Cell polarization

One of the first events that takes place when a cell (such as a neutrophil) receives an input stimulus is creation of an internal 'map', whereby many signalling molecules (proteins, lipids) get reorganized. Some of these will concentrate in what becomes the front of a cell, while others concentrate in the back.

Many studies have been aimed at explaining this and associated phenomena, generally termed "polarization".

Some terminology and defn's:

direction sensing (D.S.) - internal chemical redistrib. in response to input stimulus (directional)

adaptation - transient response to spatially uniform stimulus

polarization - here used interchangeably with (D.S.), but in some works used to denote changes in cell shape.

motility / protusion - rearrangement of the cytoskeleton that leads to forward motion of the cell

chemotaxis - ability to follow external gradient of chemical(s).

Some preliminary information.

Typical sizes of eukaryotic cells — $10\,\mu m$ (small fragments) e.g. of keratocytes — $150\,\mu m$ large cells e.g. fibroblasts

Typical gradients cells can respond to: ~ 2% across their diam.

Typical response time:

chemical polarization: · 10's of sec.
amoeba
white blood cells

fibroblasts: 10's of minutes ~ 1 hr.

Typical rate of diffusion of GTPases in membrane: $D_m \approx 0.1\,\mu m^2/s$

in cytosol: $D_c \approx 10\,\mu m^2/s$

$D_m \ll D_c$

Typical (total) conc. of GTPases in cells: $2 - 10\,\mu M$

Typical membrane residence time of active GTPase: ~ 2 sec

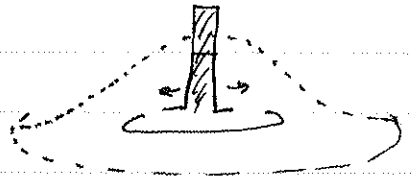
% in active form: 40%

Note: See Jilkine et al (2007) Bull Math Biol

(1) Relationship to Pattern Formation

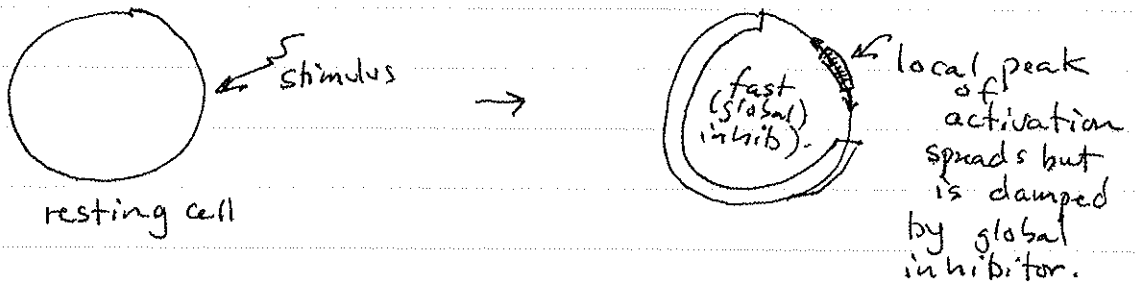
Lateral Inhibition can give rise to spatial pattern/and/or chemical redistribution.

Idea:



Local peak of activation self-reinforces locally, but some inhibitory effect has wider 'reach', damps out activation in the surround.

This idea has been exploited (in one way or another) in many models for cell polarization, essentially as follows:



Activator - identified as any of several types of signalling molecule
e.g. G proteins (Meinhardt 99; Levine et al 06
PI3K (Levchenko + Iglesias 07; Onsum + Rao 07; Krishnan + Iglesias 07;

Inhibitor - not yet found but ideas include:
Ca^{++} (Meinhardt 99
PTEN (Levchenko + Iglesias 07 etc.

Many models are described as "capability" (or "functionality") models, i.e. not based on identified components.

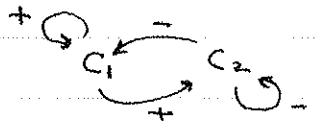
Relationship to Turing RD pattern formation

We have seen earlier that a reaction-diffusion system of the form

$$\frac{\partial c_1}{\partial t} = f(c_1, c_2) + D_1 \frac{\partial^2 c_1}{\partial x^2} \qquad \text{"activator"}$$

$$\frac{\partial c_2}{\partial t} = g(c_1, c_2) + D_2 \frac{\partial^2 c_2}{\partial x^2} \qquad \text{"inhibitor"}$$

can give rise to spatial pattern provided certain conditions are satisfied, namely



$D_2 > D_1$
(inhib. diffuses faster than activator)

$$\left|\frac{D_1}{a}\right| < \left|\frac{D_2}{e}\right|$$

(range of activation < range of inhibition)

where $a = \left.\frac{\partial f}{\partial c_1}\right|_{HSS}$ are jacobian coeffs eval. at hom. S.S.

$e = \left.\frac{\partial g}{\partial c_2}\right|_{HSS}$

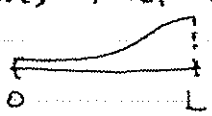
Moreover, the ~~wavenumber~~ pattern that grows first (i.e. close to the Turing bifurcation) has wavenumber q satisfying

$$q^2_{min} = \frac{1}{2}\left(\frac{e}{D_2} + \frac{a}{D_1}\right) > 0$$

i.e. the spacing between peaks of such a chemical pattern are roughly

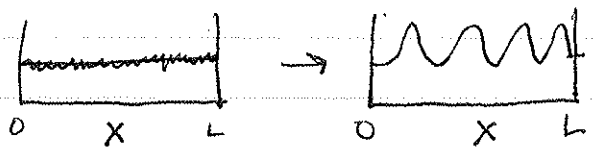
$$d = \frac{2\pi}{q_{min}}$$

Exercise 1 Given a cell of diameter L, find conditions that would assure that only a polar pattern could form via the above mechanism.



Properties of Turing-type pattern formation

- Instability to arbitrarily small noise



(this means the homogeneous "rest state" is easily disrupted)

→ Domain size will affect the pattern that forms
(as domain size increases, more peaks fit in)
This stems from the fact that the RD eqns give rise to a (small) range of unstable wavenumbers, i.e. a prefered wavelength, esp. near the Turing bifurc.

- Can obtain spots/stripes and other patterns in 2D
(some of these inappropriate for polarization)

- Near the bifurcation, the time needed for pattern to grow is long - the reaction time is correspondingly slow.

Polarized pattern from Turing RD system: How fast does it grow?

Close to a Turing bifurcation, the eigenvalue σ changes sign, becoming a small, positive value. But σ satisfies the characteristic equation

$$\sigma^2 - \beta\sigma + \gamma = 0$$

where $\beta = \text{Trace}(J_0)$

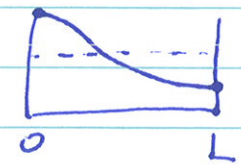
$\gamma = \det(J_0)$

At the bifurcation and near it:

For small $\sigma \ll 1$, $\sigma^2 \ll 1$ $\Rightarrow$ $-\beta\sigma + \gamma \approx 0$

$\sigma \approx \gamma/\beta$ ← approximate growth rate of perturbation

A "polarized" pattern has wavenumber $q = \frac{\pi}{L}$ (i.e. corresponds to ½ wavelength.)



What are candidate molecules involved in cell polarization?

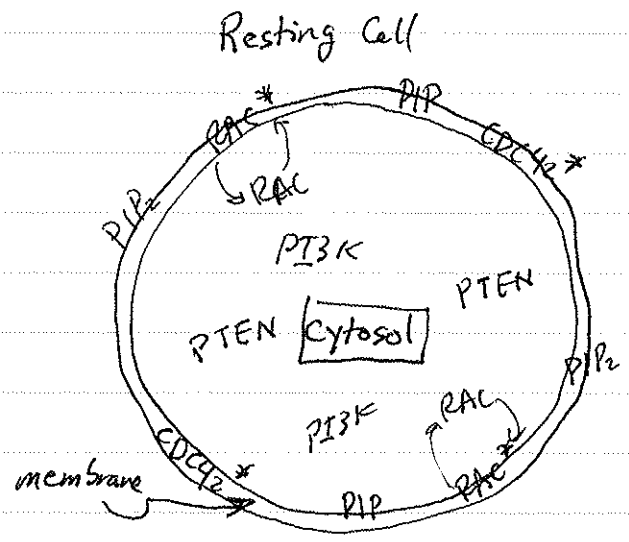
- Rho proteins (also called small GTPases): Rac, Cdc42, Rho

  These are "molecular switches" that have both active and inactive forms. Active forms are exclusively bound to the cell membrane.

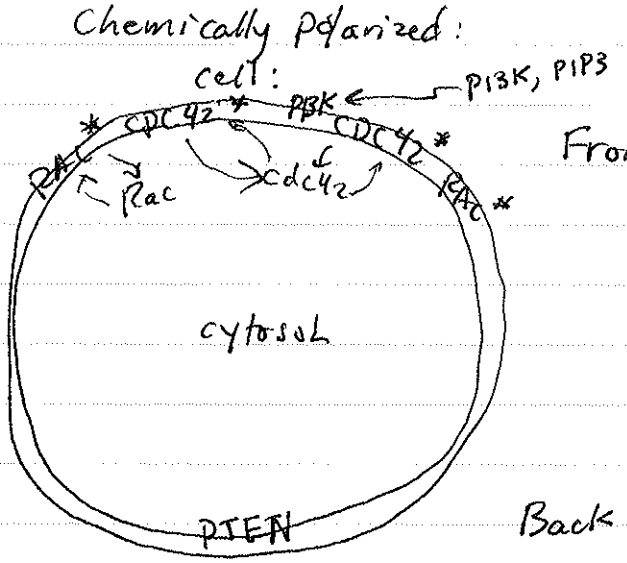
- Lipids (phosphoinositides): PI, PIP, PIP_2, PIP_3
  These are embedded in the cell membrane, and differ from each other by addition of phosphate group. Rarest form: PIP_3. This, and PIP_2 have a high effect in cell polarity

- kinases/phosphatases: PTEN and PI3K are proteins that remove (phosphatase) and add (kinase) a phosphate group to the PIP's:

$$PIP_2 \underset{\text{PTEN}}{\overset{\text{PI3K}}{\rightleftharpoons}} PIP_3$$

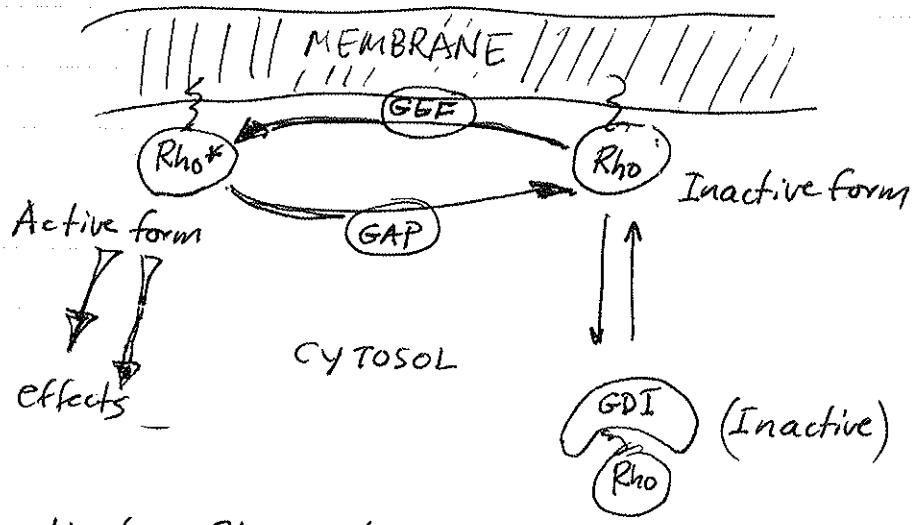


"uniform" distribution of most of the above



Active cdc42, Rac concentrate at front, as do PI3K, PIP_2, PIP_3.

GTPases (=Rho proteins)



GEF: activates Rho protein
GAP: inactivates " "
GDI: removes " " from membrane, holds it in inactive form.

- Proteins can diffuse laterally in the cell membrane
- " " in the cytosol
- " " bind and unbind from membrane

- Proteins can only interact with other targets or produce some effects in their active form.

Typical effects include: - positive or negative feedback on GEFs, GAPs, GDIs of same or other GTPases
- nucleation of actin filaments (Cdc42, Rac)
- myosin contraction (Rho)

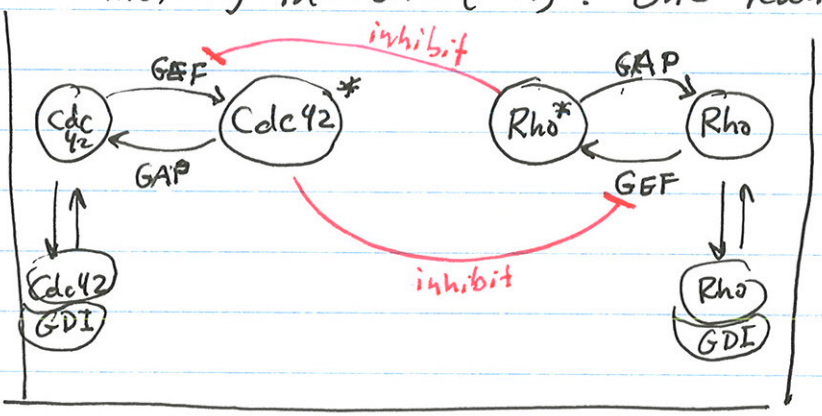
Note: there are many other "downstream signals" associated with the same Rho proteins.

Minimal "mutual inhibition" model

Evidence from H. Bourne's lab had suggested that Cdc42 and Rho mutually inhibit one another. Here is a small mini-model that was developed by Jilkine to depict such interactions

Cdc42 ⟶⟵ Rho

Note: this scheme means that the active forms of each will mutually inhibit (⊣). One realization:



Here we assume all "crosstalk" between Cdc42 and Rho is via inhibition of GEF's

(But there are many other possibilities)

Minimodel: Consider only the active forms as variables

$C(t)$ = conc. Cdc42 in active form*

$\rho(t)$ = conc Rho " " "

Lump together inactive form of each, assume ≈ constant

C_i, ρ_i = " " "

It is convenient to define C_{tot}, ρ_{tot} = total amt of each

Then eqns would be of the form

$$\begin{cases} \frac{dC}{dt} = \text{rate of activation} - \text{rate of inactivation} \\ \frac{d\rho}{dt} = \text{" " "} \end{cases}$$

Assume: • interactions via GEFs $\Leftrightarrow$ crosstalk is in rate of activation terms

• simple linear "decay" $\Leftrightarrow$ inactivation is of form δC where $[\delta] = \text{time}^{-1} = s^{-1}$

• activation requires presence of inactive material
↳ will be important later.

Obtain:

$$\frac{dC}{dt} = I_{activC}(\rho) \cdot \frac{C_i}{C_{tot}} - \delta C$$

- $I_{activC}(\rho)$: amt C activated per unit time, depends on ρ inhibition
- $\frac{C_i}{C_{tot}}$: constant parameters for now
- δC: constant rate of inactivation (by GAP)

similarly

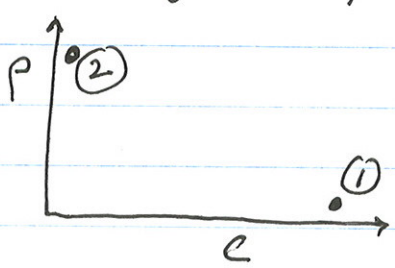
$$\frac{d\rho}{dt} = I_{activ\rho}(C) \cdot \frac{\rho_i}{\rho_{tot}} - \delta\rho$$

(Note: negative feedback Cdc42 ⊣ Rho is in the $I_{activ.}$ terms.)

Question: What kinds of "biochemically reasonable" functions $I_{activC}(\rho)$ and $I_{activ\rho}(C)$ would result in "mutual exclusion" (i.e. states with either all C or all ρ). ?

⇒ Need multiple stable steady states

① (high C, low ρ) ② (low C, high ρ)



"Biochemically Reasonable" terms:

(a) linear $\quad I(x) \sim \alpha x$

(b) Michaelian $\quad I(x) \sim \dfrac{Kx}{k_b + x}$

(c) Sigmoidal $\quad I(x) \sim \dfrac{Kx^2}{k_b^2 + x^2}$

(d) Constant $\quad I(x) \sim K$

or some combination thereof.

Example: Sigmoidal GEF inhibition model

One example that works (but by no means the only one);

- basal activation rate + crosstalk of type (c)

$$\begin{cases} \dfrac{dc}{dt} = \hat{I}_c \left(1 - \dfrac{\rho^2}{k_\rho^2 + \rho^2}\right) \dfrac{c_i}{c_{tot}} - \delta c \\ \dfrac{d\rho}{dt} = \hat{I}_\rho \left(1 - \dfrac{c^2}{k_c^2 + c^2}\right) \dfrac{\rho_i}{\rho_{tot}} - \delta \rho \end{cases}$$

↑ basal rate

↑ inhib. due to crosstalk

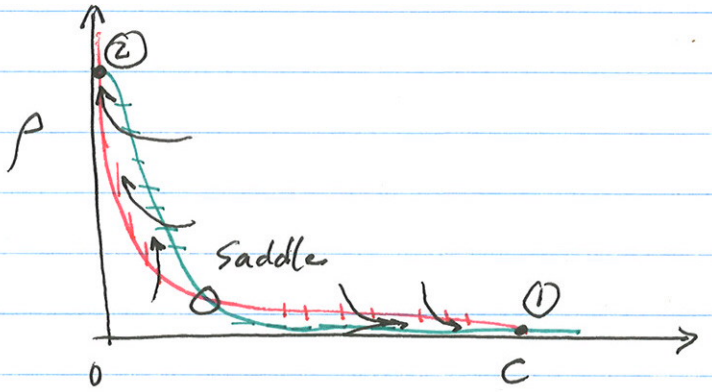
Rewrite as:

$$\begin{cases} \dfrac{dc}{dt} = \bar{\bar{I}}_c \left(\dfrac{1}{k_\rho^2 + \rho^2}\right) \dfrac{c_i}{c_{tot}} - \delta c \\ \dfrac{d\rho}{dt} = \bar{\bar{I}}_\rho \left(\dfrac{1}{k_c^2 + c^2}\right) \dfrac{\rho_i}{\rho_{tot}} - \delta c \end{cases}$$

or, grouping constants:

$$\begin{cases} \dfrac{dc}{dt} = \tilde{I}_c \left(\dfrac{1}{k_\rho^2 + \rho^2}\right) - \delta c \\ \dfrac{d\rho}{dt} = \tilde{I}_\rho \left(\dfrac{1}{k_c^2 + c^2}\right) - \delta c \end{cases} \qquad \tilde{I}_c = \bar{\bar{I}}_c \dfrac{c_i}{c_{tot}}$$

etc.



Phase plane portrait.

Nullclines:

c nullcline: $c = \frac{\tilde{I}_c}{\delta}\left(\frac{1}{k^2+\rho^2}\right)$

ρ nullcline: $\rho = \frac{\tilde{I}_\rho}{\delta}\left(\frac{1}{k^2+c^2}\right)$

Observe that we arrived at the appropriate "exclusion" of Cdc42 and Rho.

Next on the agenda:

Include diffusion (in membrane and cytosol) to produce a reaction-diffusion model

Examine the behaviour of this model

See how the limitation on total amt of protein affects the dynamics (← make inactive forms variables too).

Exercise: (1) Consider the crosstalk Cdc42 ⟂⟶ Rho

- sketch a few other realizations of such mutual inhibition.
- For each one, propose a set of ODE's to describe the interactions
- show phase-plane portrait and dynamics.

(2) Another "reasonable" biochemical term is $I_n(x) = \frac{Kx^n}{k_0^n + x^n}$

where $n \gg 1$. This type of relationship produces a sharper switch-like response.

(a) Sketch $I_n(x)$ for $n = 1, 2, 4$ on same graph. How do these curves relate?

(b) Explore the sigmoidal GEF inhibition model with $I_4(x) = \frac{Kx^4}{k_0^4 + x^4}$. How does this change the phase-plane diagram?

(3) Use XPP or Matlab to simulate the model in (2). Are there always 3 steady states? Consider a bifurcation diagram of the Rho steady state with $\bar{\bar{I}}_p$ as bifurc. parameter. Show that you get a fold bifurcation.

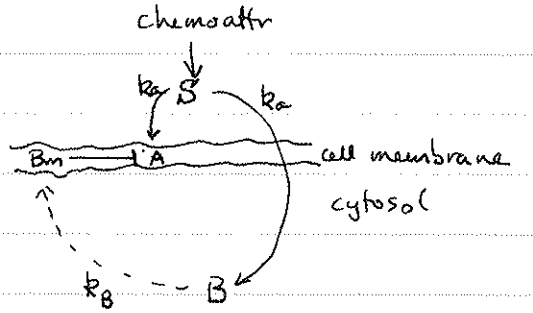
Other models for cell polarization

On the next pages, we briefly summarize a large number of recent cell polarity models from the literature.

See also the recent surveys and review articles (cited separately.)

Levine, Kessler, Wouter-Jan Rappel
(2006) PNAS 103(26): 9761-9766

Directional sensing via "balanced inactivation"



S^* = activ. receptors
A = membr. bound "activator"
B = cytosolic species
B_m = membr-bound inhibitor

assumes
- equal rates (k_a) of A, B activ.
- rapidly diffuse inhib. (B)

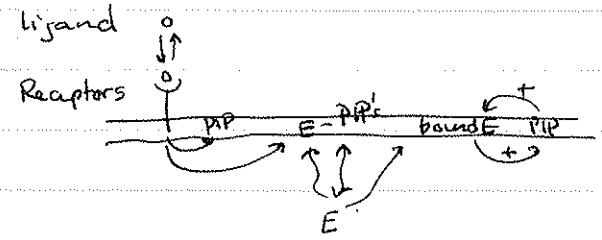
produces:
- shallow gradient → large assymetry
- can be reversed
- acts like a 'switch'

candidates:
heterotrimeric G-protein components

Postma, Van Haastert (2001)
BJ 81: 1314-1323
Diffusion/translocation for gradient sensing

- importance of diffusion ranges/lifetimes

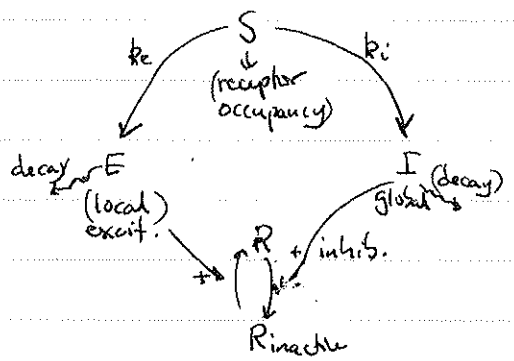
$\lambda = \sqrt{D_m / k_{-1}}$ = spatial range of 2nd messeng.

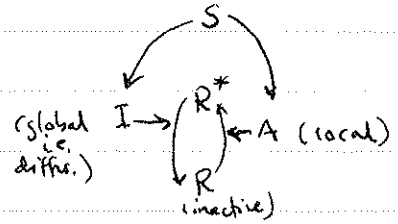


assumes: - 2nd messenger (~ PIP's)
- cytosolic (PH-domain) protein that translocates to membr. (E) (substrate depletion)
- positive feedback PIP → + via recruitment of E

Levchenko and Iglesias (and friends)
LEGI model (2002) BJ 82: 50-63

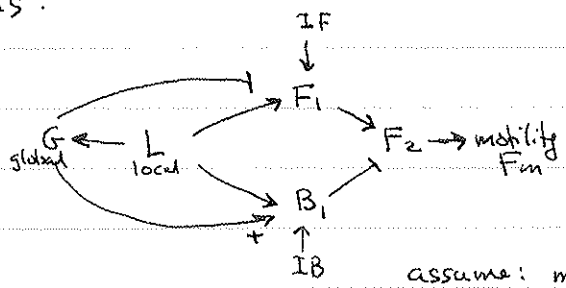
- adaptation module
- response proportional to gradient steepness
- adaptation to uniform gradient
- reversal ✓

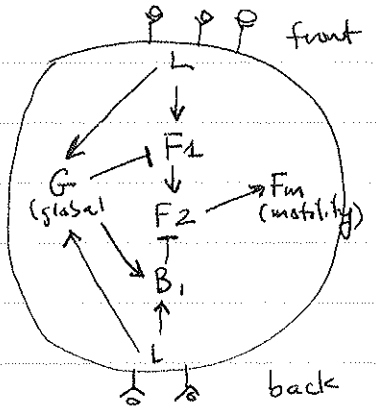




Krishnan + Iglesias (2007)
BJ 92: 816–830

intrinsic vs gradient-induced polariz.
(and their interactions.



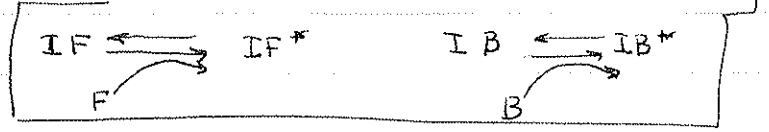


receptor-mediated:
candidates: F_1 ~ PI3K
F_2 ~ PIP_3, PIP_2
F_m ~ Factin or GTPases?
B_1 ~ PTEN

intrinsic: IF } frontness
IB } backness

goal: to check various links between intrinsic and receptor-mediated pathways (Fig 5)

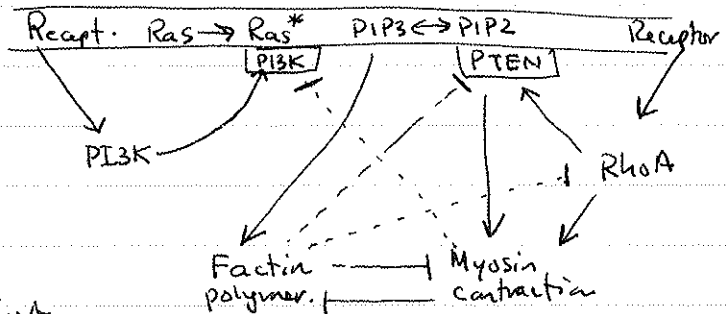
assume: membrane diffusion only for G, F_1, F_2
- Turning by 'phenomenological' model; instantaneous
$T \underset{k_{-1}}{\overset{k_1 s}{\rightleftharpoons}} T^*$
- each component has active* and inactive form.
- Diffusion coef ~ 1–5 $\mu m^2/s$ for all except G and L
- Intrinsic: other pathways, nondiffusible



Onsum + Rao (2007)
PLoS Comput. Biol 3(3) e36

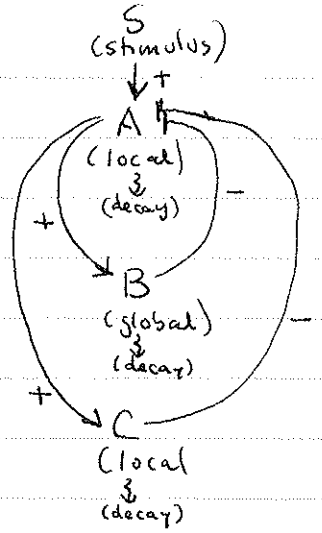
Neutrophil gradient sensing and polariz.

assumes: both Gβγ + Ras to activate PI3K
(coincidence circuit)
(⇒ PI3K activ more sensitive than RhoA to spatial gradient)
require both Ras and PI3K to transmit signal



results: polarizes, can turn (some lag)
partial adaptation.

Meinhardt (1999)
J Cell Sci 112 : 2867-2874
Orientation of chemotactic cells



"Turing based"

a - autocatalytic activator (local)

b - inhibitor (global)

c - inhibitor (local)
- needed to unlock a pattern so cell can respond to new stimuli

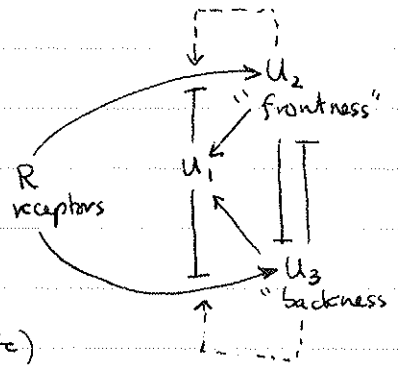
assumes: - c has longer time constant than a
- (could use depletion instead of inhib. b)

Results: amplification ✓
- locking prevented by C
- random pseudopod formation

candidates: G proteins (a) and Ca^{++} (b), and $InsP_3$ Receptor (c)

critique by Levchenko + Iglesias: the above has no adaptation (as per Dicty)
Ca^{++} conc changes do not affect gradient sensiy.

A. Narang (2005-6?)
J.T.B



Mutual inhibition of frontness/backness

A Turing mechanism
U_2 - frontness } both diffusible in cell membr
U_3 - backness }
U_1 - diffusible inhibitor in cytosol
- diffusible inhibitor (or depleted substrate)

assumes: no +ve feedback. only ⊣
- kinetics like (ecology) species competition
- $D_1 \gg D_2, D_3$

Results : spontan. polariz
- chemo attr or chemo repuls.

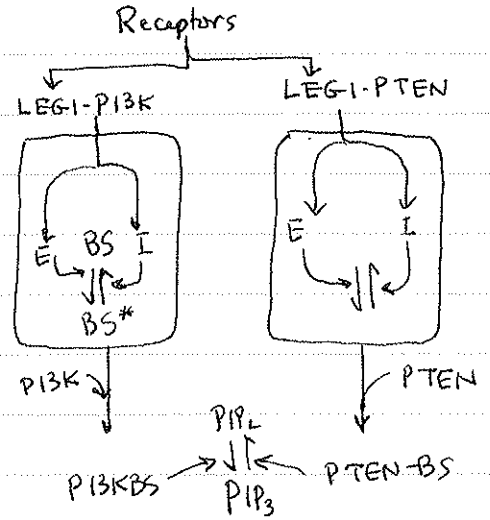
claims - this is simplest mutual inhib type model

candidates for U_1 : inositol phosphates (cytosolic)
- reactive oxygen species.

critique by Onsum+Rao : direction of polarity vs gradient can be upset if params perturbed.

Ma, Janetopoulos, Yang, Devreotes, Iglesias (2004) BJ 87: 3764-3774

Two LEGI Modules to explain Dicty temp/spatial direction sensing



BS = binding sites
BS* = activated "