Mathematical Model of Phospholipid Dynamics in Gradient Sensing

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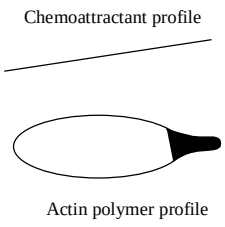
Based on Narang *et al.*, *Annals Biomed. Eng.*, **29**, 677, 2001

Properties of Gradient Sensing Mechanism

Chemoattractant gradient is *mild*, but actin polymerization is *localized*

⬇

External signal must be *amplified*



Amplification occurs only for sufficiently large gradients

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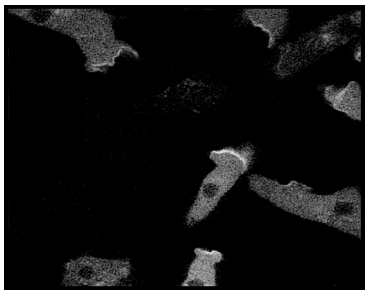
There must be a *threshold* for amplification

Response to Non-Uniform Chemoattractant Gradient

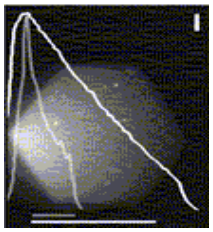
In the presence of the gradient induced by a micropipette, the PI localization appears and disappears

Parent *et al.*, 1998



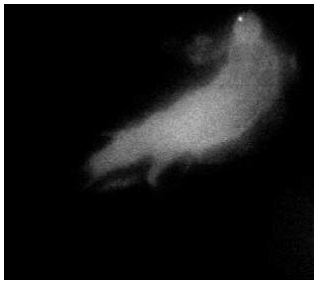
Servant *et al.*, 2000

In neutrophils, the localization of PH-Akt-GFP is 6 times the chemoattractant gradient



Response to Uniform Chemoattractant Stimulus

In *neutrophils*

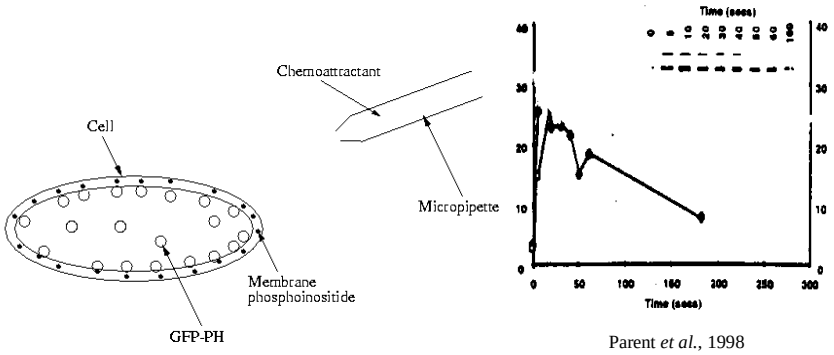


Servant *et al.*, 2000

- PH_{Akt} -GFP accumulates uniformly along the membrane within 10 secs
- But PH_{Akt} –GFP localizes ultimately and remains so for up to 8 minutes

Response to Uniform Chemoattractant Stimulus

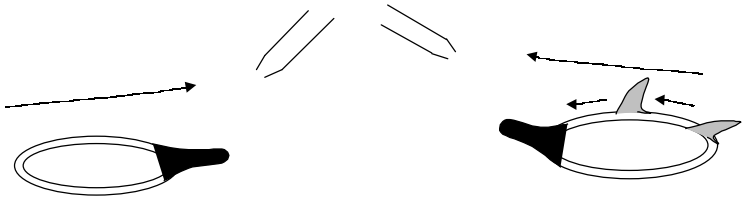
In *Dictyostelium*



Parent *et al.*, 1998

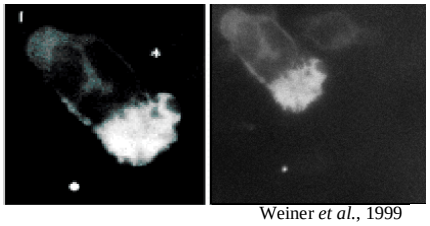
• GFP-PH immediately migrates to cell periphery, reverts to cytosol within 2 minutes, then develops a polarization.

Switch in direction of the gradient: *Shallow* Gradient



Shallow Gradient:

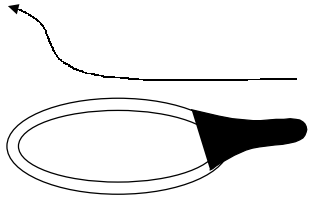
A gradient whose influence is felt all the way to pre-existing leading edge.



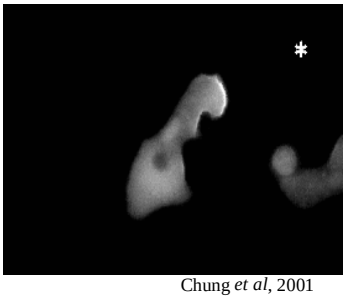
Weiner *et al.*, 1999

• New pseudopod (or Arp3 localization) does not form at the new location. Instead, existing pseudopod swivels to the new location.

Switch in direction of the gradient: *Steep* Gradient



Steep Gradient:
A gradient so localized that its influence is not felt at the pre-existing leading edge.



Chung *et al*, 2001

• Old pseudopod (PI localization) retracts and new pseudopod (PI localization) forms at the new location

The Model

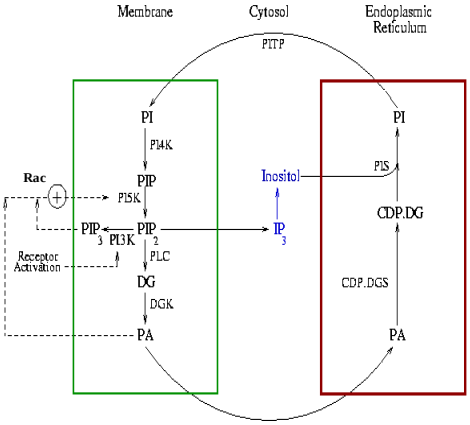
Model consists of two interacting species

Activator	Inhibitor
• Synthesized *autocatalytically*	• *Inhibits* the activator
• Diffuses *slowly*	• Diffuses *rapidly*

Wish to account for two experimental observations:

1. *Localization of PIs* in response to non-uniform *and* uniform chemoattractant gradients
2. *Movement of pre-existing PI localization* in response to changes in direction of chemoattractant gradient

PI Cycle : Intuitive explanation for PI localization

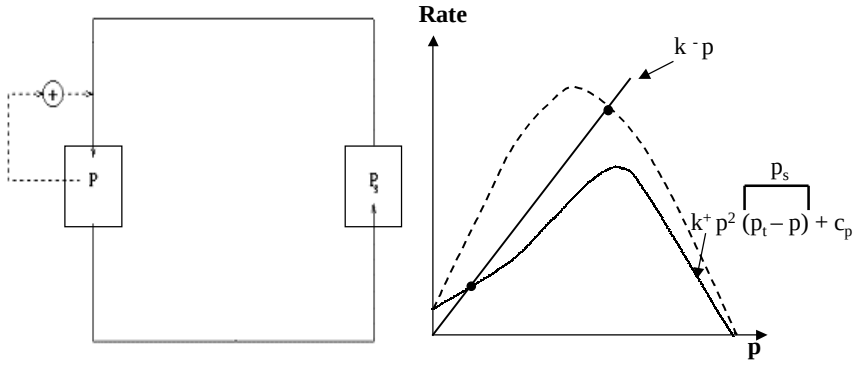


- Receptor activation activates PI3K.
- ↑ in PIP_3 ⇒ Recruitment of PI5K via Rac
- Leads to autocatalytic synthesis of PIP_2 and PIP_3. Feedback is further reinforced by PA.

The net effect is:

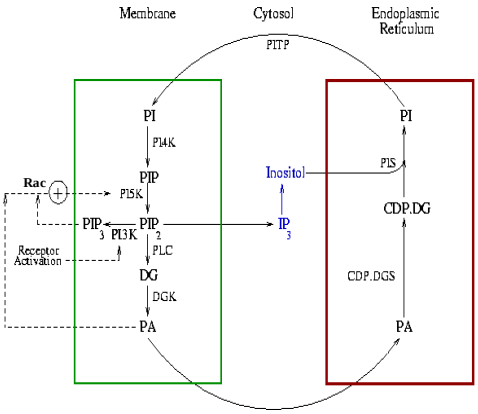
1. Membrane PI's builds up at the expense of PI's in ER
2. PI cycle turns faster

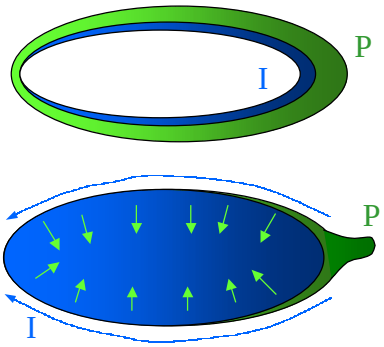
Leading Edge



- Synthesis of *P* is *autocatalytic* and *cooperative* at small *p* and *self-limiting* at large *p*.
- Receptor activation increases k^+ and "lifts" the synthesis curve.
- New steady state has higher *p* and higher turnover rate.

Intuitive explanation for PI peak stabilization

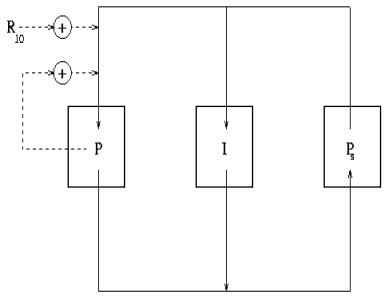


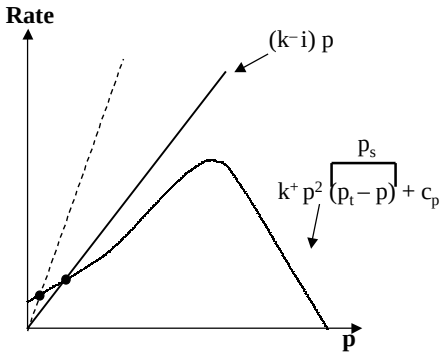


• Receptor activation causes autocatalytic build up of PI's at the leading edge which results in localized inositol formation

• Inositol diffuses away from the stimulus site and transfers PI from the membrane to the ER, thus preventing the peak from spreading

Trailing Edge





• *i* increases at the trailing edge

• Slope of removal curve increases

• Steady state *p* decreases and turnover rate is little slower

Role of PLCγ in movement of fibroblasts and neutrophils

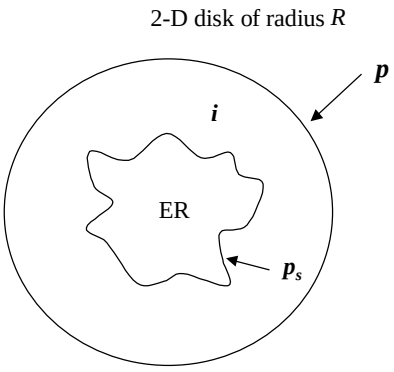
"Both the PLC and motility responses [in fibroblasts] *were decreased by expression of a dominant-negative PLC gamma-1 fragment in EGF-responsive infectant lines."* – Alan Wells *et al.*, 1994

"… cell motility [in neutrophils] *is [Ca2+]i dependent when the cells are examined on physiological substrates such as fibronectin or vitronectin. Calcium-buffered cells appear to make repeated attempts to move but are unable to detach from a fibronectin or vitronectin substrate"* – Hendey *et al.*, 1993

Mathematical Model

p : Activator

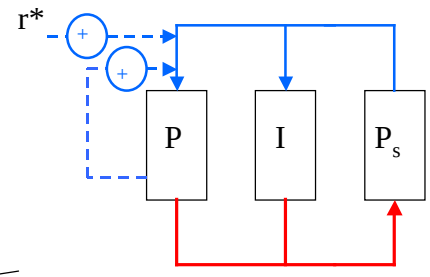
i : Inhibitor



Model Variables

- ***p*** Slow-diffusing membrane phosphoinositides
- ***i*** Fast-diffusing cytosolic inositol phosphates
- p_s Slow-diffusing phosphoinositides in ER

Model Equations



$$\frac{\partial r^*}{\partial t} = k'_{01} - k'_{01} r^* + \frac{D_r}{R^2}\frac{\partial r^*}{\partial \theta^2}$$

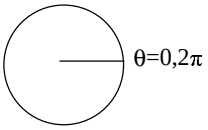
$$\frac{\partial p}{\partial t} = k_f r^*(\theta) p_s p^2 - k_r pi + c_p - k_p p + \frac{D_p}{R^2}\frac{\partial^2 p}{\partial \theta^2}$$

$$\frac{\partial i}{\partial t} = s\{k_f r^*(\theta) p_s p^2 - k_r pi\} + c_i - k_i i + \frac{D_i}{R^2}\frac{\partial^2 i}{\partial \theta^2}$$

$$p_t = \frac{1}{2\pi R}\int_0^{2\pi} (p + p_s) R d\theta$$

Periodic Boundary Conditions

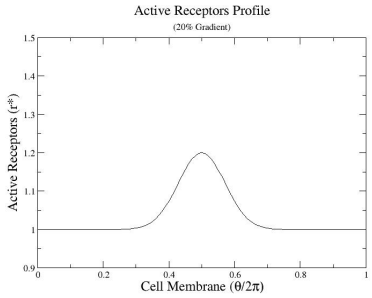
- Concentrations of *p* and *i* are equal at θ= 0, 2π
- Fluxes of *p* and *i* are equal at θ= 0, 2π

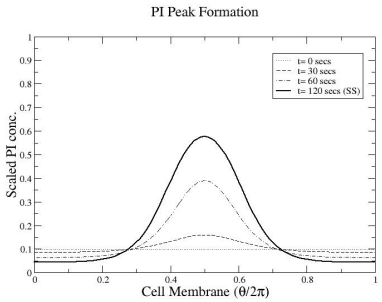


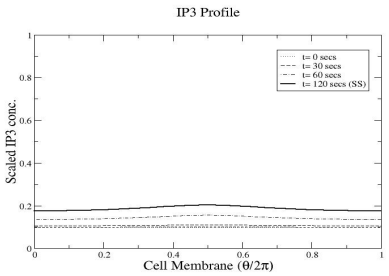
Initial Conditions

- At *t<0*, cell is in a uniform steady state corresponding to a uniform concentration of active receptors
- At *t=0*, a non-uniform profile imposed on the concentration of the active receptors

Response to chemoattractant gradient







- Non-uniform SS develops in 120 secs
- Inhibitory effect can be seen at the back of the cell
- *i* has a nearly flat profile

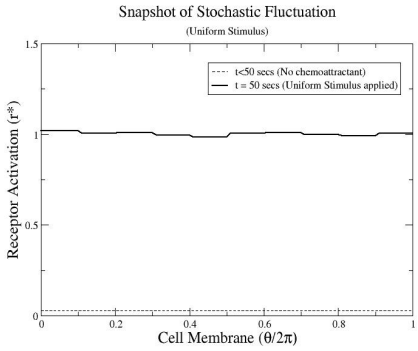
Response To Uniform ChemoattractantStimulus

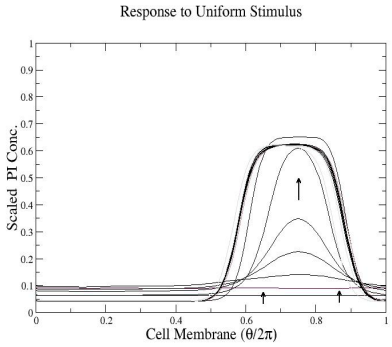
- Chemoattractant concentration may be macroscopically uniform
- But there might be significant random fluctuations in receptor-ligand binding

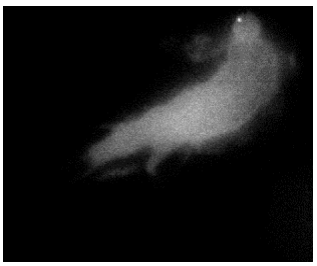
Simulating receptor-ligand binding fluctuations

- Cell membrane was partitioned into ten equal arcs.
- In each arc, fluctuations in active receptor concentrations were simulated by a stochastic model describing receptor-ligand interactions.
- Noise in the stochastic model is simulated by a Wiener process.
- Active receptors (r*) have a 1% deviation from the mean value.

Response to Uniform Stimulus



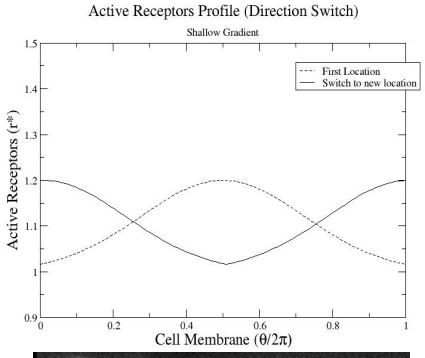


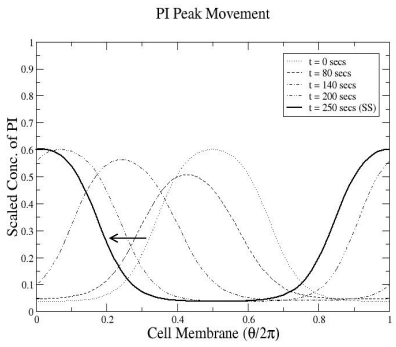


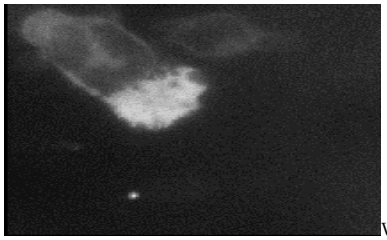
- Cells initially accumulate PI uniformly
- This is followed by the formation of a PI peak

Servant *et al.*, 2000

Switch in direction of the gradient : *Shallow* Gradient

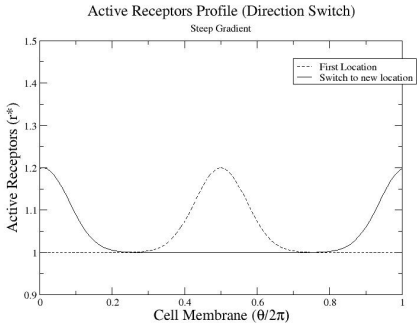


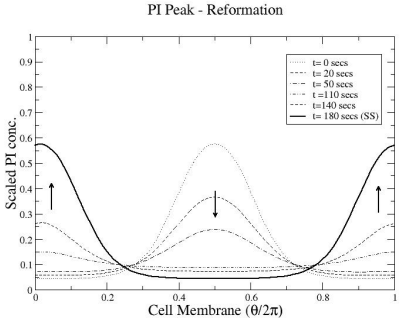


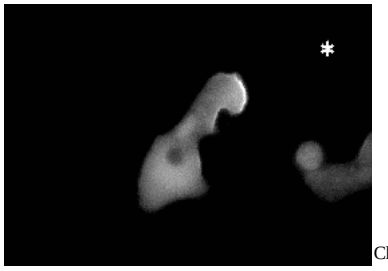


Weiner *et al.*, 1999

Switch in direction of the gradient : *Steep* Gradient

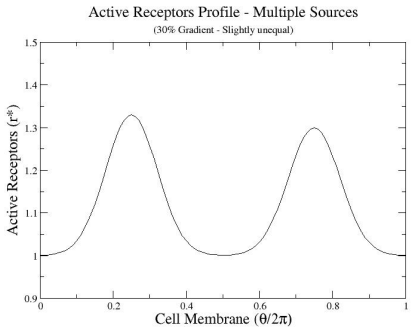


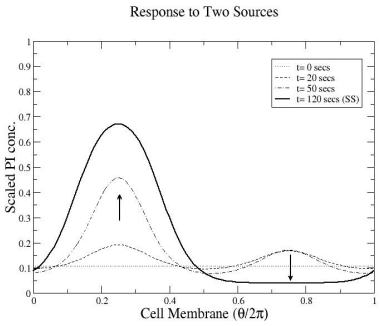




Chung *et al*, 2001

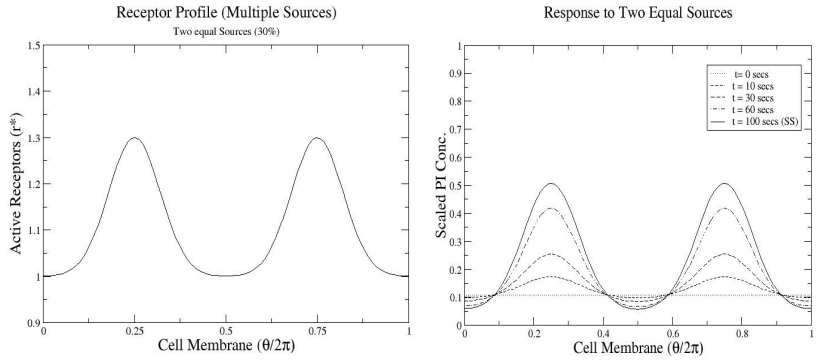
Response to Two *Unequal* Chemoattractant Sources





- Initially, localization develops at locations of both sources.
- Finally, the larger source "wins" and a *single* localization forms at the site of highest chemoattractant concentration.

Response to Equal Chemoattractant Sources

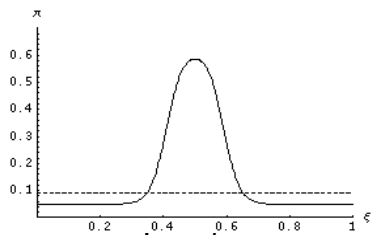


• Two peaks can form only if the difference between the two chemoattractant maxima is very small relative to the magnitude of the maxima.

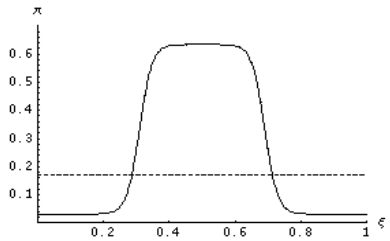
Variation of peak width with rate constants

k_f = Rate of PI formation

Initial Profile
Final Profile



Low k_f



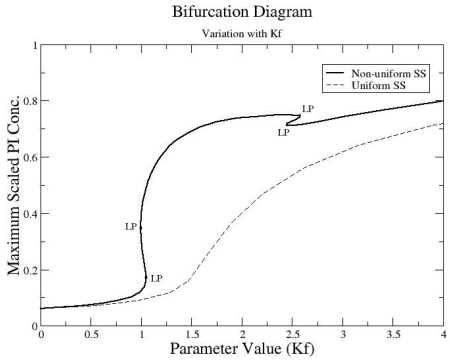
High k_f

• Width of the steady state peak increases with k_f

• PI is synthesized so fast that peak spreads before inositol phosphates can contain them

• Similar results if k_r is decreased.

Variation of steady states with k_f:



- Both uniform and non-uniform SS exist over a range of k_f
- Gradient makes system jump from uniform to non-uniform SS
- At large and small k_f, non-uniform SS merges with uniform SS

Conclusions:

A reaction-diffusion model predicts the following:

- PI's localize in response to uniform and non-uniform chemoattractant gradient

- PI's move in response to changes in direction of chemoattractant gradient
  Shallow Gradient: Existing peak moves to the new location
  Sharp Gradient: New peak forms as existing peak goes down

- Width of PI localization changes when reactions are activated or inhibited.

- Unique peak develops even in response to multiple chemoattractant sources.

Acknowledgements

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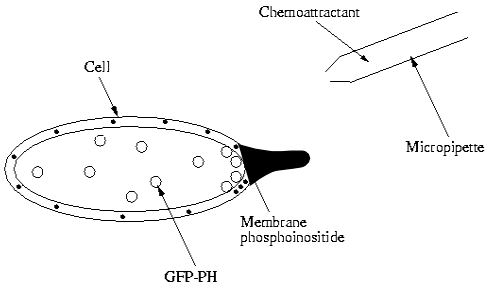
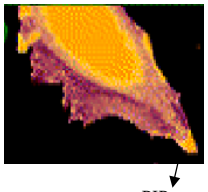
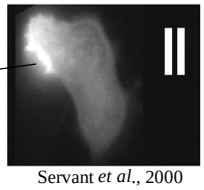
Response to Non-Uniform Chemoattractant Gradient



Tall *et al*, 2000





Servant *et al*., 2000

Observation

• Within 10 secs, GFP-PH (PIP_2 /PIP_3 marker) migrates toward highest concentration and remains there