• Tomorrow’s recitation: Problem Set 5 Support

• Problem Sets #3 and #4 have been graded

• Final PS will be posted next week Tuesday 11/29 (due two weeks later, last day of classes)

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**L21**

Stochastic Cell Polarization

*How do single cells select a random direction?*

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**Strategy, long term goals:**

1. By quantitatively exploring spontaneous cell polarization we will better understand the molecular mechanism needed for understanding directed cell motility

2. Understanding stochastic cell polarization, or general stochastic cellular behavior, will improve understanding of non-genetic individuality and its impact on the fitness of a population

Focus on ‘well characterized’ biochemical networks in ‘simple’ organisms:

**Part I:** Cell individuality in the gradient sensing response of *Dictyostelium*

**Part II:** The random budding decision in budding yeast

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![Image](image_url)

*Wedlich-Soldner and Li, NCB (2003)*
Part I

Cellular individuality in the gradient sensing response of Dictyostelium

Azadeh Samadani and Jay Mettetal

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

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.
A different technology: UV induced uncaging of cAMP

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

The spatial and temporal cAMP distribution can be accurately quantified by a two-dimensional diffusion equation

Response of a single cell to a pulse

Signal difference with respect to unstimulated cell

Quantify GFP concentration along plasma membrane
The response function $R(\theta, t)$ of a single cell

$$R(\theta, t) = \frac{I(\theta, t) - I(\theta, t = 0)}{I_{total}}$$

$R(\theta, t)$ can be characterized with 3 parameters

1. **Localization**
   $$L(t) = \langle R(\theta, t) \rangle_\theta$$

2. **Polarization**
   $$P(t) = \langle R(\theta, t) e^{i\theta} \rangle_\theta$$

3. **Polarization angle**
   $$\phi = \text{direction of maximum response}$$

A single cell responds reproducibly to multiple pulses

repeated stimulation of the same single cell

10 repeated stimulae for the same cell

$pulse duration = 1$ s
waiting time between pulses = 30 s

error bars denote the standard deviation

A single cell responds reproducibly to multiple pulses

10 repeated stimulae
for three different cells
**Single Cell Averaging vs Population Averaging**

1. **1 cell, 10 pulses**
   - Graph showing the response $R(\theta, T_{max})$ for a single cell.

2. **100 cells, 1 pulse**
   - Graph showing the response $R(\theta, T_{max})$ for a population of cells.

**Polarization $P$ and polarization angle $\phi$ vary significantly from cell-to-cell**

- Graphs showing $L(\%)$, $P_y(T_{max})$, $P_x(T_{max})$, and the distribution of $\phi(T_{max})$.

- $<P_x> = (3.6 \pm 0.4) \%$

- However, the population correctly detects the pulse direction.

**The population correctly detects the pulse direction**
Polarization $P$ and polarization angle $\phi$ vary significantly from cell-to-cell, but are correlated.

A similar behavior is observed for the localization.

The noise in directional sensing does not decrease by increasing the external concentration.

Summary Main Experimental Observations

- In response to repeated spatio-temporal pulses of cAMP, lat A-treated Dictyostelium cells display reproducible behavior, quantified by the localization $L$, polarization $P$, and polarization angle $\phi$.
- A significant cell-to-cell variability in polarization $P$ and polarization angle $\phi$ is observed.
- For a single cell the response is highly reproducible from pulse-to-pulse.
- On average the population displays a non-zero polarization in the right direction.
- Individual cells polarizing in the right direction have a about two-fold larger polarization than cells that polarize in the wrong direction.
The model reproduce the average and dynamics of localization fairly well. The model predicts a smaller polarization than observed in the experiments.

**Problems with the LEGI models**

- The model reproduce the average and dynamics of localization (not polarization) fairly well.
- Every single cell (according to the model) will polarize in the direction of the external gradient.

**What can we do to improve on LEGI models?**

There is no allowance for stochasticity in the LEGI model.

**Proposal:**

\[
S = S_{\text{external}}(cAMP) \times S_{\text{internal}}
\]

\[
S = \left( S_0 + S_1 \cos \theta \right) \left( 1 + \varepsilon \cos(\theta + \phi) \right)
\]
What happens in the case of a uniform external stimulation? $S_1 = 0$

Geometric model allows for symmetry breaking even in the case of uniform stimulation

A uniform external stimulation

$S_1 = 0$

The distribution of polarizations are uniform as predicted by the geometric model

A directed pulse

The distribution of polarizations are shifted toward the direction of the external pulse
Geometric Model Predictions

(\(\phi_e\) is uniformly distributed)

1. Cells more likely to polarize in the correct direction
2. Cells that polarize in the correct direction localize more GFP
3. Cells that polarize in the correct direction polarize stronger

\[
S = \left\{ S_0 + \frac{\varepsilon}{2} S_1 \cos(\phi_e) \right\} + \left( S_1 + \varepsilon S_0 \cos(\phi_e) \right) \cos(\theta) - \left( \varepsilon S_0 \sin(\phi_e) \right) \sin(\theta)
\]

Geometric model predicts the correlation between \(L, P\) and \(\phi\)

no external signal (\(S_1 = 0\))

external signal (\(S_1 \neq 0\))
Conclusions

Part I: Cell individuality in the Dicty gradient sensing response

Single cell reproducibly respond to multiple identical stimuli, however a significant variability in polarization is observed from cell-to-cell. On average the population detect the right direction.

We propose that the effective signal can be decomposed in an external cAMP signal and an internal static signal upstream of PH domain recruitment.

Part II

Traveling Cdc42 activity waves and spontaneous cell polarization in budding yeast

Ertugrul Ozbudak and Attila Becskei

What is the dynamics of this symmetry breaking?

Experimental probe: Gic2p(1-203)-GFP (CRIB-GFP)

Calcofluor staining

unipolar budding

haploid WT

e.g. bud1∆

random budding

(Ni and Snyder, MBC, 2001)

Chaht (1999)
Cdc42p activity waves perform a random walk at the cell periphery.

Wave mobility depends in a dose-dependent manner on Bud1p concentration.

Decreasing GAP activity immobilizes waves.
In the absence of the actin cytoskeleton symmetry breaking still occurs, but Cdc42p activity patch is immobilized.

**Summary**

**Main Experimental Observations**

- In **bud1Δ** cells in early G1 traveling waves of activated Cdc42p randomly explore the yeast cell periphery.
- This random motion stops when:
  - cells initiate budding
  - GAP activity decreases
  - GEF activity increases
  - the actin cytoskeleton is depolymerized
- Actin cytoskeleton is not necessary for symmetry breaking (consistent with Wedlich-Soldner *et al.*, JCB 2004 and Irazoqui *et al.*, NCB 2003).

Cdc42p mediated positive feedback loops allow for symmetry breaking:

- scaffold-mediated positive feedback
- actin-mediated positive feedback

Positive feedback recruitment of active Cdc42p explains the random polarization but NOT the traveling waves

\[ \frac{\partial a(x,t)}{\partial t} = \frac{\alpha}{1 + a^2(x,t)} a(x,t) - a(x,t) + \frac{\partial^2 a(x,t)}{\partial x^2} \]

Proposal
- in addition to positive regulation, a negative loop exists
- actin mediated loops are delayed

Model is consistent with observed phenotypes

\[ \frac{\partial a(x,t)}{\partial t} = \frac{\alpha}{1 + a^2(x,t)} a(x,t) - a(x,t) + \beta a(x,t - \Delta t) + \frac{\partial^2 a(x,t)}{\partial x^2} \]

\[ \begin{align*}
\beta & = 0 \\
\beta & > 0 \\
\beta & < 0
\end{align*} \]
Conclusions

Part II: Traveling Cdc42 activity waves organize random budding

In cells lacking functional landmarks Cdc42p self-organizes in traveling activity waves that randomly explore the cell periphery

We propose that the driving force of the waves is due to the competition between positive and negative feedback loops regulation Cdc42p activity

Happy Thanksgiving!