Cellular individuality in directional sensing

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How do cells make a decision?

A cell makes many decisions based on the cues from the external environment.

<table>
<thead>
<tr>
<th>Presence of a gradient</th>
<th>Absence of a gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neutrophils/Dictyostelium</strong></td>
<td>No gradient</td>
</tr>
<tr>
<td><strong>Cue-dependent symmetry breaking</strong></td>
<td><strong>Random symmetry breaking</strong></td>
</tr>
</tbody>
</table>

How does the decision making vary from cell-to-cell?
How do cells make a decision?

Bacteria (Prokaryote)  White blood cell (Eukaryote)

Movie by Nikhil Mittal & Elena Budrene

This movie is made by David Rogers. Taken from website of Tom Stossel.
Temporal gradient sensing

Absence of chemical attractant

Presence of chemical attractant

Temporal gradient sensing
Bacteria vs. Amoebae

**Bacteria (Prokaryote): Small**
- Small compared to diffusion length
- Sample over time
- Biased random walk towards the food

**Amoebae (Eukaryote): Large**
- Larger cells
- Sample the periphery of the cell
- Directed motion towards the food
Objectives and long term goals:

1. By quantitatively exploring cue-dependent cell polarization, we will better understand the molecular mechanism of directed cell motility (chemotaxis)

2. By understanding stochastic cellular behavior, we will improve our understanding of non-genetic individuality and its impact on the fitness of a population

Focus on ‘well characterized' biochemical networks in a ‘simple' organism:

The model system: *Dictyostelium* (social amoeba)
A model system: *Dictyostelium* (social amoeba)

An experimental model system for eukaryotic chemotaxis

cAMP source
A well characterized biochemical networks

GFP indicates where the leading edge of a cell would be if the cell is able to move.
Receptor distribution is uniform around cell membrane

Movie taken from P. Devreotes website

Therefore asymmetric signaling must occur downstream of the receptors
PH-CRAC-GFP is a convenient reporter of the leading edge of a cell, even when cells are immobile.

In a gradient, PH-CRAC-GFP accumulates to the leading edge of a cell.

Gradient sensing can be separated from the movement.

CRAC: Cytosolic Regulator of Adenylyl Cyclase

A different technology: UV induced uncaging of cAMP

UV (360 nm) cleaves this bond

Main advantages:
- allows well defined cAMP pulses
- pulses are reproducible
spatio-temporal cAMP concentration

\[ D_{\text{cAMP}} \sim D_{\text{fluorescein}} = 3.0 \times 10^{-6} \text{ cm}^2/\text{s} \]
Response of a single cell to a pulse

Response of the cell is polarized towards the direction of the 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

quantifying GFP concentration
Along cell membrane

Maximum of the response ~ 8 seconds
Response of a single cell to a pulse

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

maximum of the response ~ 8 seconds
A single cell responds reproducibly to multiple pulses.
The response function can be characterized with 3 parameters

1) **Localization**  mean of the response function
2) **Polarization**  amplitude of the response function
3) **Polarization angle**  direction of the maximum response
A single cell responds reproducibly to multiple pulses

10 repeated stimulation of the same cell

polar plot of the polarization vector

the error bars denote standard deviations

the pulses are separated by 2 minutes
Response to the same pulse vary significantly from cell-to-cell

Single cell vs. Population

Single cell - 10 pulses
100 cells - 1 pulse

\[ \langle P_x \rangle = (6 \pm 0.4) \% \]
The population correctly detects the pulse direction

more cells polarize in the direction of the pulse $\phi = 0$
The magnitudes of $L$ and $P$ correlate with $\phi$

“Right cells” ($\phi = 0$) larger localization; stronger polarization
“Wrong” cells ($\phi = 180$) smaller localization; weaker polarization
Can we reduce the noise by increasing the signal?

If the bound state of the receptor $t \sim (1-2 \text{ sec})$

$D$ (of cAMP) = $10^{-6} \text{ cm}^2/\text{s}$

$R \sim (D \, t)^{1/2} = 10^{-3} \text{ cm} \sim 10 \mu\text{m}$

There are between $5 \times 10^4$ to $10^5$ receptors/cell

In the sampling volume there are:

<table>
<thead>
<tr>
<th>C</th>
<th>molecules</th>
<th>molecule/receptor</th>
<th>Noise/signal $\frac{1}{\sqrt{N}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-10}$ M</td>
<td>$6 \times 10^2$</td>
<td>0.01</td>
<td>cells do not respond</td>
</tr>
<tr>
<td>$10^{-9}$ M</td>
<td>$6 \times 10^3$</td>
<td>0.1</td>
<td>1%</td>
</tr>
<tr>
<td>$10^{-8}$ M</td>
<td>$6 \times 10^4$</td>
<td>1</td>
<td>0.5%</td>
</tr>
<tr>
<td>$10^{-7}$ M</td>
<td>$6 \times 10^5$</td>
<td>10</td>
<td>0.1%</td>
</tr>
<tr>
<td>$10^{-6}$ M</td>
<td>$6 \times 10^6$</td>
<td>100</td>
<td>0.01%</td>
</tr>
</tbody>
</table>
The noise in directional sensing does not decrease by increasing the external concentration.

The origin of symmetry breaking must be interacellular.
Summary of the main experimental observations

• The response of a single cell is reproducible from pulse-to-pulse

• The response of cells within population vary greatly from cell-to-cell

• On average the population finds the correct direction of the pulse

• Individual cells polarizing in the right direction have about two-folds larger localization and polarization than cells that polarization in the wrong direction

• The origin of the noise must be intaracellular

How can we explain the variability?
Models

Local excitation and global inhibition of the signal

Activator
Diffuses slowly
Local (leading edge)

Inhibitor
Diffuses rapidly
Global (front, back and sides)

• Diffusion-Translocation, Postma, van Haastert, Biophys. J. (2001)
• Inhibitor-Diffusion, Rappel, Thomas, Levine and Loomis, Biophys. J. (2002)
• Local excitation- Global Inhibition, Iglesias and Levchenco, Biophys. J. (2002)
Mechanism: Local Excitation-Global Inhibition

Is this a good model?
Local Excitation-Global Inhibition Model (LEGI)  
Activator Equations  
Iglesias and Levchenco (2002)

\[
\frac{dA}{dt} = k'_a S (A_{tot} - A) - k_{-a} A \\
A_{inactive} + A = A_{tot} \\
A \ll A_{tot} \\
k_a = k'_a A_{tot} \\
\frac{dA}{dt} = -k_{-a} A + k_a S
\]
Local Excitation-Global Inhibition Model (LEGI): Inhibitor Equations

Iglesias and Levchenco (2002)

\[
\frac{dI}{dt} = -k_{-i} I + k_i S (I_{tot} - I)
\]

\[
I_{inactive} + I = I_{tot}
\]

\[
I << I_{tot}
\]

\[
k_a = k_{a tot} \]

\[
\text{diffusion}
\]

\[
\frac{dI}{dt} = -k_{-i} I + k_i S + k_d \frac{d^2 I}{dx^2}
\]
Local Excitation-Global Inhibition Model

Iglesias and Levchenco (2002)

\[
\frac{dA}{dt} = -k_a A + k_a S
\]
\[
\frac{dI}{dt} = -k_i I + k_i S + k_d \frac{d^2 I}{dx^2}
\]
\[
\frac{dR^*}{dt} = -k_r I R^* + k_r R A
\]
Localization dynamics can be reproduced by the LEGI model

LEGI model fits the average and the dynamics of the localization fairly well

LEGI predicts a smaller polarization than observed experimentally
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.
- There is no allowance for stochasticity in the LEGI model.

What can we do to improve on LEGI models?
The error bars denote standard deviations, which increase 5 fold from single cell to population.
The geometric Model

**Proposal:** $S = S_{\text{ext}} \times S_{\text{int}}$

$S_{\text{ext}} = S_0 + S_1 \cos(\theta)$

$S_{\text{int}} = 1 + \varepsilon \cos(\theta + \phi_\varepsilon)$

What happens in the case of a uniform external stimulation? $S_1 = 0$
First order prediction of the geometric model

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

The distribution of polarizations are uniform as predicted by the geometric model
The distribution of polarizations are shifted toward the direction of the external pulse
Proposed Experiments:

Moving the external source around the cell

internal signal (static)
external signal (dynamic)
Geometric model fits the data with only two fitting parameters $\alpha = \varepsilon S_0/S_1$ and $\phi_\varepsilon$

small $\varepsilon$ (<< S1/S0) $S_{eff}$ will follow the extracellular signal exactly ($\phi \approx \theta_s$)

large $\varepsilon$ (>> S1/S0) the $S_{eff}$ stays in the direction of the internal signal ignoring the extracellular signal ($\phi \approx \phi_\varepsilon$)
Geometric model can quantitatively predict the fraction of cells that polarize in a specific direction using measured average value of $\alpha$ from our population measurements. The geometric model quantitatively predicts the relation between mean localization and polarization, with the polarization angle.
Summary

• The response of a single cell is highly reproducible from pulse-to-pulse

• In contrast, a large variability is observed from cell-to-cell

• Geometric model successfully predicts the observed variability

• This observed variability is the results of variation in the spatial localizations of the proteins inside a cell and cannot be explain only by the fluctuations in the number of signaling molecules from cell-to-cell
Other interesting questions:

- Single *dictyostelium* cells communicate with each other through pulses of cAMP
- Cells demonstrate rectified motion in response to traveling pulses of cAMP

![Diagram showing cAMP concentration and cell motion](image)

1. Why do cells show rectified motion?
2. How does the response of cells vary as a function of pulse frequency?
3. How do cells respond to periodic vs. chaotic or aperiodic stimuli?
4. How does the chemotactic response vary by changing the adaptation time?

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Mike Desantis  
Department of Physics Brandeis University
Experimental set up:
Making cAMP waves with different frequencies

Jay Mettetal (MIT), Mike DeSantis and Samuel Rauhal (senior thesis at Brandeis)
Chemotaxis toward pulses of cAMP

1 sec pulse every min.

~ 20 pulses 350 mm

Cell tracks as a function of wave frequency

$\Delta t = 7$ sec 15 sec 30 sec 60 sec 120 sec 240 sec
Preliminary results with tracking motile cells shows that:

1 – At least within a certain range of frequencies, time varying stimuli are more efficient than continuous stimuli.

2- Maximum response occurs for $T = 30$ sec.

Wild type Dictyostelium cells produces pulses with period of 6 min.