cyclic AMP (cAMP) is an attractant for Dictyostelium

**Dictyostelium** (social amoeba): an experimental model system for eukaryotic chemotaxis

- cAMP gradient
- CRAC-GFP (PH-GFP) is a convenient reporter of local PIP3 concentration

**CRAC-GFP (PH-GFP) is a convenient reporter of local PIP3 concentration**

- uniform increase in cAMP
- rotating cAMP gradient by moving cAMP filled pipette

Parent, Devreotes et al.
Response of Dictyostelium to cAMP

uniform step in cAMP | cAMP gradient

- initial distribution $t \sim 3 \text{ s}$
- steady-state distribution $t \to \infty$

uniform and transient | polarized and persistent

A different technology: UV induced uncaging of cAMP

Main advantages:
- allows cAMP pulses with well-defined amplitude and duration
- highly reproducible pulses

Response of a single cell to a pulse

raw data
Total time = 30 sec
$R_{cell} = 5 \mu m$

signal difference with respect to unstimulated cell

Response of a single cell to a pulse

signal difference with respect to unstimulated cell
quantify GFP concentration along plasma membrane
A single cell responds reproducibly to multiple pulses

Repeated stimulation of the same single cell

10 repeated stimulae for the same cell

$R(\theta, T_{\text{max}})$ %

$\theta$ (degrees)

Error bars denote the standard deviation

Pulse duration = 1 s
Waiting time between pulses = 30 s

What kind of models can be developed that reproduces these experimental findings?

First model to discuss:
Narang, Subramanian and Lauffenburger

Subramanian, Narang

The molecules in the model:

GFP-PH binds special lipids in membrane: PIP2 and PIP3

Geometry of cell: circular
Inside cytoplasm: well-stirred
Inside membrane: diffusion-limited
**Phosphatidylinositol Signal Cascades**

Sequence of events:
Kinase enzymes sequentially catalyze transfer of $\text{P}_i$ from ATP to OH groups at positions 5 & 4 of the inositol ring, to yield *phosphatidylinositol-4,5-bisphosphate (PIP}_2").

Phospholipase C cleaves PIP$_2$. Cleavage of PIP$_2$, catalyzed by Phospholipase C, yields two second messengers: *inositol-1,4,5-trisphosphate (IP$_3$) & diacylglycerol (DG)*.

**Diacylglycerol**, with $\text{Ca}^{++}$, activates *Protein Kinase C*, which catalyzes phosphorylation of several cellular proteins, altering their activity.
PA (phosphatidic acid) activates PI4P5K (PIP-kinase) (autocatalytic loop)

Reduced model:

Assumption:
PIP’s in membrane + PIP’s in ER = constant
Model for receptors: perfect adaptation.

Results: uniform stimulus

Results: gradient