Chemotaxis of Eucaryotic Cells

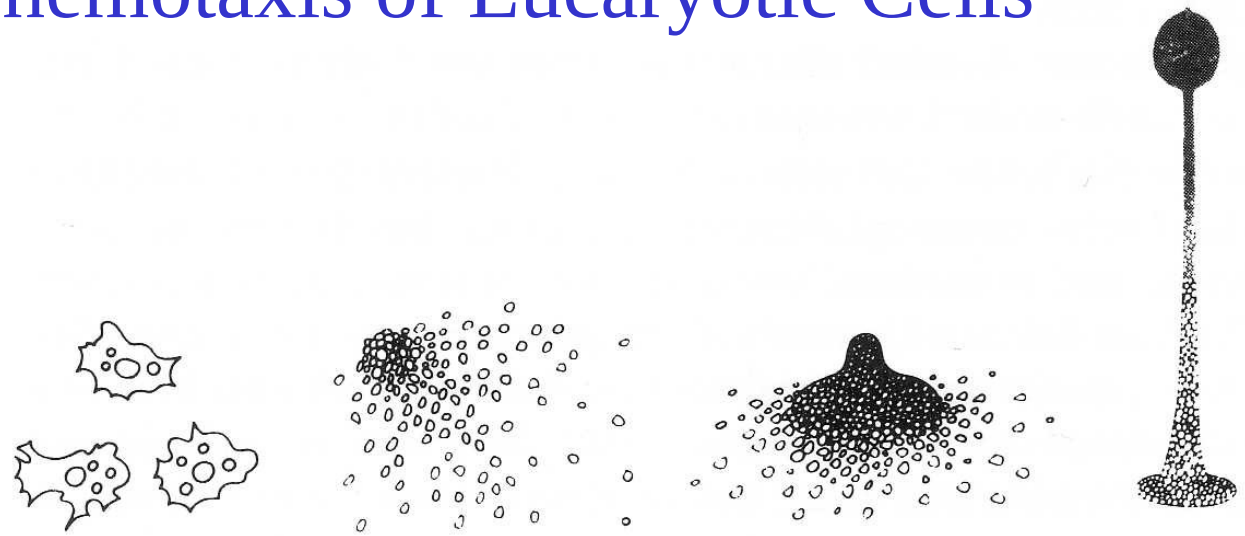
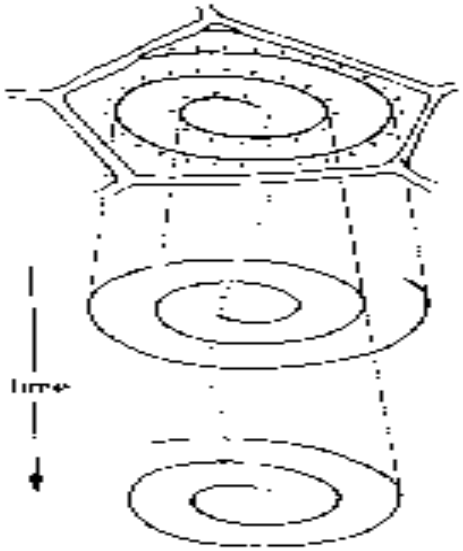


Abb. 9 Entwicklungsstadien des Schleimpilzes.



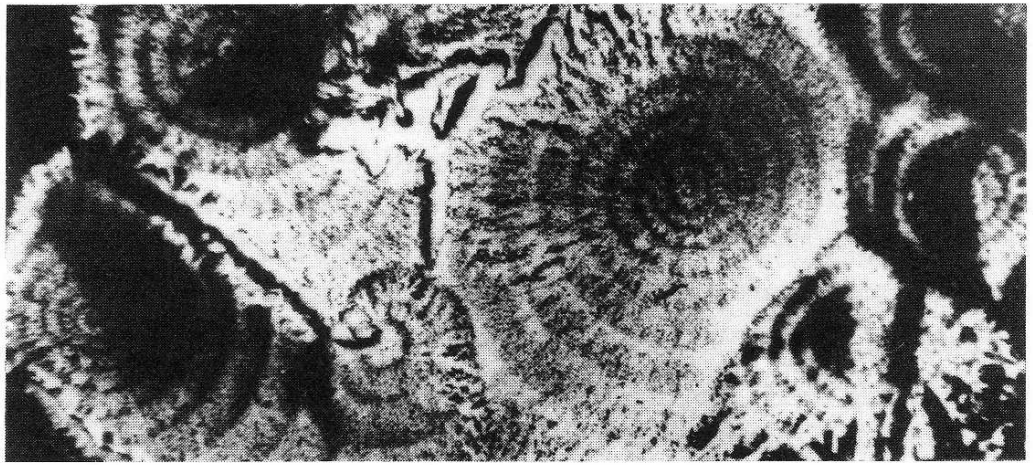


Abb. 10 Spiralwellen von cAMP beim Schleimpilz.

Der slime mould
Dictyostelium Discoideum

ing the idea of 'modular' biology. Together with cell biologist Andrew Murray, director of the Bauer centre, geneticist Leland Hartwell of the Fred Hutchinson Cancer Research Center in Seattle, and physicist Stanislas Leibler of Rockefeller University

bind to cAMP first send a repressive signal to all other receptors in the cell that prevents them from binding for about 30 seconds, Levine's model proposes[3].

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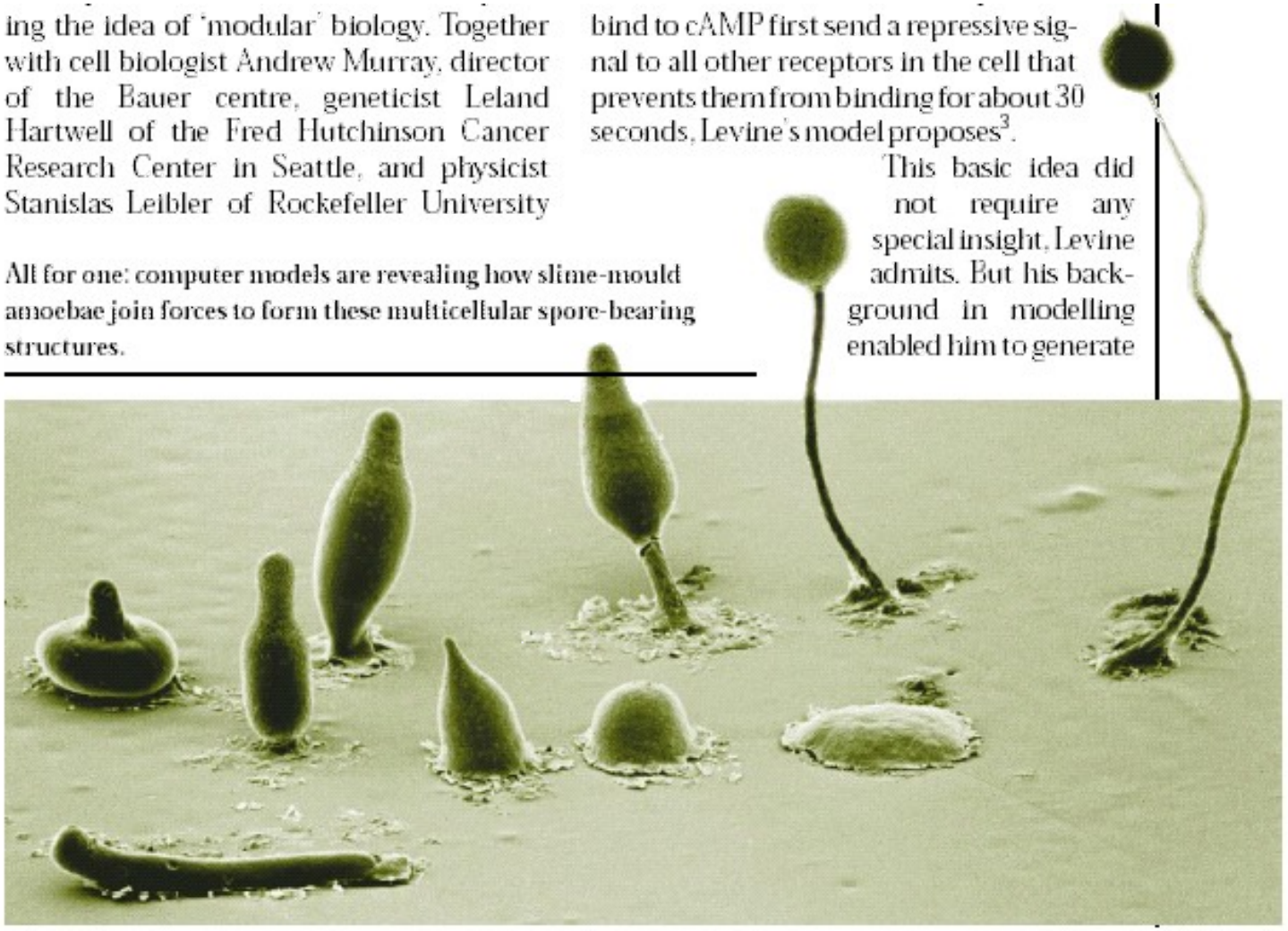
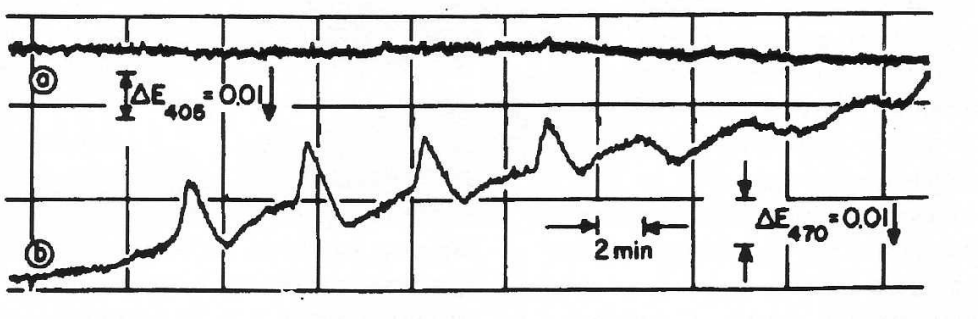
All for one: computer models are revealing how slime-mould amoebae join forces to form these multicellular spore-bearing structures.

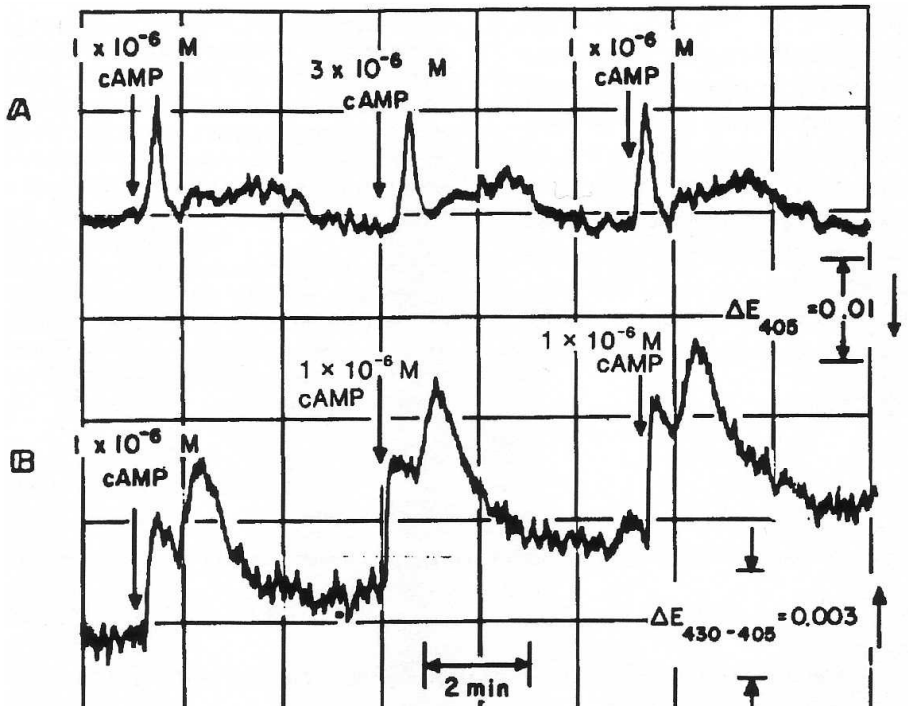


Spore Formation

Observation: Spontaneous c-AMP Oscillations

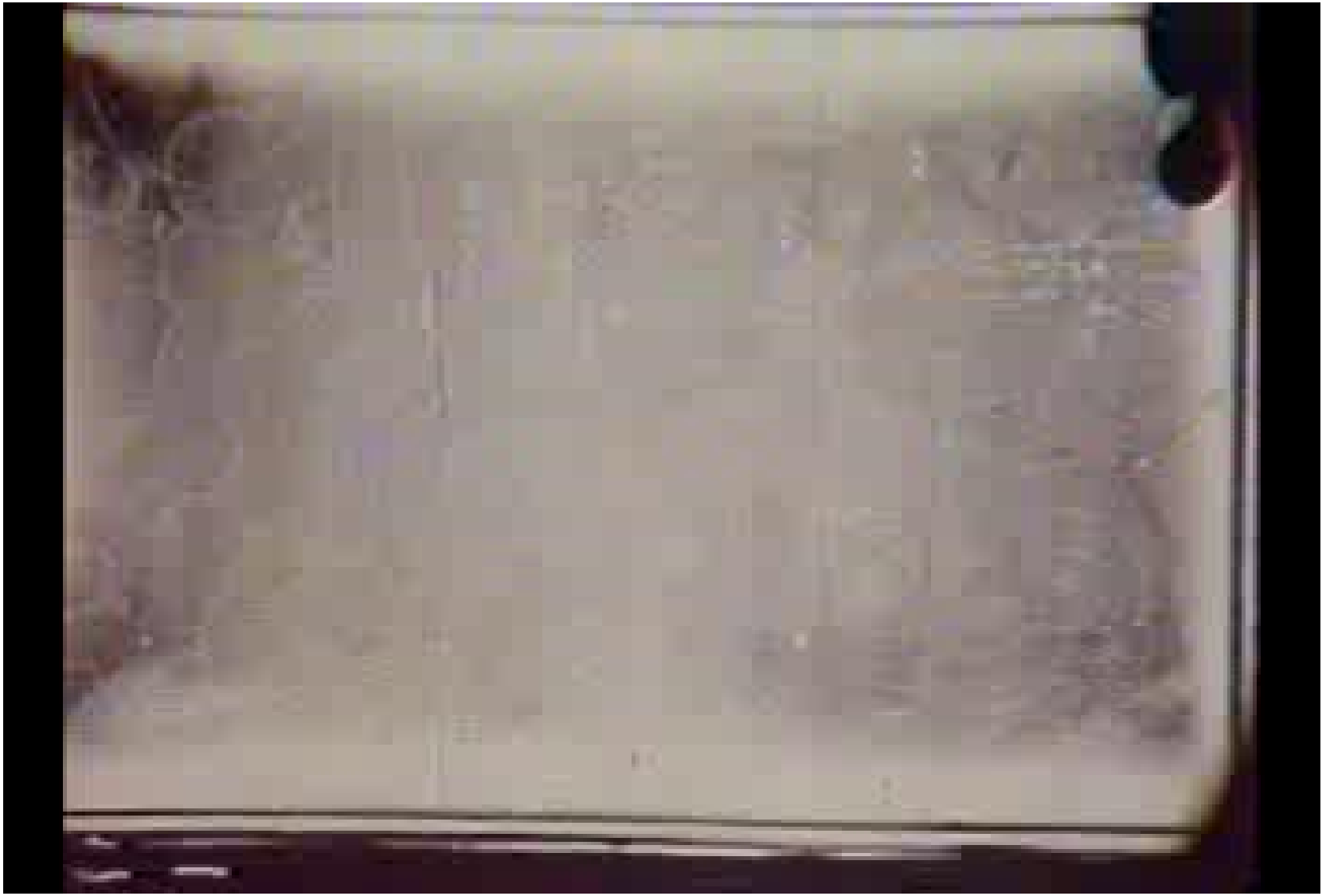
The optical density of homogeneised cell suspensions of Dictyostelium shows a collective change of the cell population which is linked to an excretion of c-AMP [G. Gerisch und B.Hess, 1973]





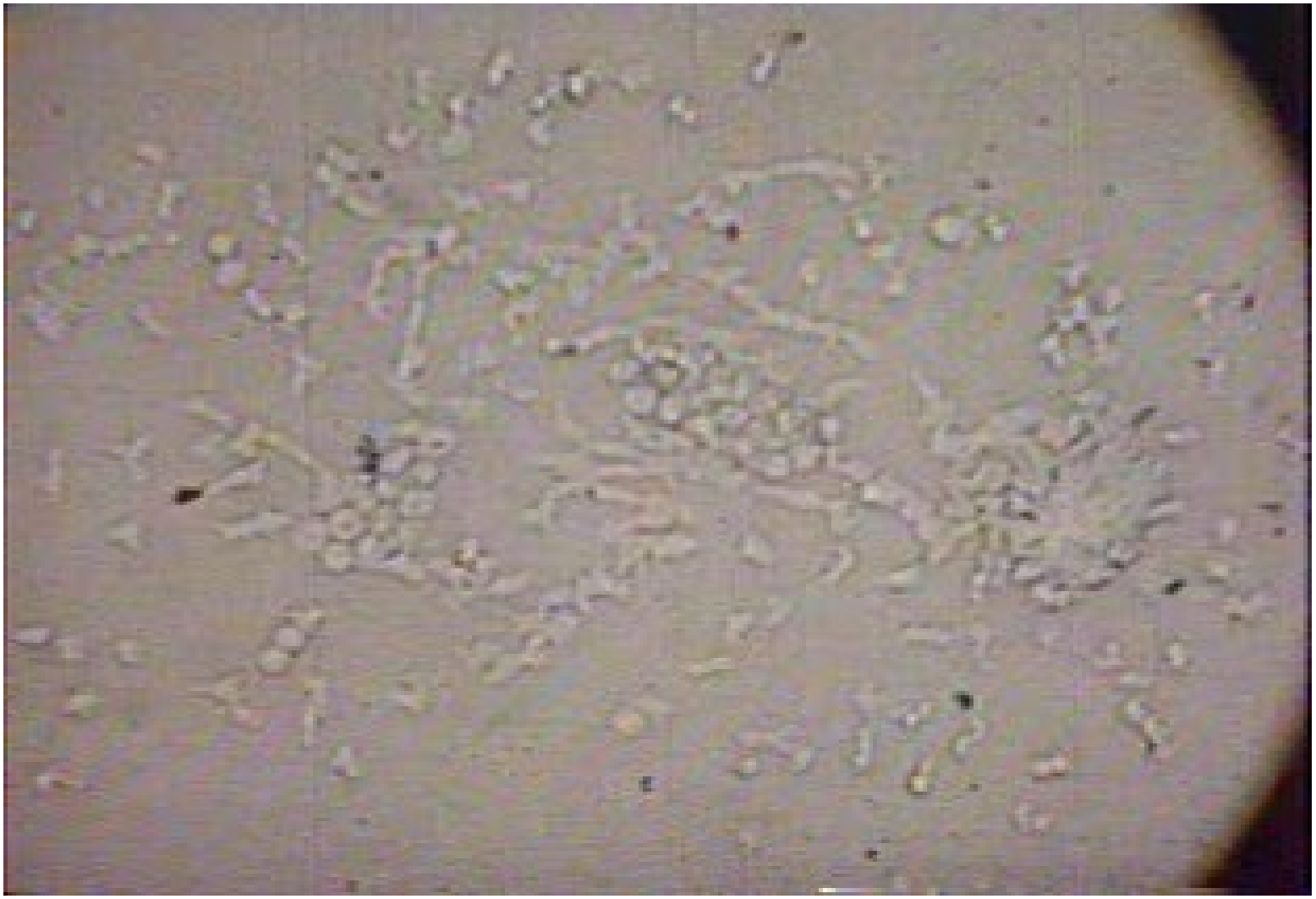
Cell suspensions which are stirred continuously (t>6h) act as autonomous oscillators and send out c-AMP signals with a period of eight minutes.

A cell response on a c-AMP pulse with a delay of one to two minutes with a 100-fold amplified c-AMP pulse.



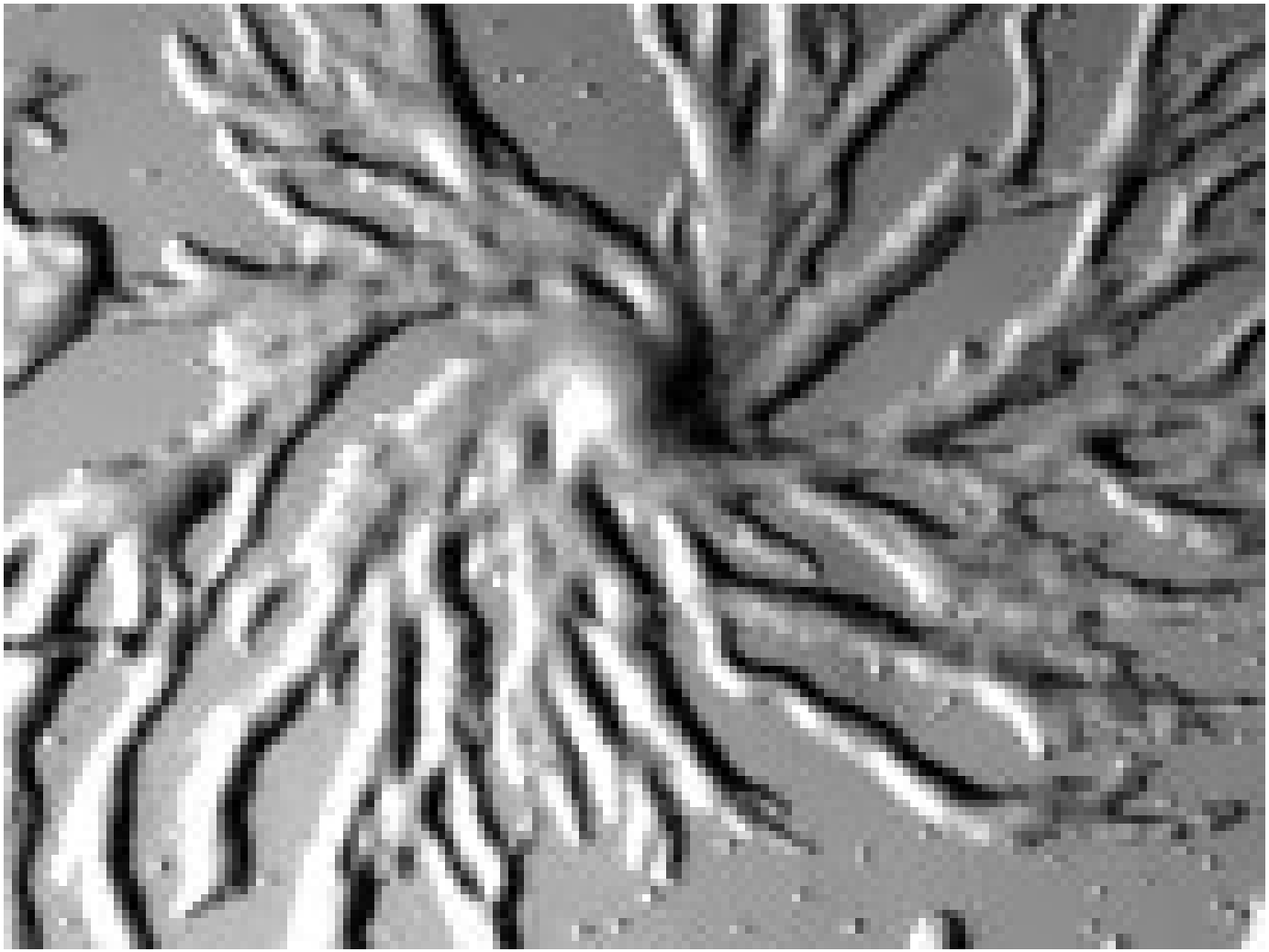
Time-lapse video of monolayer of aggregating cells filmed through a dark-field macroscope. White bands represent chemotactically oriented cells. Period of wave initiation is 6 minutes. From P. Devreotes, Johns Hopkins Medical Institutions

Cell Aggregation



Time-lapse video of small group of aggregating cells.
Interval between movement steps is 6 minute.
From P. Devreotes, Johns Hopkins Medical Institutions.

Cell Aggregation



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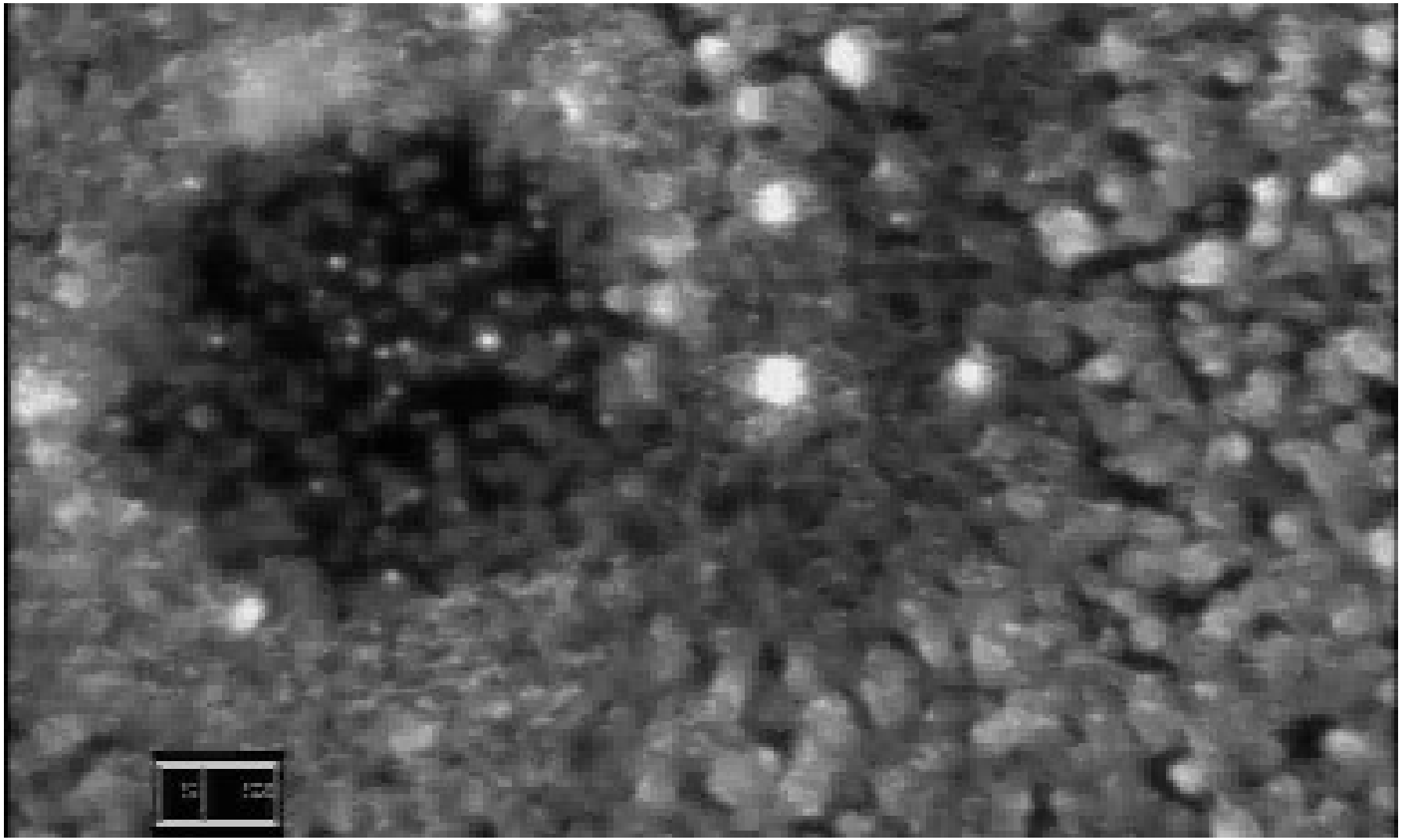
c-AMP Waves in Aggregating Cells



Core of a spiral wave in aggregating *D. discoideum* cells.
Time between images is 10 seconds.
From F. Siegert and C. J. Weijer, J. Cell Sci. **93,** 325-335 (1989).

Chemotaxis in Dictyostelium Discoideum

The **dictyostelids** are a group of cellular slime moulds. When food (normally bacteria) is readily available they take the form of individual amoebae, which feed and divide normally. However, when the food **supply is exhausted**, they aggregate to form a multicellular assembly, called a pseudoplasmodium or slug (not to be confused with slug the animal). **The slug has a definite anterior and posterior, responds to light and temperature gradients, and has the ability to migrate.** Under the correct circumstances the slug matures forming a fruiting body with a stalk supporting one or more balls of spores. These spores are inactive cells protected by resistant cell walls, and become new amoebae once food is available.

In Acytostelium, the fruiting body is supported by a stalk **composed of cellulose**, but in other dictyostelids the **stalk is composed of cells**, sometimes taking up the majority of the original amoebae. With a few exceptions, these cells die during stalk formation, and there is a definite correspondence between parts of the slug and parts of the fruiting body.

Aggregation of amoebae generally takes place in **converging streams**. The amoebae move using filose **pseudopods**, and are attracted to chemicals produced by other amoebae. **In Dictyostelium, aggregation is signalled by cAMP**, but others use different chemicals.

Dictyostelium has been used as a **model organism** in molecular biology and genetics, and is studied as an example of **cell communication**, **differentiation, and programmed cell death.**

From: en.Wikipedia.org

Der slime mould
Dictyostelium Discoideum

ing the idea of 'modular' biology. Together with cell biologist Andrew Murray, director of the Bauer centre, geneticist Leland Hartwell of the Fred Hutchinson Cancer Research Center in Seattle, and physicist Stanislas Leibler of Rockefeller University

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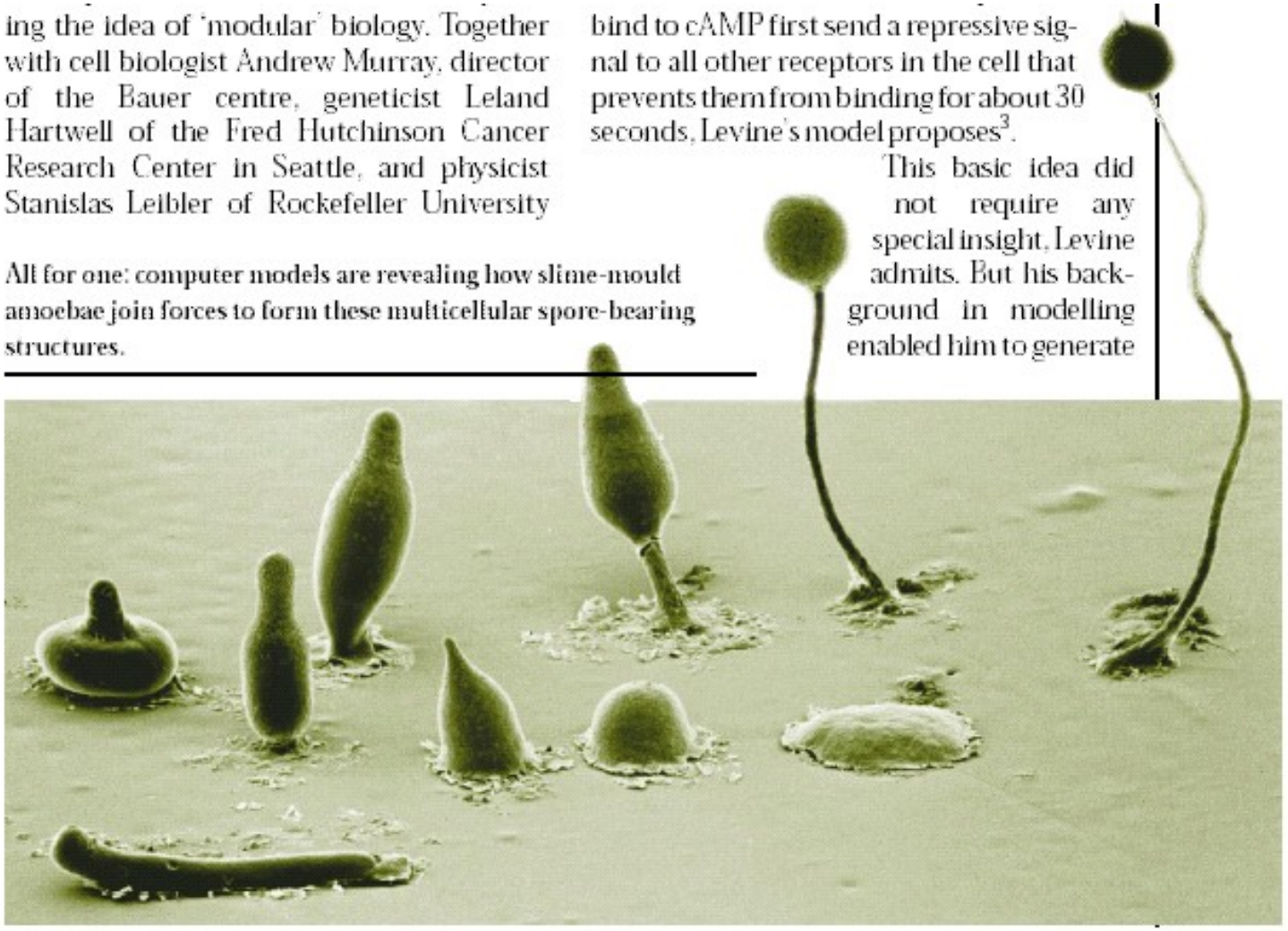
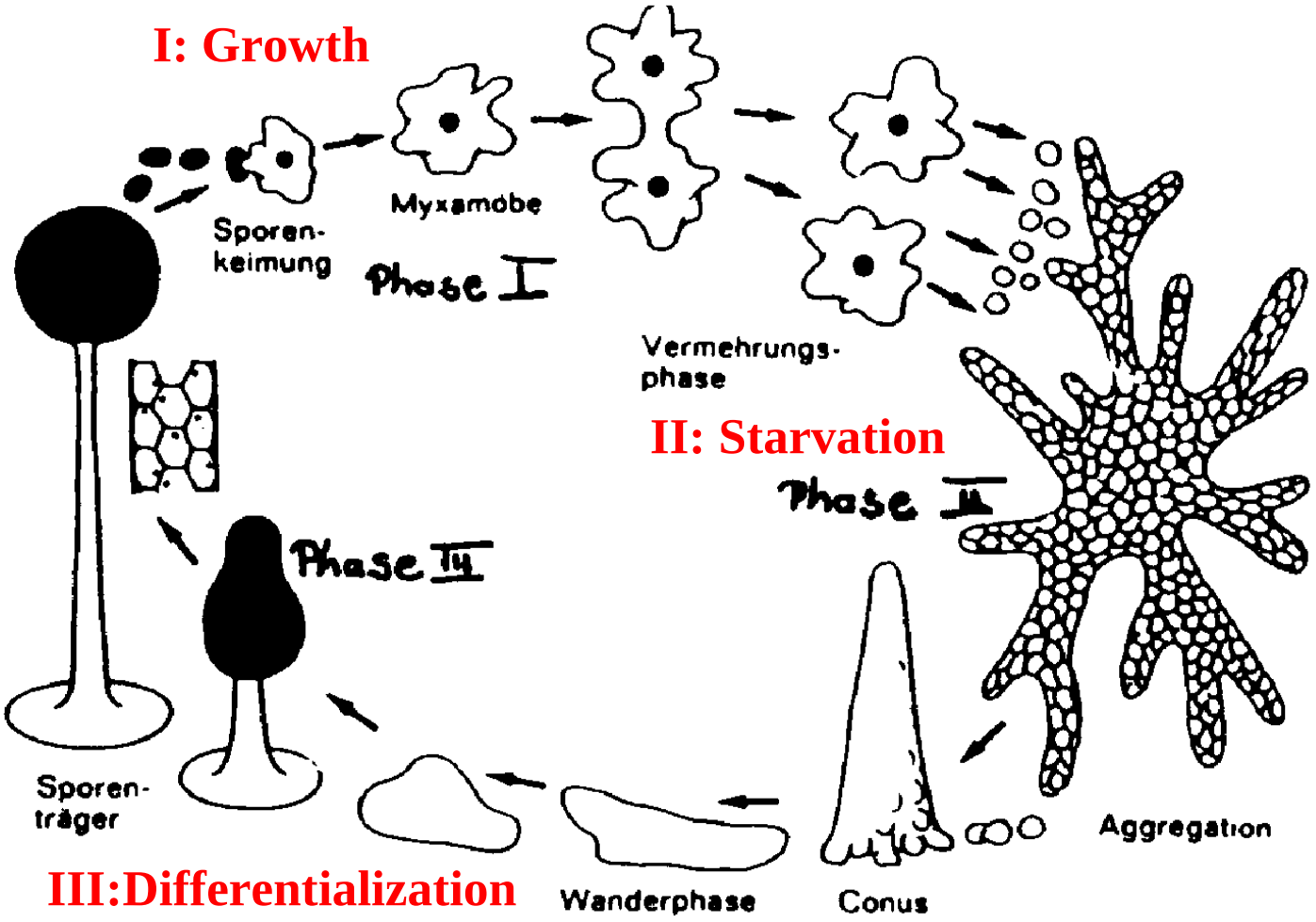


Developmental Cycle of D. Discoideum



The Life-Cycle of Didi

Phase I:

The amobae live (on agar plates) under paradise-like conditions (good relation between nutricients and population density) as single cellular organisms and grow by cell division. This phase consists of exponential growth and a stationary state of constant cell number.

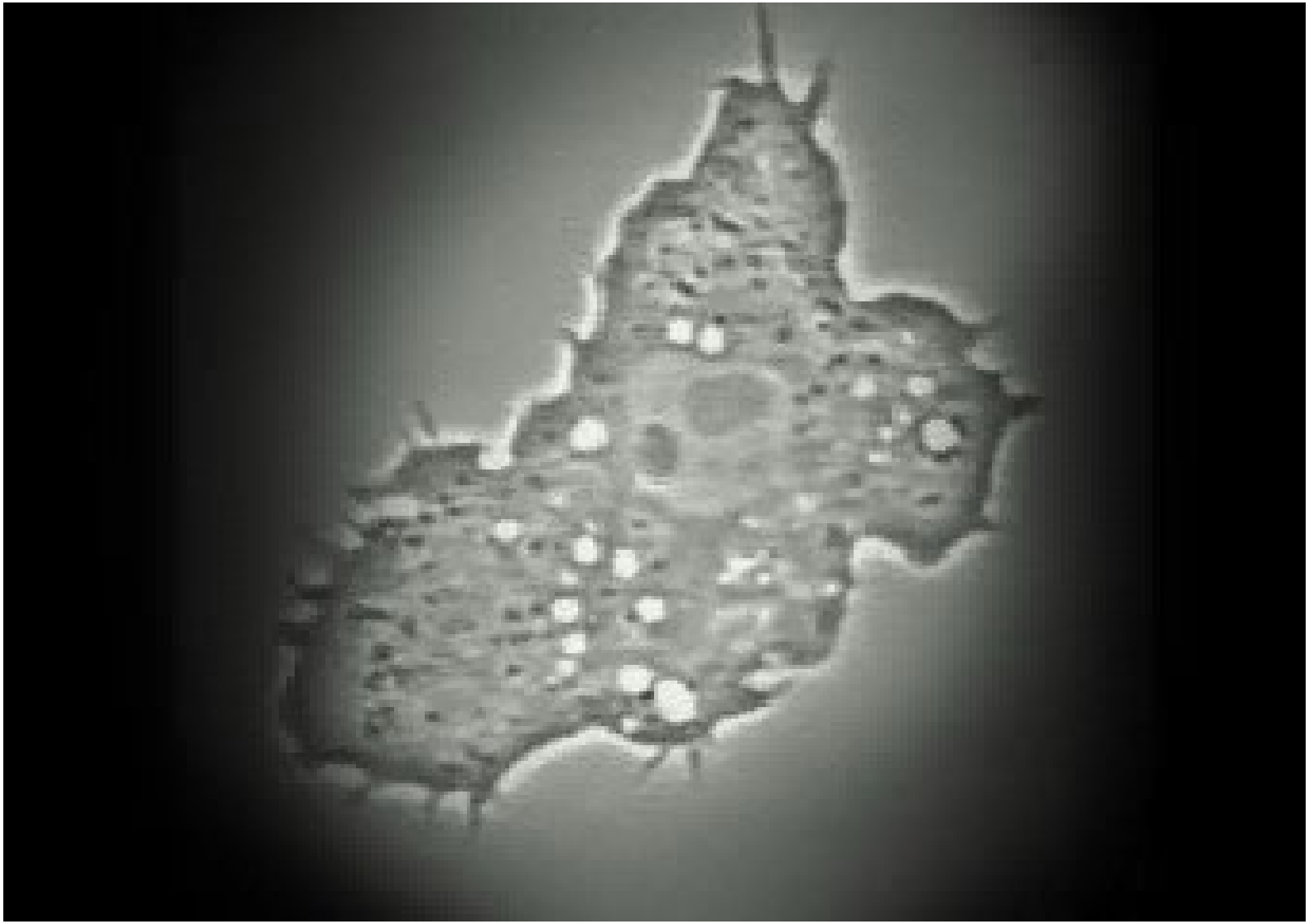
Phase II:

The ***scarcity of nutricients*** leads after eight hours to a ***collective motion*** of the cells to statistically distributed centers, guided by collective chemotaxis. We will see that this is the result of cellular signalling using the messenger c-AMP.

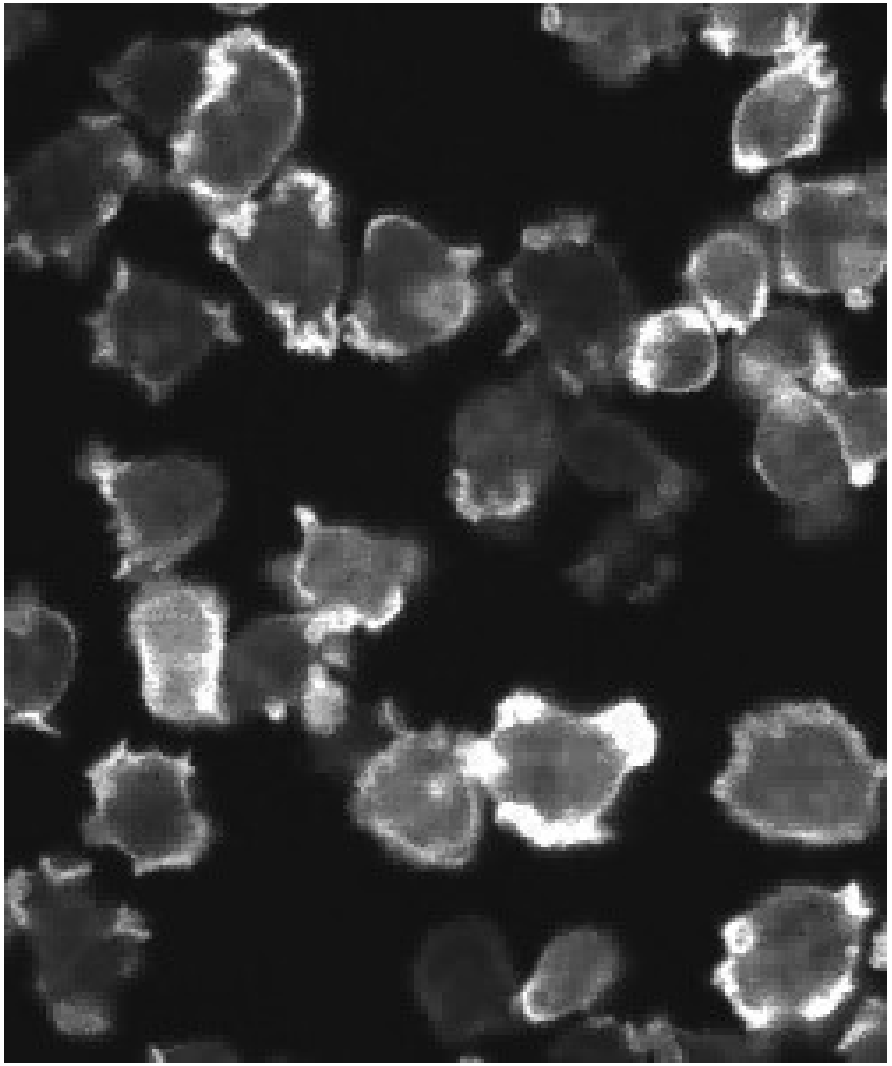
Phase III:

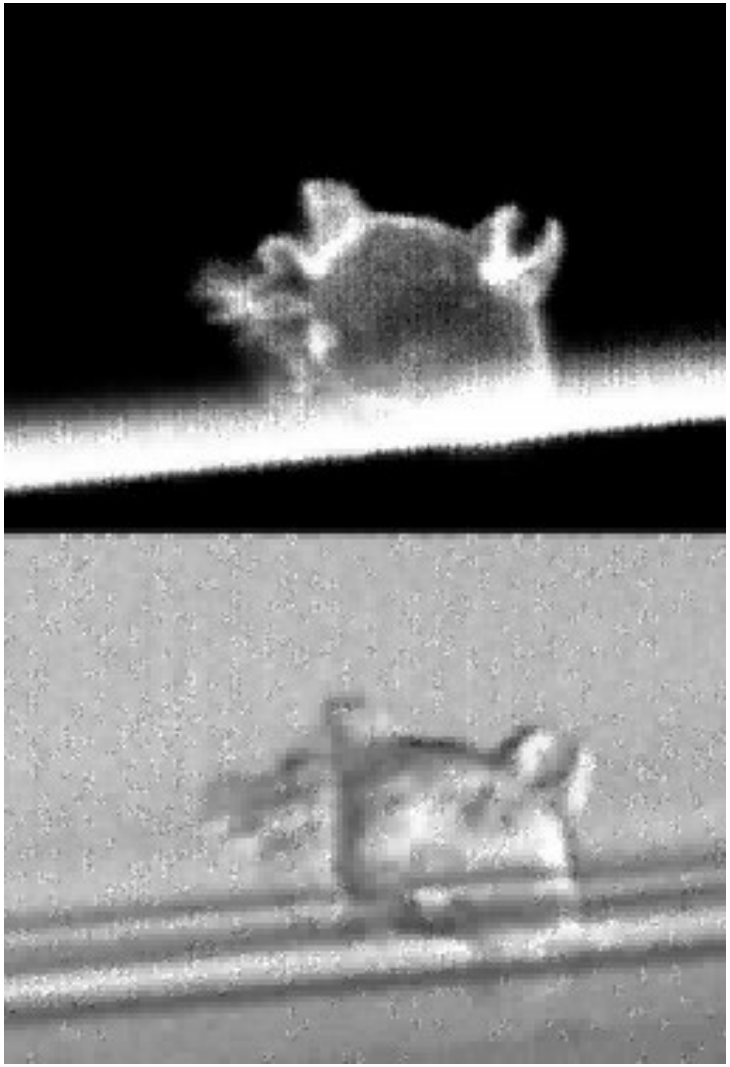
Differentialisation of the cells and growth of the slime mould. Three cell types develop: ***spores*** which are capable of subsequent cell division, stabilizing cells of the stalk and cells forming the skin of the spore head.

Chemotaxis in Dictyostelium Discoideum



Dicties move "stochastic" without c-AMP stimulation

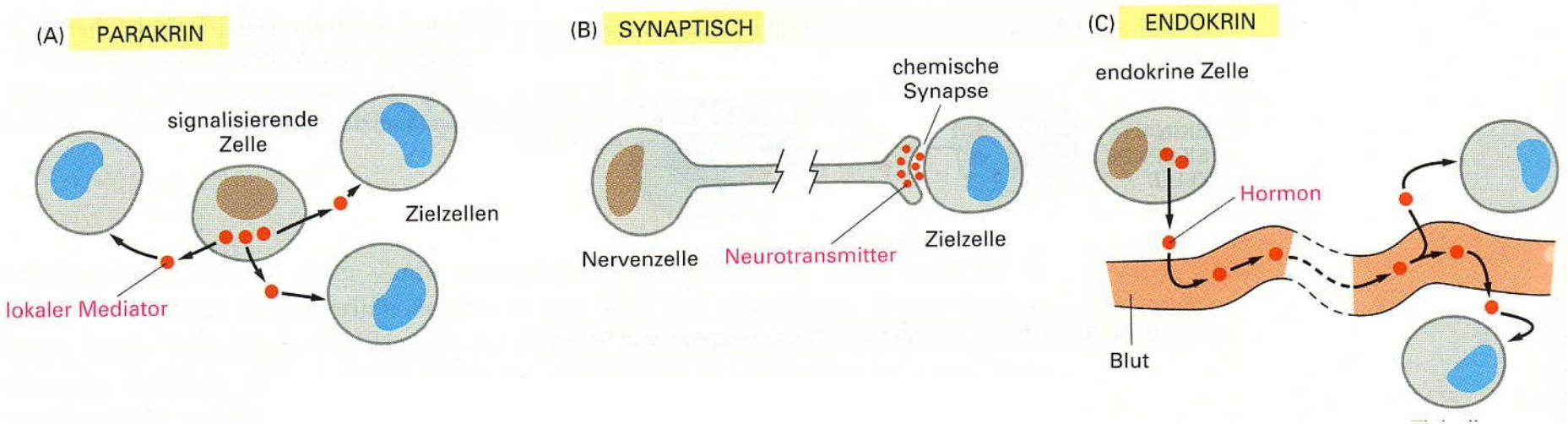


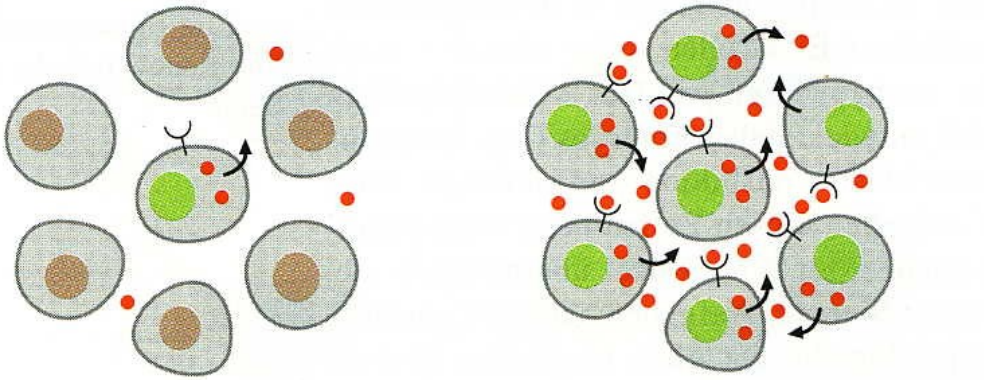


Eukaryotic Chemotaxis

- Directional sensing
  - Local excitation - global inhibition
- Polarization
  - Lipid-based signalling
- Locomotion
  - Actin polymerization

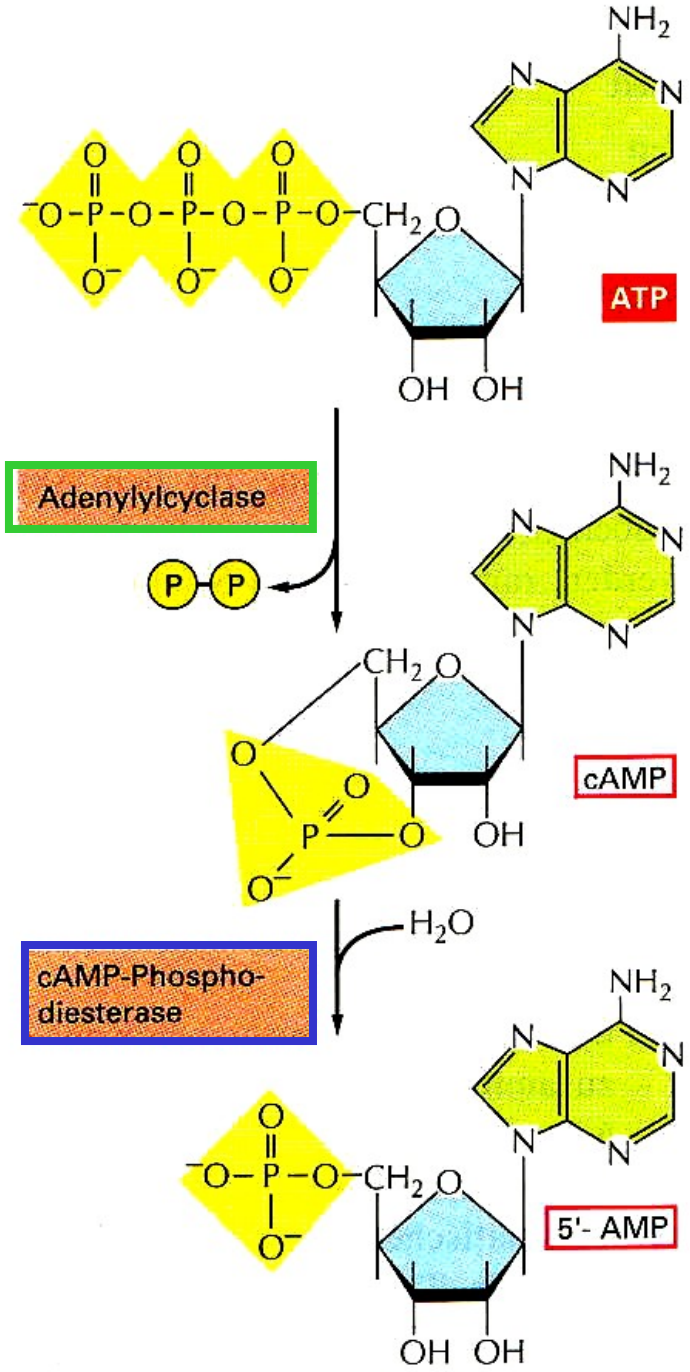
Modes of Signalling





EINE EINZELNE SIGNALISIERENDE ZELLE ERHÄLT WENIG AUTOKRINES SIGNAL

IN EINEM VERBAND IDENTISCHER SIGNALISIERENDER ZELLEN ERHÄLT JEDE EIN STARKES AUTOKRINES SIGNAL

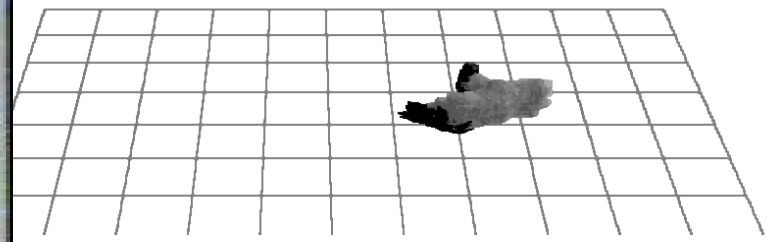


The messenger c-AMP

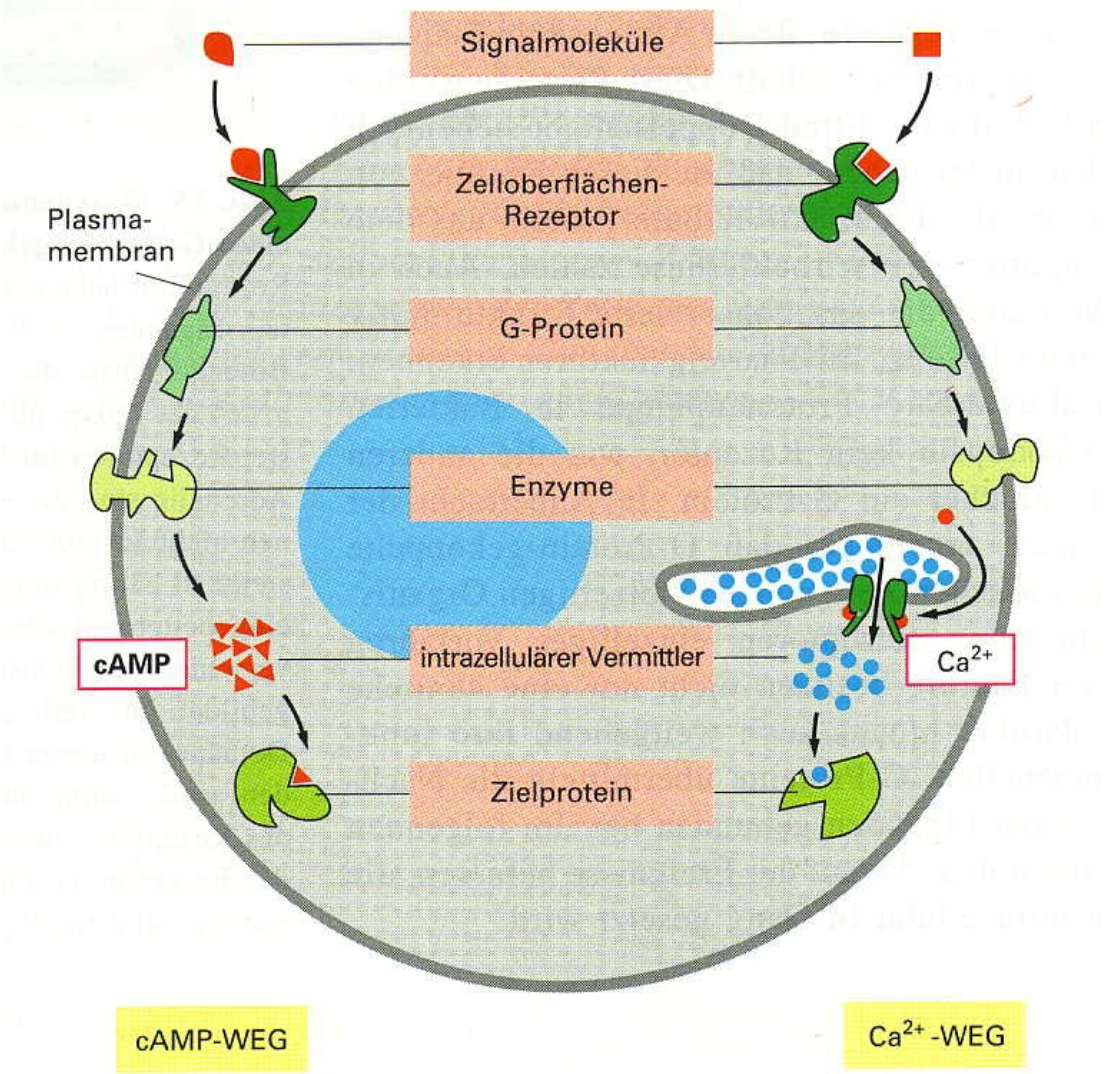
Cyclic Adenosine MonoPhosphat

1) **c-AMP** : the messenger
2) **ATP** : has the role of an energy source, for nuclic acid polymerization and as substrate for the c-AMP production
3) **Adenylylcyclase**: Enzyme which transforms ATP to c-AMP
4) **c-AMP–Phosphodiesterase**: enzyme to degrade c-AMP

Directed locomotion induced by c-AMP gradients

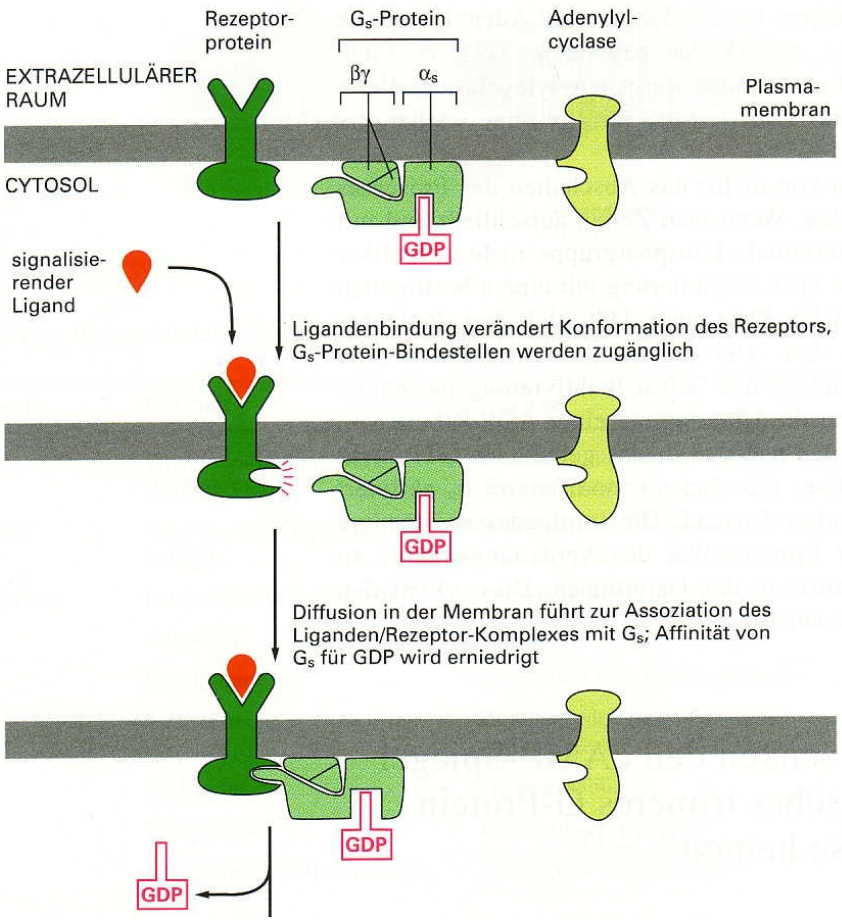


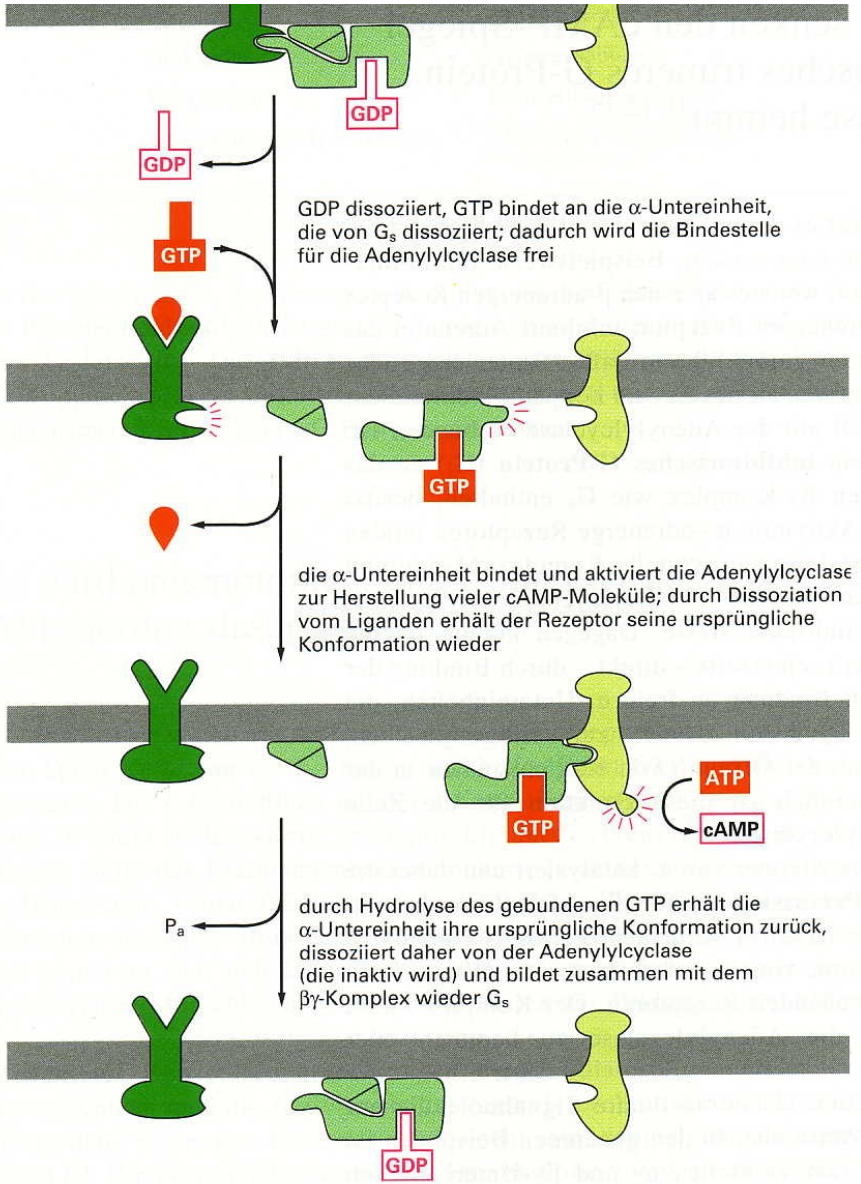
G-Protein-coupled receptors transmit the signal intracellularly by trimeric G-Proteins, which then change the concentration of intracellular „second messengers“.



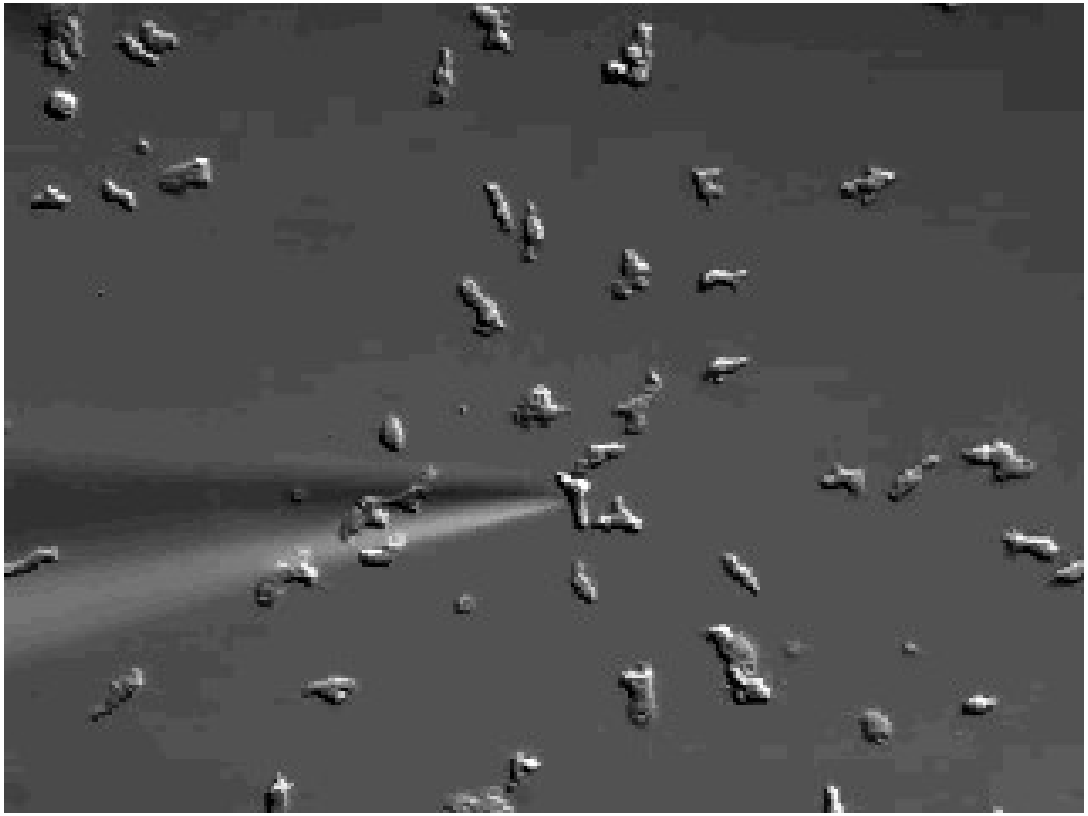
Activation of Receptor couples to Adenylylcyclase

(G-Protein-coupled receptors)





Cell-Aggregation by c-AMP Stimulus

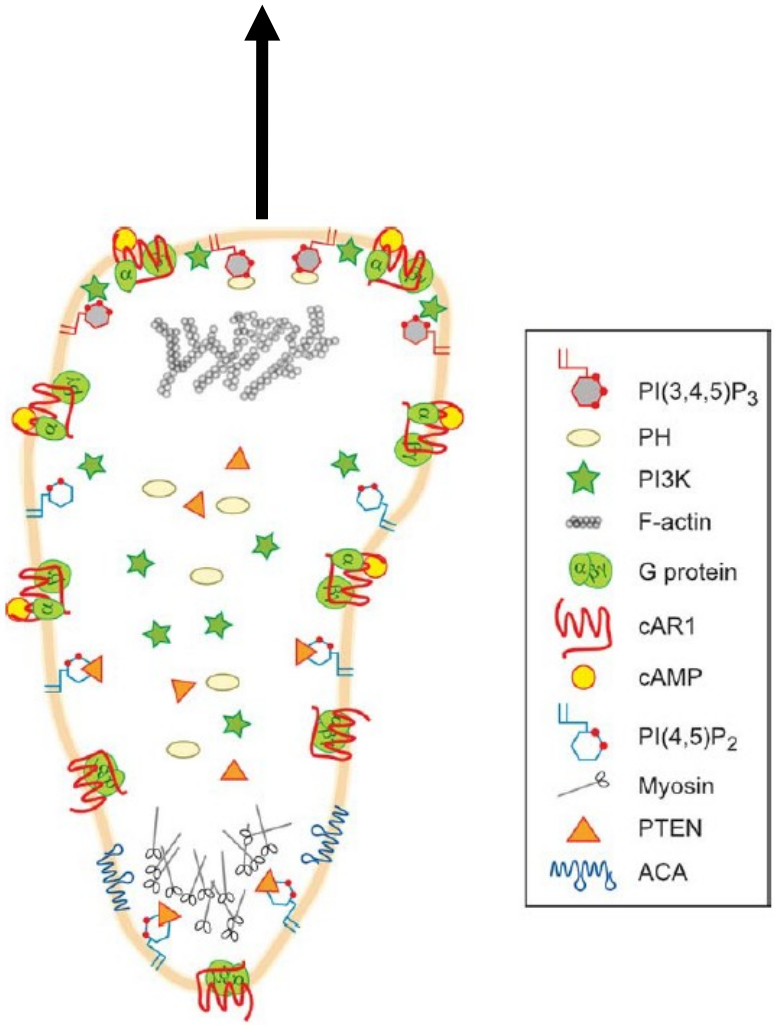


Aggregation of *D. discoideum* ameobae towards cAMP, lower magnification.
From R. Firtel, University of California, San Diego

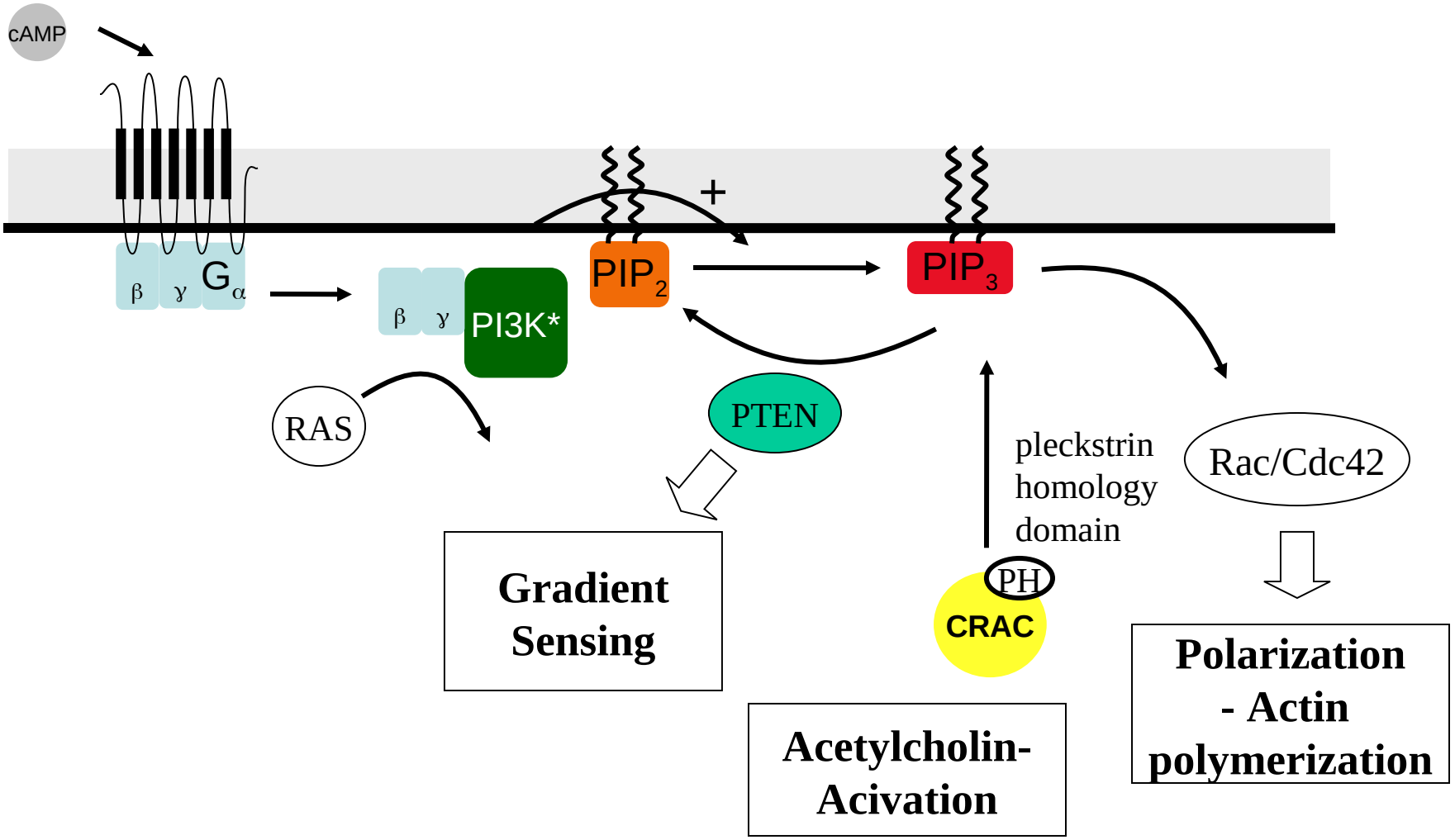
Dictyostlium Discoideum as Model System for Chemotaxis

Chemotaxis to cAMP is part of a program of differentiation where free-living amoebae aggregate to form a multicellular organism. During aggregation the cells orient and migrate directionally toward selforganizing gradients of extracellular cAMP. Studies of these events in *D. discoideum* have led to the identification and localization of key molecules in chemotactic signaling pathways and to the basic mechanisms involved in chemotaxis.

For instance, the discovery of a family of receptors, designated cAR1–cAR4, for the chemoattractant cAMP provided the first evidence that chemotactic **signaling occurs through seven helix receptors linked to heterotrimeric G proteins**. The cARs couple to a specific G protein consisting of 2, one of eleven subunits, and a unique complex. A similar system operates in **mammalian leukocytes**, where twenty chemokine receptors couple principally to the inhibitory G protein, Gi. Additional elements of the pathway are also **conserved: exposure of amoebae or leukocytes to chemoattractants results in increases in multiple second messengers, including PIs (phosphoinositides), cAMP, cGMP, IP3, and Ca2, and subsequent rearrangements in the cytoskeleton.**

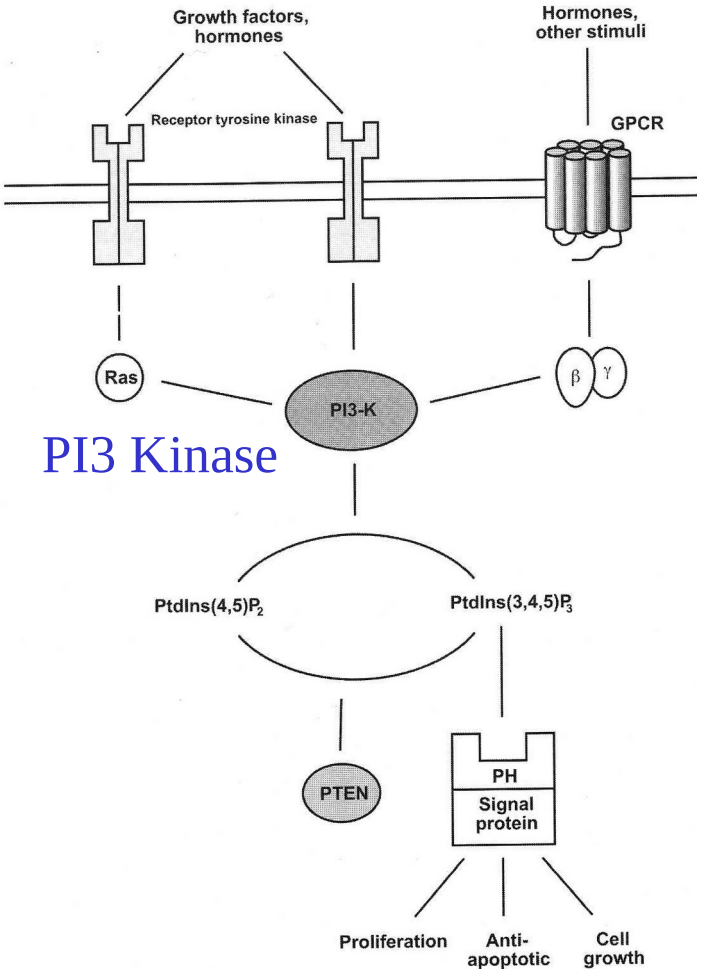


Signal pathway of di*ctyostelium discoideum*

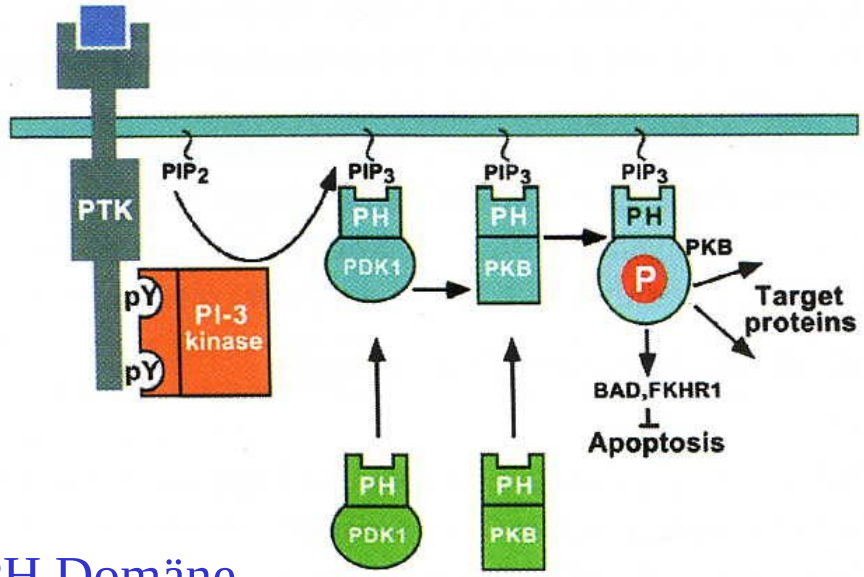


Inositol Phospholipid Signaltransduction

PIP_3 as the product of PI3 Kinase is a messenger molecule similar to cAMP. In contrast to cAMP, PIP_3 is located in the cell membrane and activates subsequent signalling molecules at the membrane surface by binding to the *Pleckstrin-Homology-Domäne*.



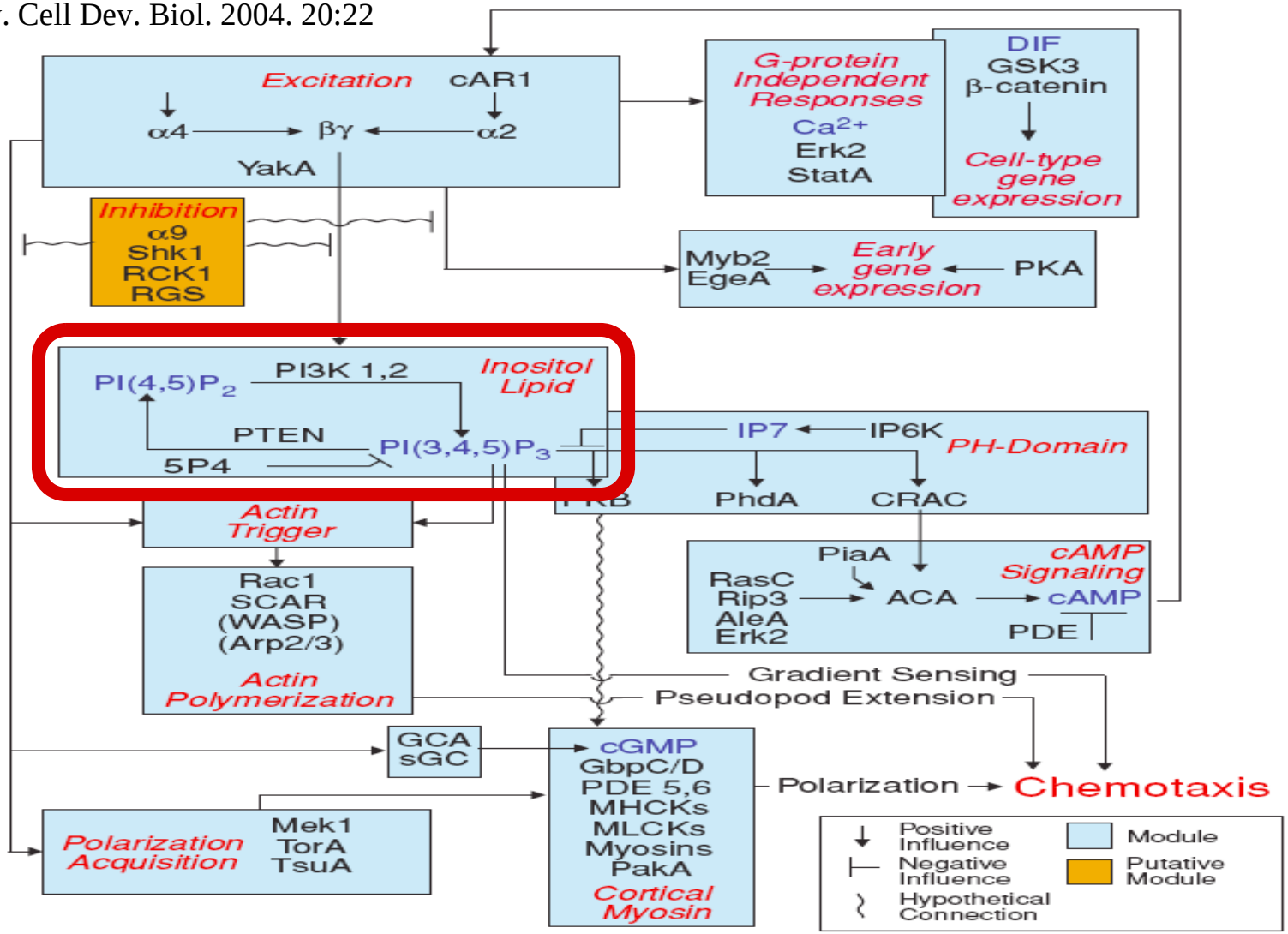
PI3 Kinase



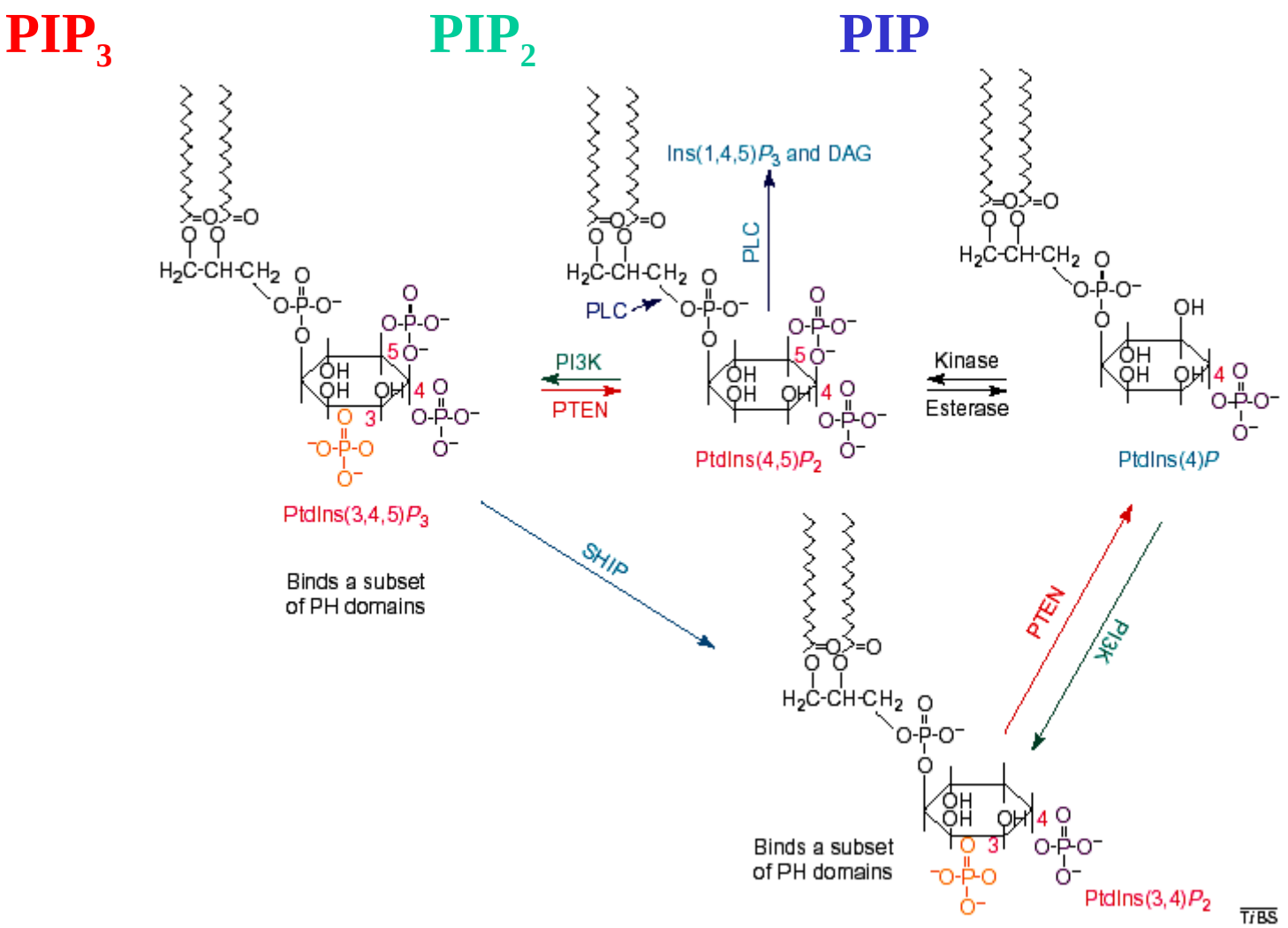
PH Domäne

Modular view of the chemoattractant-induced signaling pathway in *Dictyostelium*

Peter N. Devreotes et al.
Annu. Rev. Cell Dev. Biol. 2004. 20:22



The Inositol Phospholipid Signal Pathway



Imaging of PI3K and PTEN

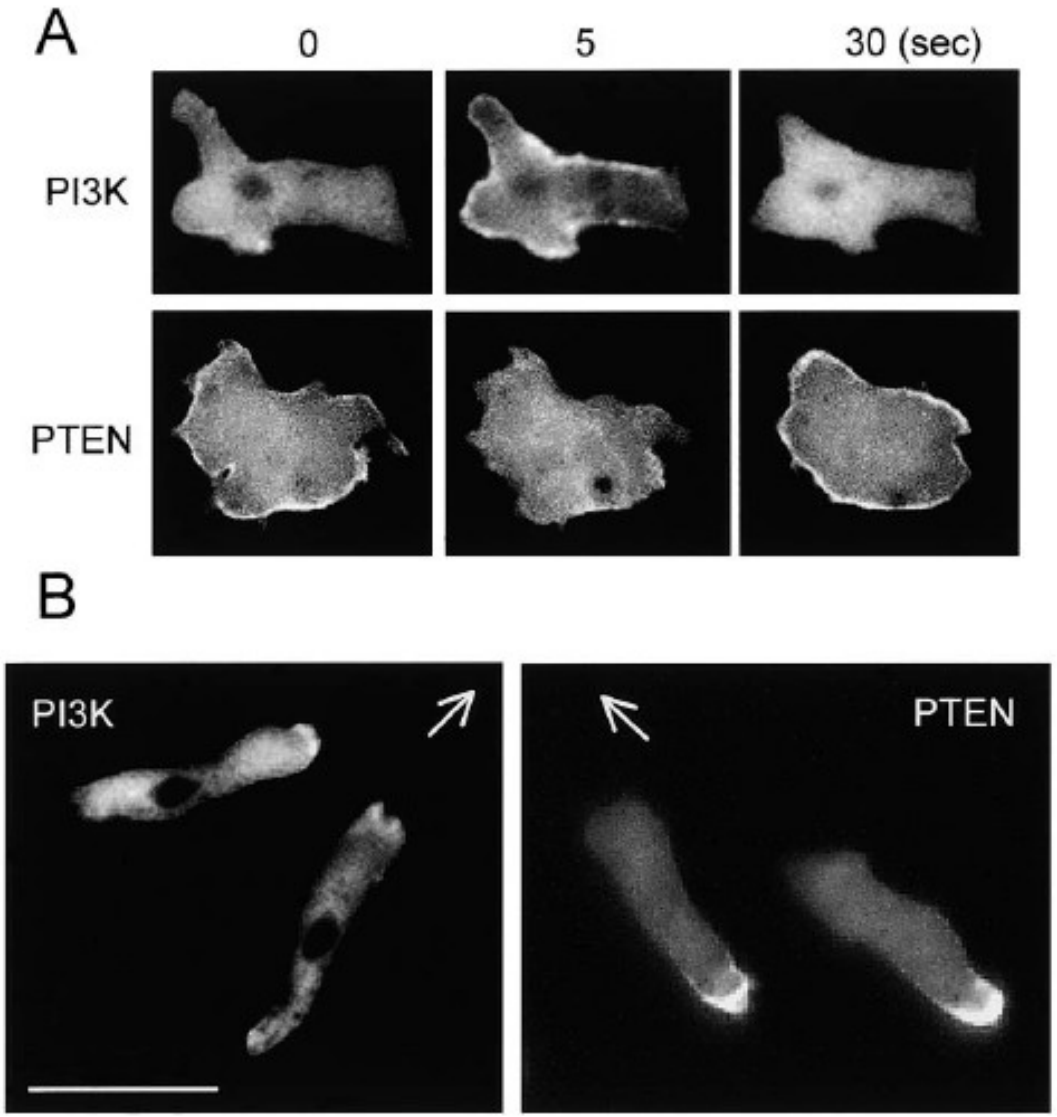


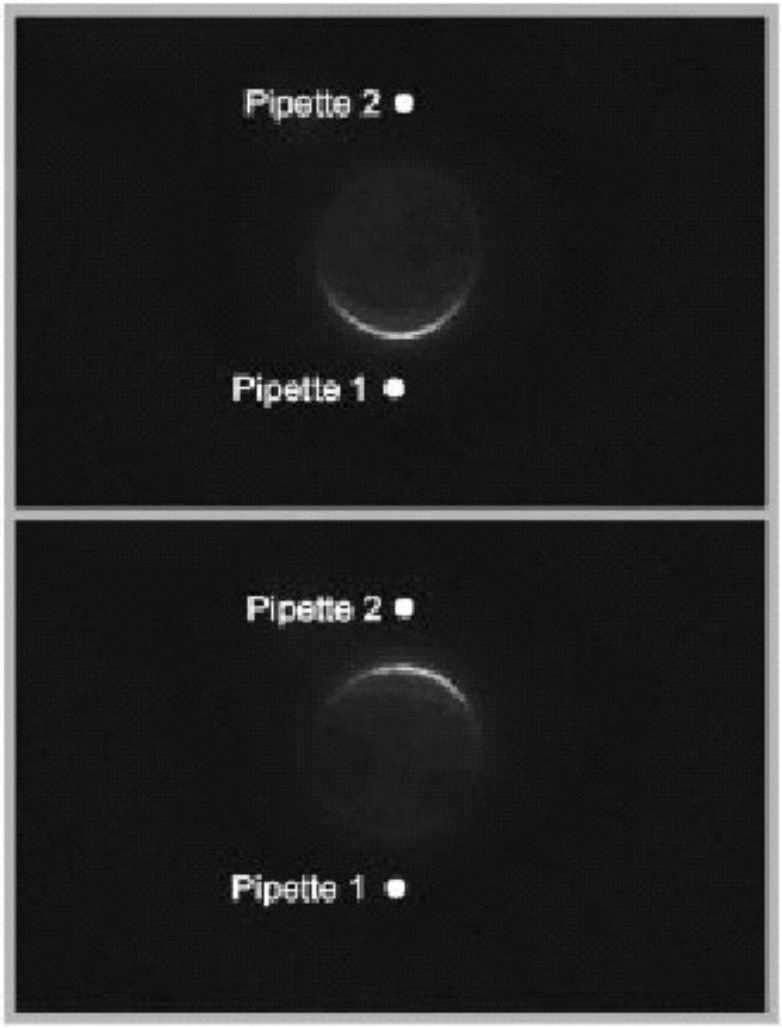
Figure 5. Changes in the Distribution of PI3K and PTEN in Response to Temporal Increases and Spatial Gradients of Chemoattractant

(A) Images show cells expressing PI3K-GFP or PTEN-GFP stimulated with a spatially uniform increase in chemoattractant. In resting cells, the majority of PI3K-GFP is located in cytosol, while PTEN-GFP is associated with plasma membrane. Upon stimulation, PI3K transiently translocates to the plasma membrane, while PTEN-GFP dissociates from the membrane. Within 20–60 s PI3K returns to cytosol, while PTEN reassociates with the membrane.

(B) Images show the distribution of PI3K-GFP or PTEN-GFP in cells moving toward a cAMP-filled micropipette. PI3K-GFP is associated with the membrane at the leading edge of cells, while PTEN-GFP is bound to the membrane at the back. The arrow indicates the direction of the micropipette. The calibration bar indicates 15 μm.

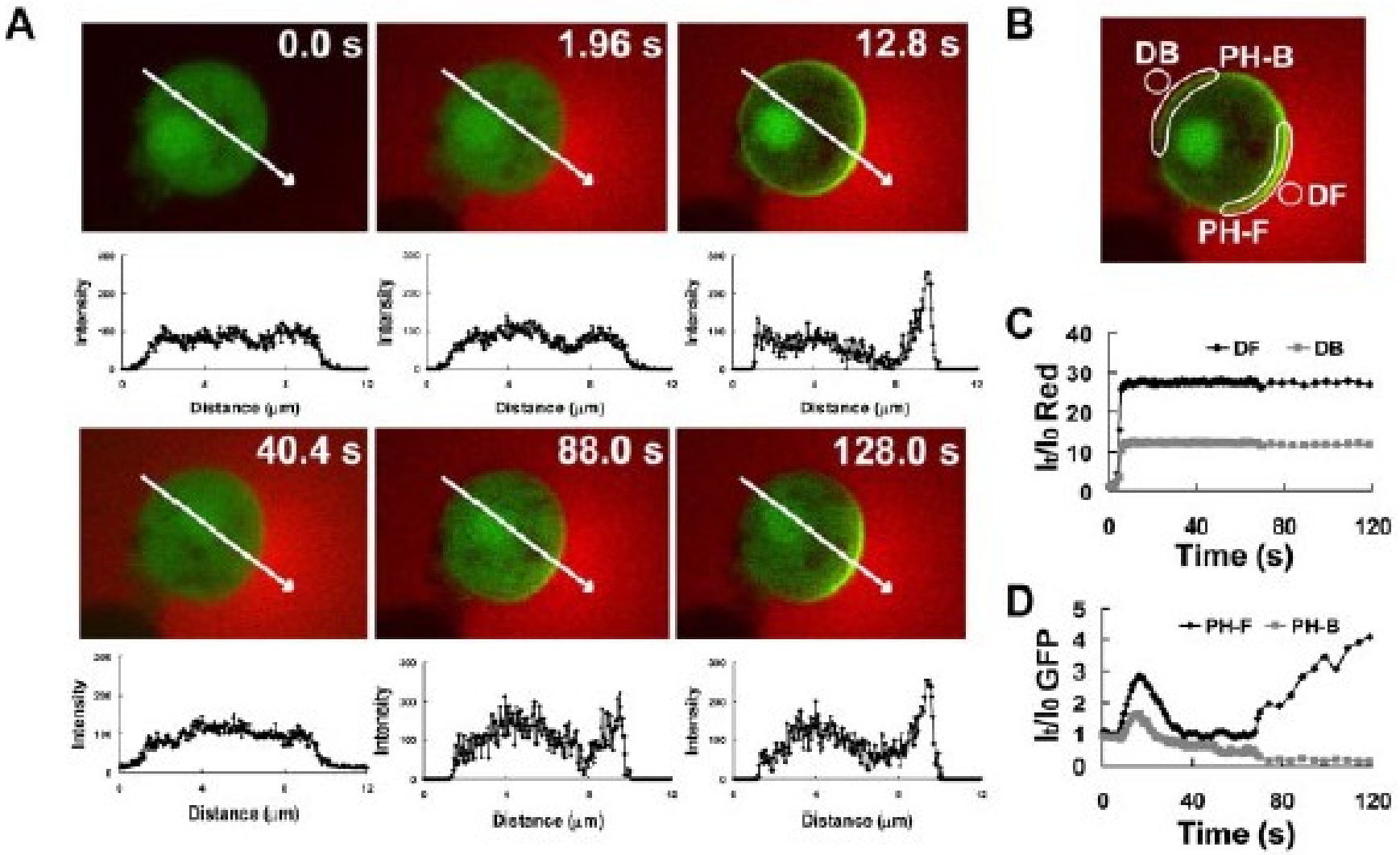
Peter N. Devreotes et al. Developmental Cell, Vol. 3, 469–478, October, 2002,

PIP_3 acts as internal compass



Cells expressing PH-Crac-GFP sense a gradient of cAMP released from a micropipette.

Work by Devreotes et al.



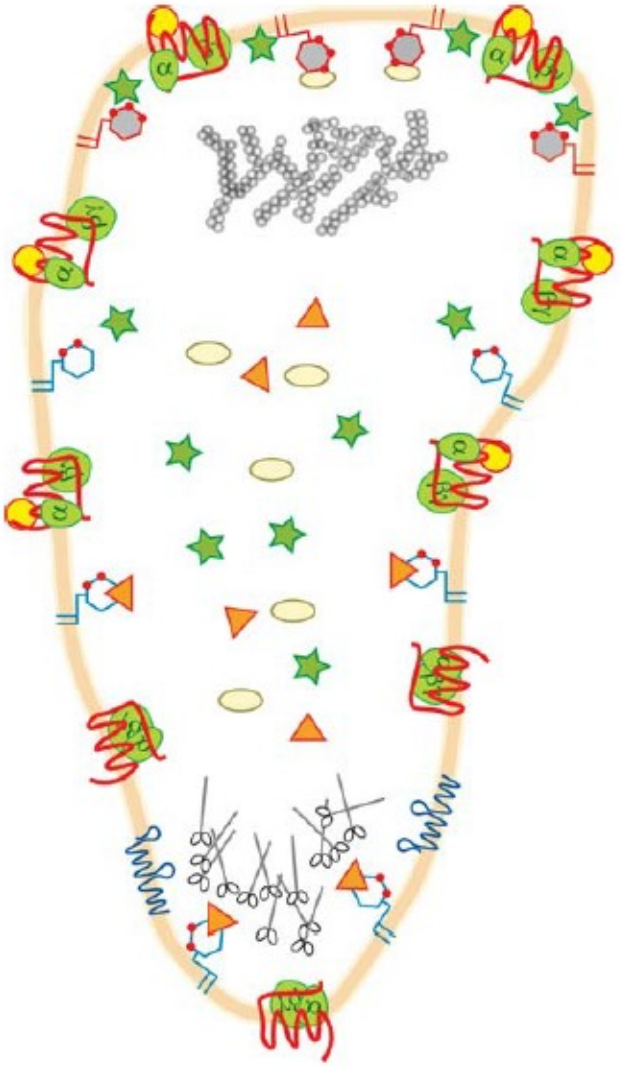
Quantitative Image Analysis
(Molecular Biology of the Cell, 16, 676–688 (2005) Tian Jin et.al.)

The Inositol Phospholipid Signal Pathway: Gradient Sensing

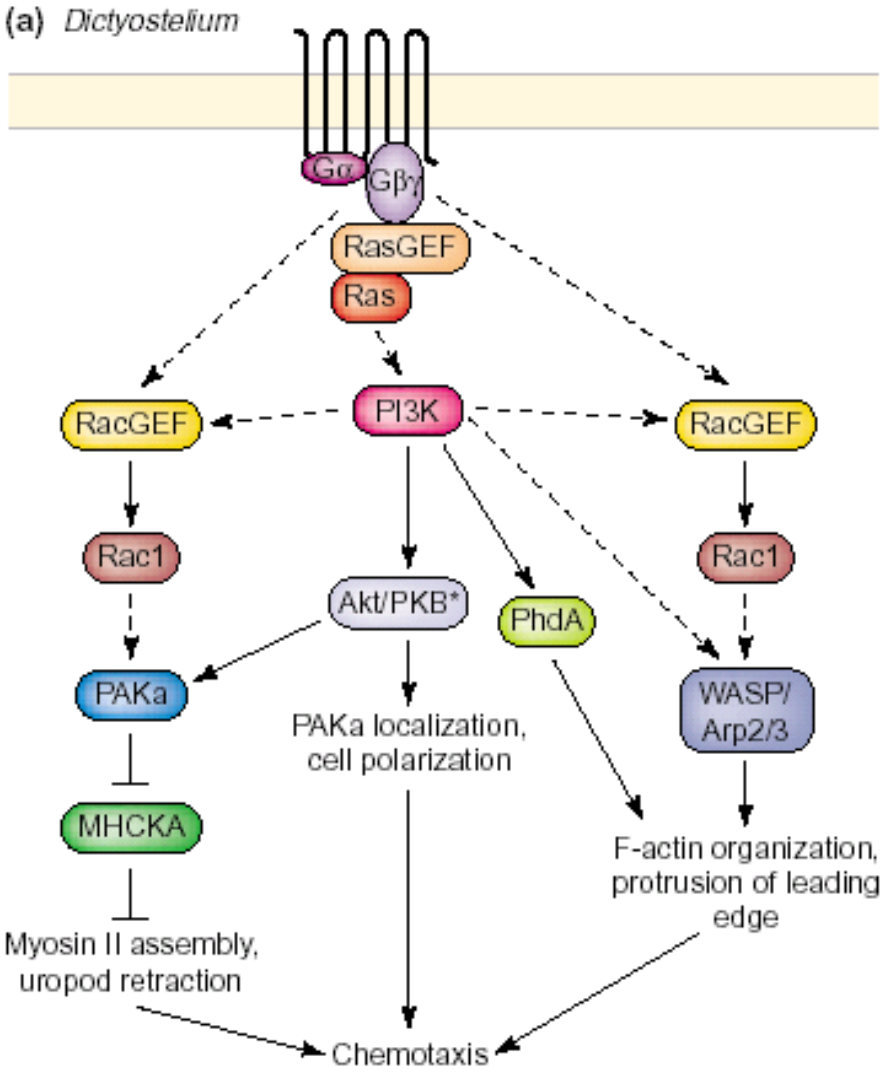
The ability to sense and respond to shallow gradients of extracellular signals **is remarkably similar** in *Dictyostelium discoideum* amoebae and mammalian leukocytes. **Chemoattractant receptors and G proteins** are fairly evenly distributed along the cell surface. Receptor occupancy generates **local excitatory and global inhibitory processes** that balance to control the chemotactic response.

-**Uniform** stimuli transiently recruit PI3Ks to, and release PTEN from, the plasma membrane, while gradients of chemoattractant cause the two enzymes to bind to the membrane at the front and back of the cell, respectively.

- **Counteracting signals** from the upstream elements of the pathway converge to regulate the key enzymes of PI metabolism, localize these lipids, and direct pseudopod formation.

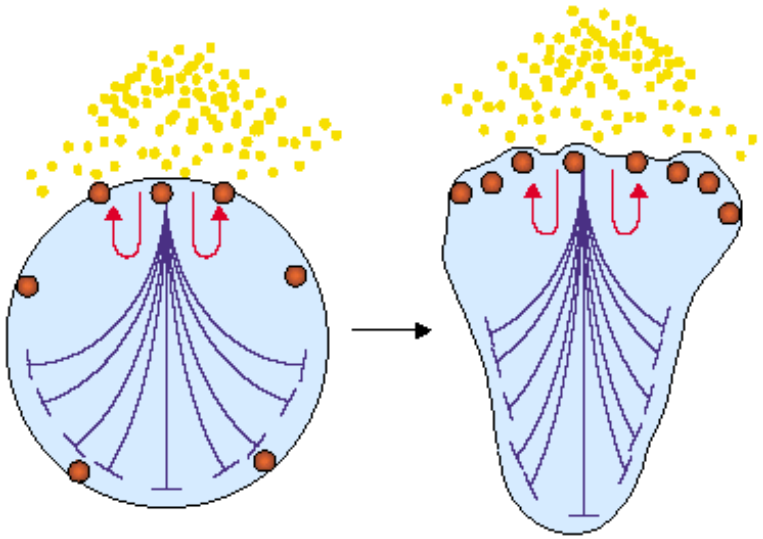


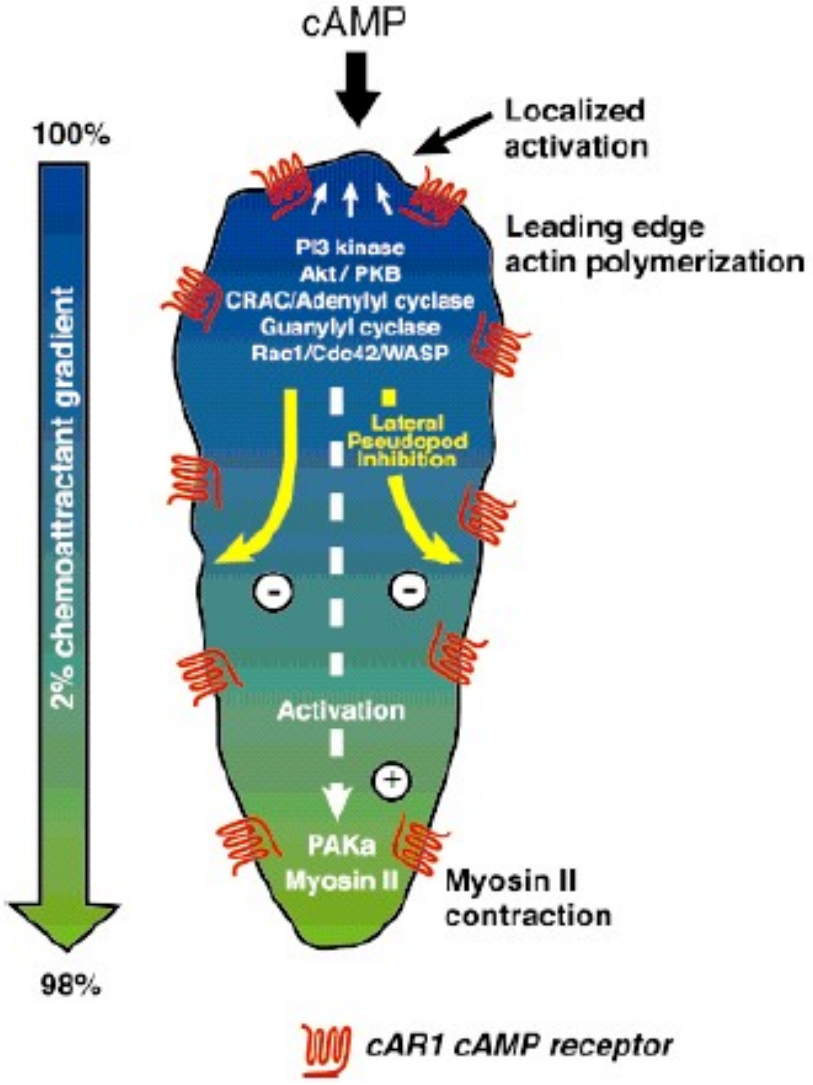
Regulation of Actin Polarization



Polarisation

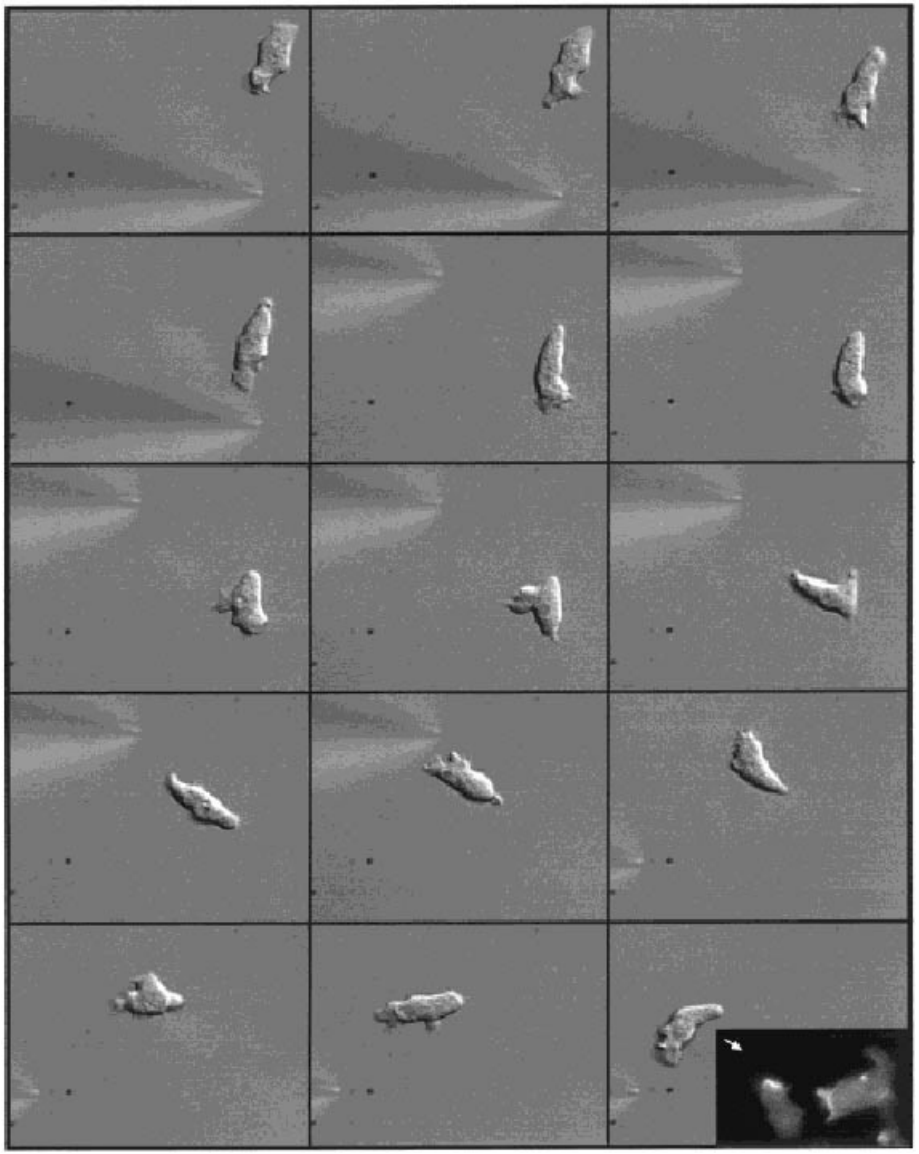
Change of cell shape by activation of the cytosceleton

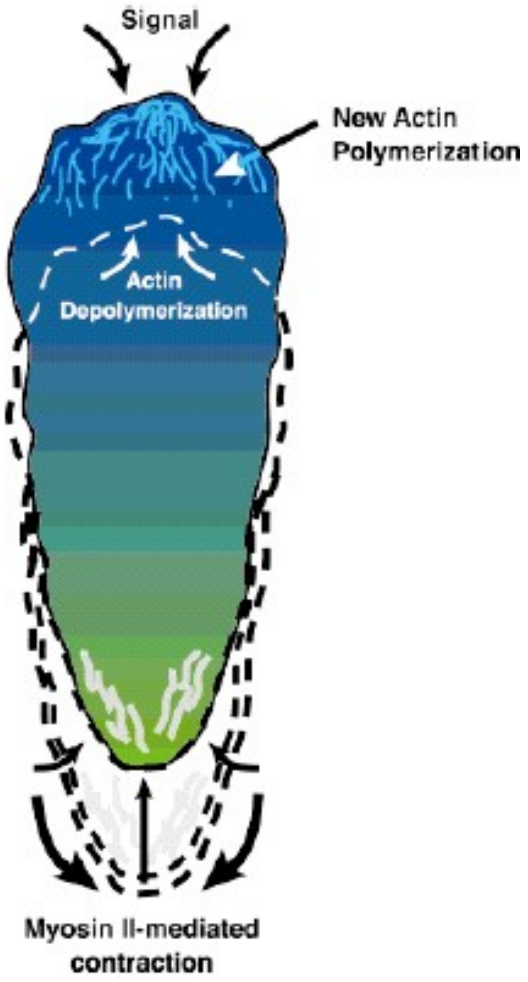




Review by Weiner

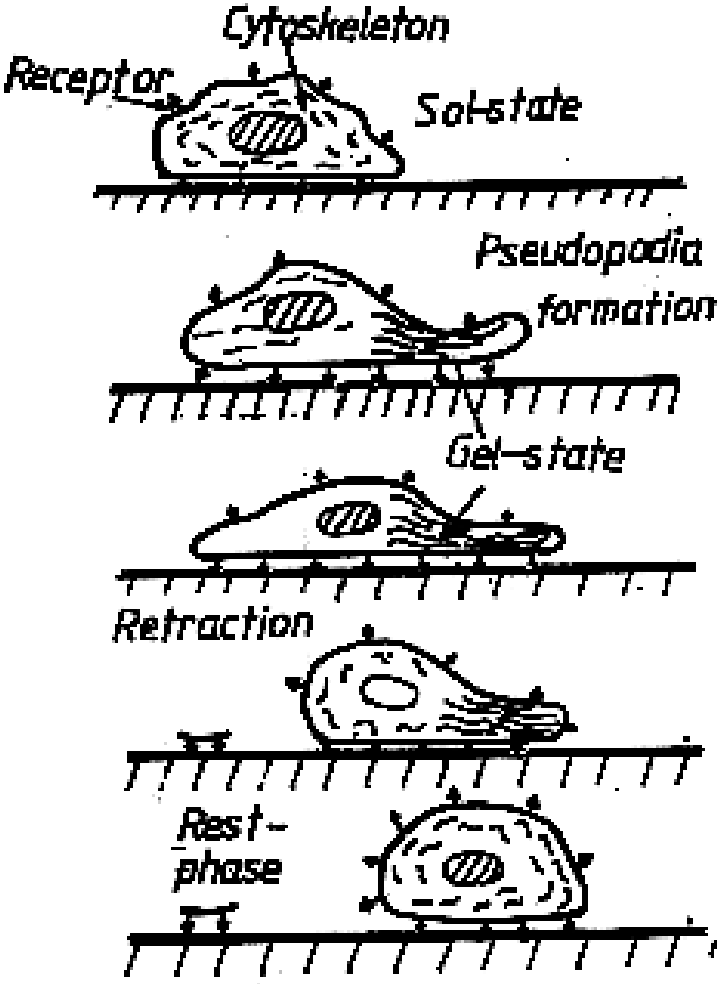
Eucaryotic Chemotaxis : localized response to chemoattractant

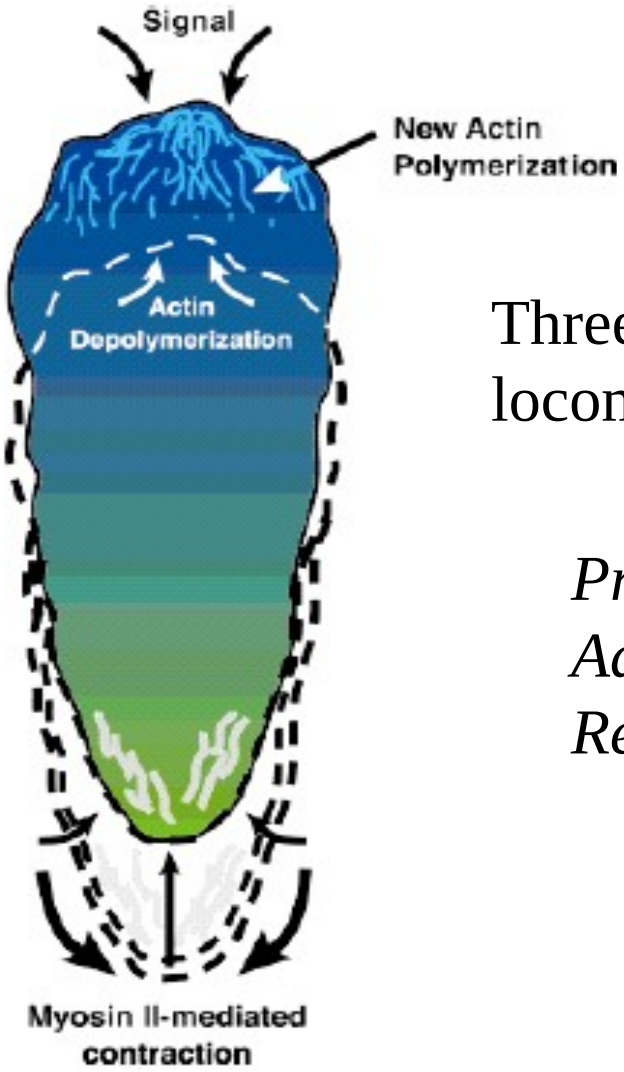




Figures : Firtel Gerisch

Zell motility is driven by Actin

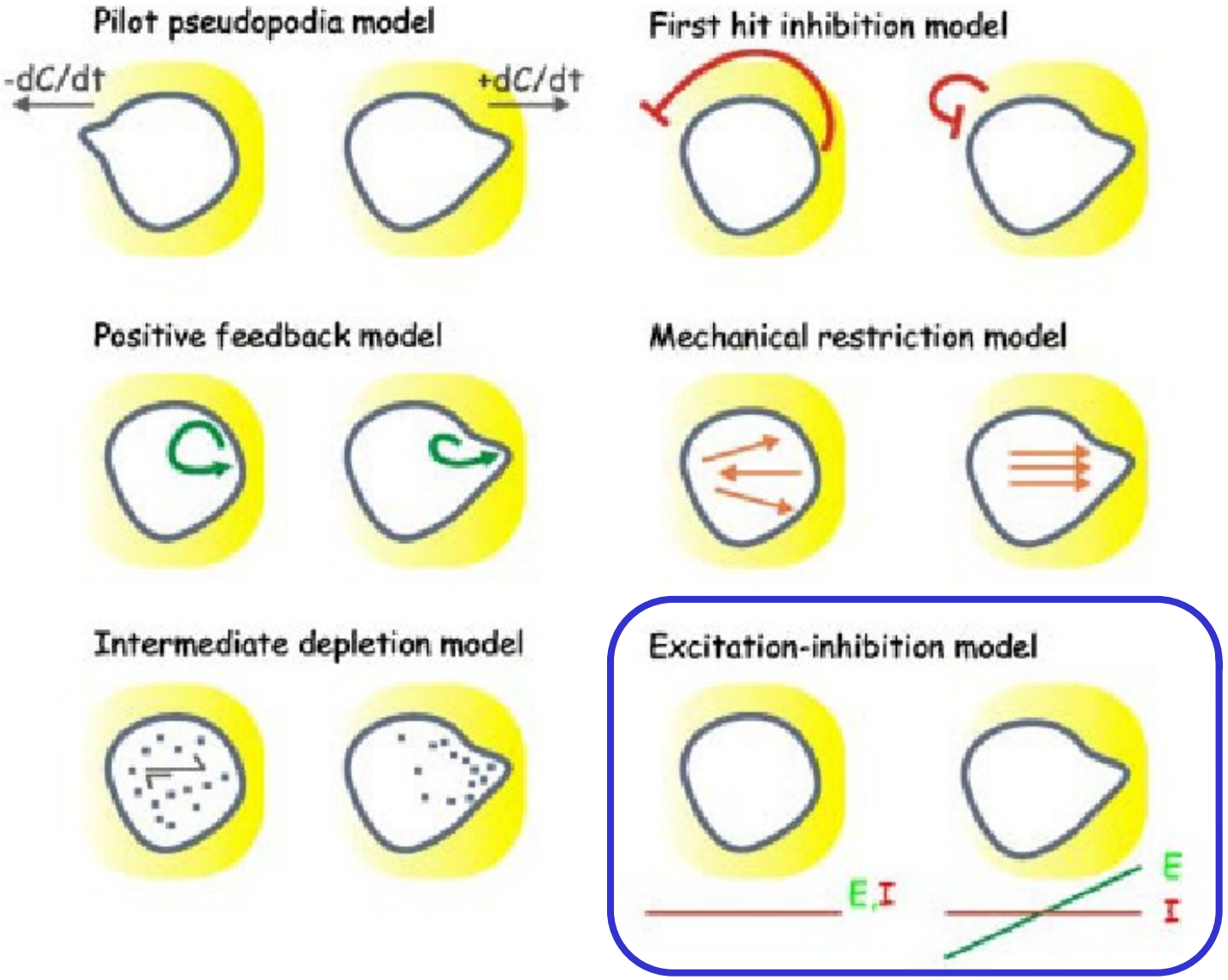




Three steps of locomotion:

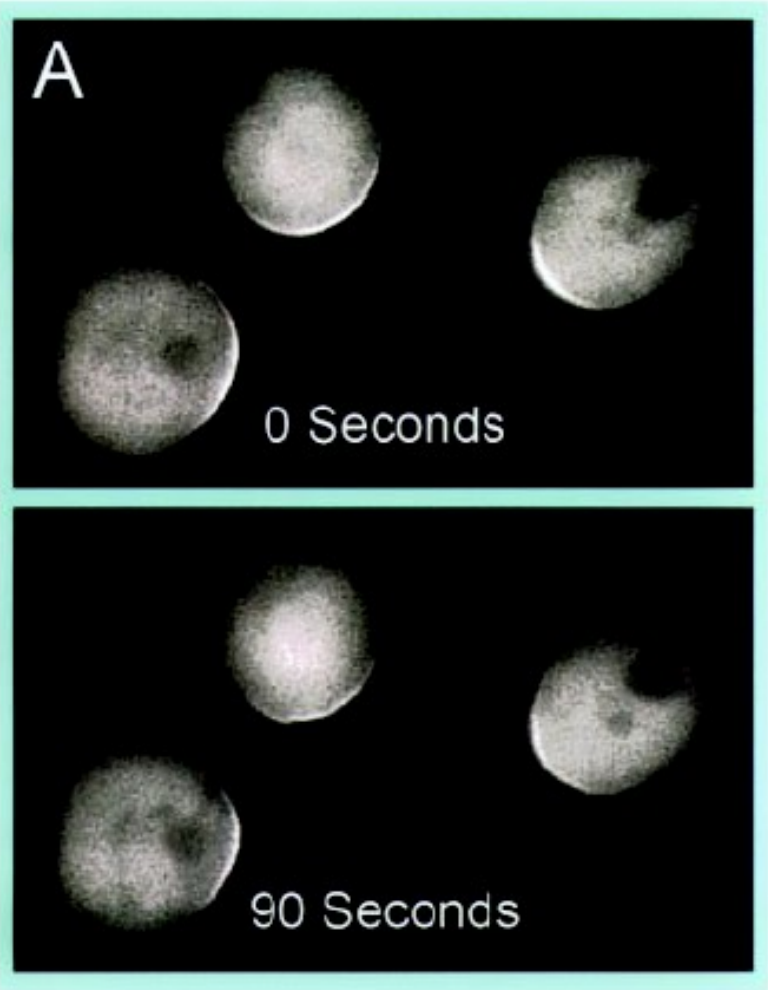
Protrusion
Adhesion
Retraction

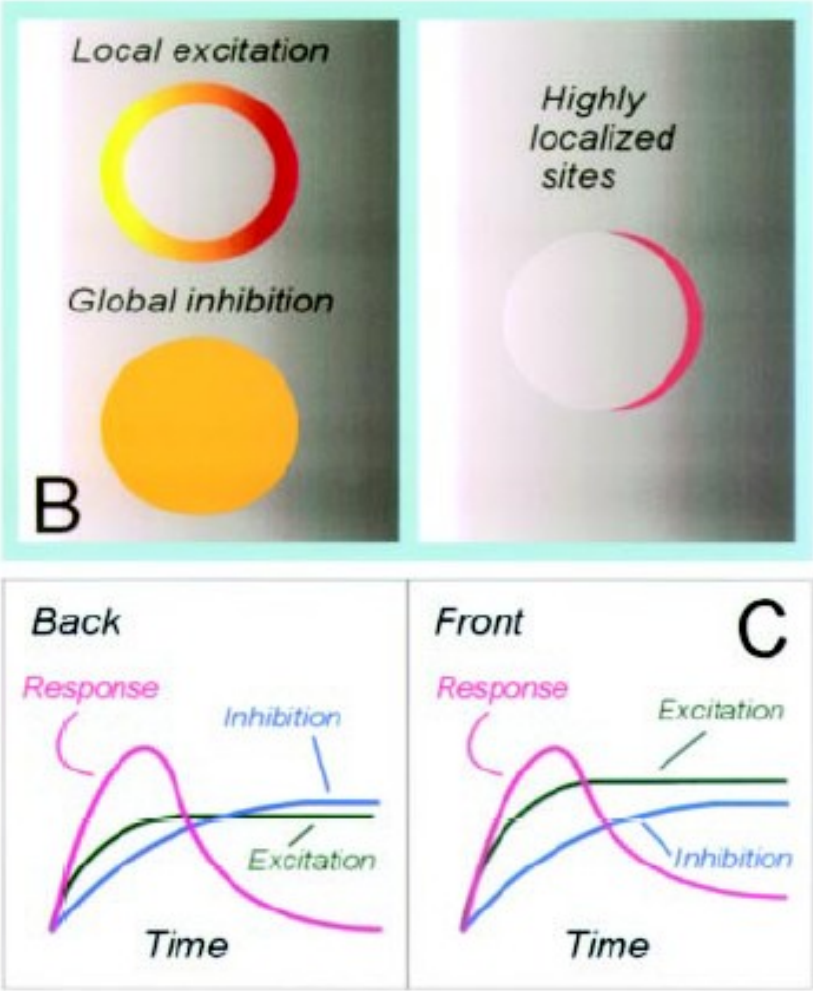
Models to explain chemotaxis



Local excitation global inhibition

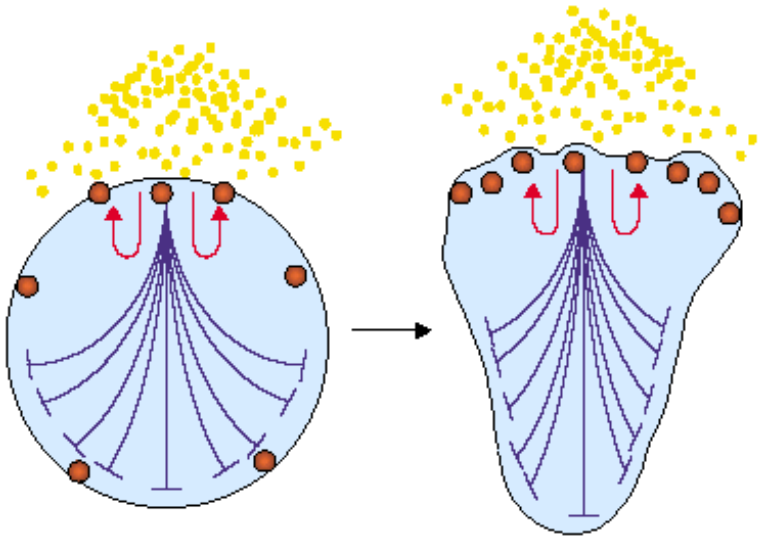
The chemotactic polarization is persistent



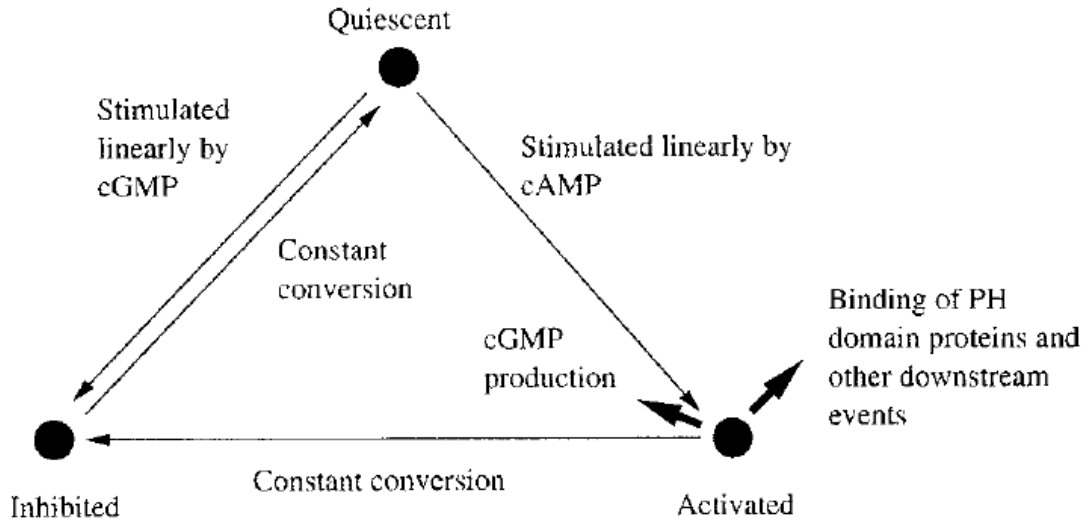


from Parent et al.

Local activation - Global inhibition



Three-State model
by Rappel et al. (2002)



The membrane has three states:
Q (quiescent), I (inhibited) and A (activated)

(The inhibition is stimulated by cGMP)

Modelling the intracellular messenger distribution

Linear Rate Equations

ρ=Density of States
c=conc.(cAMP)
g=conc.(cGMP)
q: quiescent, a: activated,
i: inhibited

$$\frac{\partial \rho_q}{\partial t} = -\alpha c \rho_q + \beta_f \rho_i - \beta_r g \rho_q,$$

$$\frac{\partial \rho_a}{\partial t} = \alpha c \rho_q - \delta \rho_a,$$

$$\frac{\partial \rho_i}{\partial t} = -\beta_f \rho_i + \beta_r g \rho_q + \delta \rho_a,$$

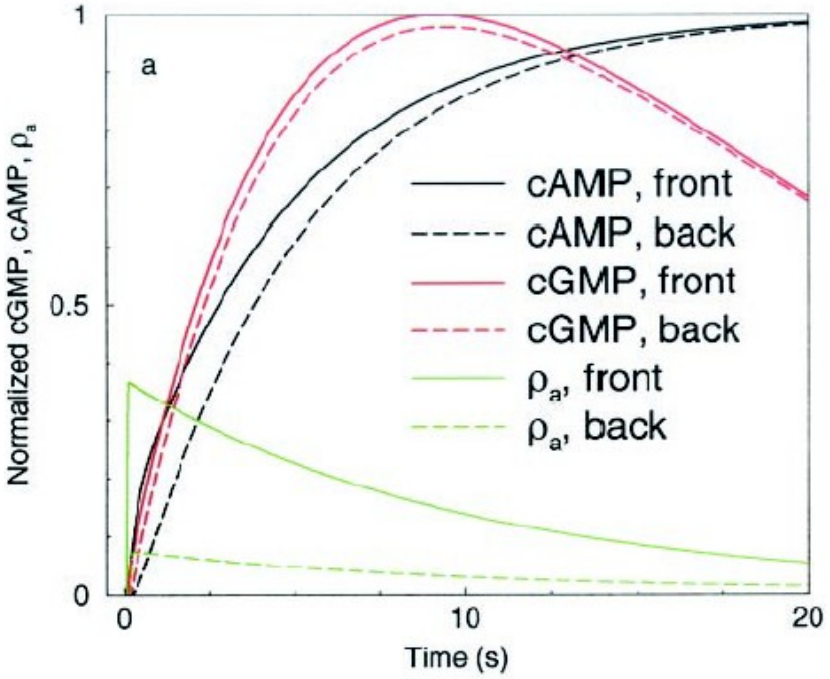
Diffusion Equations

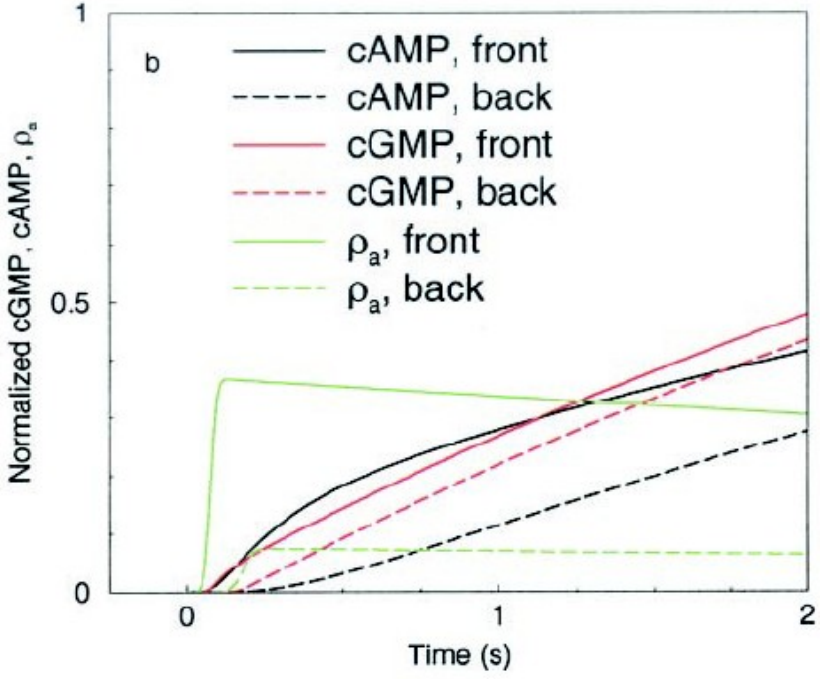
$$\frac{\partial c}{\partial t} = D_c \vec{\nabla}^2 c - \mu_c c \qquad \frac{\partial g}{\partial t} = D_g \vec{\nabla}^2 g - \mu_g g,$$

Boundary Conditions

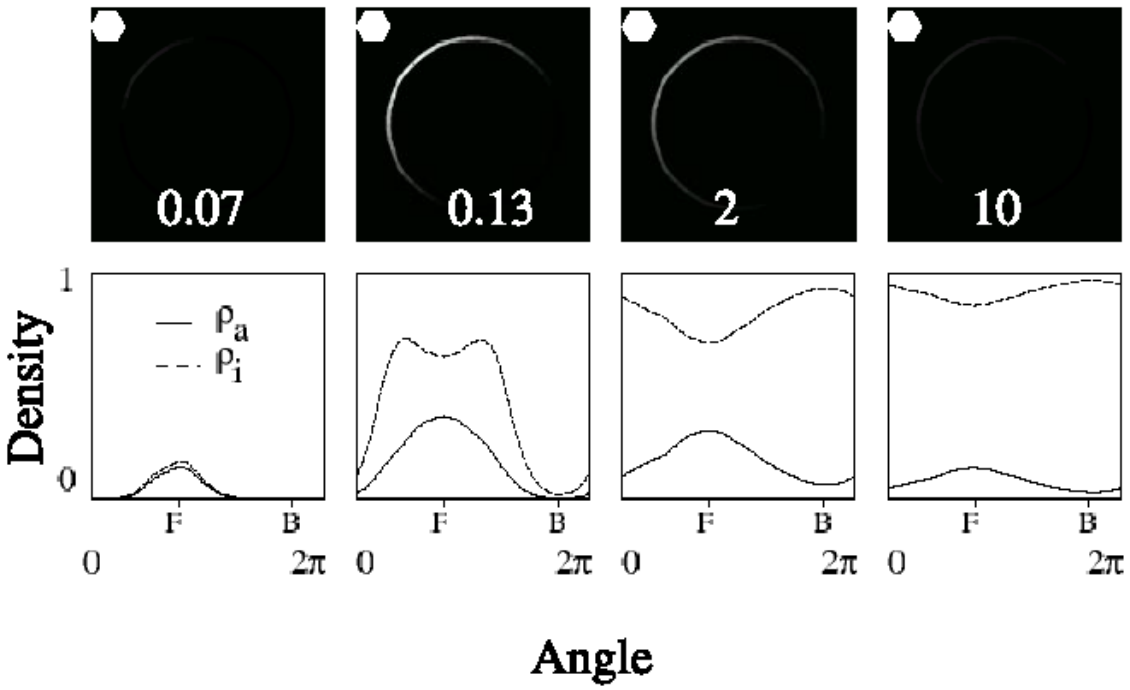
$$\frac{\partial g_{\text{boundary}}}{\partial t} = \sigma_g \rho_a + \text{bulk terms}.$$

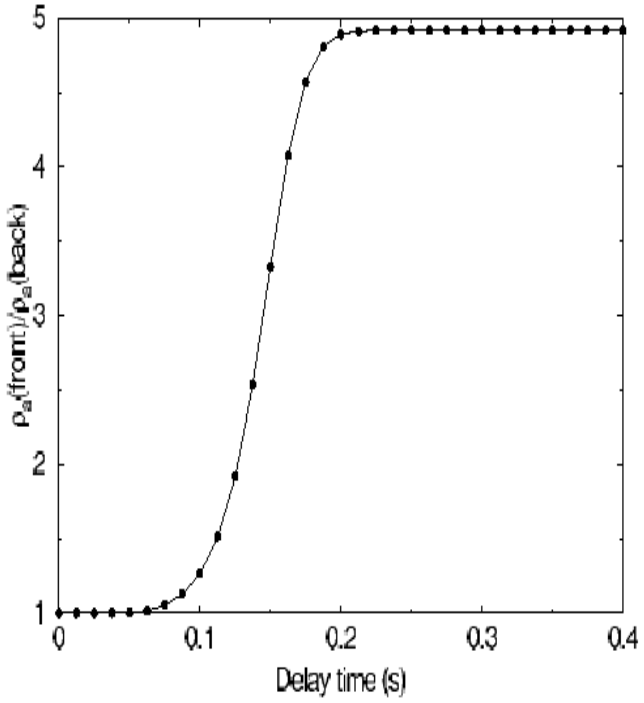
Numerical solutions of the differential equations





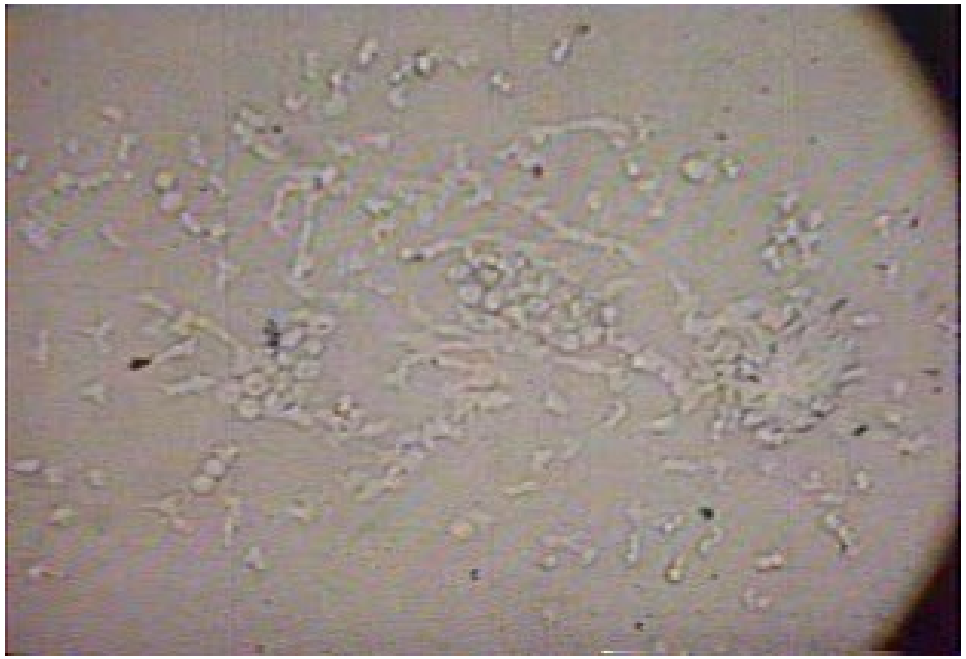
Simulation Results

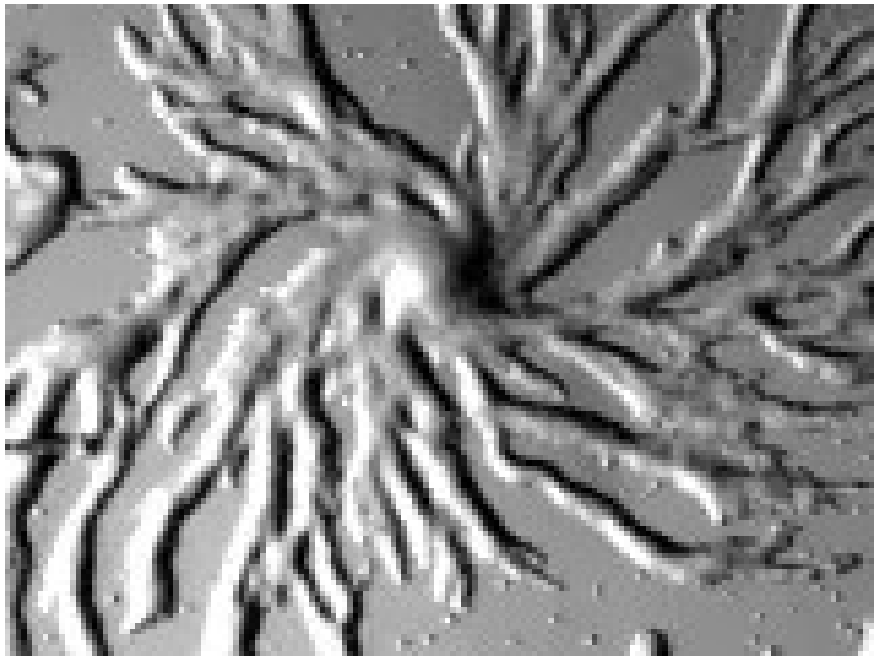




Excursion: Neurons

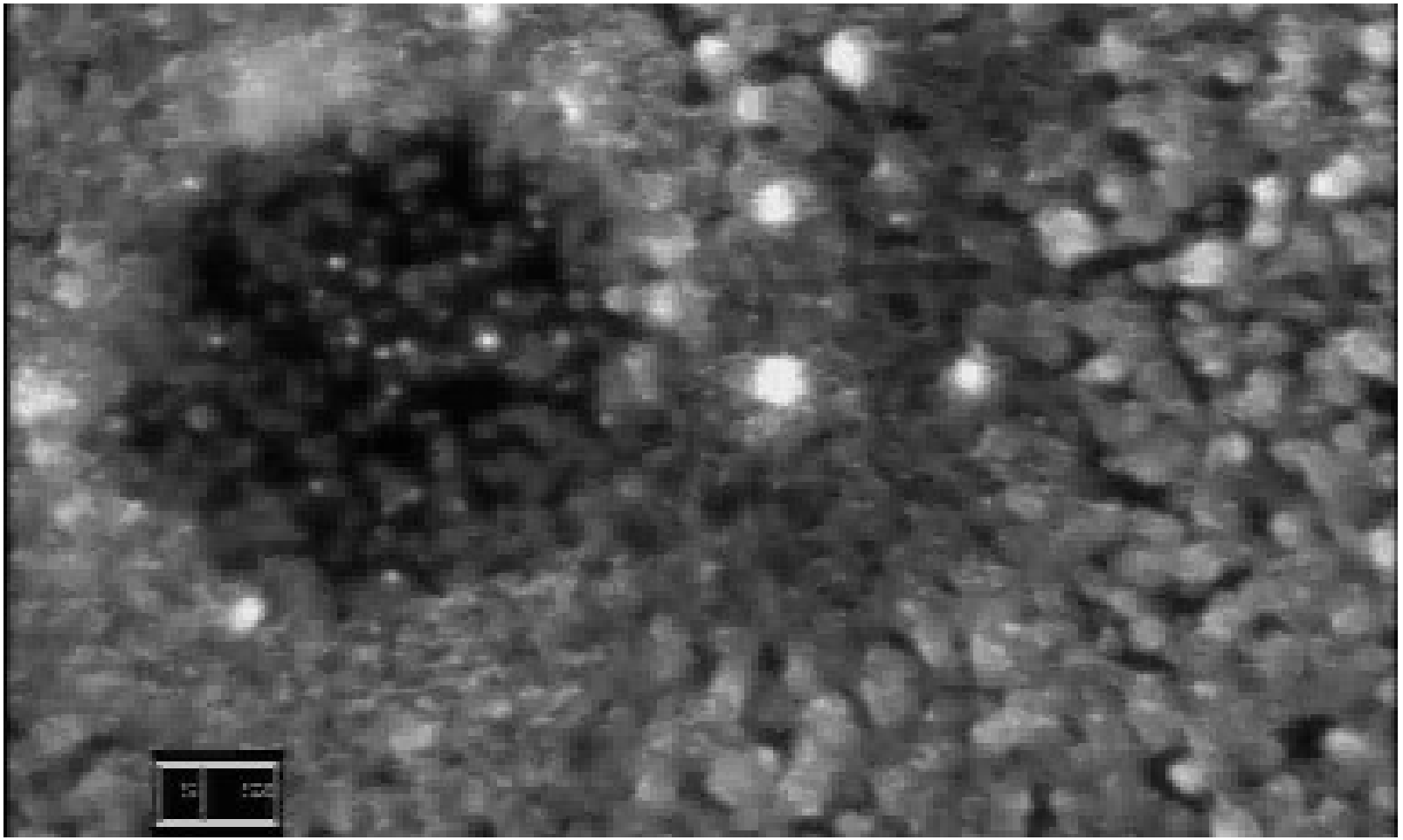
Cell Aggregation





Time-lapse video of small group of aggregating cells.
Interval between movement steps is 6 minute.
From P. Devreotes, Johns Hopkins Medical Institutions.

c-AMP Waves in Aggregating Cells

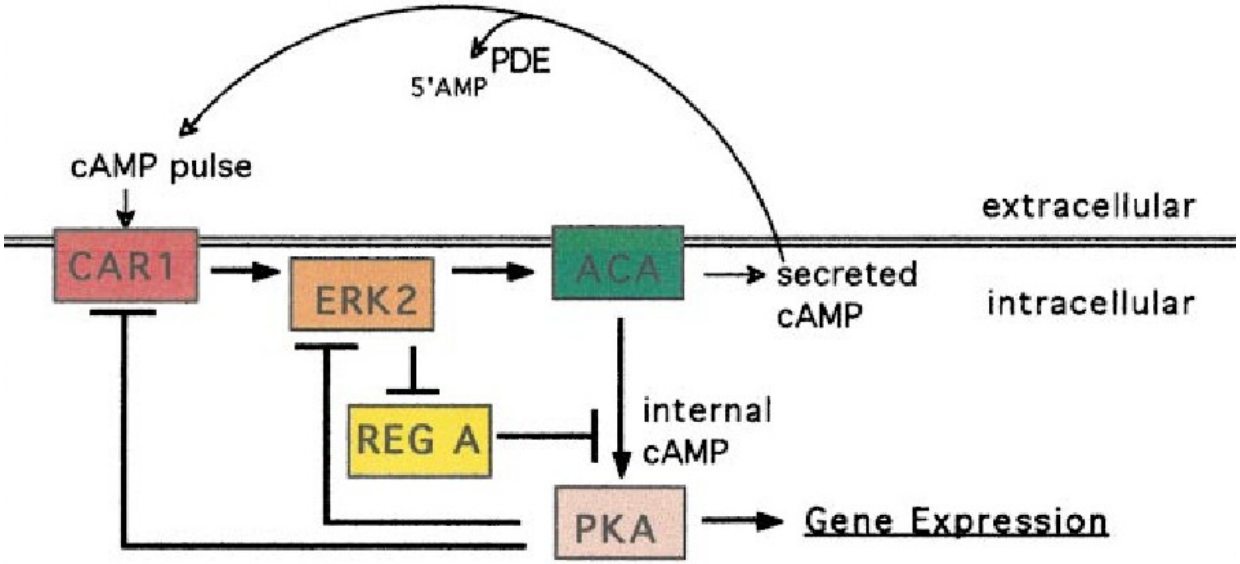


Core of a spiral wave in aggregating *D. discoideum* cells.
Time between images is 10 seconds.
From F. Siegert and C. J. Weijer, J. Cell Sci. **93,** 325-335 (1989).

Video Cell Aggregation

Biochemical Network of Cell Aggregation

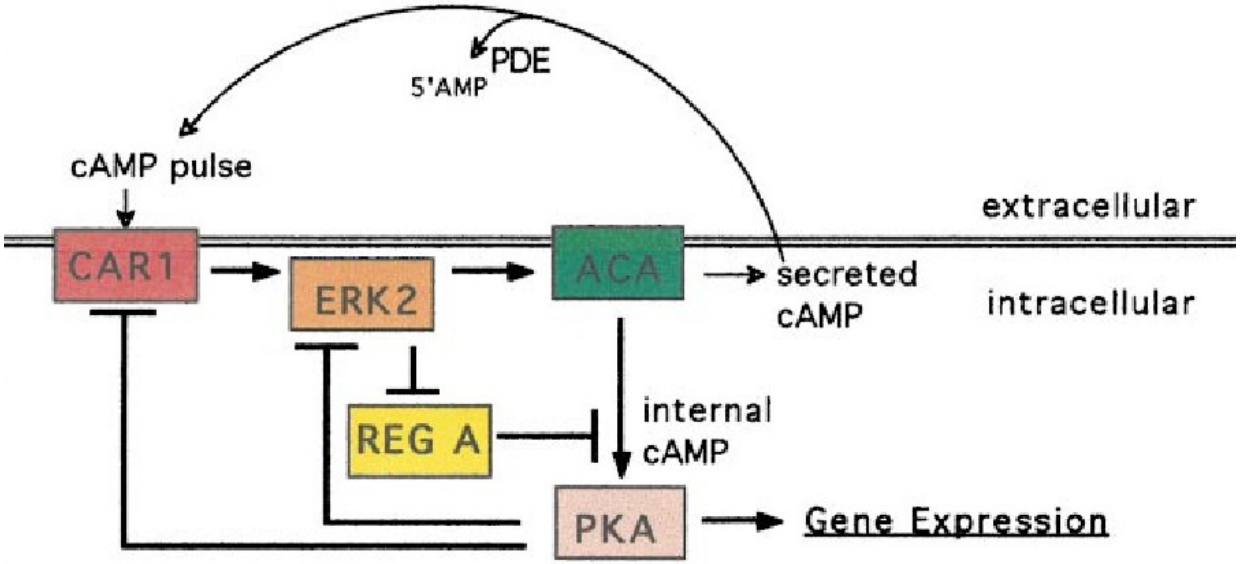
(Laub&Loomis, 1998)



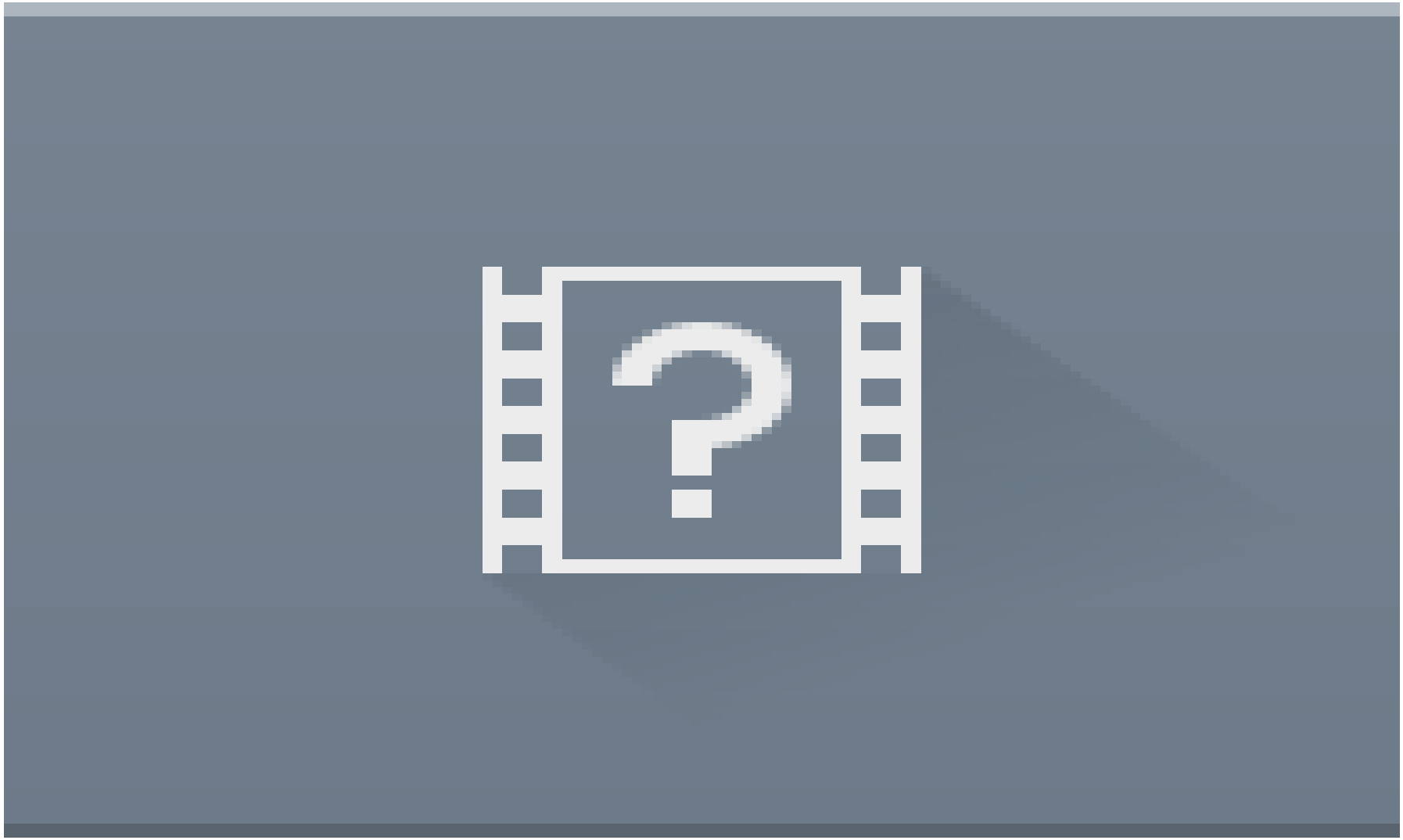
a rapid positive feedback activation of CAR1, via ACA, followed by a delayed negative feedback inhibition of CAR1, via PKA, produces the observed oscillations in *Dictyostelium* cells developed for 4 h.

Biochemical Network of Cell aggregation

(Laub&Loomis, 1998)



Negative feedback comes from PKA, inhibiting CAR1. However this positive feedback does not continue unabated. Two to three minutes after the stimulation of cells with external cAMP and the activation of ACA, there is a **rapid 5- to 10-fold reduction in the affinity of CAR1 to cAMP** and a consequent reduction in ACA activity. CAR1 returns to its original unphosphorylated and high-affinity state shortly after the removal of external cAMP, indicating that the phosphates are rapidly removed by a phosphatase. When cAMP is removed, the modification of CAR1 is reversed, and CAR1 is again able to bind cAMP with high affinity. Thus, ligand binding oscillates in response to the levels of external cAMP.



Dark field waves of *D. discoideum* cells on caffeine agar.
Time between images is 36 seconds.
From F. Siegert and C. J. Weijer, J. Cell Sci. **93,** 325-335 (1989).

Autocatalytic Oscillators

$$\frac{dx}{dt} = v$$

$$m \cdot \frac{dv}{dt} = -\mu v - kx$$

$$\frac{dx}{dt} = y$$

$$\frac{dy}{dt} = -\Phi(x) \cdot y - x(t)$$

Der Van der Pol Oscillator

$$\ddot{x}(t)-\varepsilon\left(1-x^2(t)\right)\dot{x}(t)+x(t)=0$$

Van-der-Pol Equation

Classical Model of an autocatalytic oscillator with $\Phi(t)=\varepsilon(x^2-1)$.

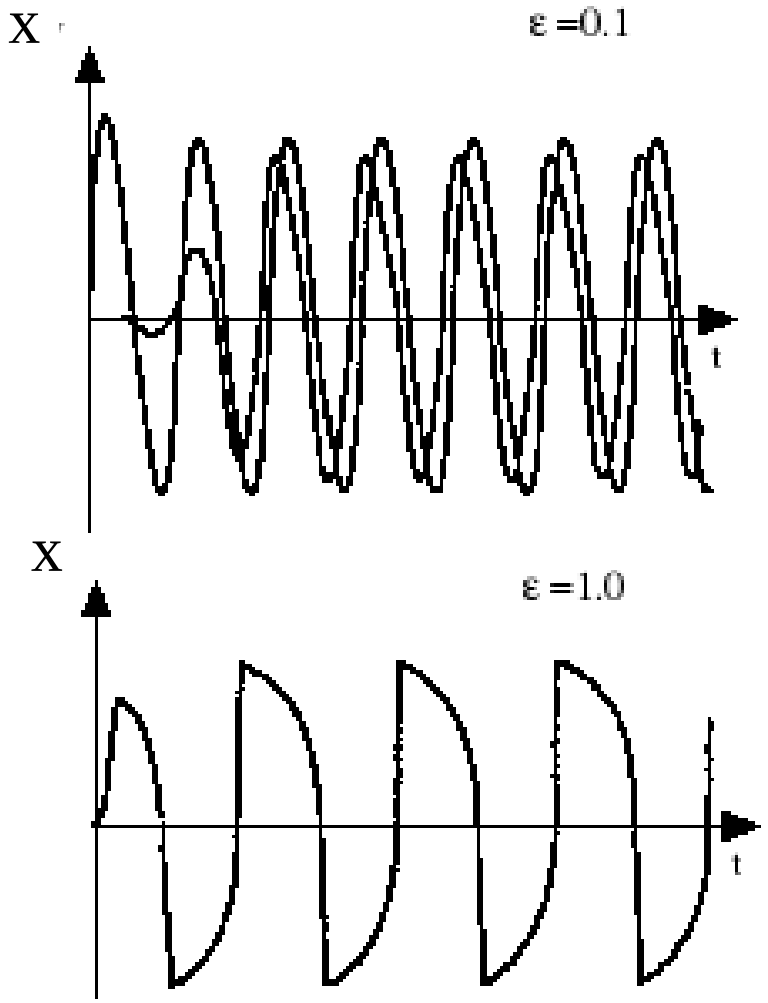
An equivalent system of equations in normal form is:

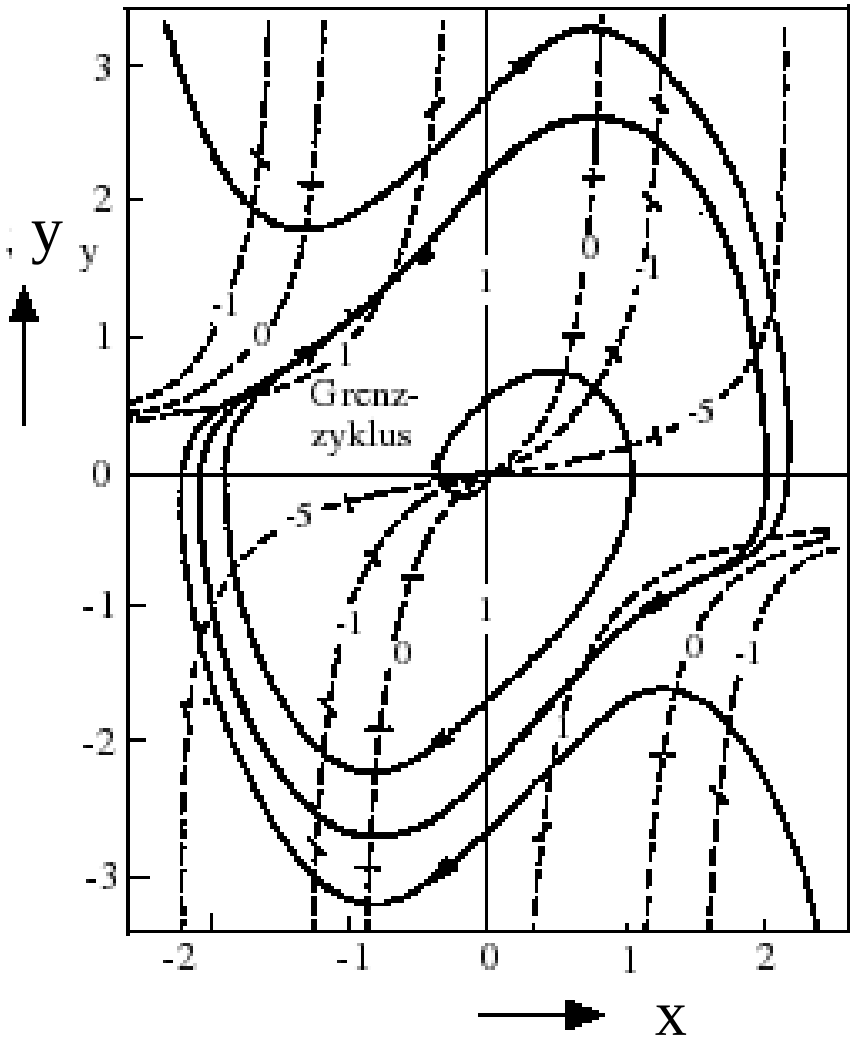
$$\frac{dx}{dt}=y$$

$$\frac{dy}{dt}=\varepsilon\left(1-x^2(t)\right)y-x(t)$$

Solving the Van der Pol Equation

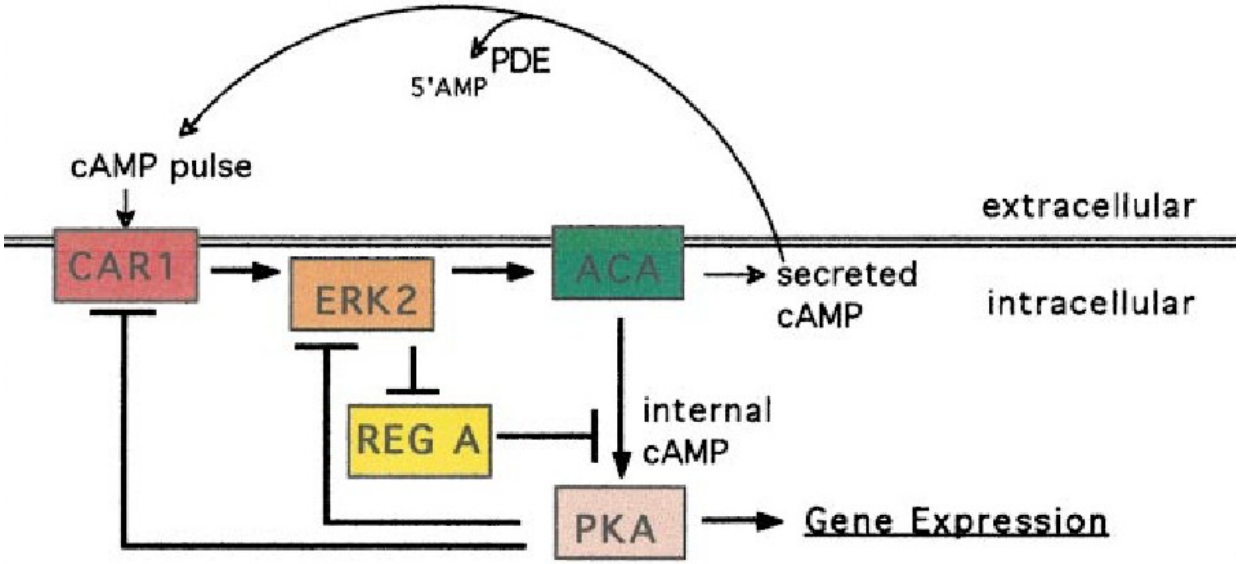
The system behaves as self-excitatory oscillator



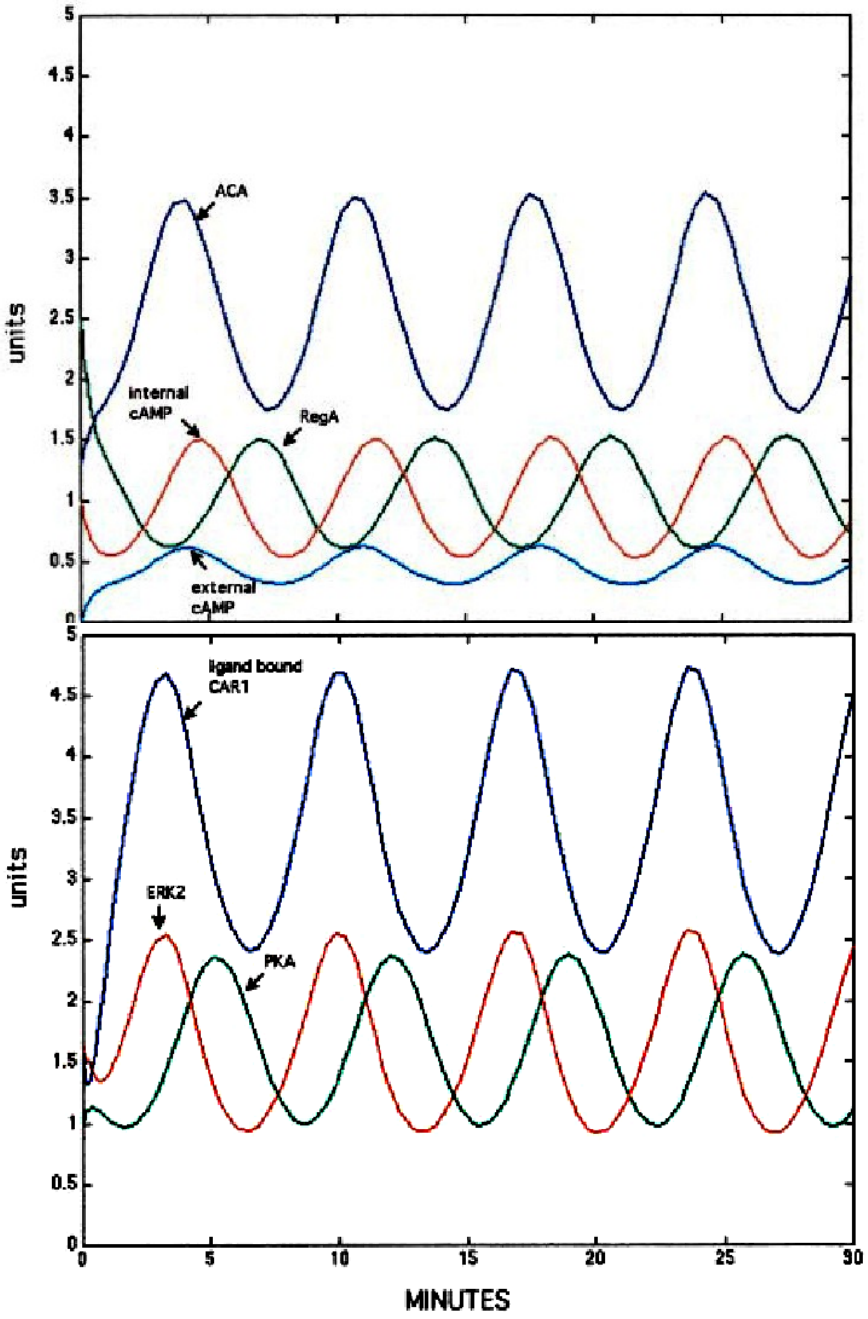


Biochemical Network of Cell aggregation

(Laub&Loomis, 1998)



$$d[ACA]/dt = k_1[ERK2] - k_2[ACA] \quad (1)$$
$$d[PKA]/dt = k_3[\text{internal cAMP}] - k_4[PKA] \quad (2)$$
$$d[ERK2]/dt = k_5[CAR1] - k_6[ERK2]\,[PKA] \quad (3)$$
$$d[\text{REG A}]/dt = k_7 - k_8[\text{REG A}]\,[ERK2] \quad (4)$$
$$d[\text{internal cAMP}]/dt = k_9[ACA] - k_{10}[\text{REG A}]\,[\text{internal cAMP}] \quad (5)$$
$$d[\text{external cAMP}]/dt = k_{11}[ACA] - k_{12}[\text{external cAMP}] \quad (6)$$
$$d[CAR1]/dt = k_{13}[\text{external cAMP}] - k_{14}[CAR1]\,[PKA] \quad (7)$$



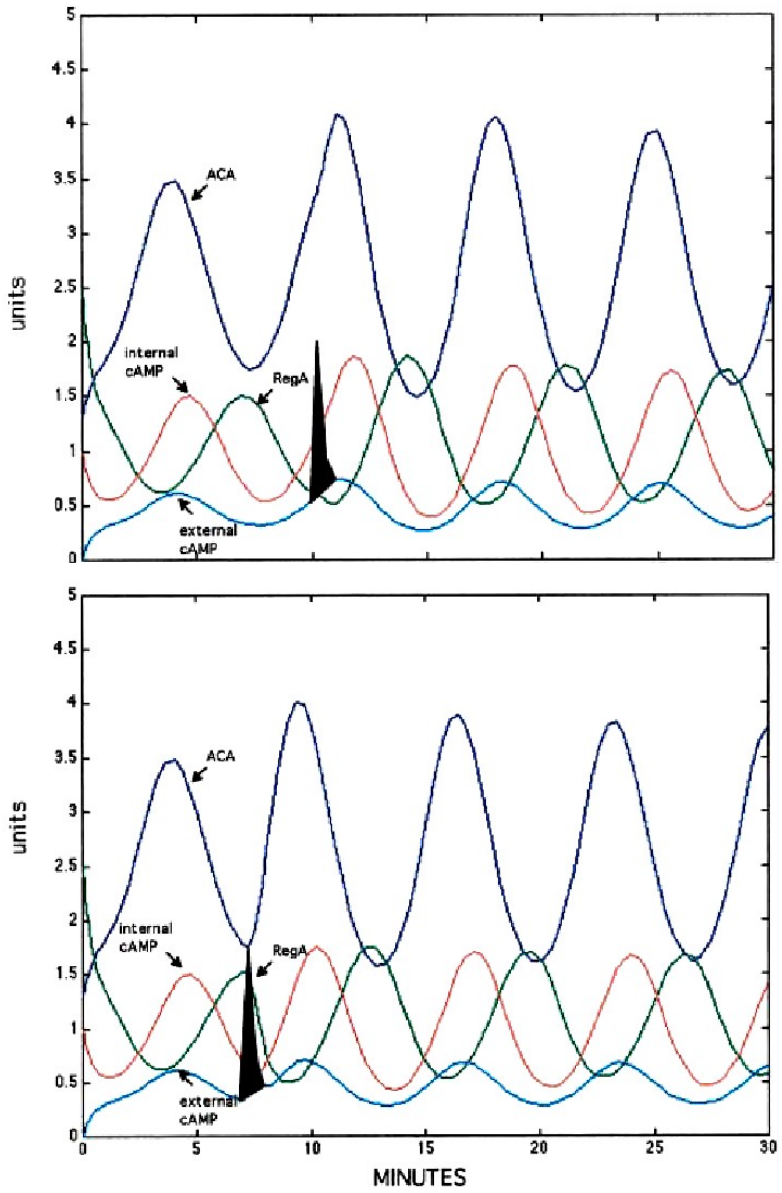
Choice of kinetic constants and results of simulation

Measured

Estimated

Parameter	Value
k_1	1.4 min^{-1}
k_2	0.9 min^{-1}
k_3	2.5 min^{-1}
k_4	1.5 min^{-1}
k_5	0.6 min^{-1}
k_6	0.8 min^{-1} μM
k_7	2.0 min^{-1} μM
k_8	1.3 min^{-1} μM
k_9	0.7 min^{-1}
k_{10}	1.0 min^{-1} μM
k_{11}	0.3 min^{-1}
k_{12}	3.1 min^{-1}
k_{13}	1.8 min^{-1}
k_{14}	1.5 min^{-1} μM

Influence of a c-AMP spike on the phase of the oscillation



Addition of c-AMP (black peak) has virtually no effect on the phase in the ACA-Peak.

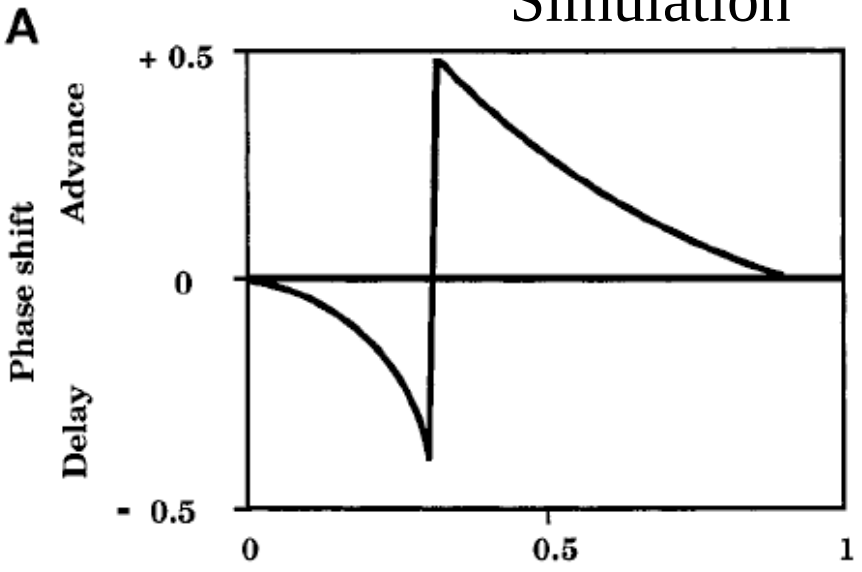
Addition of c-AMP (black peak) at the time of a low levvel of ACA and internal c-AMP makes the cycle jump by almost half a period in front.

In both cases, the added c-AMP was immediately degraded by the phosphodiesterase.

=> The network leads to a synchronization of the cells.

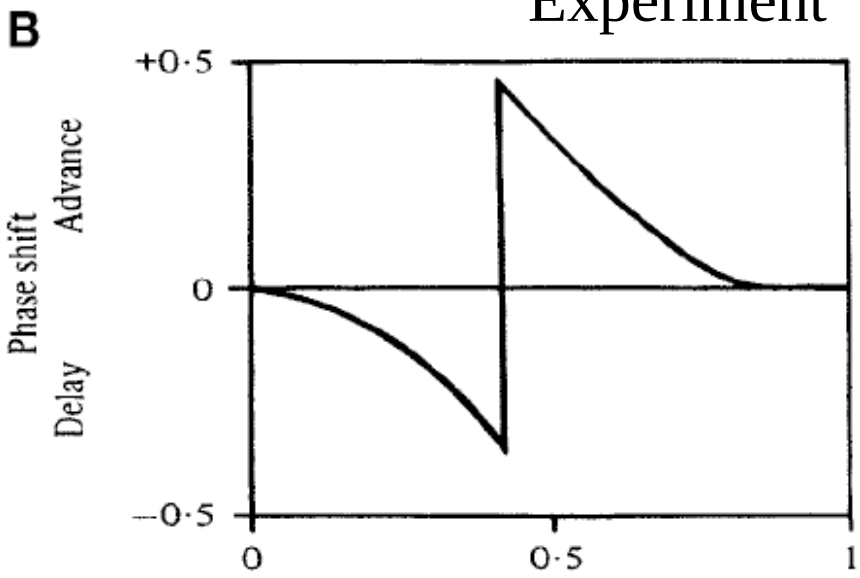
Phase Shift by adding cAMP

Simulation



Phase at which cAMP was added

Experiment



Phase at which cAMP was added

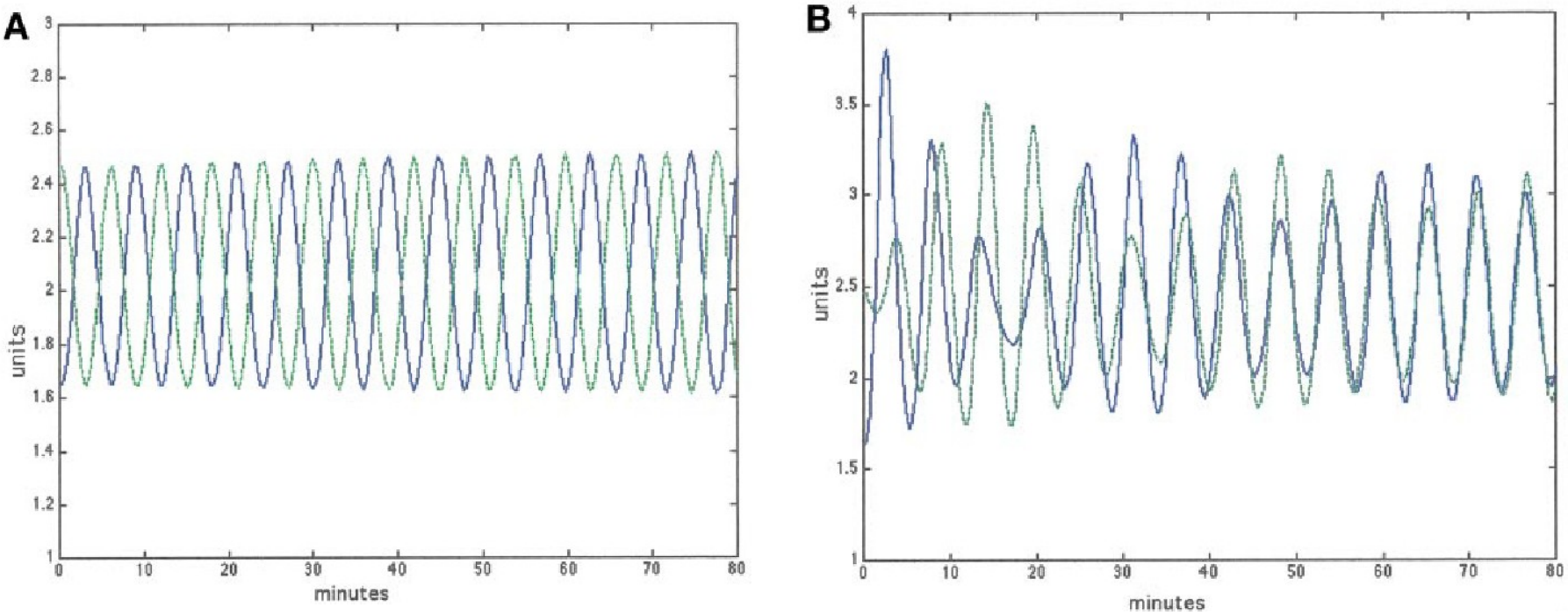
Adding cAMP to an oscillating cell leads to no change if done at zero phase shift, but either delays the cell or advances the cell in ist cycle. As result, the cells synchronize within maximally 1h.

(Laub&Loomis, 1998)

Phase Shift synchronizes cell populations

Uncoupled by external cAMP

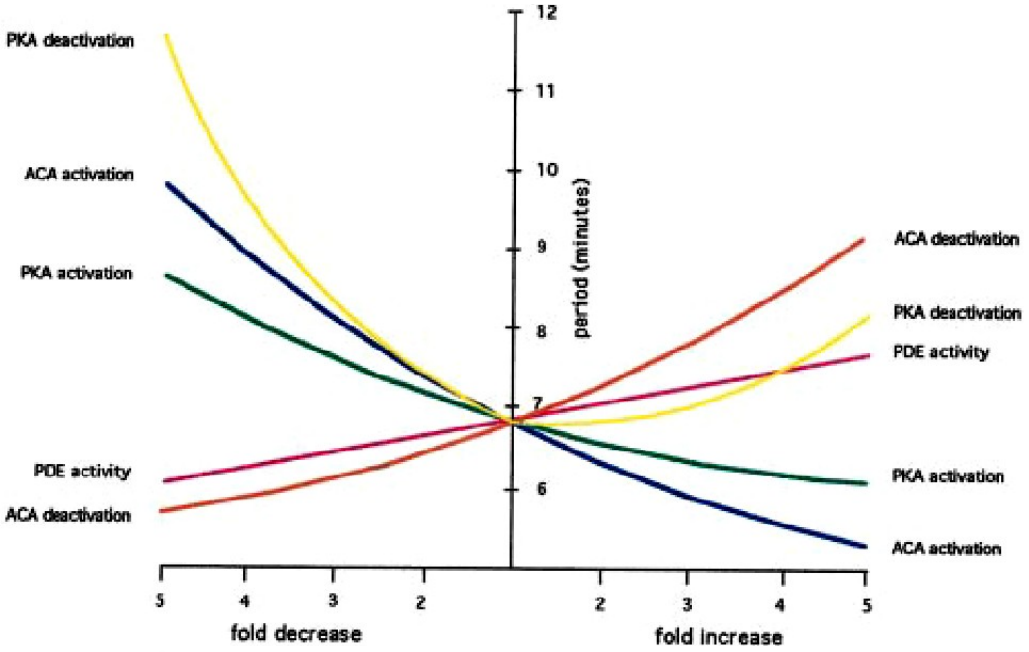
Coupled by external cAMP



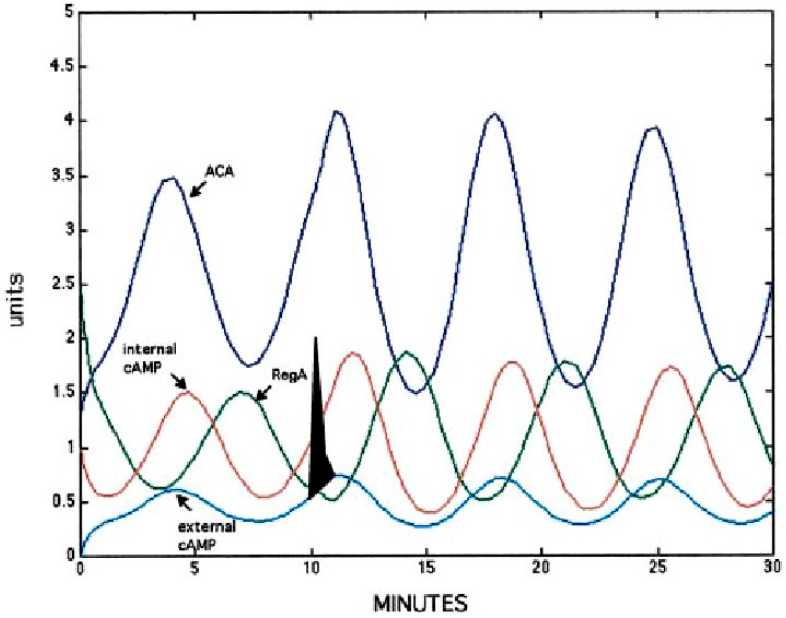
Synchronization in the computer model. If external cAMP does not mix between cells (hypothetical control case), cells do not synchronize. But even two cells which oscillate at maximal phase difference, start to synchronize within 1h.

(Laub&Loomis, 1998)

Robustness of the biochemical network



The frequency of the oscillation depends only weakly on changes of the kinetic constants.



... It has been argued that any biologically significant circuit that has survived selective surveillance must be robust in the face of random perturbations, and our circuit supports such an hypothesis (Barkai and Leibler, 1997). ...

The cyclic phosphorylation of the kinase ERK2

Phospho-ERK2 is detected by specific Phospho-ERK2-Antibodies with the help of Western blotting.

(Maeda et al. *Science* 2004)