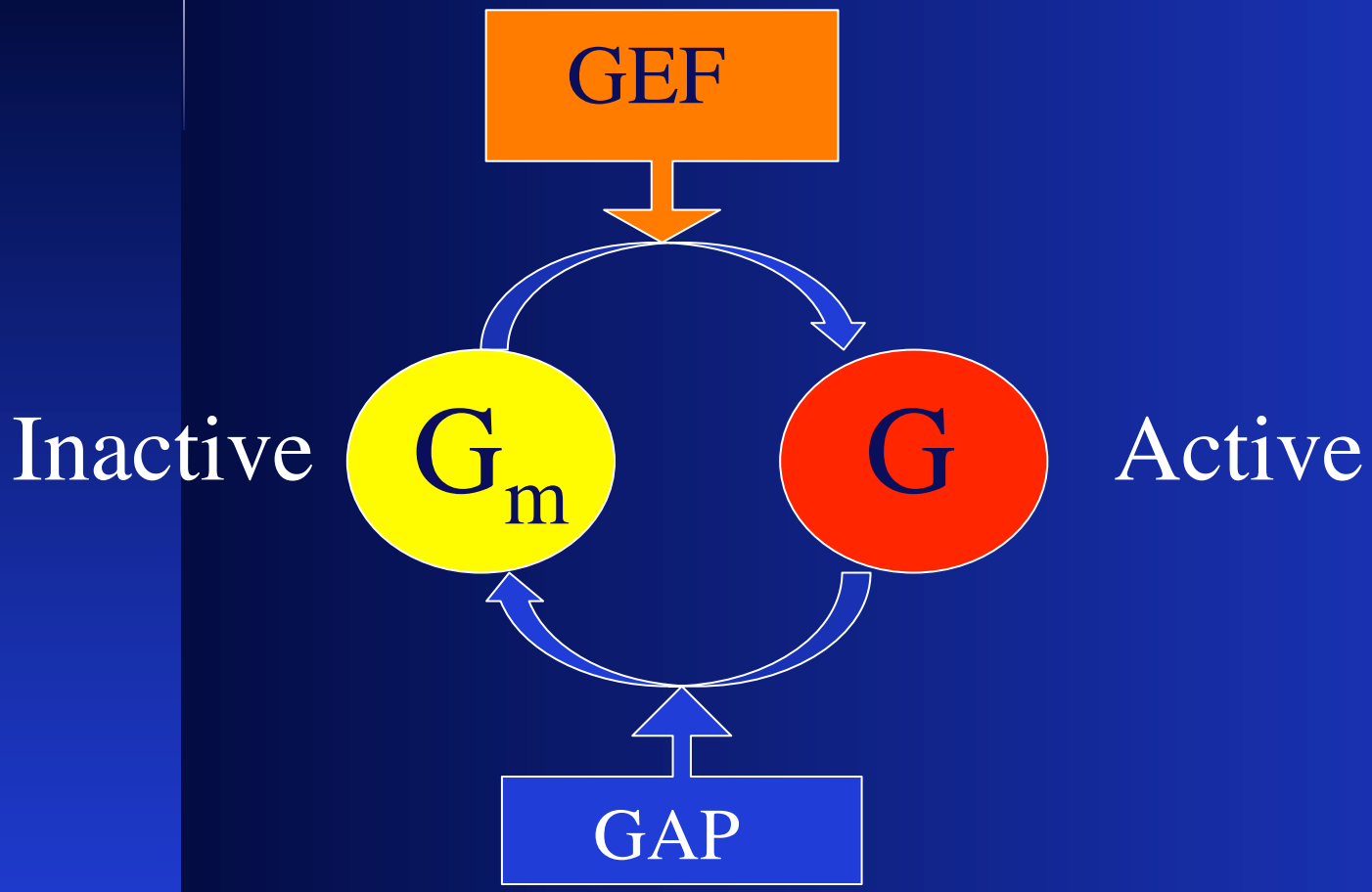
Now look at properties of a GTPase

Related to previously studied phosphorylation cycle



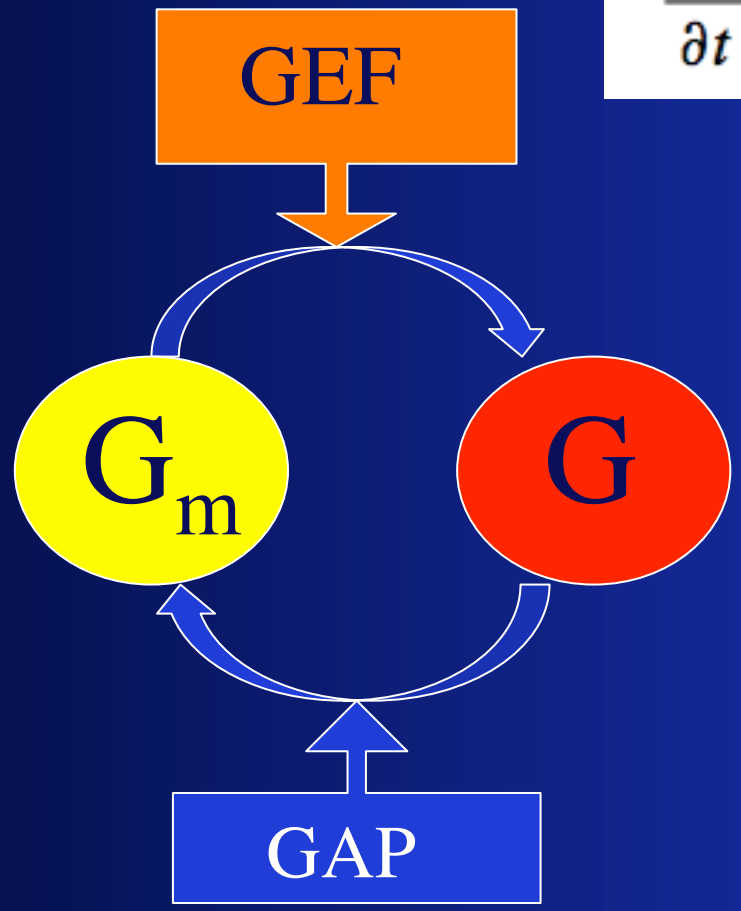
$$M + M_p = M_{tot}$$

GTPase cycle



GTPase cycle

$$\frac{\partial G}{\partial t} = k_G^+ \mathrm{GEF}_G\, G_m - k_G^- \mathrm{GAP}_G\, G$$



More detailed:

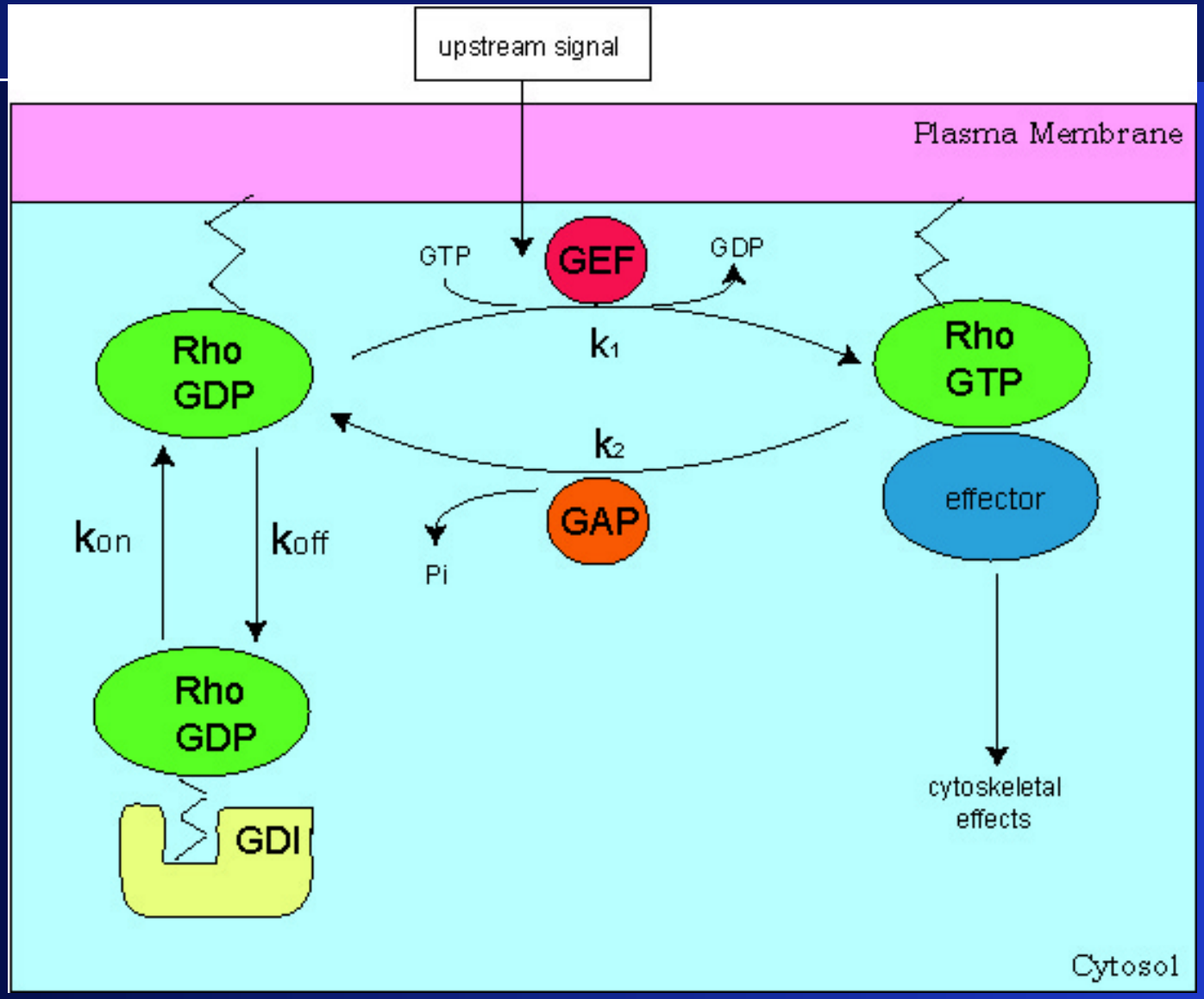
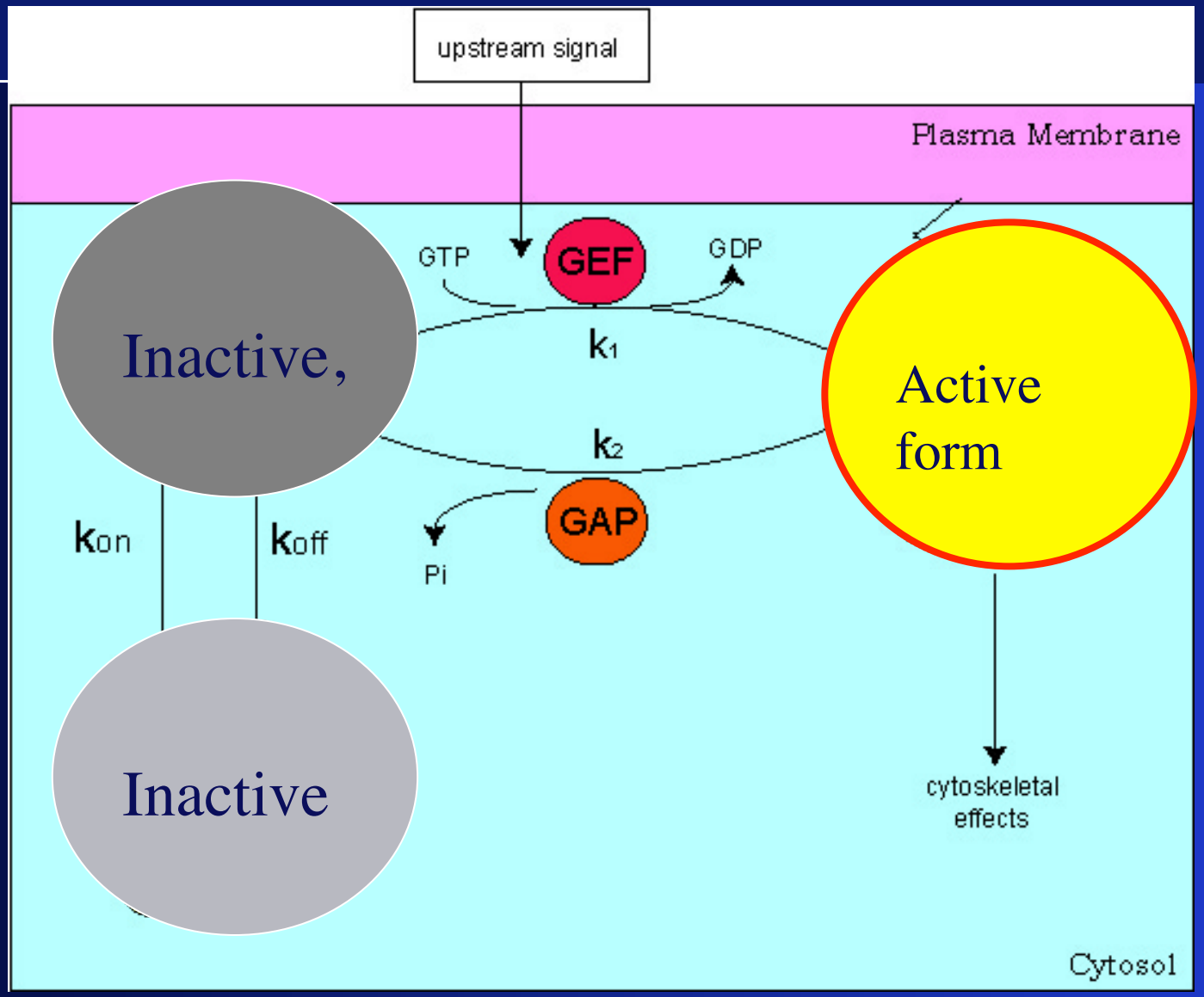
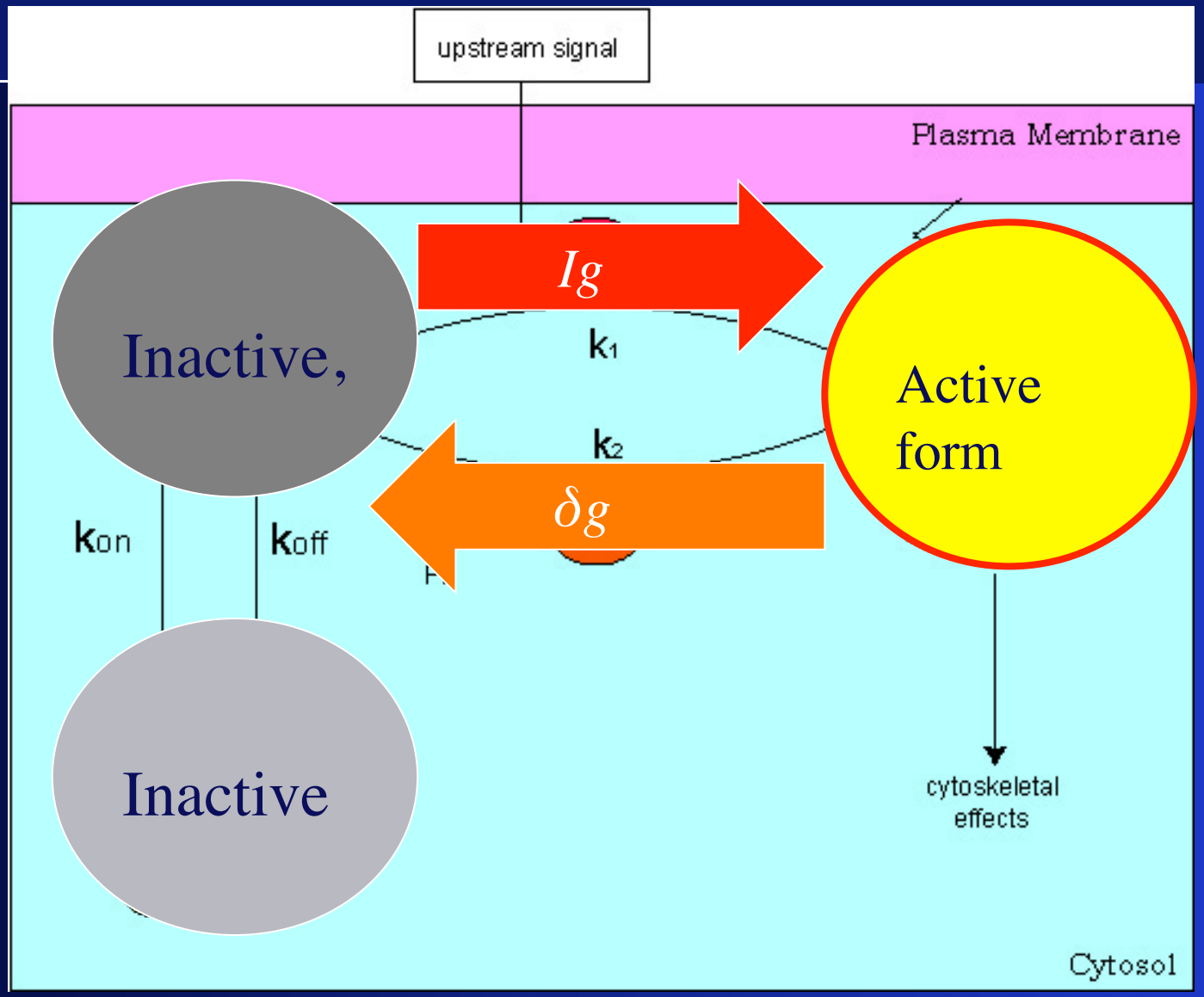


Fig courtesy: A. Jilkine


upstream signal
Plasma Membrane
GTP
GEF
GDP
Inactive,
k1
Active
form
k2
GAP
kon
koff
Pi
Inactive
cytoskeletal
effects
Cytosol


upstream signal
Plasma Membrane
Ig
Inactive,
k1
k2
δg
Active
form
kon
koff
Inactive
cytoskeletal
effects
Cytosol

Equations for 1 GTPase

Active

$$\frac{\partial G}{\partial t} = \hat{I}_G \frac{G_m}{G_{\text{tot}}} - \delta_G G$$

Inactive membrane

$$\frac{\partial G_m}{\partial t} = -\hat{I}_G \frac{G_m}{G_{\text{tot}}} + \delta_G G - k_{\text{off}} G_m + k_{\text{on}} G_c$$

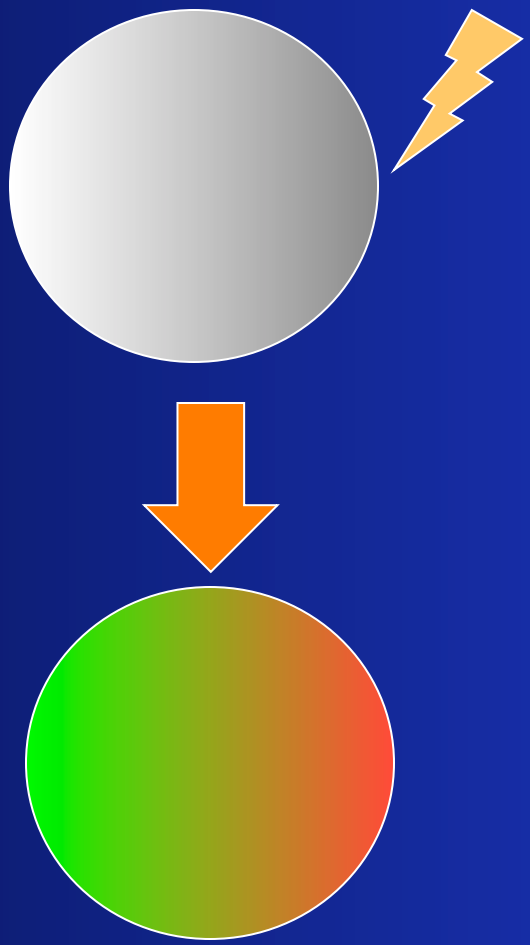
Cytosolic

$$\frac{\partial G_c}{\partial t} = k_{\text{off}} G_m - k_{\text{on}} G_c$$

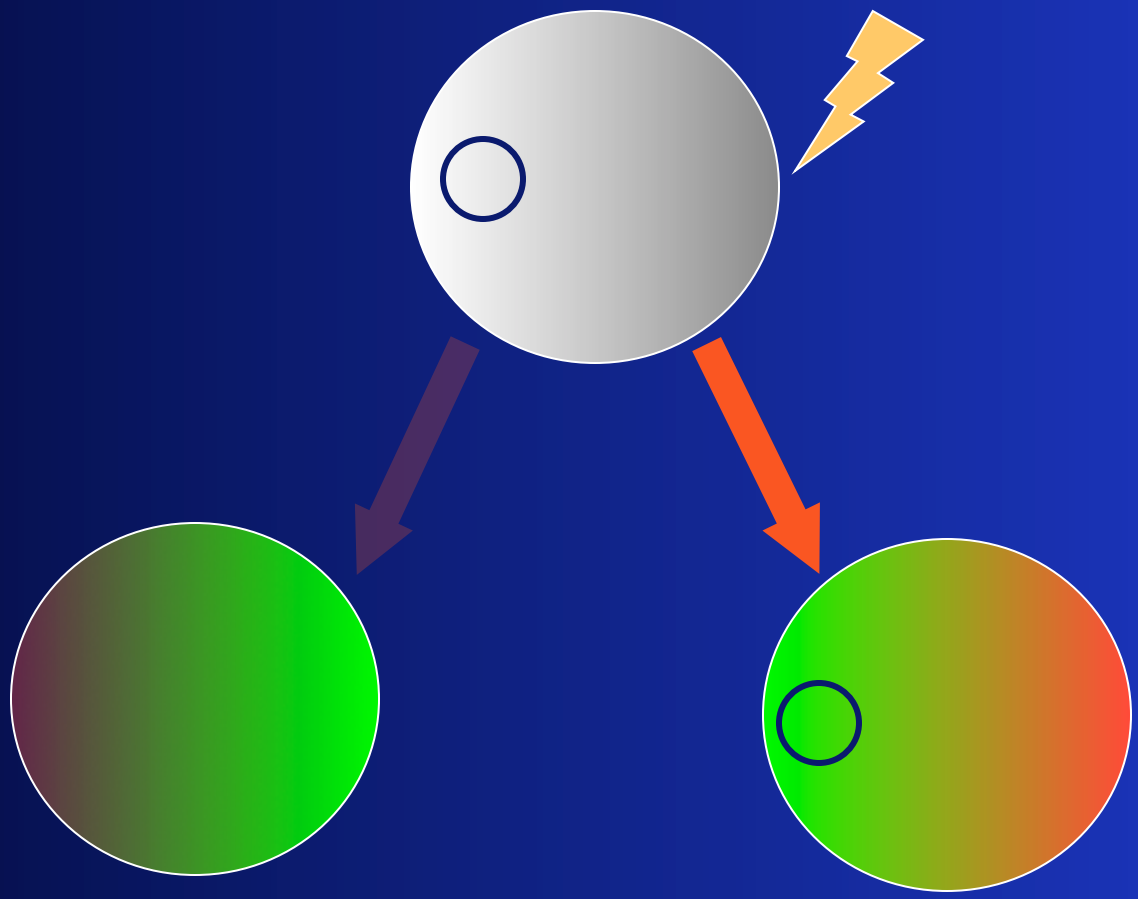
Plus diffusion..
But (as we see later) this will get simplified

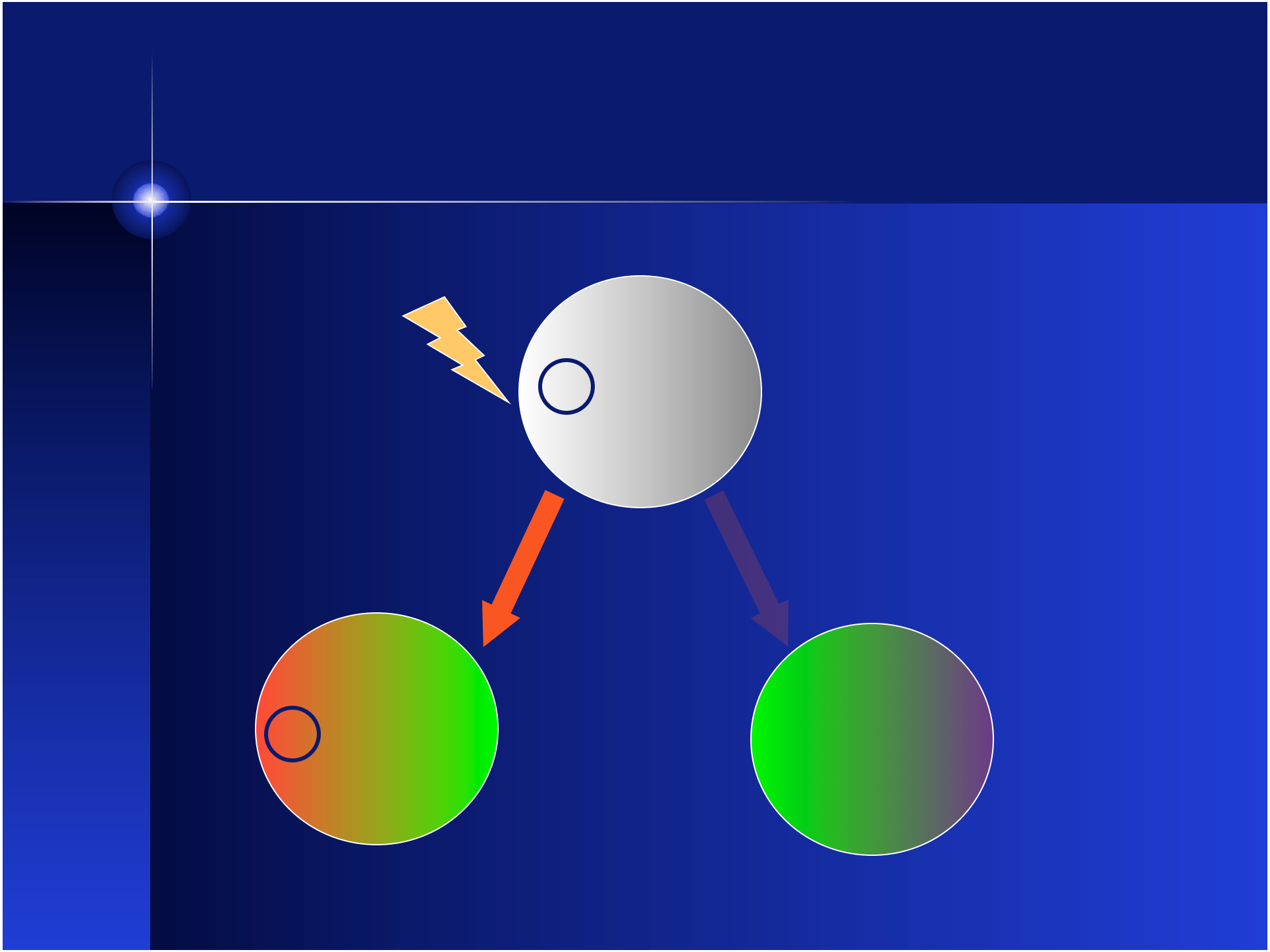
Why bistability?

Polarization

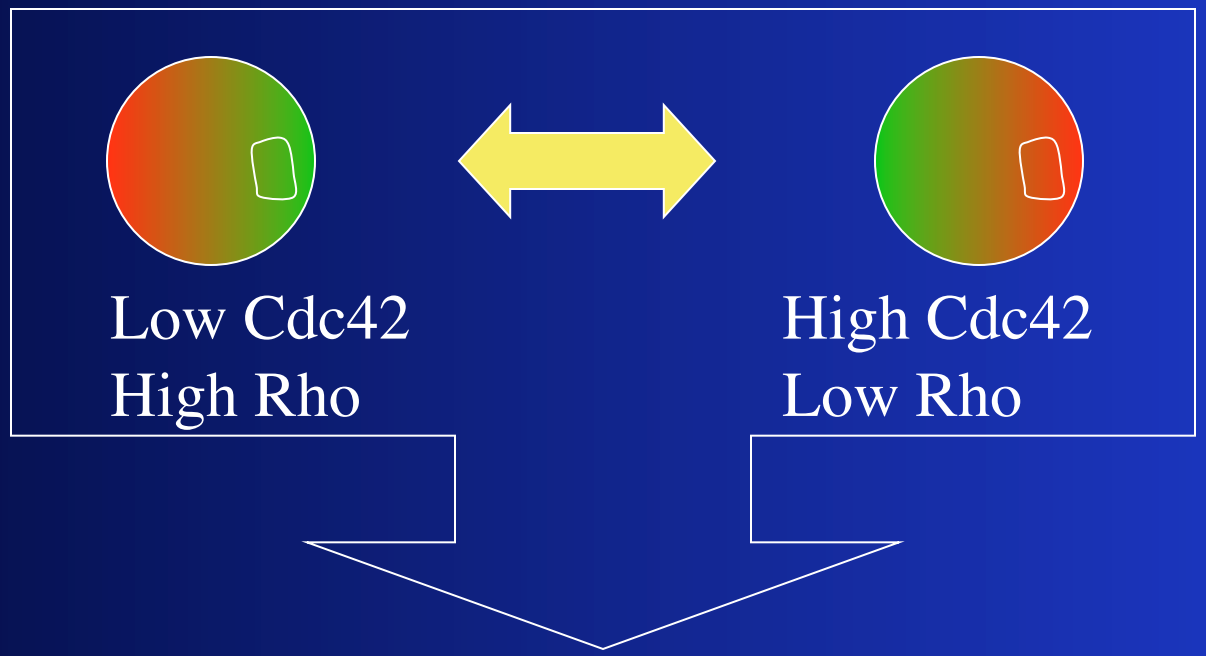


Bistable behaviour





The need for bistability



More than one persistent state possible:
"bistability"

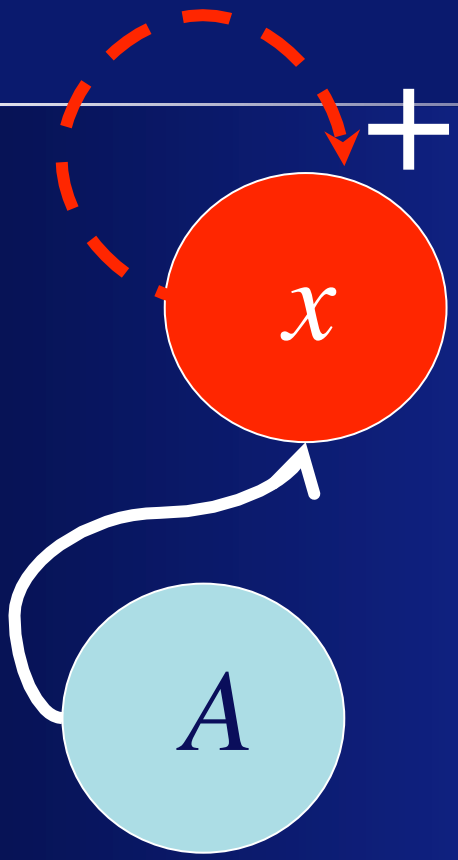
How to get bistability

For multi-stability to be possible, a positive feedback circuit is essential. (Alternately, an even number of negative interactions is equivalent to a positive feedback circuit.)

Ferrell, J.E., Jr., 1996. Tripping the switch fantastic. Trends Biochem. Sci. 21, 460–466.

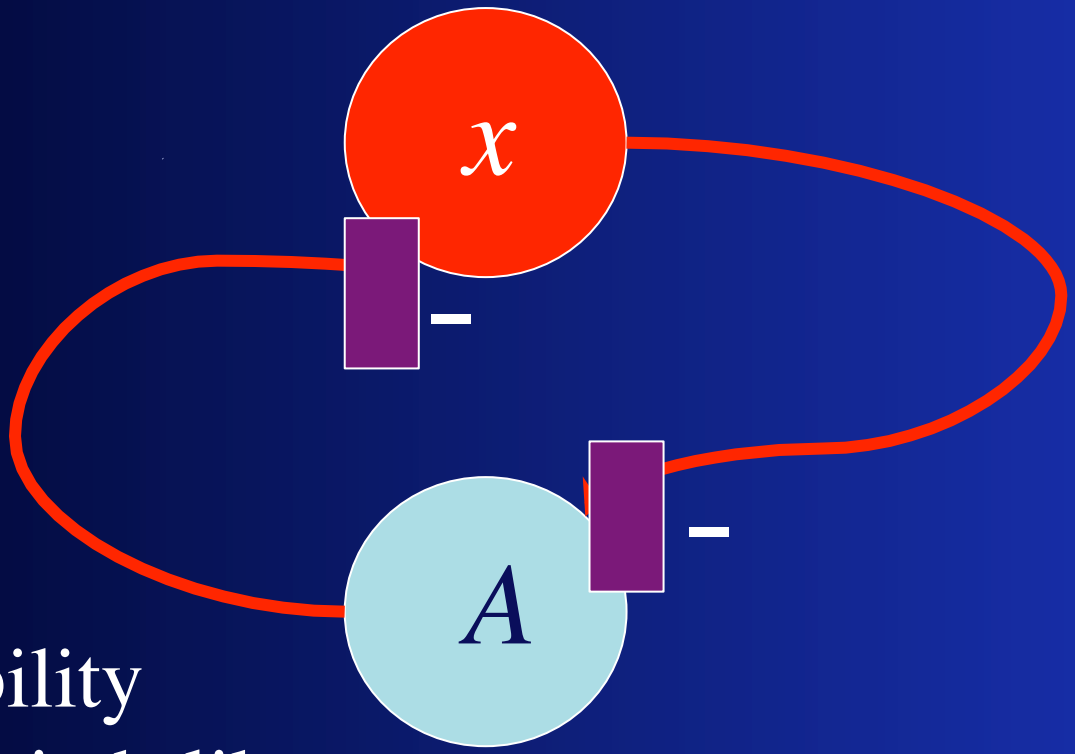
Ferrell, J.E., Jr., 2002. Self-perpetuating states in signal transduction. Curr. Opin. Cell Biol. 14, 140–148.

Positive feedback



Bistability and switch-like behaviour possible

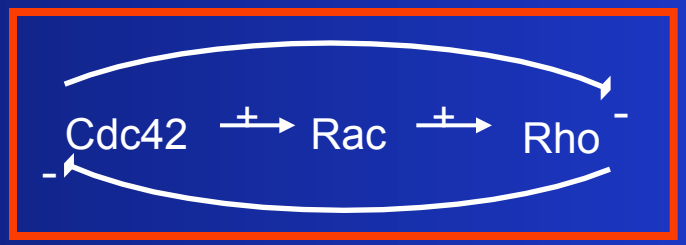
Double negative feedback



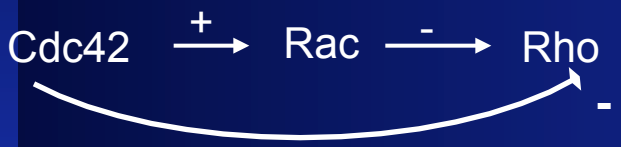
Bistability and switch-like behaviour possible

Only one scheme consistent with bistability

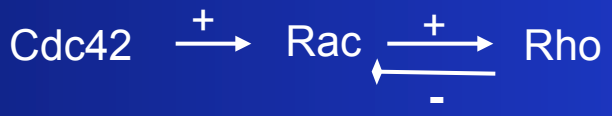
Hall 1995



Giniger 2002



Evers 2000, Sanders 1999

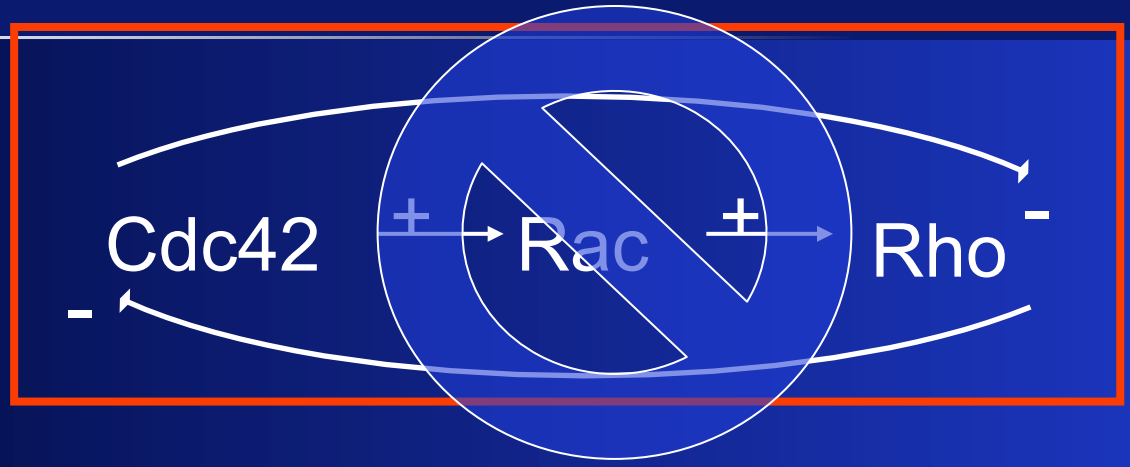


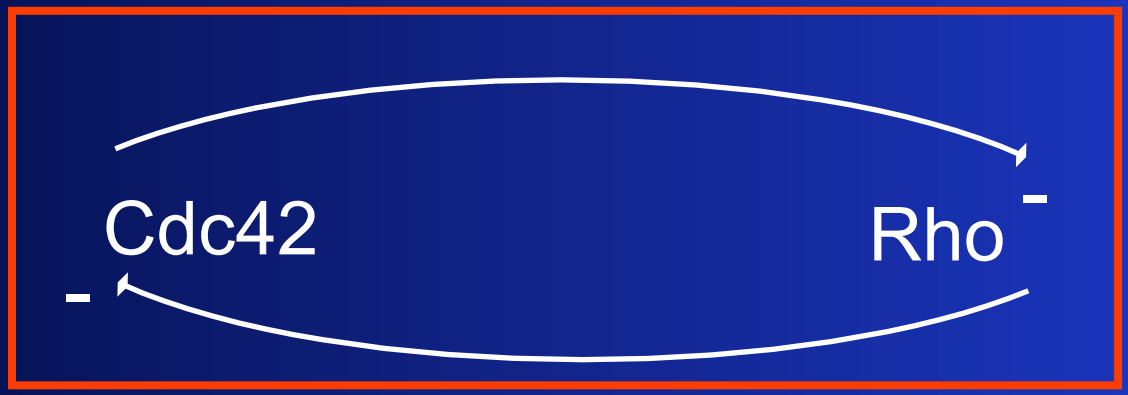
Van Leeuwen 1997

Li 2002

Formulating a set of equations

First consider just Cdc42 and Rho





Simplest Variant

- Cdc42 |-------| Rho (mutual inhibition)

$$\frac{dC}{dt} = g_C(\rho) - \delta_C C,$$

$$, g'_\rho(C) < 0$$

$$\frac{d\rho}{dt} = g_\rho(C) - \delta_\rho \rho,$$

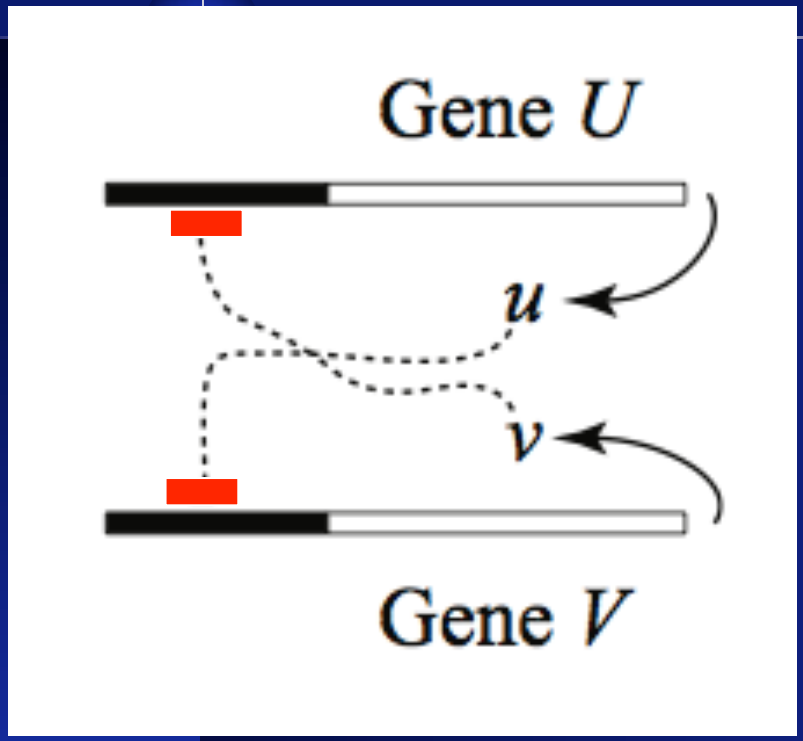
$$g'_C(\rho) < 0,$$

Specific Expressions

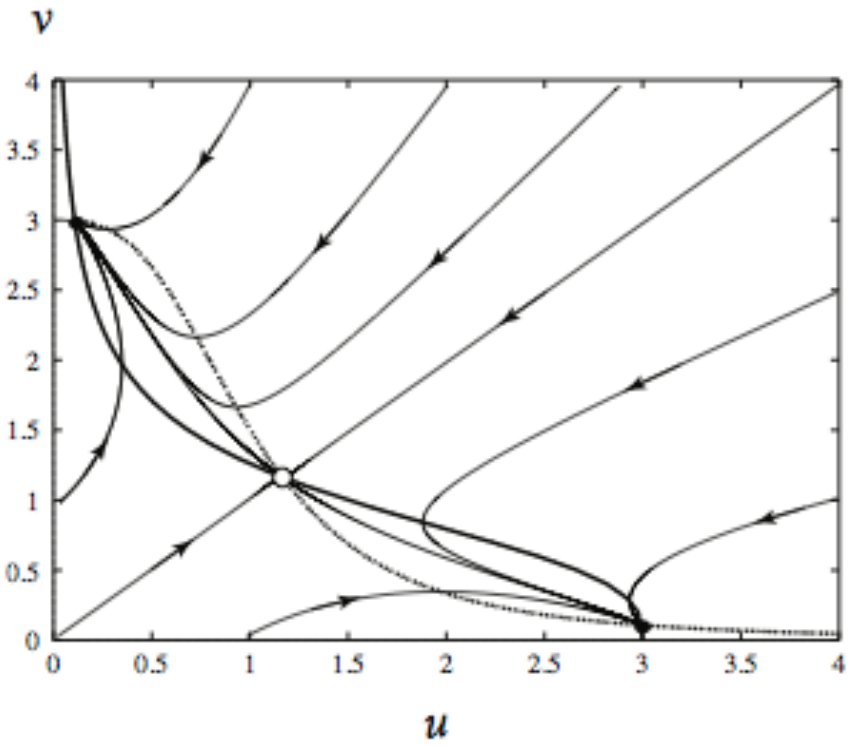
$$\frac{dC}{dt} = \frac{I_C k_C^m}{k_C^m + \rho^m}\left(\frac{C_i}{C_{\text{tot}}}\right) - \delta_C C,$$

$$\frac{d\rho}{dt} = \frac{I_\rho k_\rho^n}{k_\rho^n + C^n}\left(\frac{\rho_i}{\rho_{\text{tot}}}\right) - \delta_\rho \rho,$$

Compare with Toggle Switch:



$$\frac{du}{dt} = \frac{\alpha_1}{1+v^n} - u,$$
$$\frac{dv}{dt} = \frac{\alpha_2}{1+u^m} - v.$$

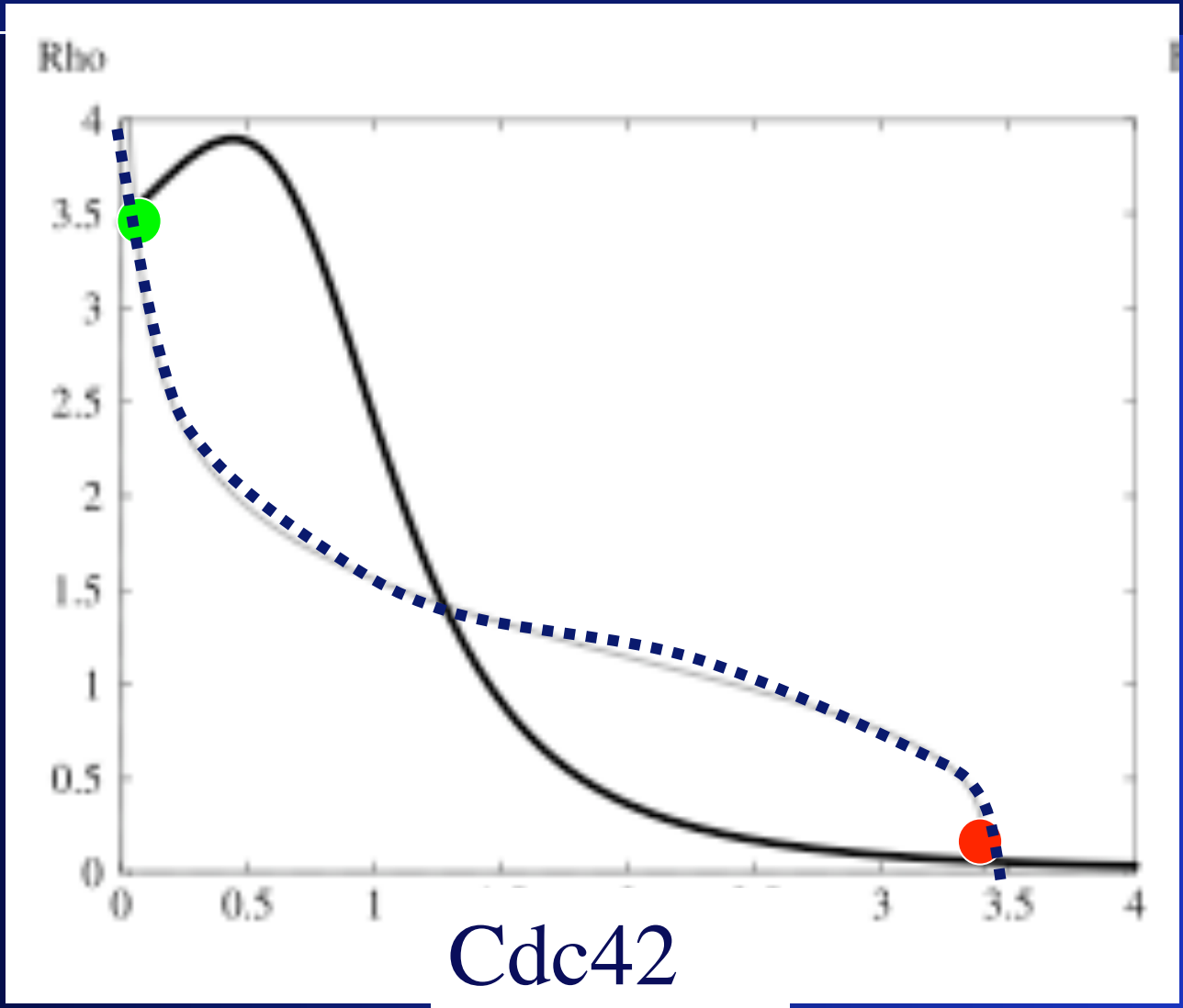


Gardner et al (2000)
Nature 403

Bistability obtained

High Rho

Rho

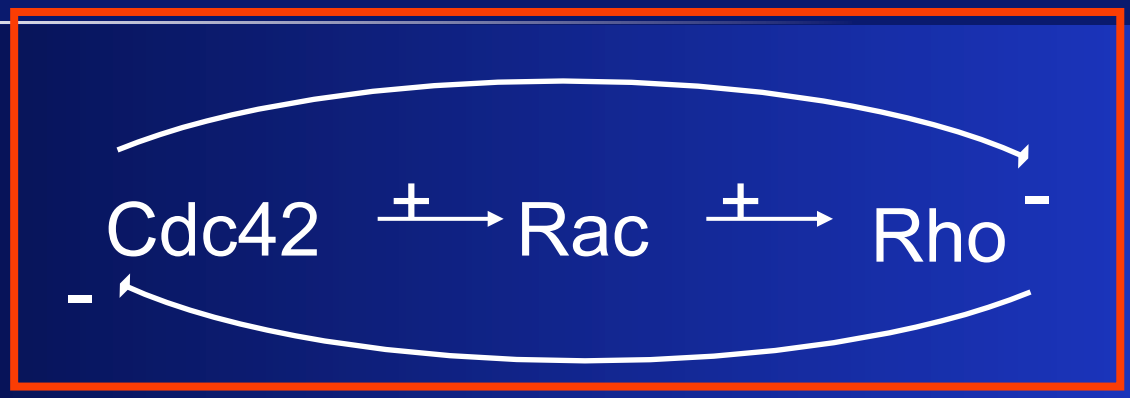


High Cdc42

Nullclines must be curved (nonlinearity)

For multiple steady states, at least one of the interactions should have some degree of "cooperativity," i.e., a Hill coefficient greater than 1.

Now add the third GTPase, Rac

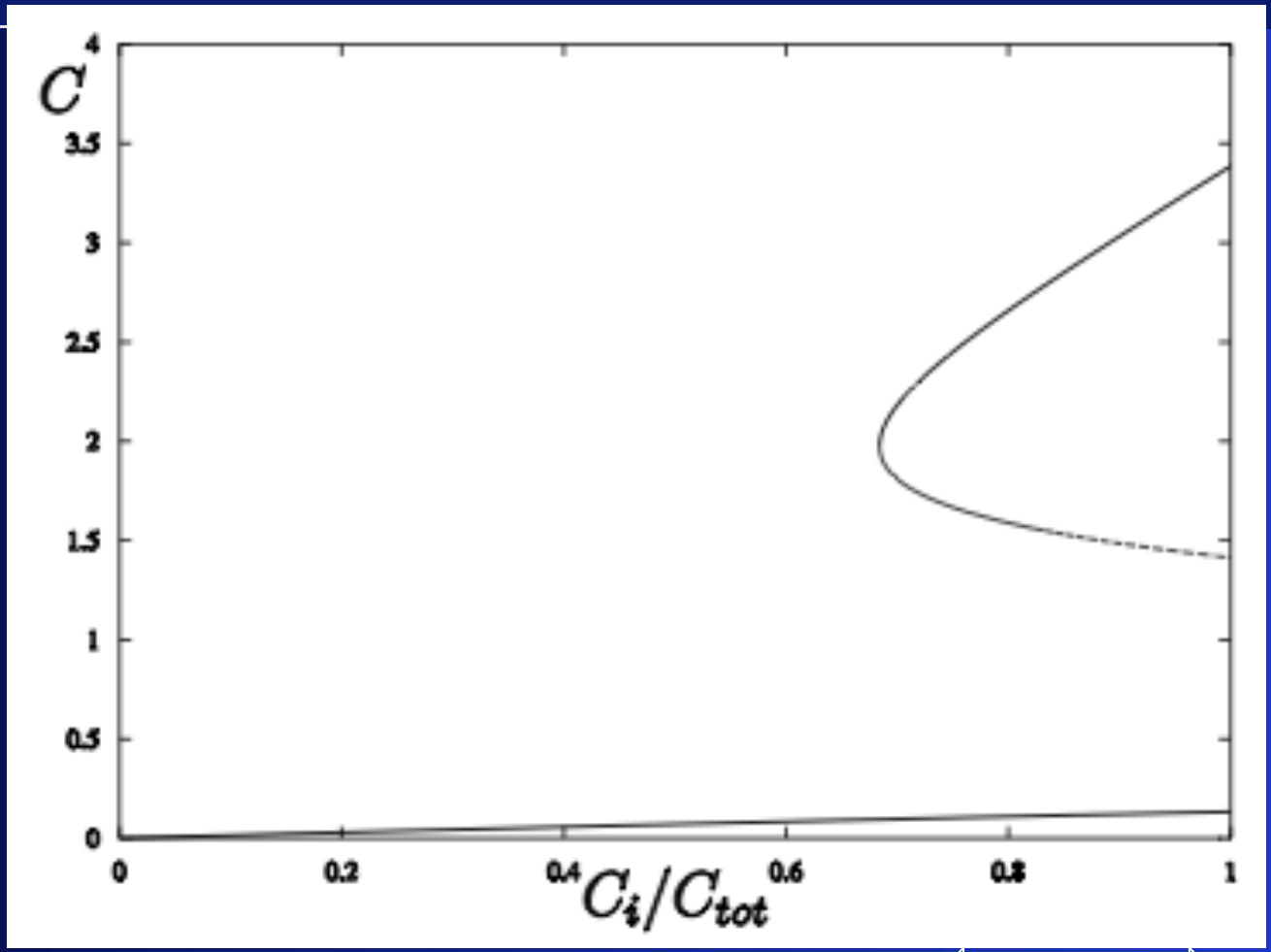


$$\frac{\partial C}{\partial t} = \frac{I_C}{1 + (\rho/\beta_\rho)^n}(C_i/C_{\text{tot}}) - \delta_C C,$$

$$\frac{\partial R}{\partial t} = (I_R + \alpha_C C)(R_i/R_{\text{tot}}) - \delta_R R,$$

$$\frac{\partial \rho}{\partial t} = \frac{(I_\rho + \alpha_R R)}{1 + (C/\beta_C)^n}(\rho_i/\rho_{\text{tot}}) - \delta_\rho \rho.$$

Bifurcation diagram



Rates and timescales: How do we find parameter values?

Parameter estimates

Michaelson et al. (2001), immunoblotting, fibroblasts: the total amount of small G-proteins is 34, 82, and 26 ng/106 cells, for ϱ, R, and C

The average membrane lifetime of an activated Rac molecule is 2 s (Sako et al., 2000), giving a decay rate of 0.5 /sec

Based on estimated decay rates and estimates of steady state concentrations, we compute approximate activation rates.

Parameter values

Parameter	Meaning	Values	Units
I_C	Cdc42 activation input rate	3.4	$\mu M\,s^{-1}$
I_R	Rac activation input rate	0.5	$\mu M\,s^{-1}$
I_ρ	Rho activation input rate	3.3	$\mu M\,s^{-1}$
β_ρ	Rho level for half-max inhibition of Cdc42	1.25	μM
β_C	Cdc42 level for half-max inhibition of Rho	1	μM
n	Hill coefficient of Cdc42-Rho mutual inhibition response	4	–
α_C	Cdc42-dependent Rac activation rate	4.5	s^{-1}
α_ρ	Rac-dependent Rho activation rate	0.3	s^{-1}
$\delta_C, \delta_R, \delta_\rho$	Decay rates of activated small G-proteins	1	s^{-1}

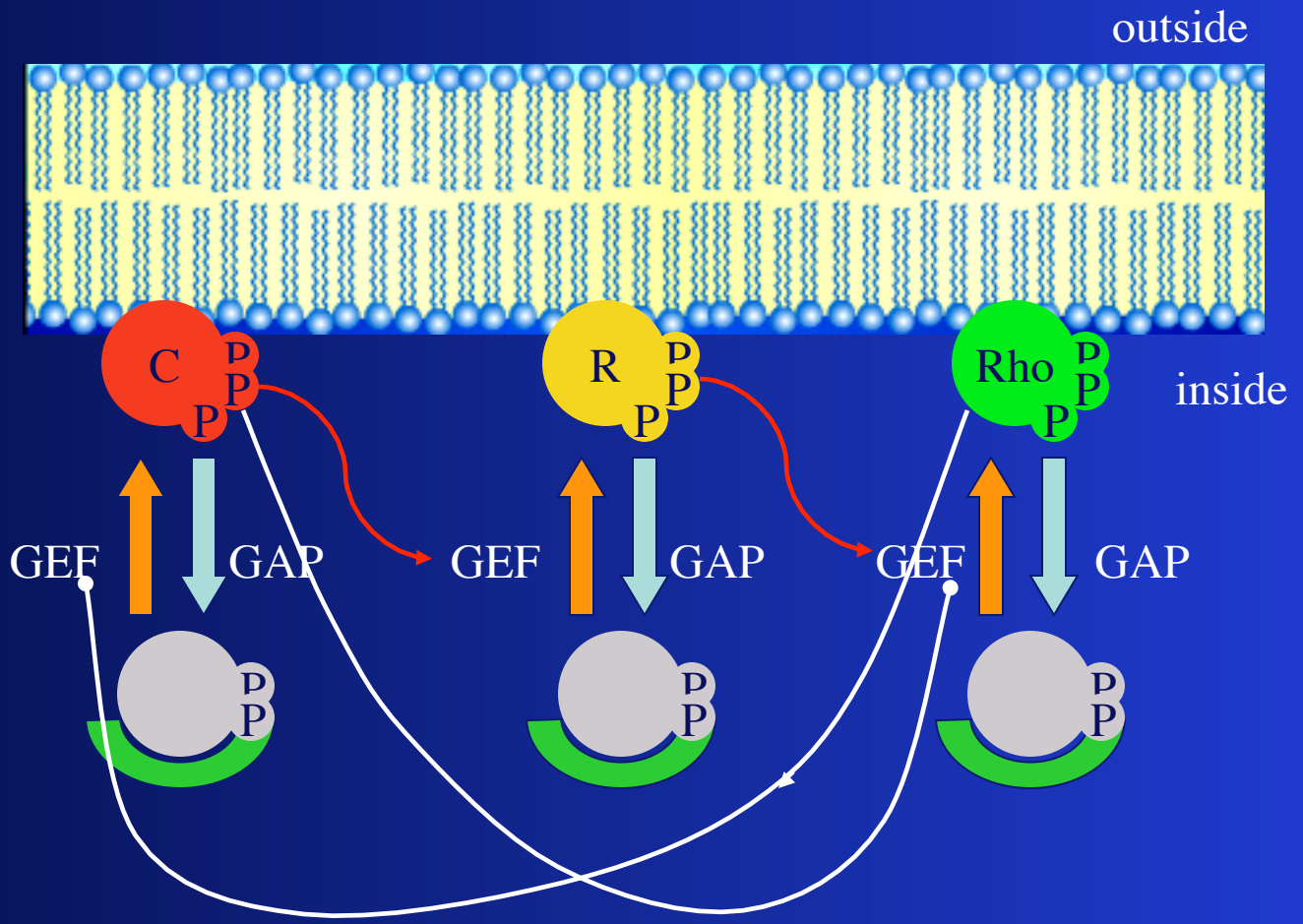
How to estimate??

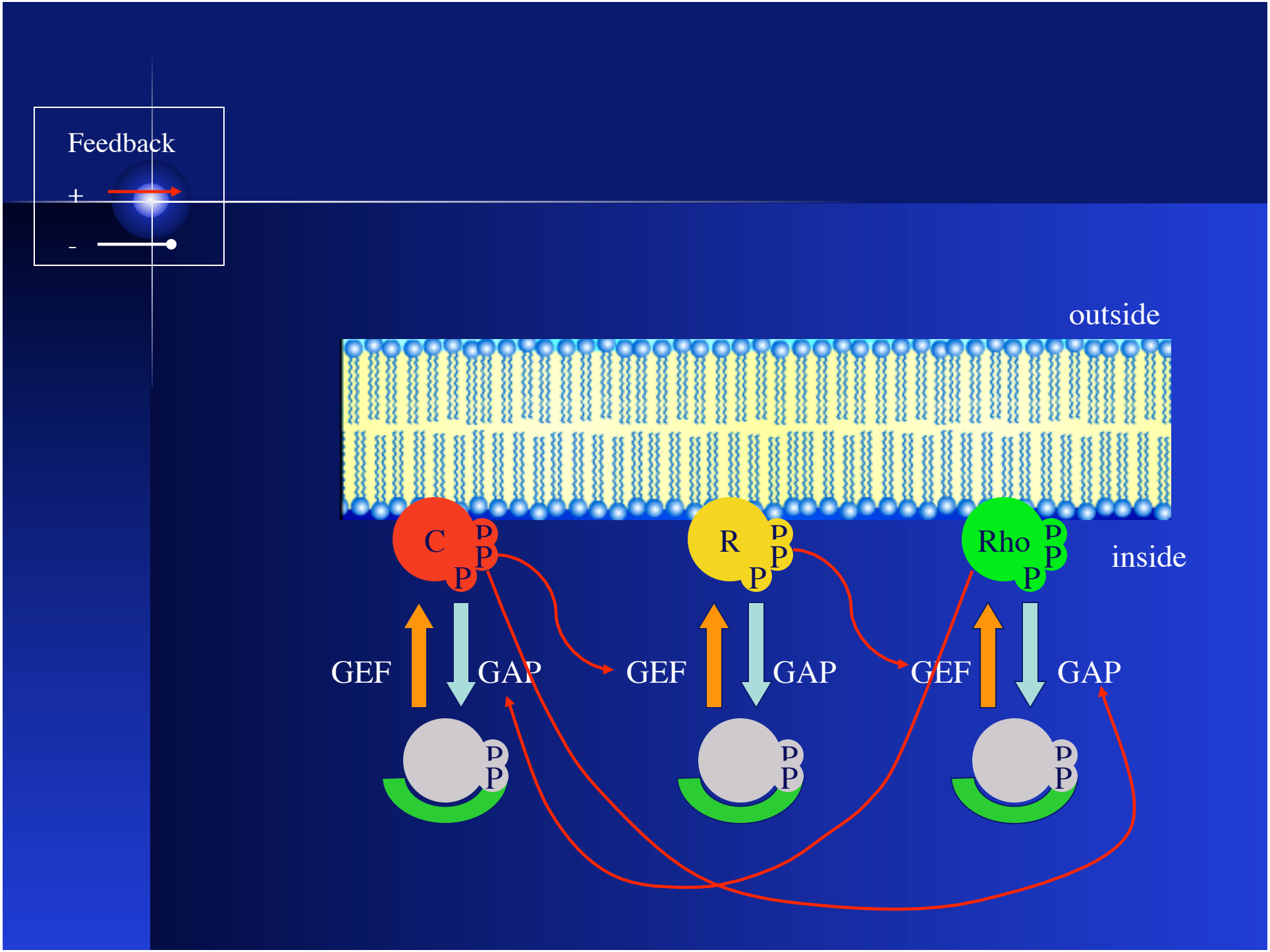
Do we “know the truth” now?

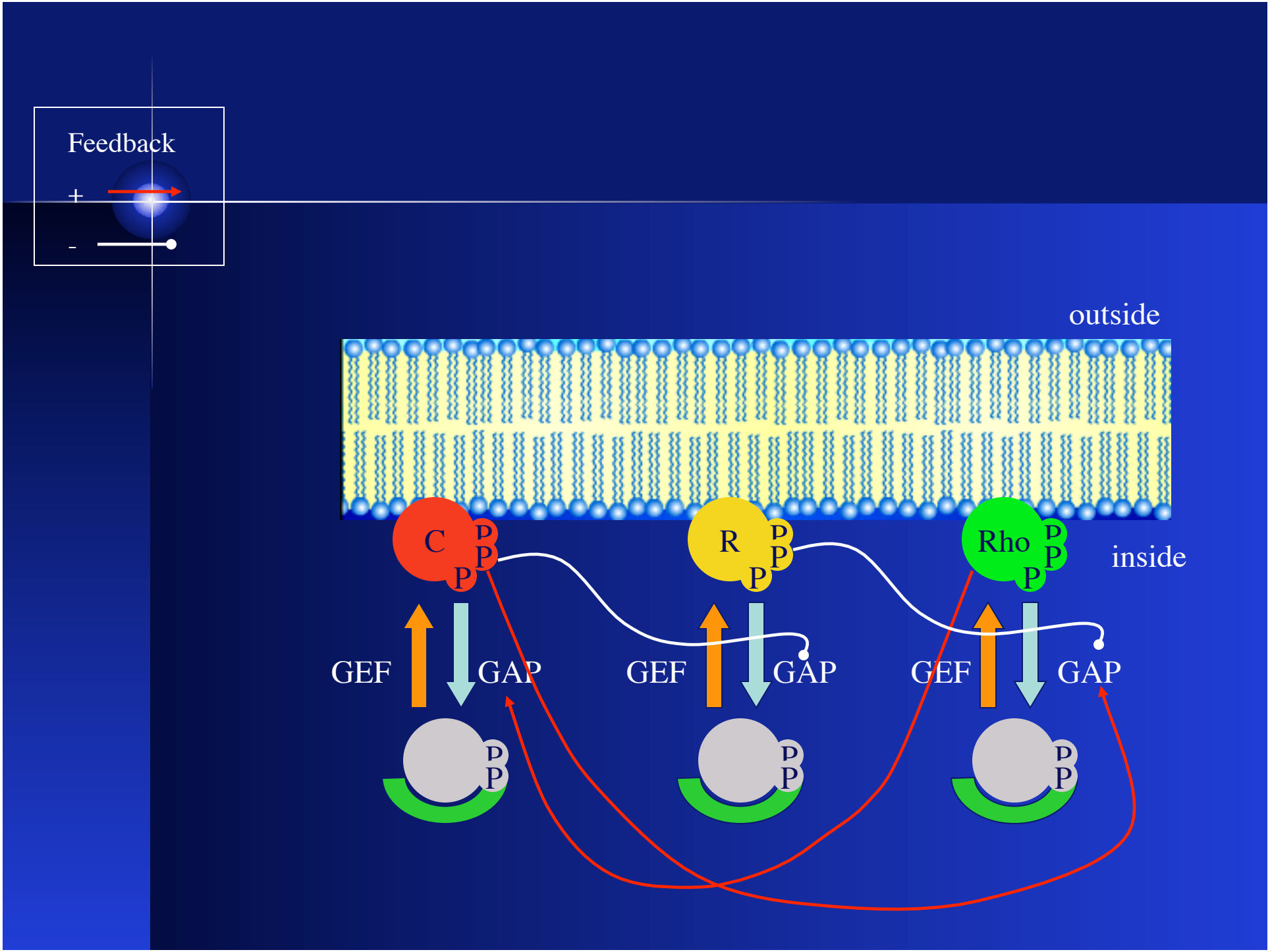
Not really!

There are other ways of getting bistability

Other possible ways to get same behaviour:

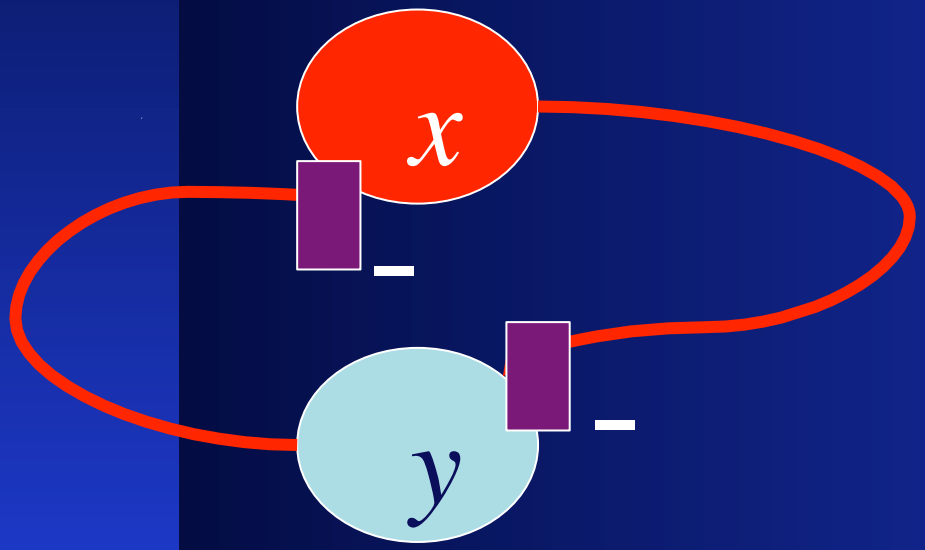



Feedback
+
-
outside
inside
C
R
Rho
P
P
P
GEF
GAP
GEF
GAP
GEF
GAP

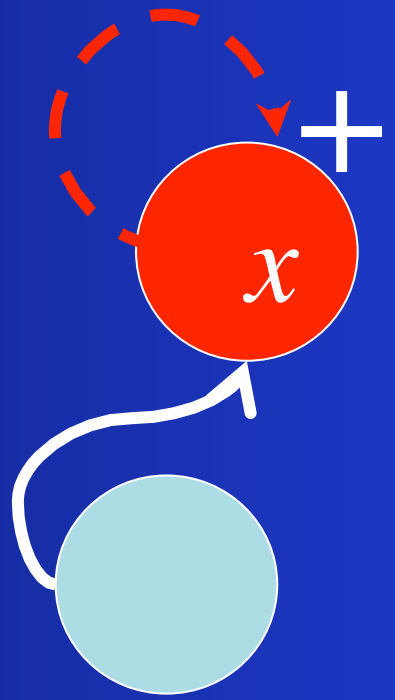

Feedback
+
-
outside
inside
C
P
P
P
R
P
P
P
Rho
P
P
P
GEF
GAP
GEF
GAP
GEF
GAP
P
P
P
P
P
P

Two (simple) ways to get a bistable system:

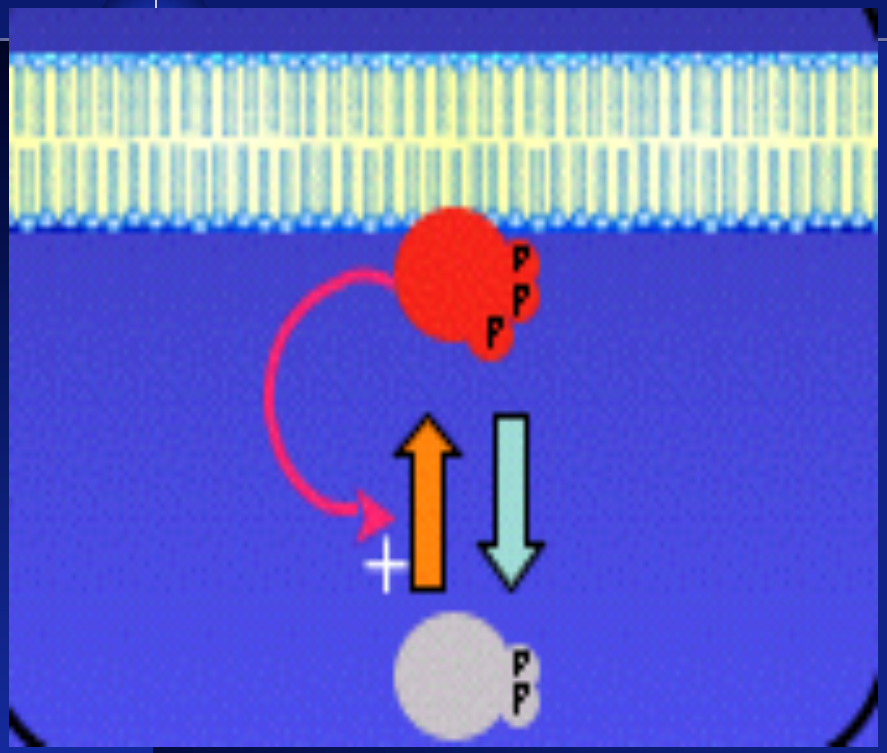
Double negative feedback



Positive feedback



Even simpler model



Bistability with a single positive feedback loop

Mori Y, Jilkine A, LEK (2008) Biophys J, 94: 3684-3697.

Mori Y, Jilkine A, LEK (2011) SIAM J Appl Math

What's next?

Put in spatial distribution (diffusion)

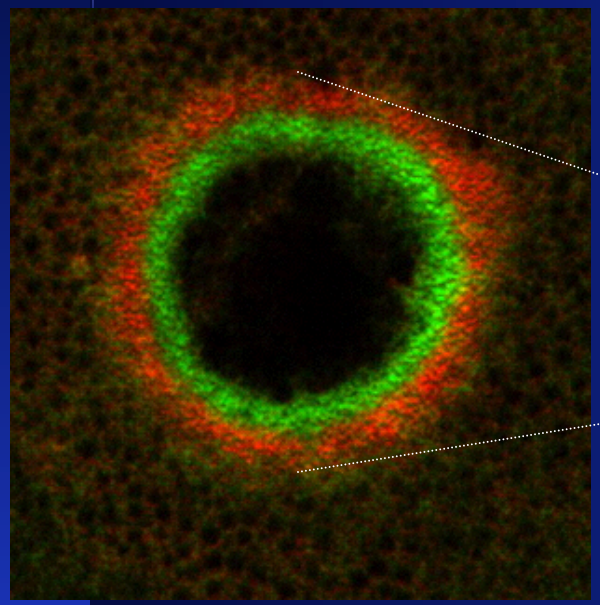
Check if such kinetics will allow for spatial polarization

Go back to include additional signaling layers

And now: some related work with a link to experiments…

Related project: Cory Simon and Bill Bement: Rings of Cdc42 and Rho

Benink & Bement (2005) JCB 168



RhoA Cdc42