CHEMOTACTIC SIGNALS AND RESPONSES ARE COORDINATED BY AN OSCILLATORY CIRCUIT IN DICTYOSTELIUM
Dictyostelium is a social amoeba that separated from plants and animals about 1 billion years ago. Many genes and pathways are well conserved.

**ADVANTAGES**
The 34 Mb genome is sequenced. There are many well defined mutant strains. It grows well and billions of cells can be induced to develop synchronously. After 4 hours of development cells signal each other with cAMP and respond.

Dynamics of spiral waves seen by dark-field microscopy; the cells contract when a cAMP wave passes over them but there is no net cellular movement.
The Dicteostelium Motility Cycle

OSCILLATIONS IN LIGHT SCATTERING AND ADENYLYL CYCLASE

![Graph showing oscillations in light scattering and adenylcyclase activity](image)

**cAMP RELAY**

- cAMP
- Gα2
- CRAC
- ACA

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The Dictostelium Motility Cycle

SIGNALING CIRCUIT

AGGREGATION STAGE NETWORK

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INTERACTIVE NONLINEAR DIFFERENTIAL EQUATIONS WITH ACTIVATING AND DEACTIVATING TERMS

\[ \text{[ACA']} = k_1\text{[CAR1]}-k_2\text{[ACA]}\text{[PKA]} \]
\[ \text{[PKA']} = k_3\text{[cAMPi]}-k_4\text{[PKA]} \]
\[ \text{[ERK2']} = k_5\text{[CAR1]}-k_6\text{[PKA]}\text{[ERK2]} \]
\[ \text{[RegA']} = k_7-k_8\text{[ERK2]}\text{[RegA]} \]
\[ \text{[cAMPi']} = k_9\text{[ACA]}-k_{10}\text{[RegA]}\text{[cAMPi]} \]
\[ \text{[cAMPe']} = k_{11}\text{[ACA]}-k_{12}\text{[cAMPe]} \]
\[ \text{[CAR1']} = k_{13}\text{[cAMPe]}-k_{14}\text{[CAR1]} \]

\[ ' = \text{differentiation with respect to time} \]

CIRCUIT OSCILLATES WITH A ROBUST 7 MINUTE PERIOD

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**PHASE SHIFT**

G. Gerisch and others

Time of addition of cAMP pulse during 7 minute period

**ENTRAINMENT**

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APPEARANCE OF OSCILLATIONS DURING DEVELOPMENT

AGGREGATION STAGE NETWORK

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OSCILLATIONS DURING EARLY DEVELOPMENT

<table>
<thead>
<tr>
<th>Enzyme Levels</th>
<th>Start</th>
<th>Rate Constants</th>
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</thead>
<tbody>
<tr>
<td>constant 10.0</td>
<td>0.0</td>
<td>k1=1.0 k2=1.0</td>
</tr>
<tr>
<td>constant 7.0</td>
<td>0.0</td>
<td>k3=0.2 k4=0.0</td>
</tr>
<tr>
<td>constant 3.0</td>
<td>0.0</td>
<td>k1=0.0 k2=0.0</td>
</tr>
<tr>
<td>constant 1.0</td>
<td>0.0</td>
<td>k1=0.0 k2=0.0</td>
</tr>
<tr>
<td>constant 1.0</td>
<td>0.0</td>
<td>k1=0.0 k2=0.0</td>
</tr>
<tr>
<td>constant 0.1</td>
<td>0.0</td>
<td>k1=0.0 k2=0.0</td>
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<tr>
<td>constant 1.5</td>
<td>0.0</td>
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<tr>
<td>constant 0.1</td>
<td>0.0</td>
<td>k1=0.0 k2=0.0</td>
</tr>
</tbody>
</table>

CHEMOTACTIC PROPERTIES OF VARIOUS STRAINS

<table>
<thead>
<tr>
<th>Mutant</th>
<th>cAMP Signaling</th>
<th>Directional Sensing</th>
<th>Lateral pseudopods</th>
</tr>
</thead>
<tbody>
<tr>
<td>AX4 (none)</td>
<td>periodic</td>
<td>excellent</td>
<td>few</td>
</tr>
<tr>
<td>CAR1</td>
<td>none</td>
<td>none</td>
<td>---</td>
</tr>
<tr>
<td>ACA</td>
<td>none</td>
<td>poor</td>
<td>---</td>
</tr>
<tr>
<td>ERK2</td>
<td>none</td>
<td>poor</td>
<td>many</td>
</tr>
<tr>
<td>REGA</td>
<td>poor</td>
<td>poor</td>
<td>many</td>
</tr>
<tr>
<td>PKA-R</td>
<td>poor</td>
<td>poor</td>
<td>many</td>
</tr>
</tbody>
</table>

AGGREGATION STAGE NETWORK

- CAMP pulse
- 5’AMP
- PDE
- CAR1
- ERK2
- REG A
- ACA
- CAMP
- PKA

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CHEMOTACTIC MIGRATION OF CELLS IN PURE POPULATIONS OF WILD TYPE (AX4) AND MUTANT (regA-) STRAINS

A. regA- cell in regA- territory

B. Labeled AX4 cell in regA- territory

CELLS LACKING RegA NEITHER SIGNAL NOR RESPOND

F. Labeled regA- cell in AX4 territory

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CELLS LACKING RegA MAKE MORE LATERAL PSEUDOPODS

MYOSIN IS NOT RECRUITED TO THE CORTEX OF CELLS LACKING RegA
CHEMOTACTIC MIGRATION OF WILD TYPE AND PKA R⁻ CELLS IN A PREDOMINANTLY WILD TYPE POPULATION

CELLS LACKING PKA MAKE MORE LATERAL PSEUDOPods
The Dicteostelium Motility Cycle

3D RECONSTRUCTION OF A WILD TYPE CELL IN A CHEMOTACTIC WAVE

new projections shown in red

position of cAMP source *

single anterior pseudopod in the front of a wave

antior pseudopod retracted at peak of a wave

multiple pseudopods in the back of a wave

In the front of a wave a cell experiences a spatial as well as a temporal gradient. Direction is established.

Aggregation center

cAMP

direction of movement (slow relative to wave)
At the peak of the wave, the cortical layer of actin/myosin is dismantled and net movement ceases.

Distal cells are stimulated to secrete cAMP.

In the back of the wave, the spatial gradient is reversed. The cell does not backtrack due to loss of cortical rigor and the formation of lateral pseudopods.
The Dictostelium Motility Cycle

MODEL OF THE CHEMOTACTIC STAGES

cGMP mediated basolateral inhibition of PI3K

- threshold cAMP (1 sec.)

basolateral myosin II-based cortical rigor

- front of a wave (3 min.)

loss of cortical rigor; lateral pseudopod formation

- back of a wave (3 min.)

recruitment of PH domain proteins; CRAC, PhdA etc.

actin assembly; pseudopod formation

no further net movement

SIGNALING AND MOTILITY CIRCUIT

CONTROL OF CORTICAL RIGOR (directionality)
The Dicteostelium Motility Cycle

Collaborators

UCSD
Michael Laub
Sam Payne
Wouter-Jan Rappel
Gad Shaulsky
Adam Kuspa
Mineko Maeda
Rick Firtel

University of Iowa
David Soll
Deb Wessels

The Albert Einstein
Jeff Segall

DYNAMICS OF CELLULAR RESPONSES TO A NATURAL WAVE

Direction of migrating wave

Phase A
Direction towards aggregation center: cell polarity established (response to positive spatial gradient of cAMP)

Phase B
Rapid, directed movement towards source: suppression of lateral pseudopods (response to increasing temporal gradient of cAMP)

Phase C
Cessation of translocation; loss of cell polarity and rounding; cessation of pseudopodal extension (response to peak concentration of cAMP)

Phase D
Maintenance of depolarized state; random pseudopod extension; no net movement in any direction (response to decreasing temporal gradient of cAMP)

Front of Wave
Incipient lateral pseudopod formation

Basic Motile Behavior
(assessed in buffer, in the absence of a cAMP signal)

Back of Wave
Decreasing formation of negative spatial gradient of cAMP
The Dicteostelium Motility Cycle

A human neutrophil chasing a bacterium (S. aureus)

A time-lapse movie made by Dr. David Rogers at Vanderbilt in the 1950’s