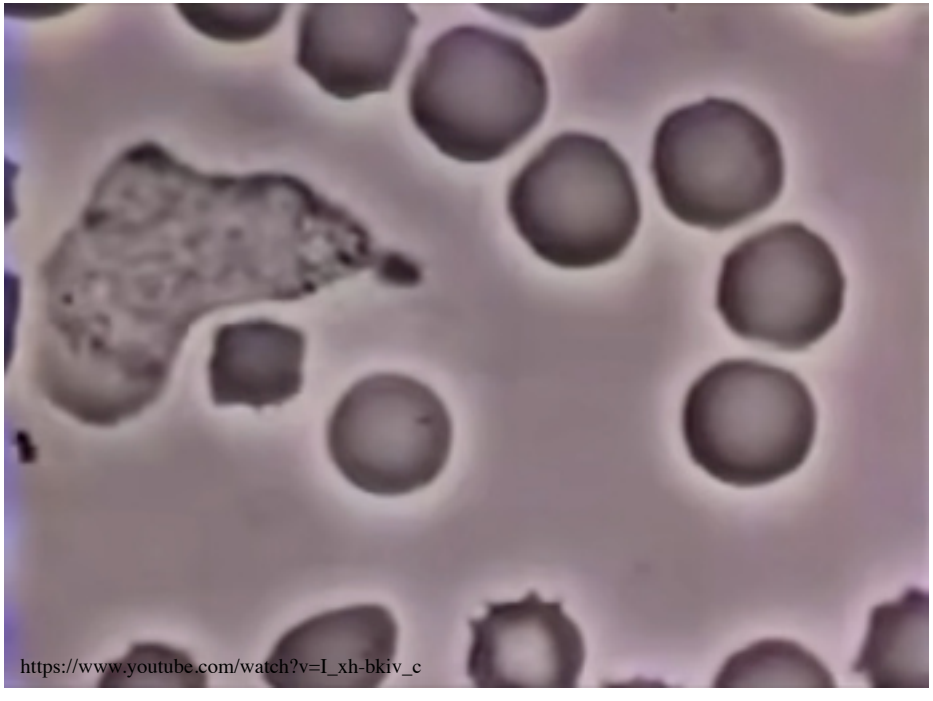
Cellular Motility



David Rogers at Vanderbilt University.

Course 6: Chemotaxis 2 – Eukaryotes

Thomas Lecuit

chaire: Dynamiques du vivant

COLLÈGE DE FRANCE 1530

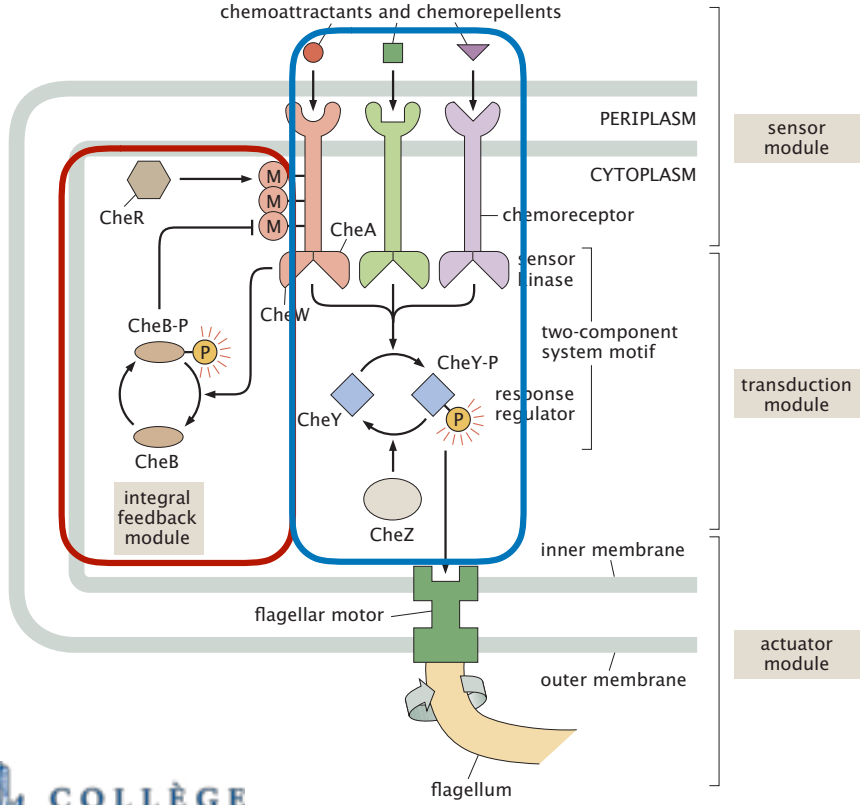
Two general classes of Mechanisms

- Temporal mechanism: comparison of chemoattractant at different positions and memory.

- Spatial mechanism: comparison of chemoattractant concentration along cell length

Conclusions – Bacterial chemotaxis

- Biased random walk in a spatial gradient
- *Temporal gradient* sensing
- Memory (short term)

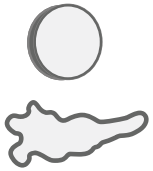


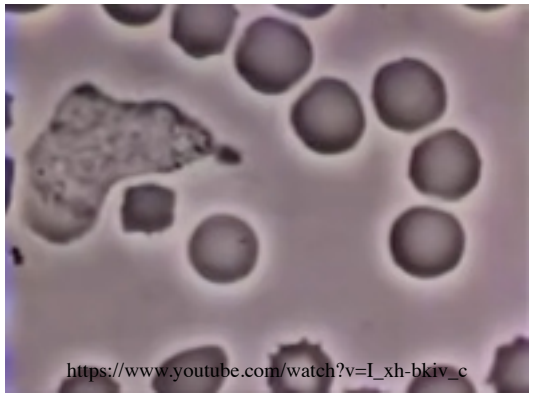
Key properties of chemotactic network

- Sensitivity - Gain : output/input ratio
- Adaptation: reset after input
- High amplitude range

Chemical guidance – Chemotaxis

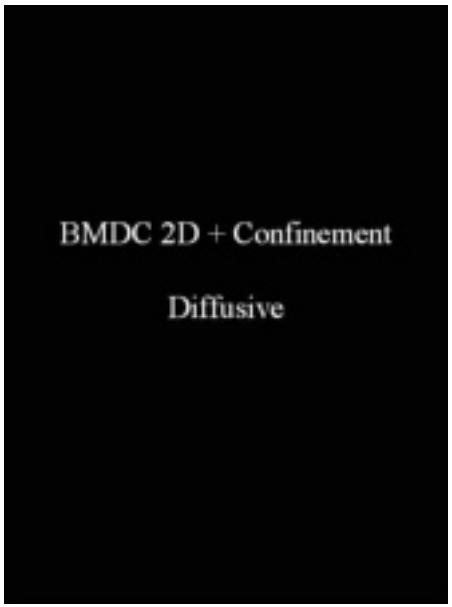
1. Bacterial chemotaxis
2. Eukaryote chemotaxis
   - Directional sensing
   - Polarity





David Rogers at Vanderbilt University.

Determinism



P. Maiuri, JF. Rupprecht, et al. *Cell 161*, 374–386 (2015)

Stochasticity - Persistence

Chemical guidance of cell motility

Key features of chemotaxis:

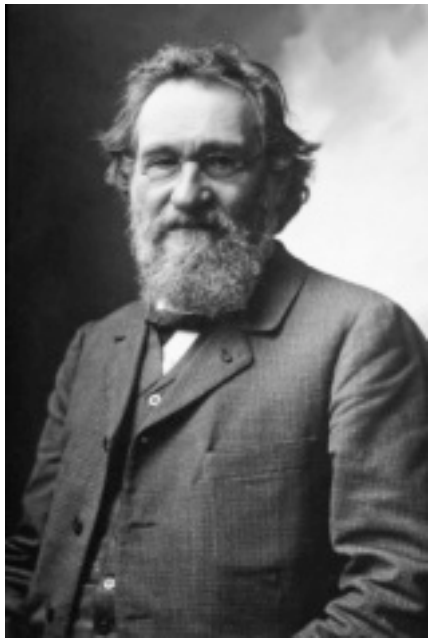
- Specificity
- Cell surface sensing (receptors)
- Sensitivity to ratio (gradient)
  but *not difference* in
  concentration of attractant

How can cells respond to a chemoattractant gradient?

Problems:
Bacteria can go up an an exponential gradient, over 20mm at mM range of concentration.
For a 2µm cell to detect such a gradient, they would need to detect 0.0001% difference on both ends

Eukaryotic cell, e.g. *Dictyostelium,* maximal chemotactic response between ~10 and 100 nM, corresponding to relative spatial gradients of ~15–30% across a 10µm cell (see later).

Chemotaxis of immune cells



Ilyia Metchnikoff (1845-1905)

Metchnikoff observed in Sicily (Messina) starfish larvae, noticed motile cells and hypothesized that this might underly response to external agents.

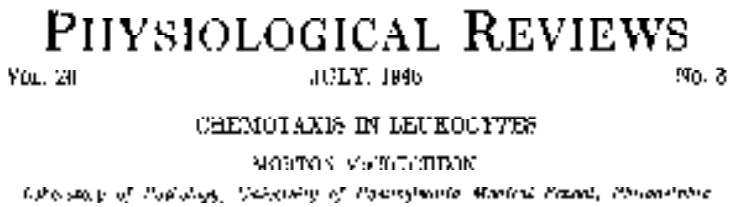
Used rose thorns under larval skin and observed leukocyte chemotaxis and phagocytosis

Metchnikoff's drawing of phagocytes at a site of inflammation (induced by silver nitrate) in the caudal fin of a Triton embryo.

Metchnikoff, E. *Lectures on the Comparative Pathology of Inflammation*. (Reprinted by Dover, New York, 1968).

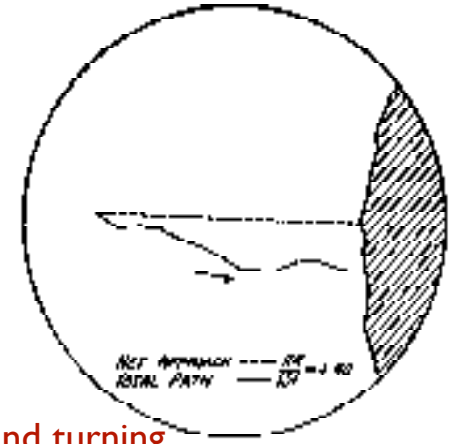
Cited in: G. A. Dunn and G. E. Jones *Nature Reviews Mol. Cell Biol*. 5:667-672 (2004)

Chemotaxis of immune cells


PHYSIOLOGICAL REVIEWS

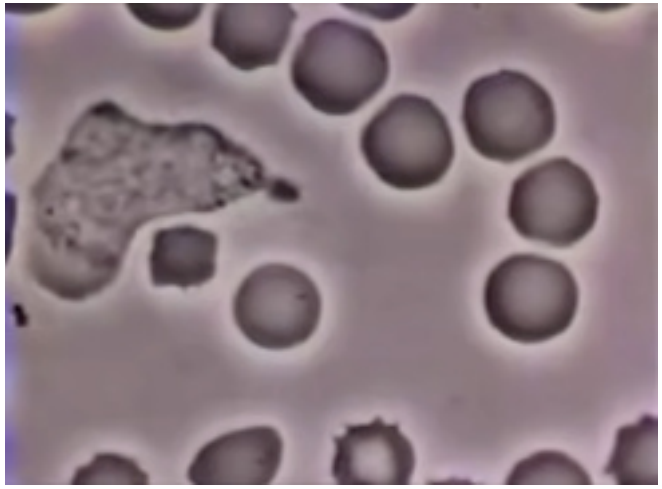
VOL. 20 JULY, 1946 NO. 3

CHEMOTAXIS IN LEUKOCYTES



- Leukocytes chemotaxis: attracted by bacteria, phagocytose bacteria
- Chemotaxis index: net path/total path
  0.61-0.97, mean 0.85, s.e.m. 0.01.
- Leukocytes paths alternate runs and turns
- Chemotactic and non-chemotactic cells do not have different velocities
- Cells that do not perform chemotaxis have a higher probability of stopping and turning

McCutcheon, M., *Physiol. Rev.*, 20, 319 (1946).

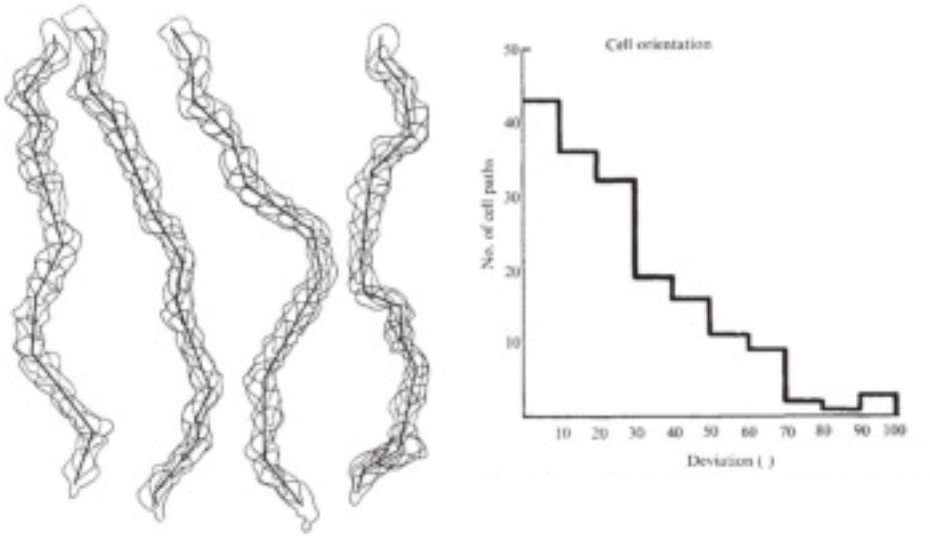


Leukocyte chasing a bacterium (*Staphilococcus aureus*)
David Rogers at Vanderbilt University. https://www.youtube.com/watch?v=I_xh-bkiv_c

Spatial sensing of chemical gradient

Mechanisms of sensing chemical gradients by polymorphonuclear leukocytes

As the cells tend to move in straight lines for short periods of time, the traced paths could be broken into segments of straight movement (that is movements having less than a 10° change in direction which were separated by relatively abrupt turns of varying magnitude).



- Leukocytes (PMN - granulocyte) alternate runs and turns

65% of segments are oriented within 30° of most direct path to the source

- **Run length statistics: no evidence of bias** (unlike Bacteria such as *E. coli*)
  —Distance between turns (length of runs) is *independent of direction* with respect to the source of attractant (coeff corr. -0.169, N=68 P>0.1).
- **Turns angle statistics: evidence of directional bias** (unlike *E. coli*)
  — angle of turn was correlated in magnitude with angle of run prior to run with respect to direct path to attractant (coeff corr. 0.35, N=68 P<0.01)
  — when angle prior to turn was >30° with respect to direct path to the source of attractant, the next direction was towards source (P<0.001)

The angle bias is not compatible with a simple temporal comparison at two time points (unless with memory of directions)

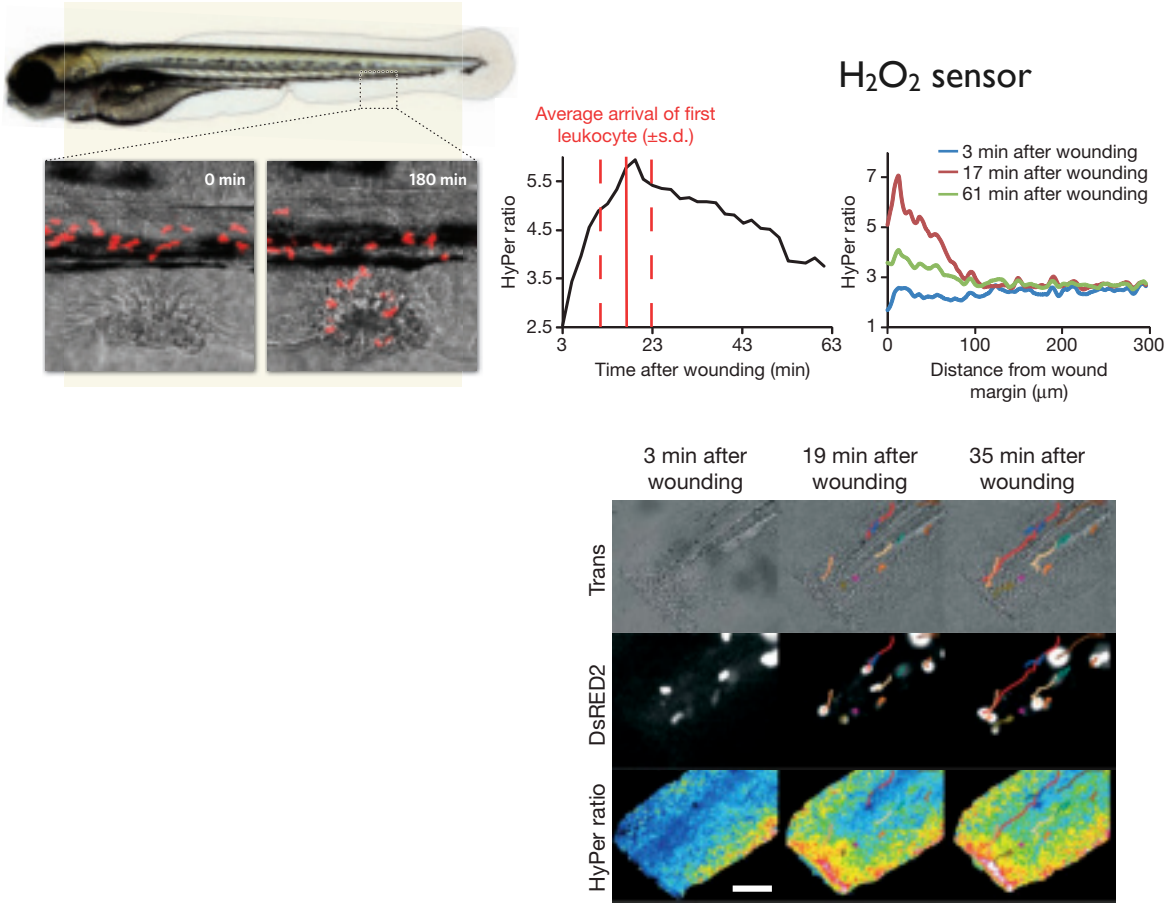
Consistent with a spatial model of chemotaxis

Consistent with this, cells extend pseudopode in direction of source with higher probabilities before they move

Zigmond SH: *Nature* 249:450-452. (1974)

Chemotaxis of immune cells

- Neutrophiles are attracted to wound sites by a gradient of peroxyde:



Niethammer, P., Grabher, C., Look, A. T. & Mitchison, T. J. *Nature* **459,** 996–999 (2009)

Chemotaxis of immune cells

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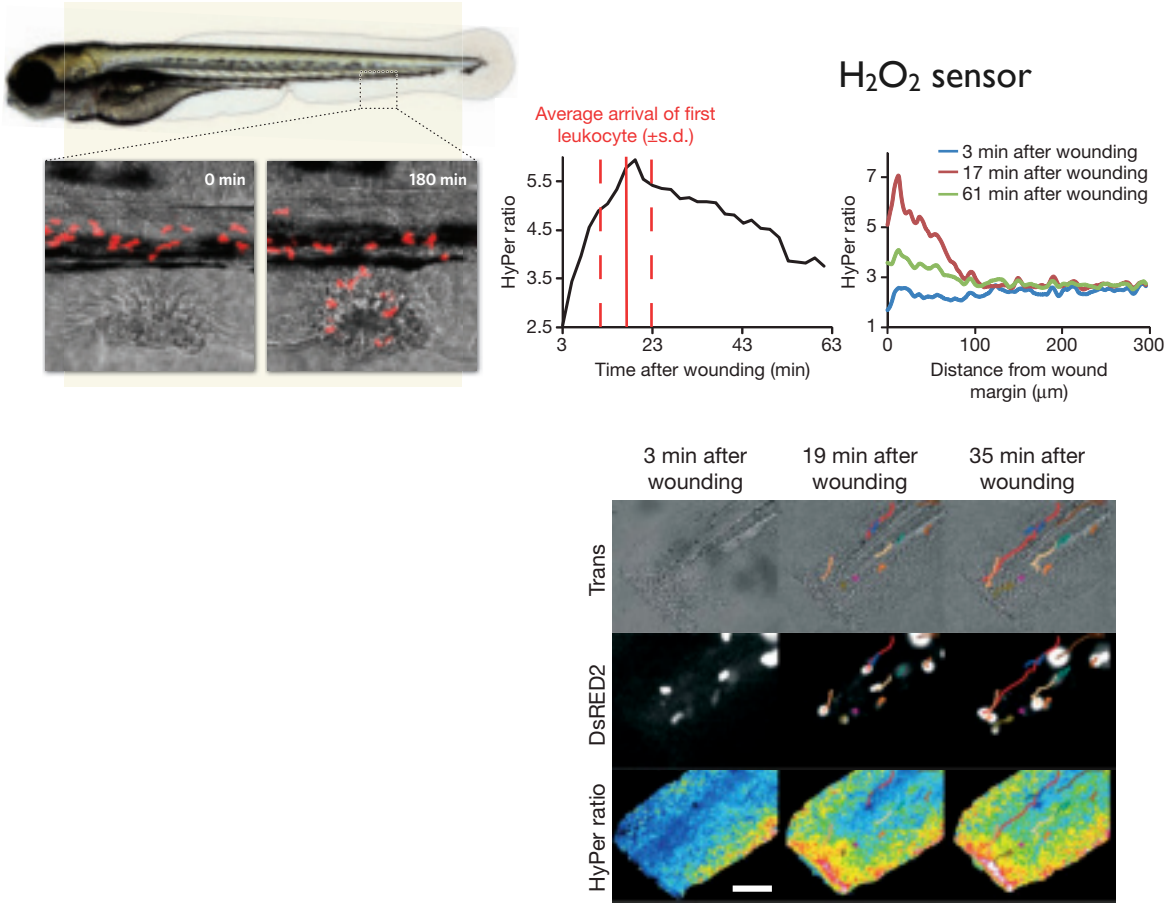
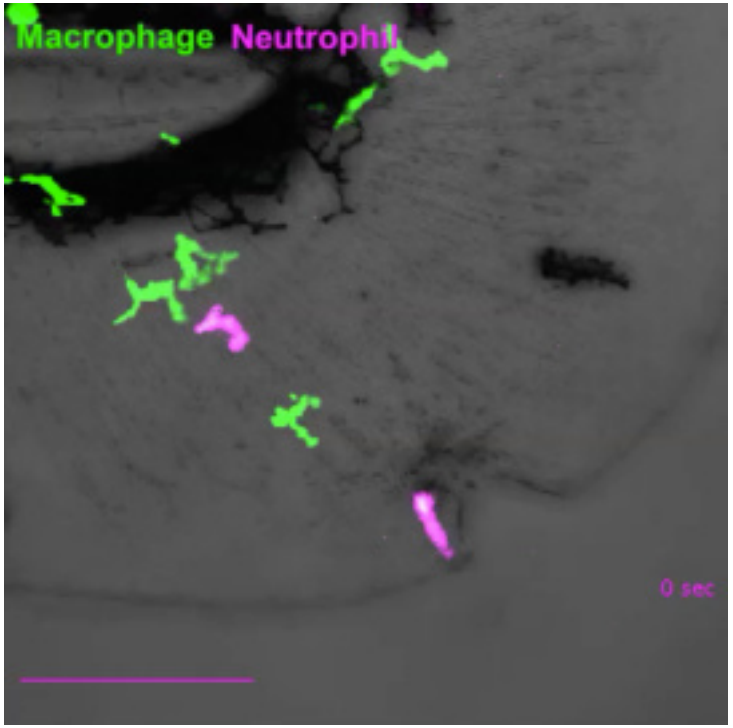


Niethammer, P., Grabher, C., Look, A. T. & Mitchison, T. J. *Nature* **459,** 996–999 (2009)



Kinetics of neutrophile (magenta)–macrophage (green) interactions at wounds in zebrafish

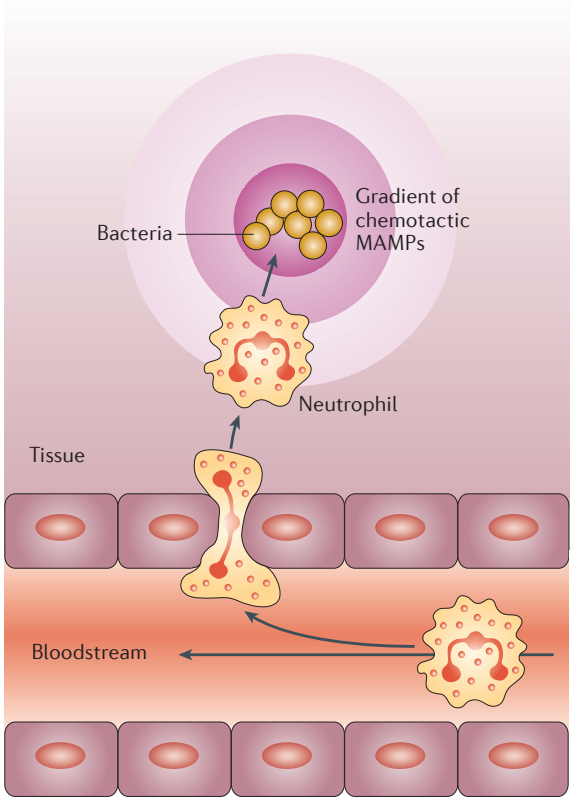
S. Tauzin et al. *J Cell Biol* (2014) 207 (5): 589–598.

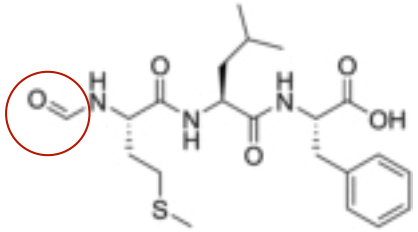
Chemotaxis of Neutrophils

Cells respond to a gradient of bacterial peptides

N-formyl oligopeptides: produced and released by bacteria

formyl-methionyl-leucine-phenylalanine (fMLP) induces polarization and motility of Neutrophils





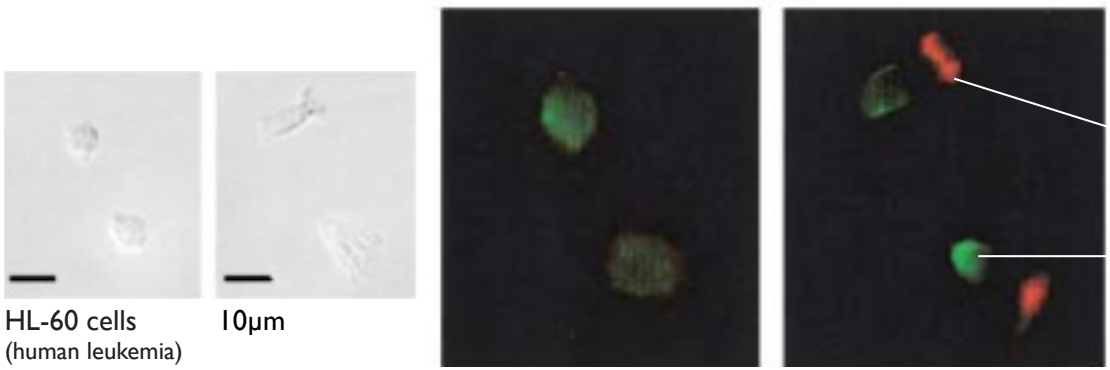
fMLP in micropipette

O. Weiner, J. Sedat and H. Bourne

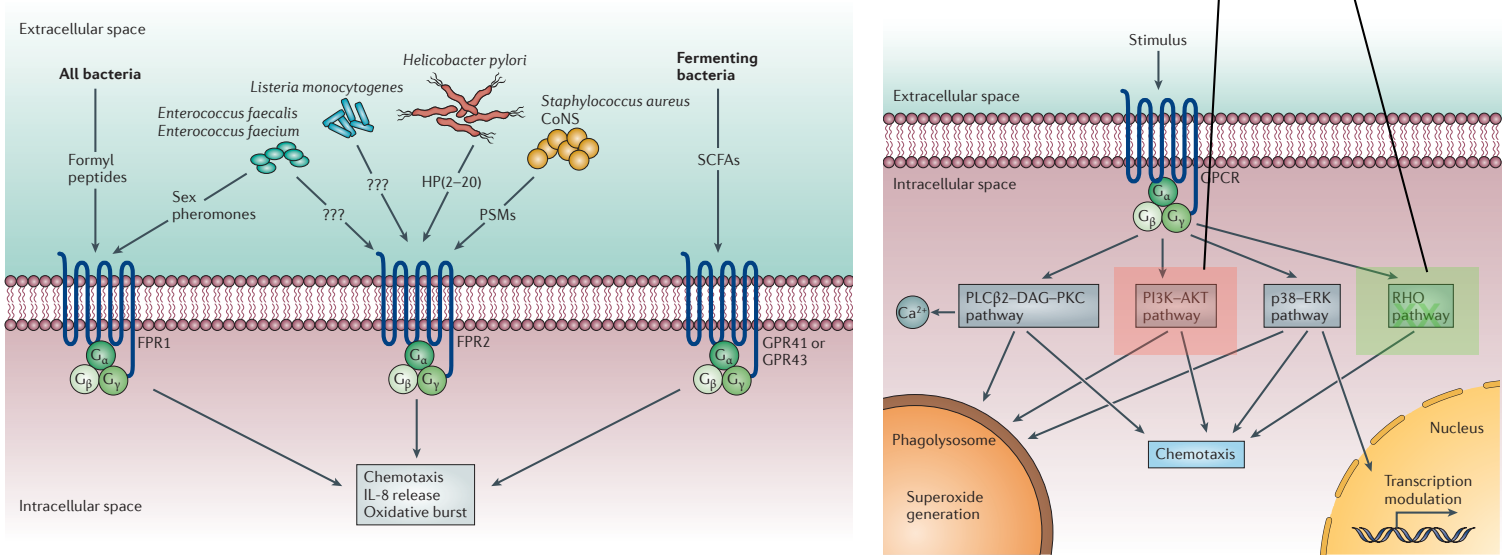
D.A. Bloes et al. *Nature Reviews Microbiology* (2014) doi:10.1038/nrmicro3390

Chemotaxis induces cell polarization

Neutrophils



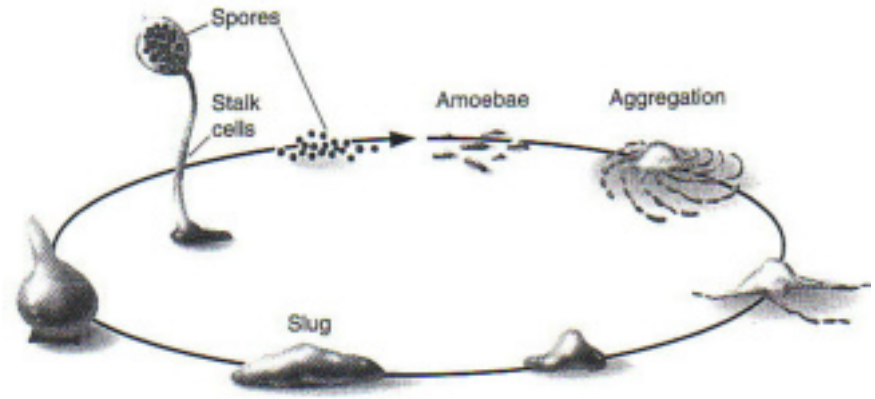
J. Xu et al. T. Mitchison and HR. Bourne. *Cell*, 114, 201–214 (2003)



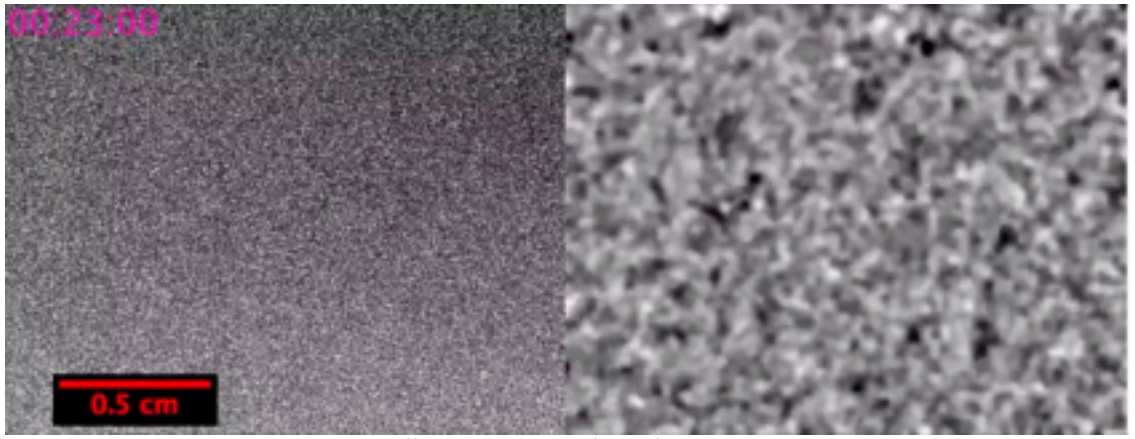
D.A. Bloes et al. *Nature Reviews Microbiology* (2014) doi:10.1038/nrmicro3390

Chemotaxis of the slime mold *Dictyostelium*

Individual *Dictyostelium discoideum* (Mycetozoa) cells are attracted by bacteria (folic acid)
During swarming induced by starvation, cAMP is released by cells that attract neighboring cells
Waves (period T ~ 6 min) of cAMP induce wave of cell polarization and motility

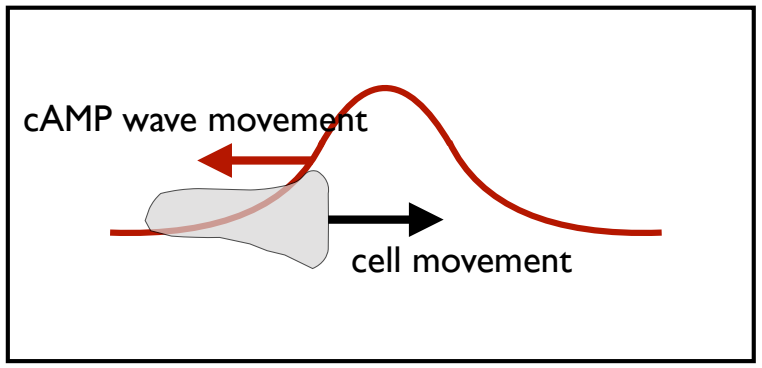


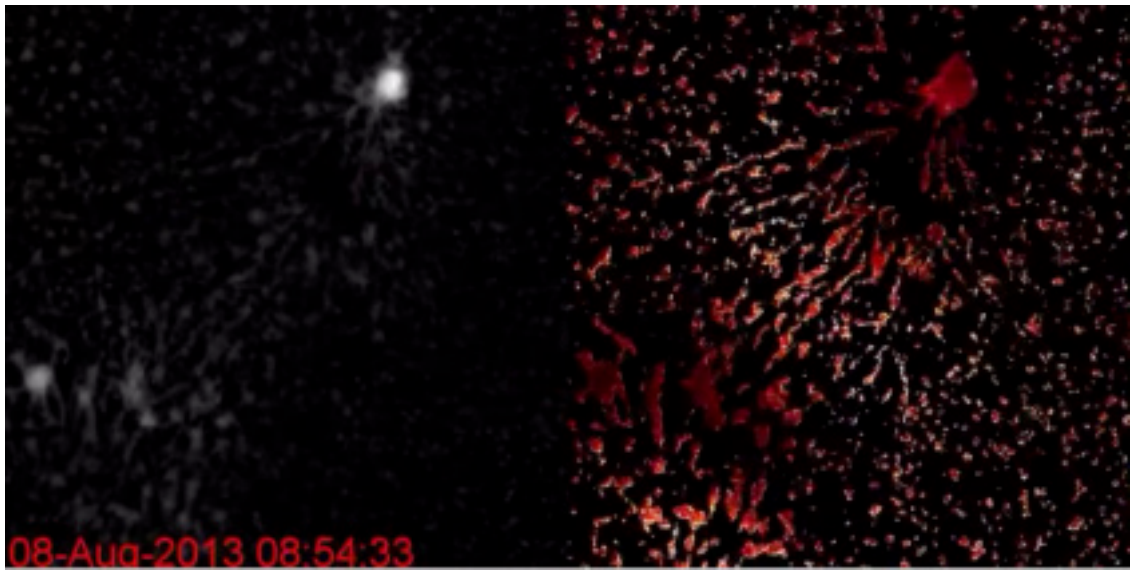
Life cycle



https://www.youtube.com/watch?v=oYRF7BaaaJY

MPI Dynamics and Self-Organisation (Goettingen) - Bodenschatz and Westendorf labs





Thomas Gregor lab - Institut Pasteur/ Princeton Univ

proxy of cAMP concentration via the heat colormap

Chemotaxis of the slime mold *Dictyostelium*

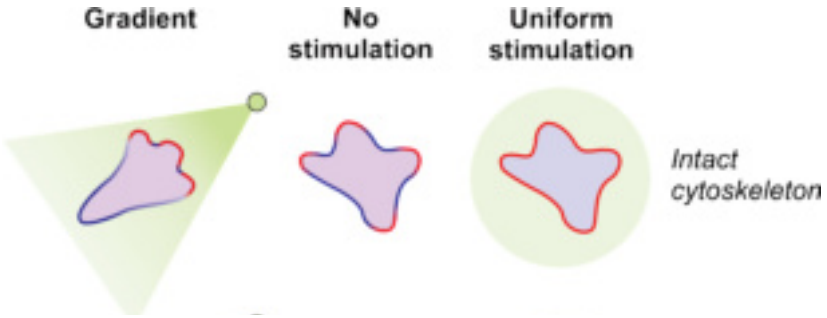
Cells respond to a gradient of cAMP

Carole Parent lab at the University of Michigan Life Sciences Institute.

Dictyostelium discoideum cells are attracted by cAMP released in a gradient from a pipette

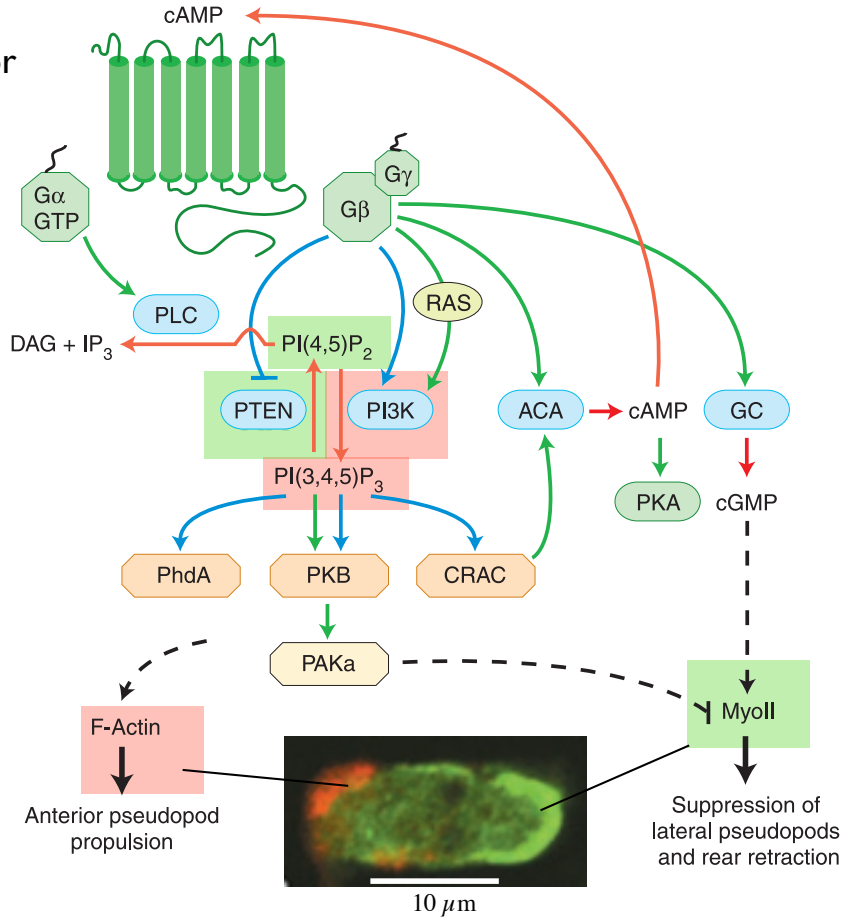
Chemotaxis induces cell polarization

Dictyostelium



Folate

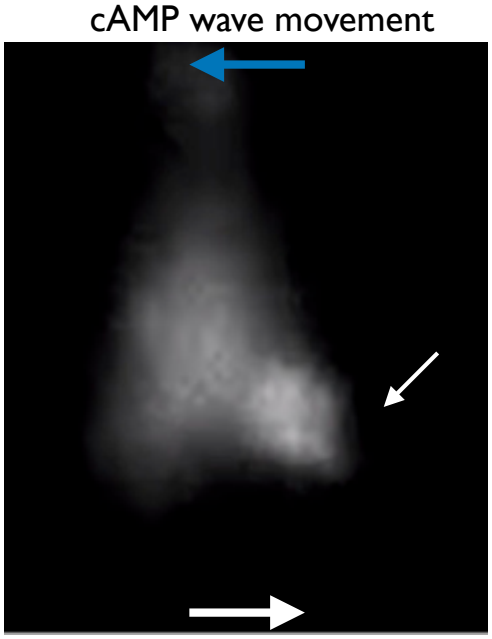
Folate receptor (GPCR)



AR. Kimmel and CA. Parent *Science* 300:1525-1527 (2003)

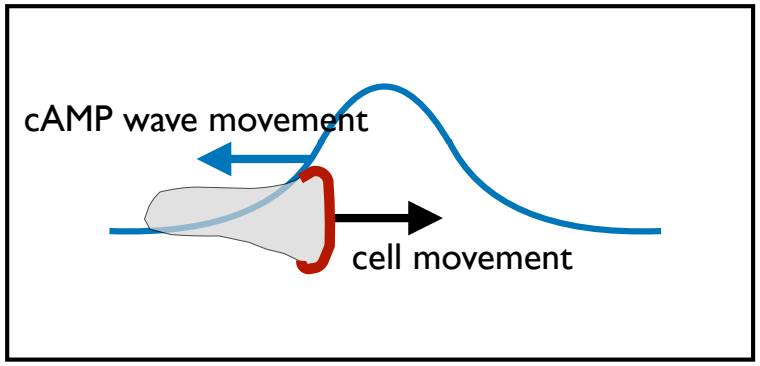
Chemotaxis induces cell polarization

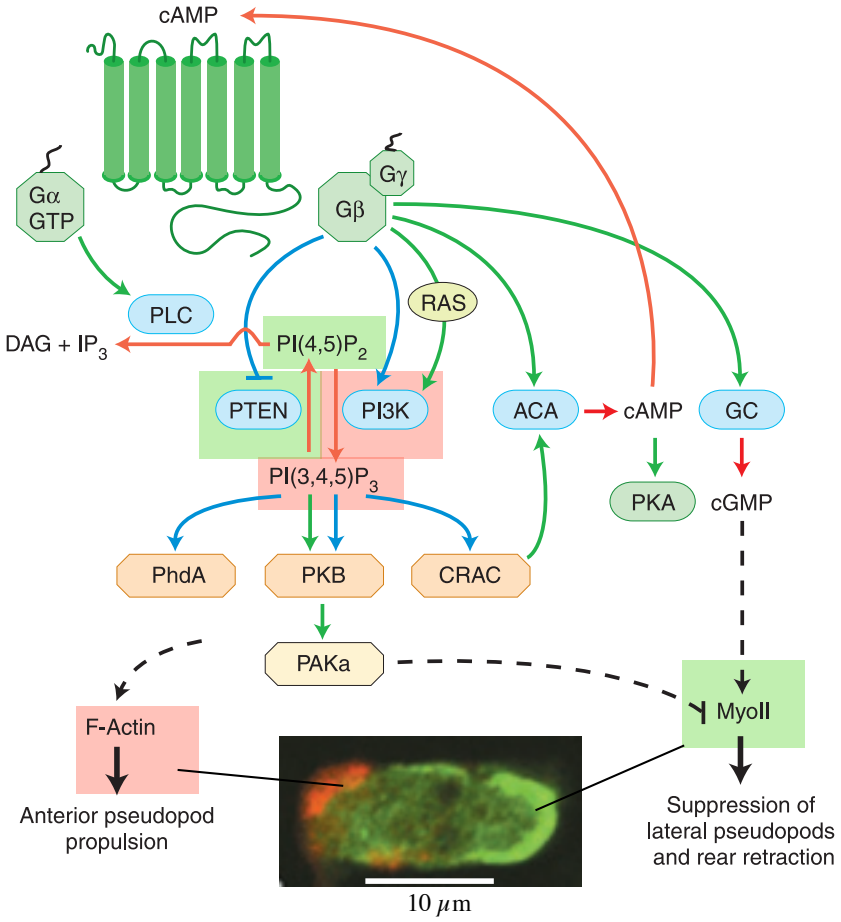
Dictyostelium



PIP_3 sensor
(PH-GFP)

P. Devreotes lab
(Johns Hopkins)





AR. Kimmel and CA. Parent *Science* 300:1525-1527 (2003)

The chemotactic response pathways

signal sensing

receptor

↓

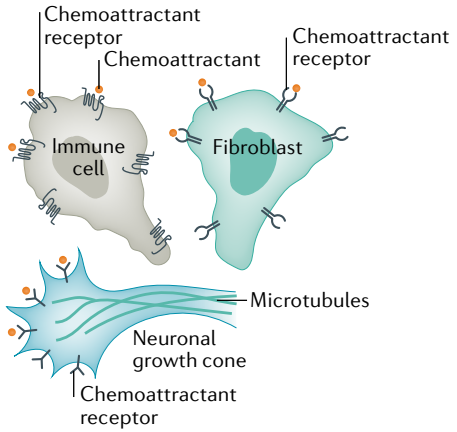
transduction network

↓

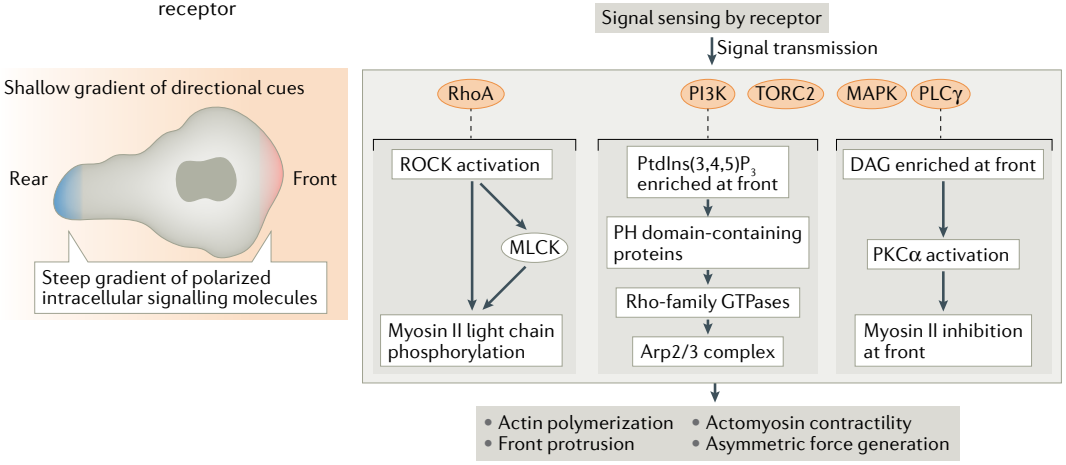
cell polarization

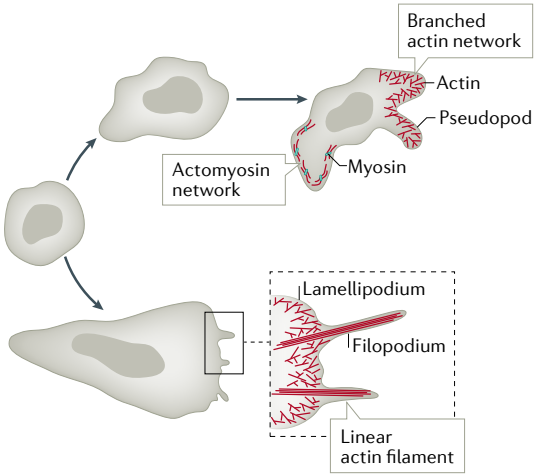
↓

mechanical response



S. SenGupta, C. A. Parent and J. E. Bear, *Nature Rev Mol. Cell Biol.* (2021)





Cell polarization

signal sensing

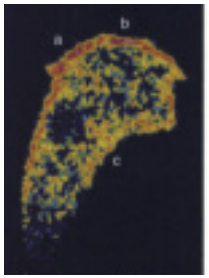
receptor

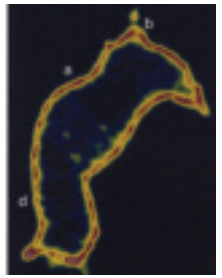
transduction network

cell polarization

mechanical response

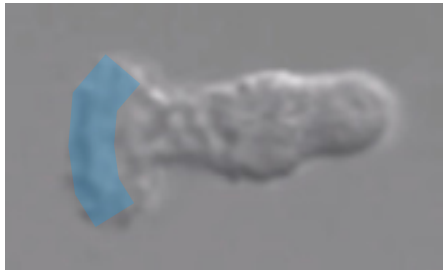
Gβ

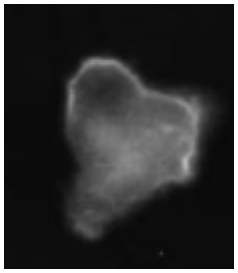




Gβ-GFP cAR1-GFP

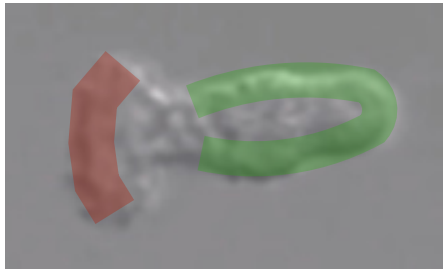
PIP_3





AktPH-GFP

F-actin



Myosin2

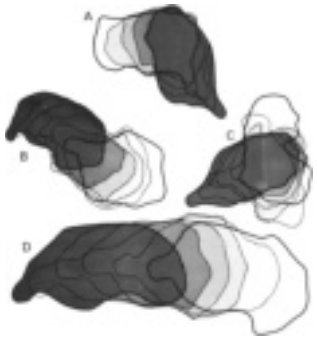
T. Jin. et al. P. Devreotes *Science*18:2092–2105 (1999)
Meili et al, R.A. Firtel. *EMBO Journal* 18:2092–2105 (1999)

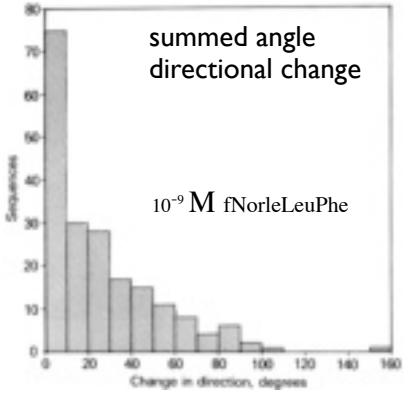
Cell polarity affects sensitivity to chemical gradient

- Cells exhibit a behavioral polarity, i.e. their capacity to respond to a chemical cue, which reflects/depends on their morphological polarity/locomotion

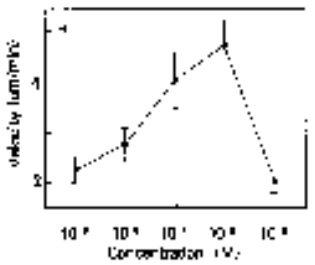
1. **Cell persistence** in moving cells with uniform chemoattractant:
   —Cell protrusions form near preexisting ones

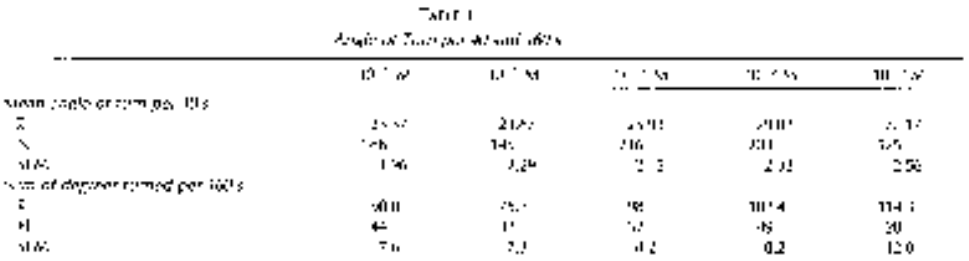
10^{-7} M fNorleLeuPhe



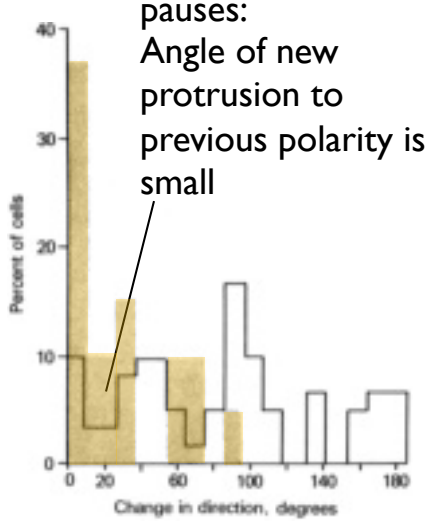


2. Increasing the concentration of chemoattractant increases the production of protrusion at the front (but the frequency of turn does not change)





Resumption of polarity after cell pauses:
Angle of new protrusion to previous polarity is small



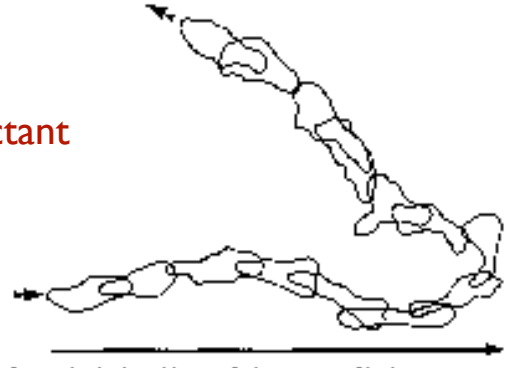
S. Zygmond et al. *JCB*. 89: 585-592 (1981)

Cell polarity affects cells sensitivity to chemical gradient

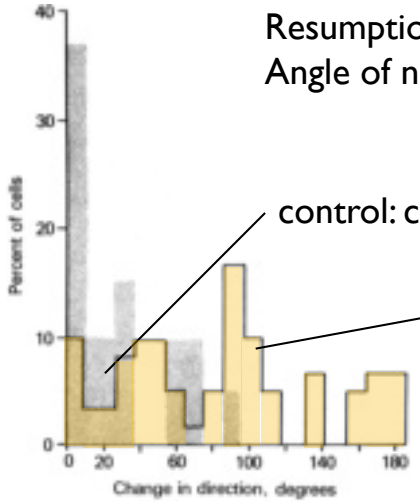
- Cell exhibit a behavioral polarity, their capacity to respond to a chemical cue, which reflects/depends on their morphological polarity/locomotion

3. Cells U-turn when an opposite gradient is formed, instead of formation of pseudopod at the back

The trailing edge (uropod) is insensitive to chemoattractant



Migrating neutrophils (PMNs) exhibit persistent motility, and their morphological polarity (eg. front-back cytoskeletal polarity for instance, contractile at the back and protrusive at the front) biases their capacity to repolarise in response to external chemical cues.



Resumption of polarity after pause:
Angle of new protrusion to previous polarity

control: cell kept polarity

cells rounded up and lost trailing edge (uropod)

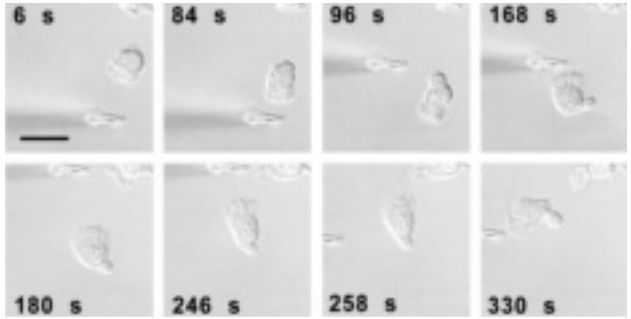
S. Zygmond et al. *JCB*. 89: 585-592 (1981)

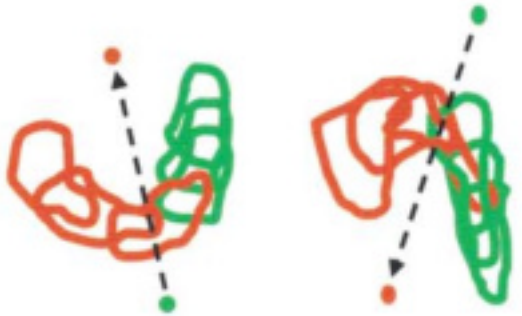
Cell polarity affects cells sensitivity to chemical gradient

- Insensitivity of the Neutrophils' trailing edge to chemoattractant is prevented by inhibition of MyosinII activation

control

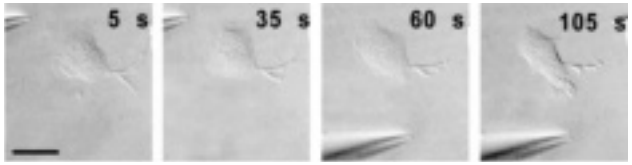
F-actin
MyosinII

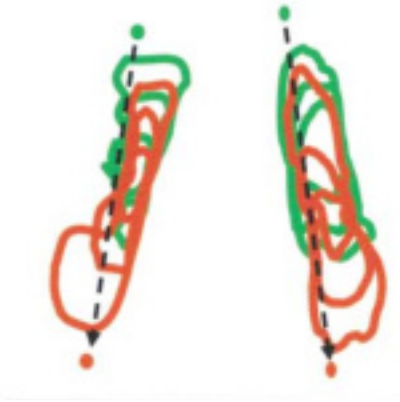




Rock inhibition
(>MyosinII inhibition)

F-actin
MyosinII

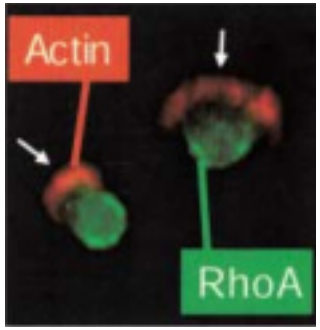


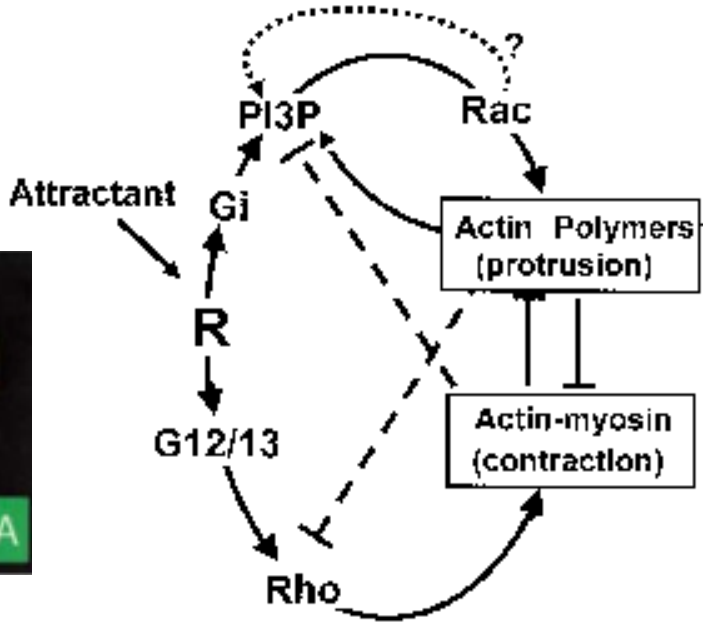


J. Xu et al. T. Mitchison and HR. Bourne. *Cell*, 114, 201–214 (2003)

Self-organizing cell polarity

- Actin cytoskeleton assemblies (contractile and protrusive networks) are not simple readouts of cell polarity, but feedback on cell polarity and sensitivity to chemoattractant
- Amplification of signal detection and processing





Frontness

Backness

Mechanochemical Turing like instability

Local excitation (positive feedback loop)

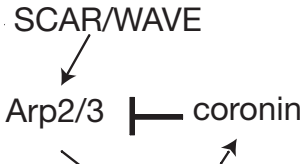
Long range inhibition

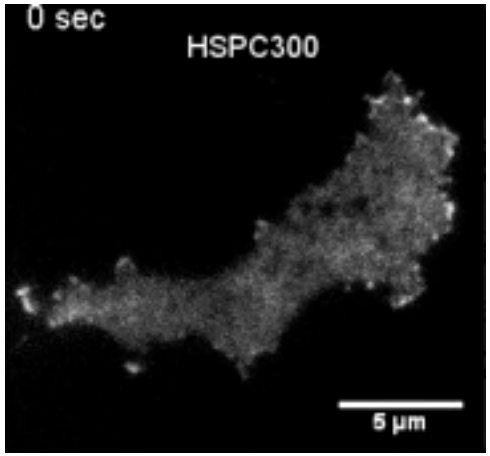
J. Xu et al. T. Mitchison and HR. Bourne. *Cell*, 114, 201–214 (2003)

Self-organizing cell polarity

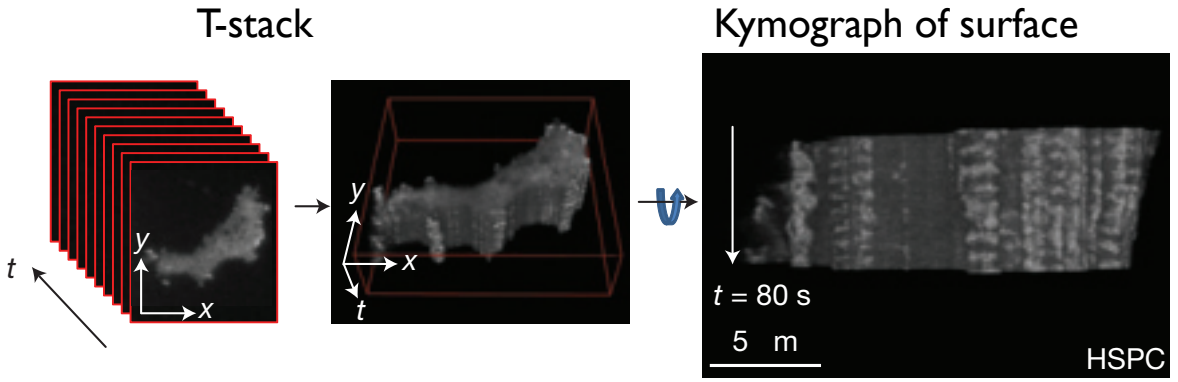
Oscillatory actin dynamics at the leading edge

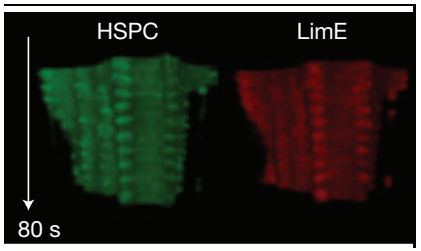
- Actin polymerization at the leading edge is dynamic in space and time (waves and oscillations)
- This is a fast oscillator (5-10s period)

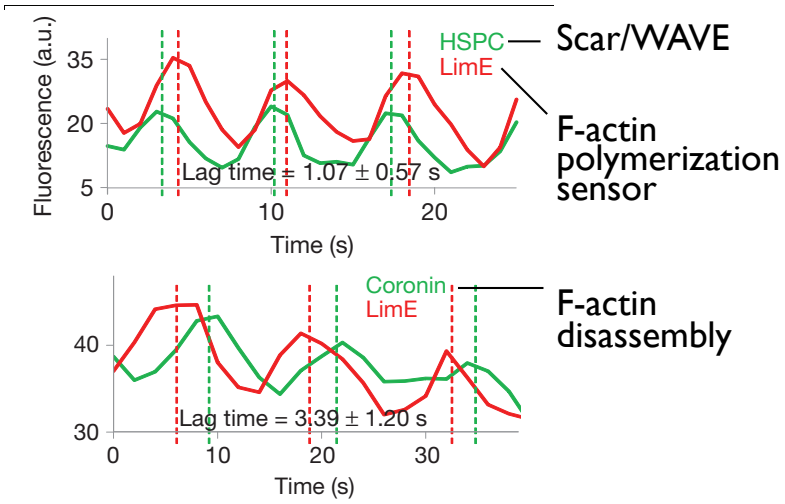




Dictyostelium cells



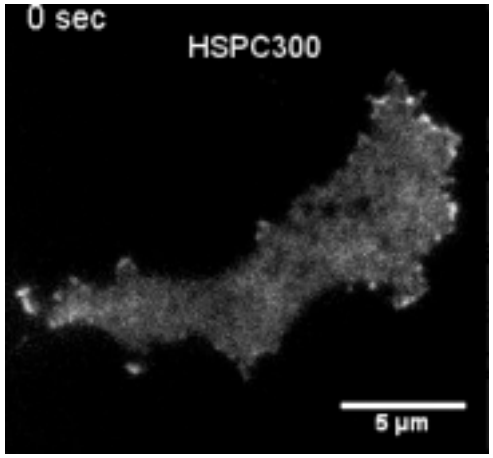


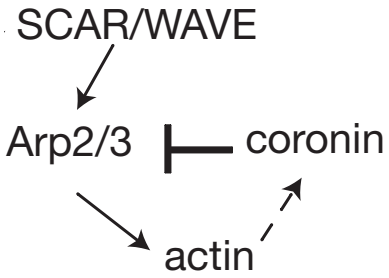


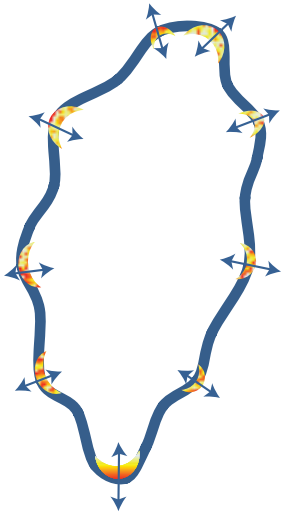
C.H. Huang et al. P. Iglesias and P. Devreotes. *Nat Cell Biol*. 15(11):1307-16 (2013)

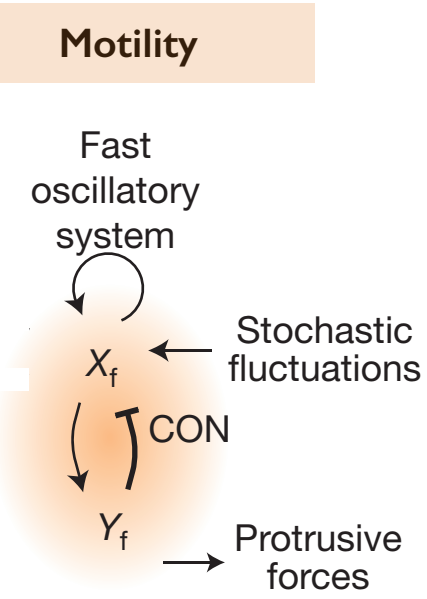
Self-organizing cell polarity

Cytoskeletal Oscillatory Network - Fast







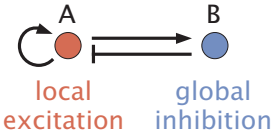


Fast oscillator underlies small undulations to the cell boundaries and behaves as an **idling motor system**

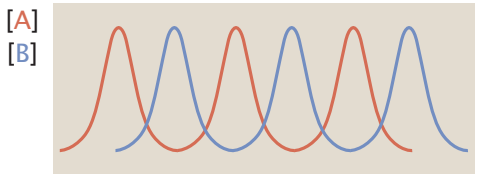
C.H. Huang et al. P. Iglesias and P. Devreotes. *Nat Cell Biol*. 15(11):1307-16 (2013)

Excitability: Bistability, Excitability and Oscillations.

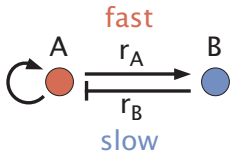
Spatial instability



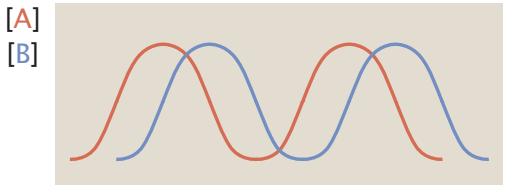
$D_A < D_B$

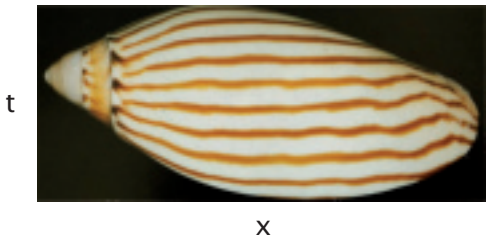


Temporal instability



$r_A > r_B$





(Note: the shells are a formal analogy, patterns are not driven by chemical instability, but by neural network Turing instabilities)

Hans Meinhardt. *The algorithmic beauty of sea shells*. Ed. *Springer-Verlag* (2009)

Boettiger A, Ermentrout B, Oster G. *The neural origins of shell structure and pattern in aquatic mollusks*. *PNAS*. 106(16):6837-42 (2009)

Self-organization in nonequilibrium chemical systems:

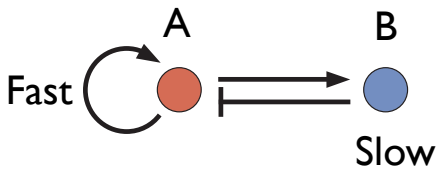
- activator auto-activation
- inhibitor induction

Decay rate:

the decay rate of inhibitor must be lower than that other activator ($r_b < r_a$)

Existence of refractory period: time needed to clear inhibitor (or to re-synthesise depleted substrate)

Excitability: Bistability, Excitability and Oscillations.



- FitzHugh-Nagumo Model :

(heuristic model adapted for study of action potential)

$$\frac{dA}{dt} = A - A^3 - B$$

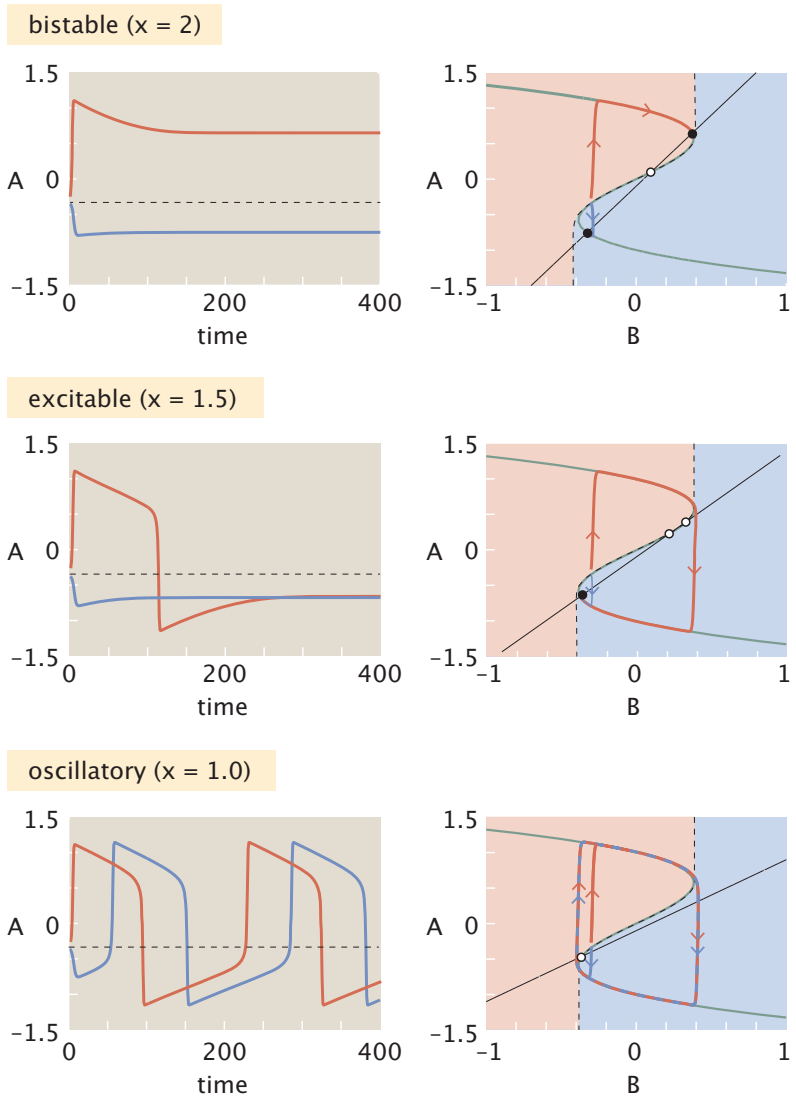
Fast reaction

$$\frac{dB}{dt} = \varepsilon(A - xB + y)$$

Slow reaction

($\varepsilon << 1$)

(nullclines in AB plane: steady state response of A to B and of B to A)



L. Gelens, G.A. Anderson and James Ferrel. *MBoC* 25:3486-3493. 2014

Excitability: Bistability, Excitability and Oscillations.

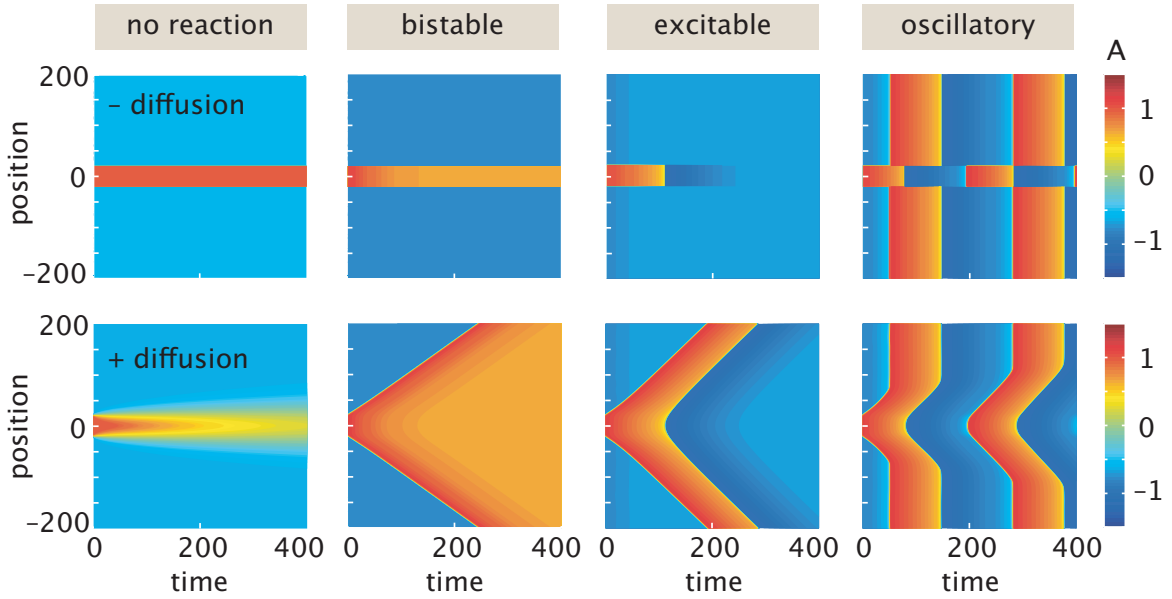
Spatial temporal patterns of activity: Trigger waves

- Spatial coupling by diffusion — Synchronization

$$\frac{\partial A}{\partial t} = D\frac{\partial^2 A}{\partial x^2} + A - A^3 - B$$

$$\frac{\partial B}{\partial t} = D\frac{\partial^2 B}{\partial x^2} + \varepsilon(A - xB + y)$$

diffusion reaction

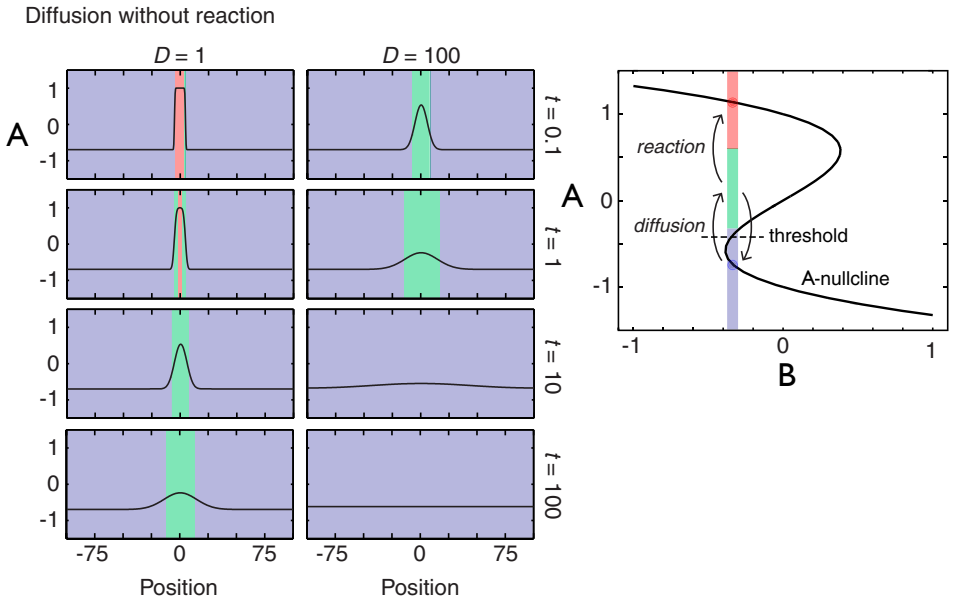


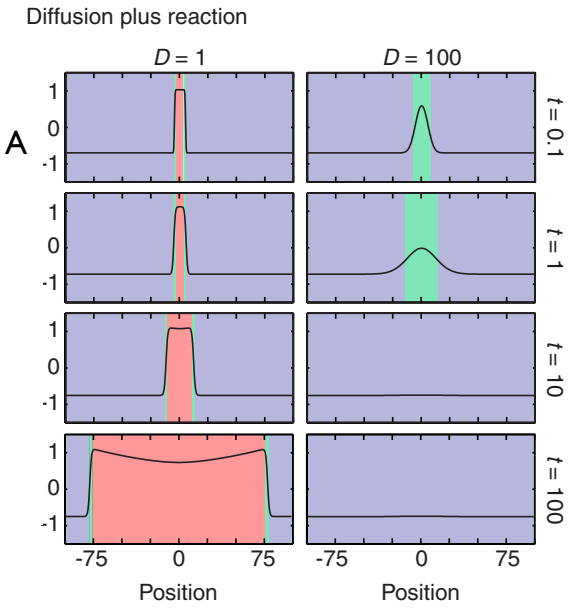
L. Gelens, G.A. Anderson and James Ferrel. *MBoC* 25:3486-3493. 2014

Excitability: Bistability, Excitability and Oscillations.

Spatial temporal patterns of activity: Trigger waves

- Diffusion is a mechanism for crossing the threshold in space





- a low A value state will increase its A concentration due to diffusion of nearby high A state but will relax back
- Higher diffusion increases rate of local propagation but makes it more transient

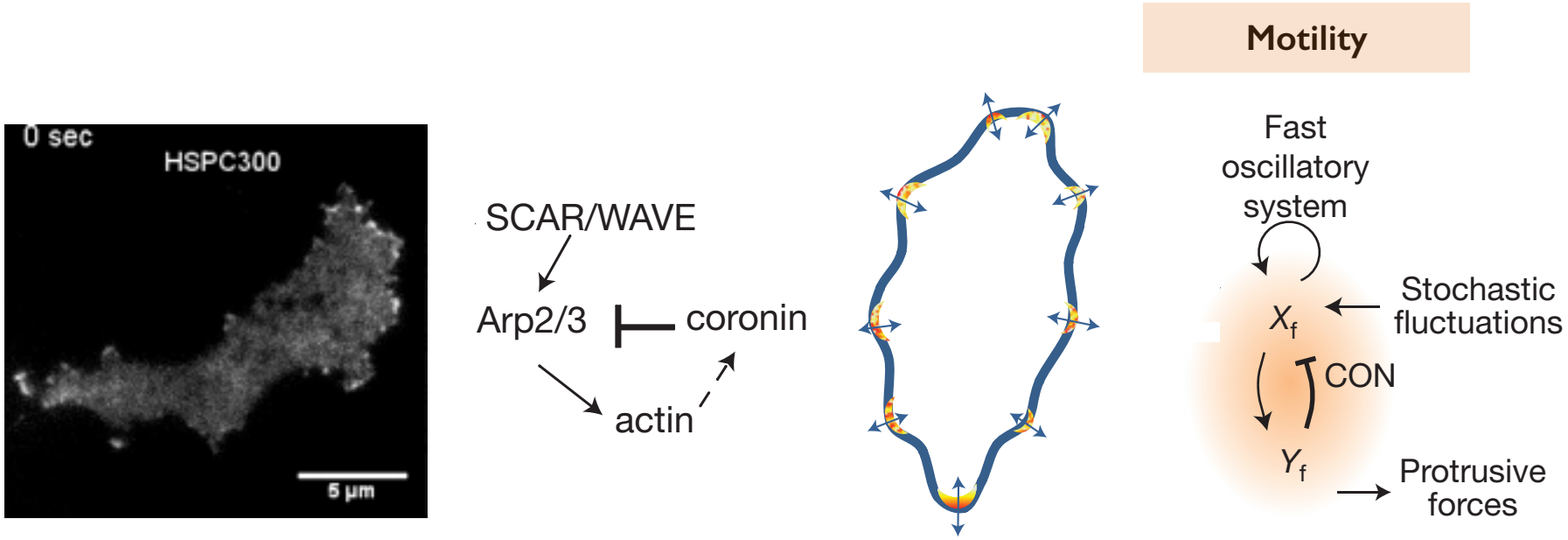
- The presence of a reaction captures supra-threshold state and move to high A state
- This depends on the relative time scale of diffusion and reaction

- Speed: $s = 2\sqrt{\frac{D}{\tau}}$ doubling time of + feedback

L. Gelens, G.A. Anderson and James Ferrel. *MBoC* 25:3486-3493. 2014

Self-organizing cell polarity

Cytoskeletal Oscillatory Network - Fast



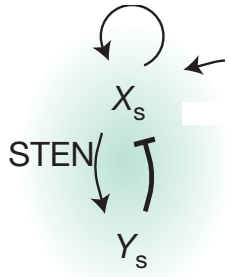
Fast oscillator underlies small undulations to the cell boundaries and behaves as an idling motor system

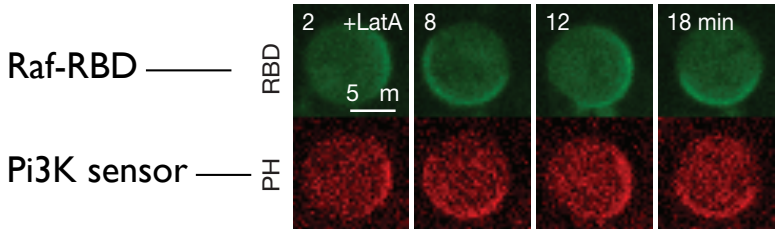
C.H. Huang et al. P. Iglesias and P. Devreotes. *Nat Cell Biol*. 15(11):1307-16 (2013)

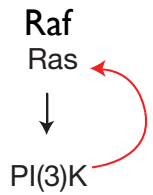
Self-organizing cell polarity

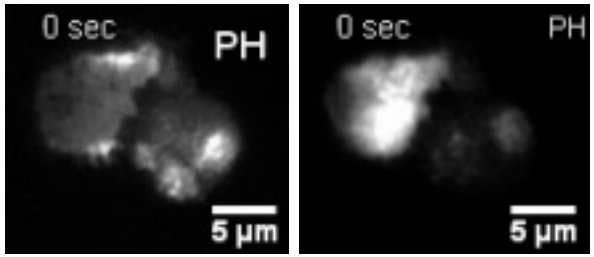
Slow excitable transduction network of polarisation

- **Signatures of excitable system:**
- Wave dynamics of **directional sensing network** (in absence of actin)



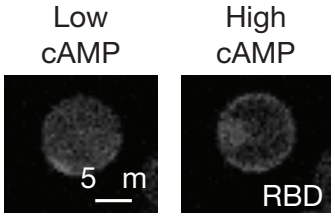






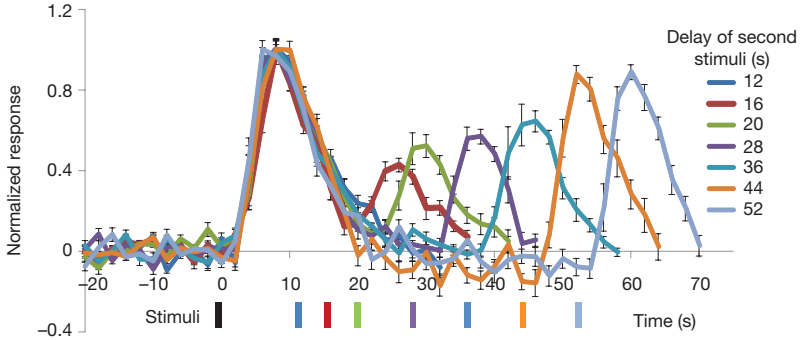
Pi3K sensor

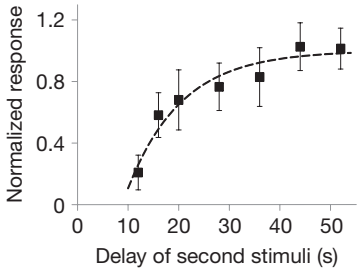
- Low intensity stimulation gives maximal response



Intensity ratio: 1.3 ± 0.2
(*n* = 7)

- Refractory period of ~9s:
  — pair of stimulations at 2 successive time points. The amplitude of the response to second cAMP stimulation increases with time delay





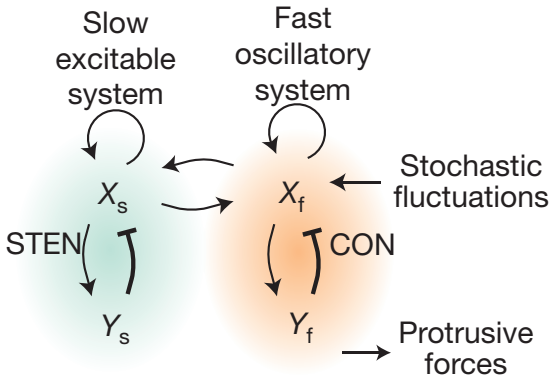
C.H. Huang et al. P. Iglesias and P. Devreotes. *Nat Cell Biol*. 15(11):1307-16 (2013)

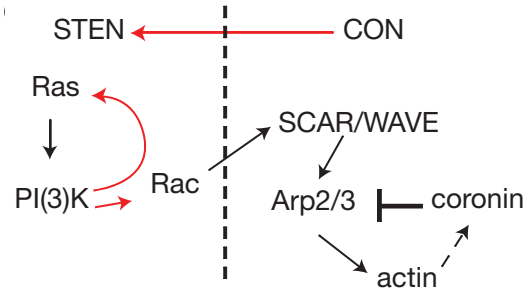
Self-organizing cell polarity

Coupling between two excitable networks

Directional sensing

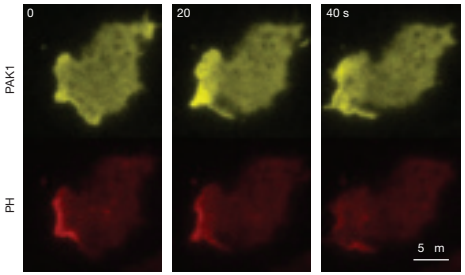
Motility

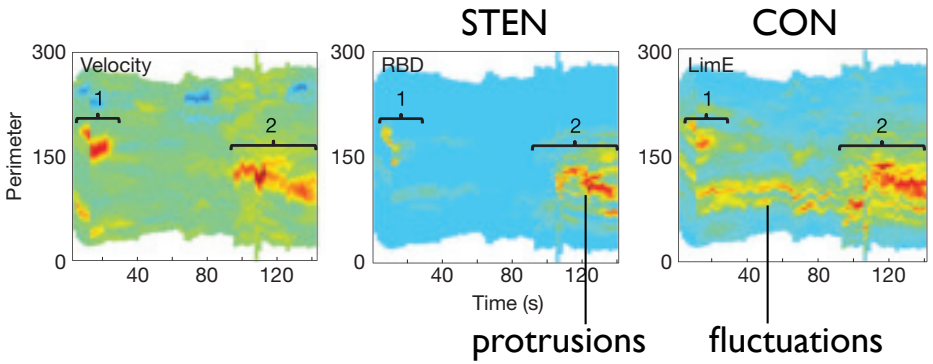




output of
STEN: ~Rac1

STEN: ~PI(3)K



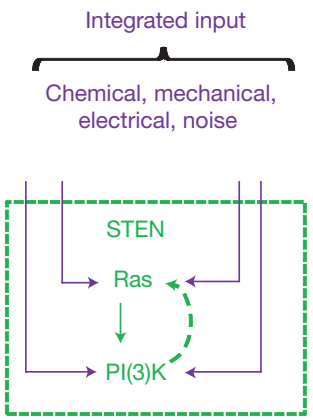


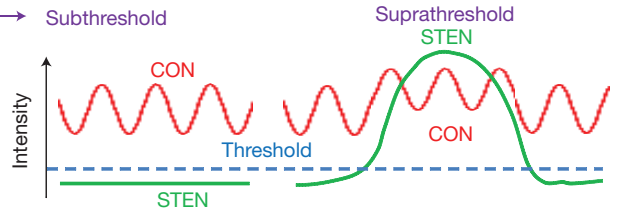
STEN and CON are correlated in cell protrusions but not
In small fluctuations of the plasma membrane

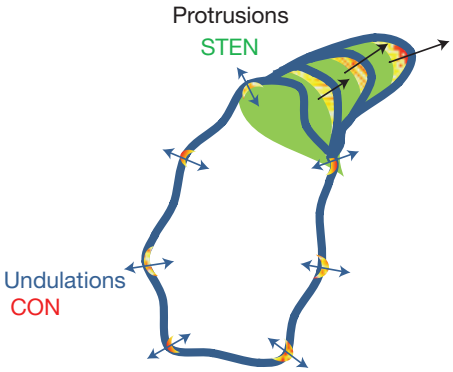
C.H. Huang et al. P. Iglesias and P. Devreotes. *Nat Cell Biol*. 15(11):1307-16 (2013)

Self-organizing cell polarity

Coupling between two excitable networks



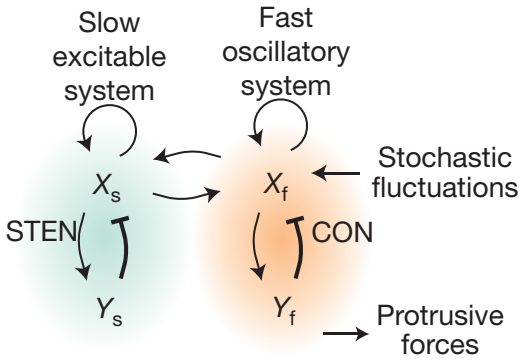


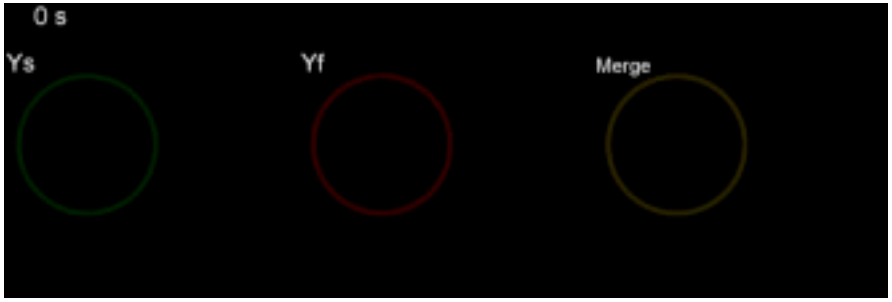


- Integrated inputs, external chemical signals and internal noise, bias components of STEN and cause STEN to pass the threshold of activation
- CON is entrained to a larger more stable state associated with actin polymerization and cell protrusion

Directional sensing

Motility





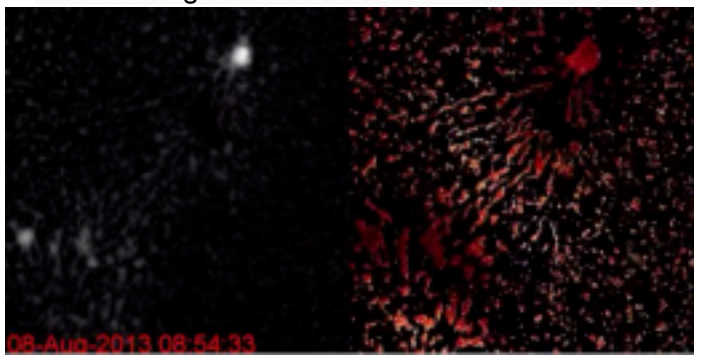
- Activation of the STEN by stochastic noise underlies random cell motility
- A cue that affects the threshold for excitation differently on opposite sides of a cell would be expected to guide cell migration. (e.g., inhibitor of PI(3)K can act as chemorepellent)

C.H. Huang et al. P. Iglesias and P. Devreotes. *Nat Cell Biol*. 15(11):1307-16 (2013)

Excitability, *memory* and *persistence* of motility

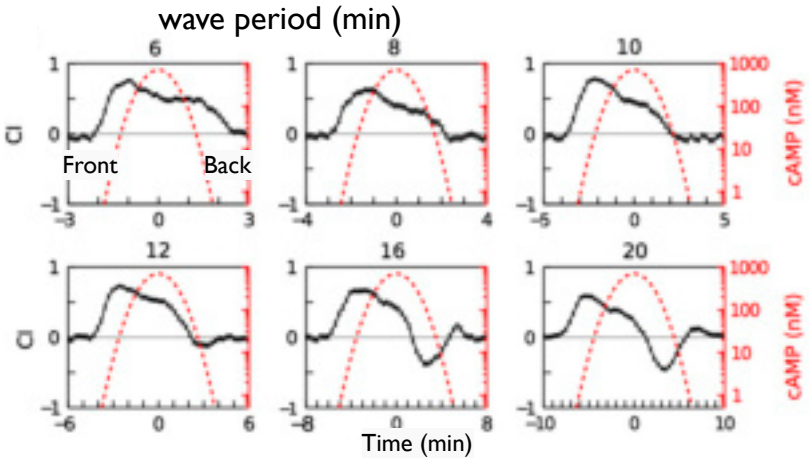
- Cells are exposed to a dynamic gradient of cAMP
- **Why don't cells move backward in the back of the wave?**
- This is not by temporal gradient measurement but by a mechanism of **cell memory**

Thomas Gregor lab - Institut Pasteur/ Princeton Univ



proxy of cAMP concentration via the heat colormap

- When exposed to a dynamic gradient in microfluidic chamber, *Dictyostelium* cells show 9 min persistence of motility and polarity: the chemotactic index is lower but positive in the back of the wave up to a wave period T~10 min. This persistence of migration is indicative of a memory with a timescale similar to the *Dictyostelium* wave period (~6 min)

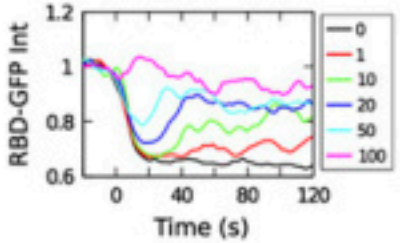


mean concentration of 50 nM with a relative spatial gradient of 17% across 10 µm

chemotactic index $CI = V_x/V$,

- **Cell memory and persistence** can be explained by refractory period intrinsic to excitable dynamics of polarity network: time scale of degradation of inhibitor.
- Cells are persistent when exposed to rapid and abrupt changes in chemoattractant, but depolarize in presence of slow, shallow gradient changes

Raf-GFP polarized recruitment is maintained when cells in a 0-100nM gradient are exposed to a uniform concentration of cAMP equal or greater than the concentration they had in the gradient



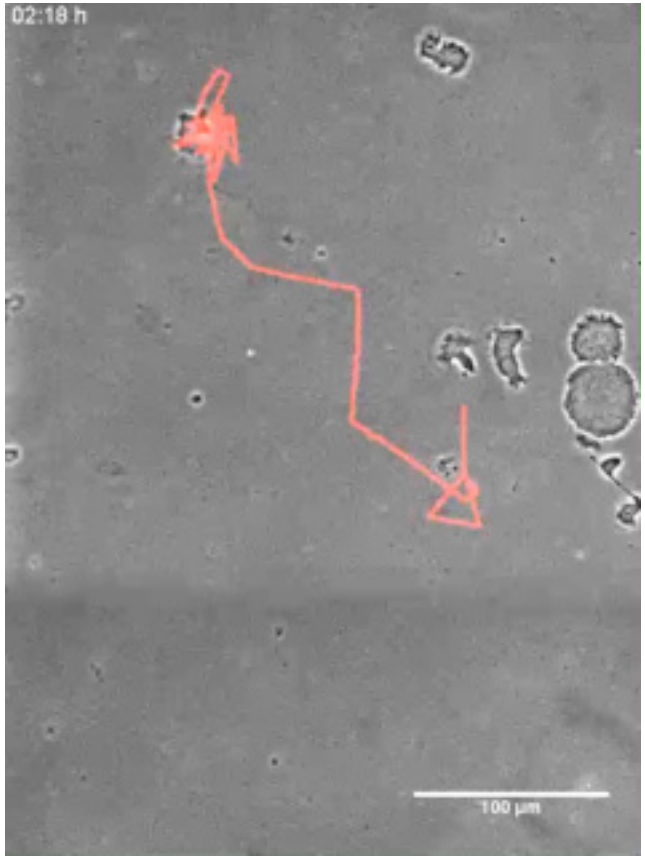
Skoge M, et al. (2014) *PNAS* 111(40):14448–14453 (2014)

Persistent random walk underlies eukaryotic chemotaxis

- Chemotaxis in Eukaryotic cells change the lifetime of persistent motility
- The default state is a random walk
- Chemotaxis causes a biased, persistent random walk

—*Dictyostelium*: In uniform chemoattractant, cells undergo a random walk with a persistence time of ~3–10 min

—In absence of chemoattractant, bone marrow derived dendritic cells (BMDCs) have variable persistence time



- What underlies the variable persistence time in motility?

P. Maiuri, JF. Rupprecht, ..., M. Sixt, R. Voituriez 2015. *Cell 161*, 374–386

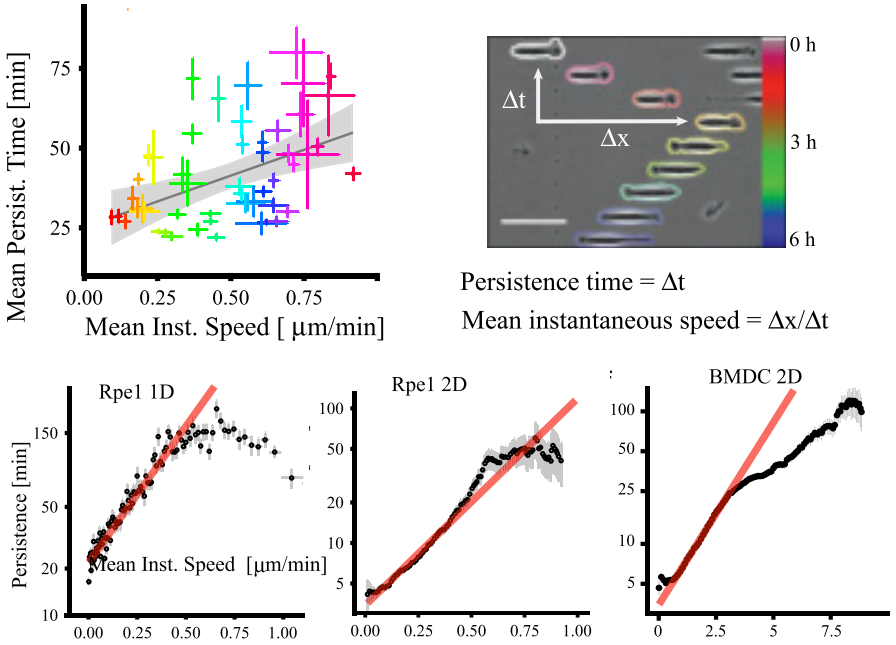
What underlies variable persistence?

Persistence is coupled to cell speed

In all conditions, 1D, 2D, 3D, on adhesive or non-adhesive substrates, cells consistently show the same relation between persistence time and speed. It applies to mesenchymal and amoeboid motility

$$\tau = Ae^{\lambda v}$$

persistence — speed

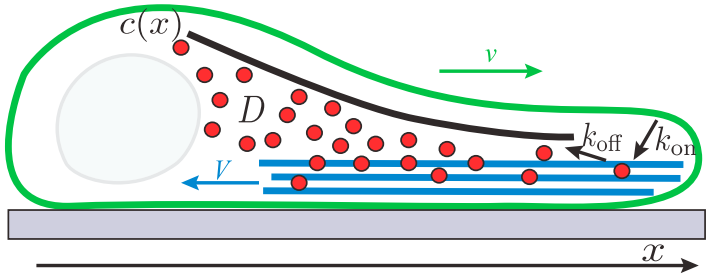


Bone Marrow derived Dendritic Cells (BMDC)
Retinal pigment epithelial cells (Rpe)

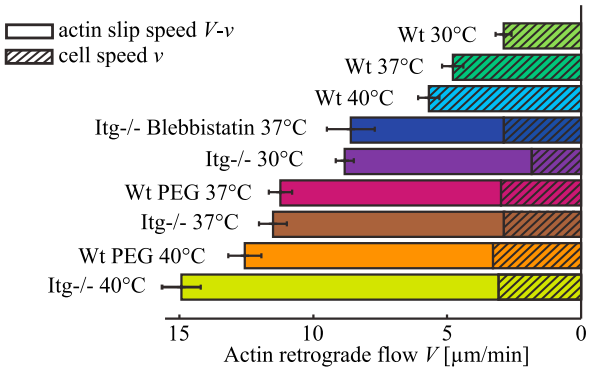
P. Maiuri, JF. Rupprecht, ..., M. Sixt, R. Voituriez 2015. *Cell 161*, 374–386

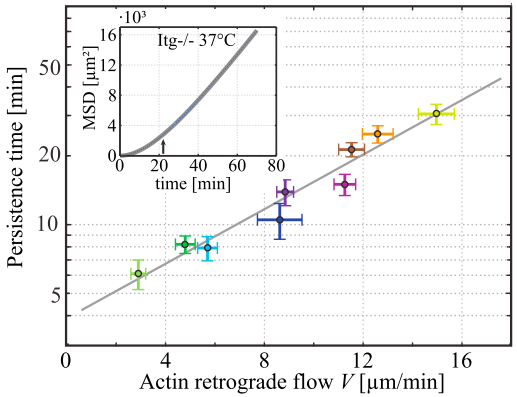
Cell persistence is coupled to actin flow speed

- The most commun feature of adhesive and non-adhesive motility is the existence of an **actin retrograde flow** driven by actin polymerization at the front and depolymerization at the back mediated by MyosinII contractility.
- Frictional or adhesion based coupling to the substrate enables forward cell movement



- Experimental perturbations lead to different retrograde actin flow at *constant/similar cell motility*
- Across conditions, the persistence time of motility scales exponentially with actin flow speed.





$$\tau = A' e^{\lambda' V}$$

- Cell motility v and actin flow speed V are linearly proportional, but across conditions the constant of proportionality is different.

P. Maiuri, JF. Rupprecht, ..., M. Sixt, R. Voituriez 2015. *Cell 161*, 374–386

Cell persistence is coupled to actin flow speed

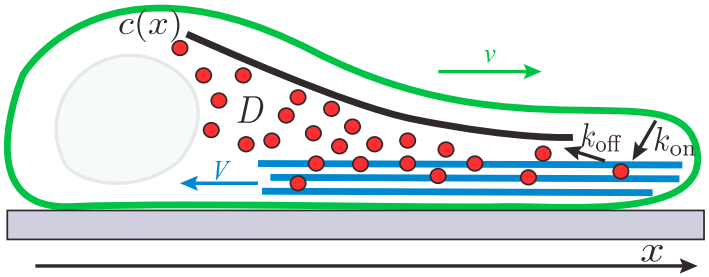
Advection of flow regulators

- actin flow reinforces cell polarity by enhancing the asymmetry of polarity cues by advection

$$\partial_t c(x,t) - \partial_x\left[\tilde{V}c(x,t)\right] = \tilde{D}\partial_x^2 c(x,t) + \partial_x \zeta_c,$$

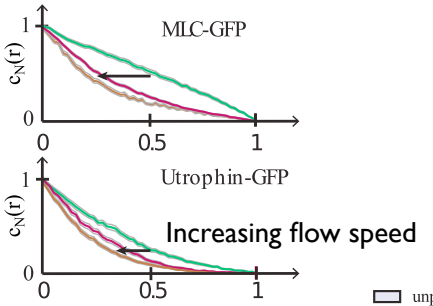
advection | diffusion | noise

$$\tilde{V} = Vk_{on}/(k_{on}+k_{off})$$
$$\tilde{D} = Dk_{off}/(k_{on}+k_{off})$$

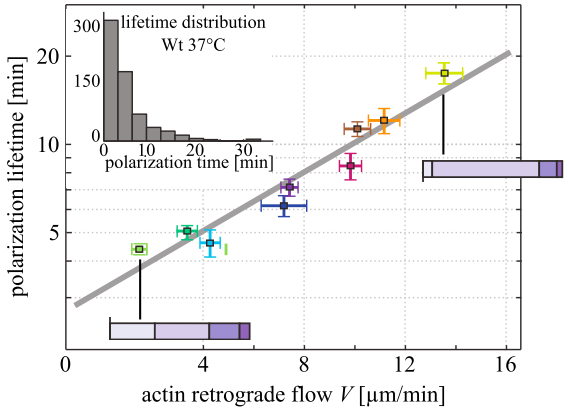


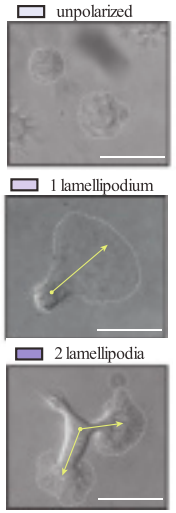
At steady state: $\overline{c}_V(x) = Ce^{-\tilde{V}x/\tilde{D}}$,

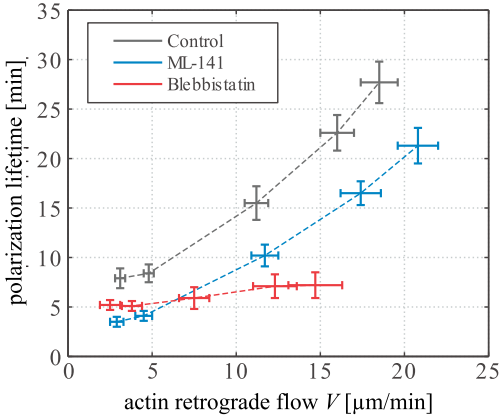
- Concentration profiles depend on retrograde flow



- Polarization lifetime is enhanced by retrograde flow And requires Myosin2 activity







P. Maiuri, JF. Rupprecht, ..., M. Sixt, R. Voituriez 2015. *Cell 161*, 374–386

A model of flow dynamics predicts 3 regimes of motility

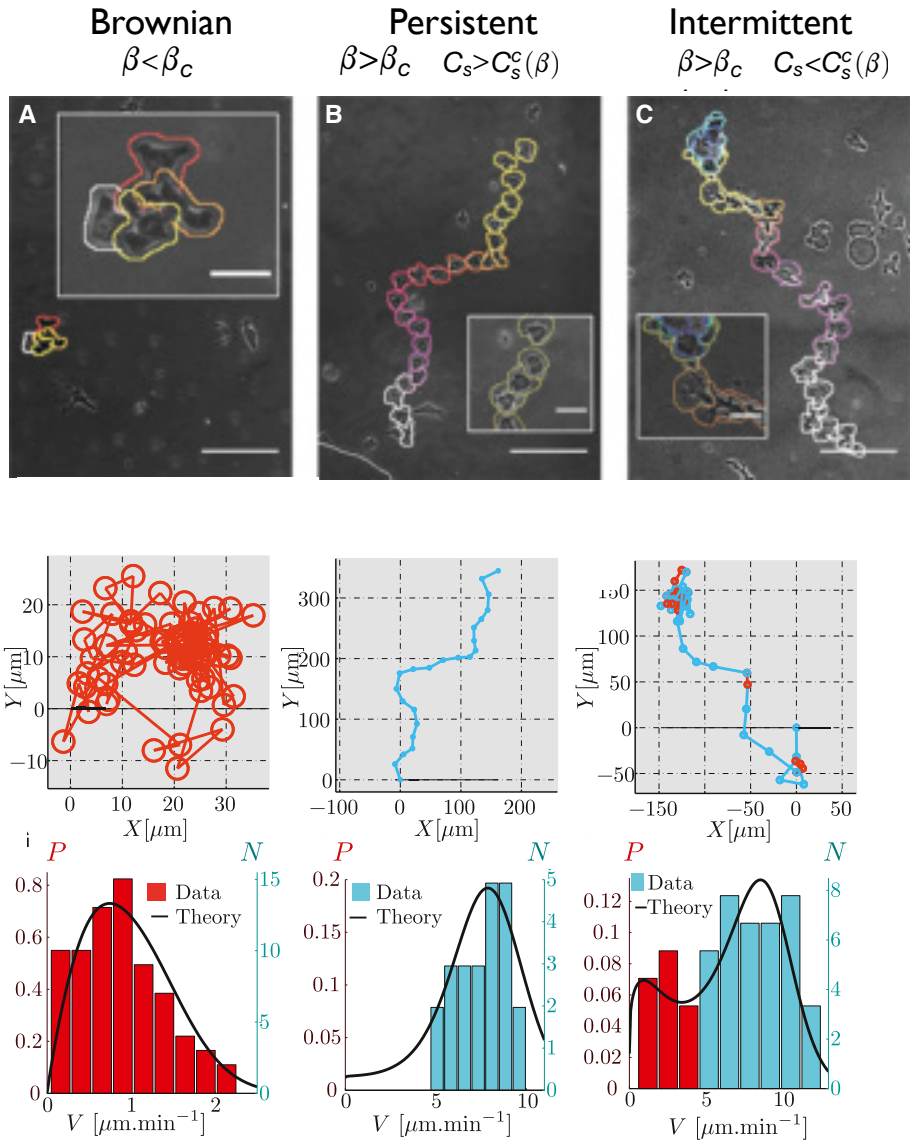
- The model which predicts the probability distribution of velocity, yields the exponential relation between velocity and polarity persistence time
- Prediction of 3 regimes based on 2 key parameters:

  —the coupling strength β_c between asymmetry of regulator $c(t)$ and flow speed V. $V^* = \beta(c^*(0,t) - c^*(L,t))$,

  —the concentration of cues C_s above which activity is saturated

$$c^*(x,t) = \frac{c^n(x,t)}{C_s^n + c^n(x,t)}.$$

- Experimental data are well fit by the model using these 2 free parameters:



P. Maiuri, JF. Rupprecht, ..., M. Sixt, R. Voituriez 2015. *Cell 161*, 374–386

Deterministic vs Self-organised Guidance

How do cells navigate over long range in situ (development, immune system, cancer)?

>>Interaction between cells and environment:

cells generate/modify their own guidance cue through such interactions

The structure of the environment matters (eg. confinement)

Decoding the chemical environment

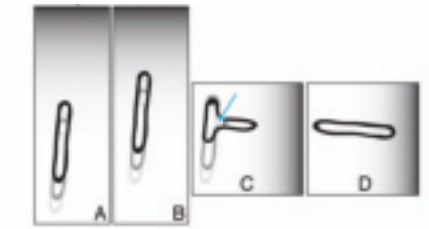
Precision

Deterministic vs Stochastic decoding

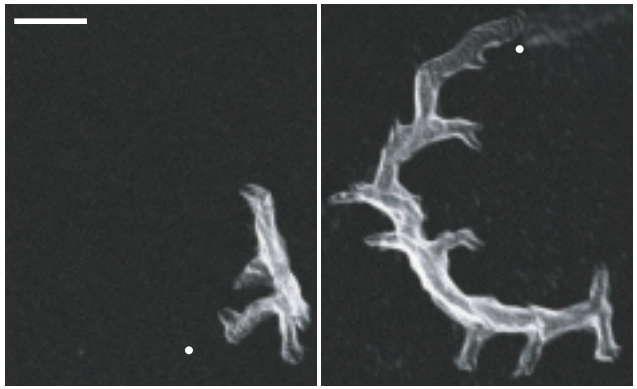
P. Maiuri, JF. Rupprecht, ..., M. Sixt, R. Voituriez 2015. *Cell 161*, 374–386

Deterministic vs Stochastic Guidance

Informed choice model

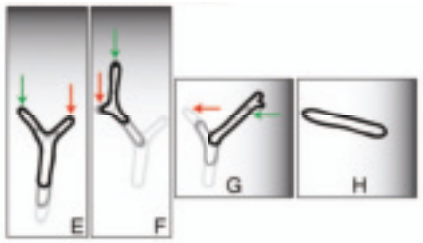


compass choice model

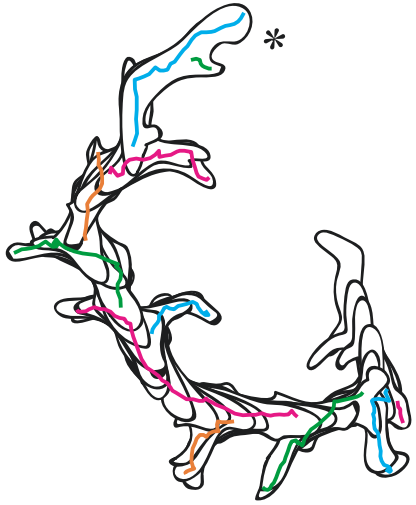


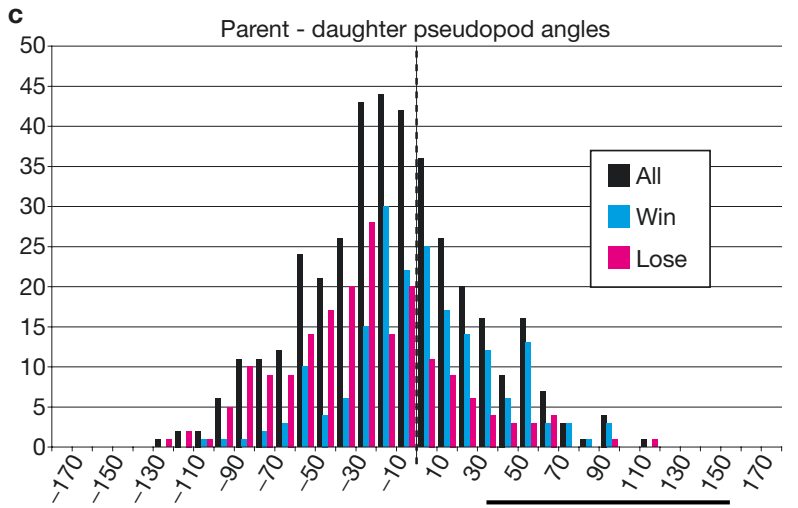
Dictyostelium discoideum

- Cells make many pseudopods are regular intervals and select the better ones up the gradient
- The pseudopod that are better aligned with gradient have an increased survival (bias for survival)



Informed choice model
Reinforcement of most up gradient protrusion





improves alignement with gradient

N. Andrew and R. Insall. *Nature Cell Biol*..2: 193-200 (2007)

Why do cells often breakdown chemoattractants?

Chemotactic cells have a variety of mechanisms for depleting attractants:

- Receptor-ligand endocytosis
- **Decoy receptors:** the CXCR7 receptor competes with CXCR4 for binding to the ligand SDF1 in the zebrafish lateral line. CXCR7 does not signal and traps the ligand.

  Dona E, et al. and D. Gilmour. *Nature* 2013, 503:285-289.
- **Cell surface enzymes that degrade attractants**

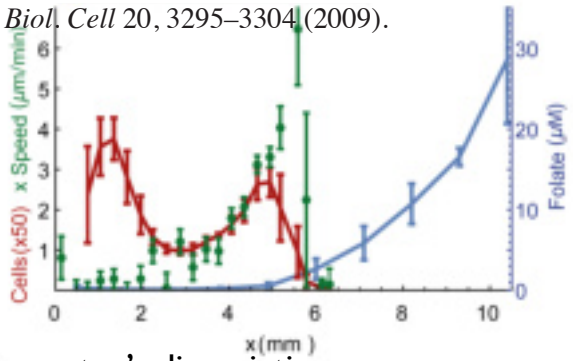
  Melanoma metastasis: lysophosphatidic acid (LPA) degradation in melanoma cells by Lipid Phosphatases

  O. Susanto et al., *J. Cell Sci*. 130, 3455–3466 (2017).

  Dictyostelium: cAMP chemoattractant is degraded by the extracellular phosphodiesteras PdsA

  G. L. Garcia, et al and C. A. Parent, *Mol. Biol. Cell* 20, 3295–3304 (2009).

- **Attractant breakdown leads to steep local, self-generated gradients**



Chemotaxis occurs most efficiently when the ligand is present at close to the receptor's dissociation constant (K_d). At substantially higher concentrations, the difference in receptor occupancy between the front and back of responding cells drops as the receptors become saturated, and chemotaxis becomes inefficient. Thus high attractant concentrations are incompatible with imposed gradients.

Self-generated gradients work best when the chemoattractant concentration is saturating, and cells break it down to a such a level that their can resolve the steepness locally.

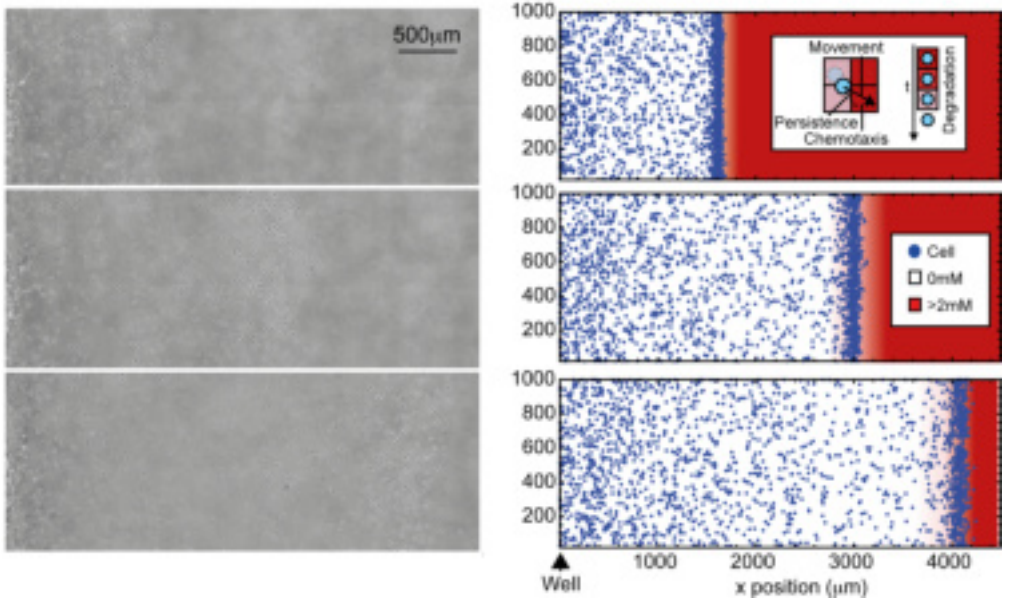
in *Dictyostelium*, strong chemotactic responses to cAMP occur with 10 μM of attractant, and the receptor K_d is in the nM range

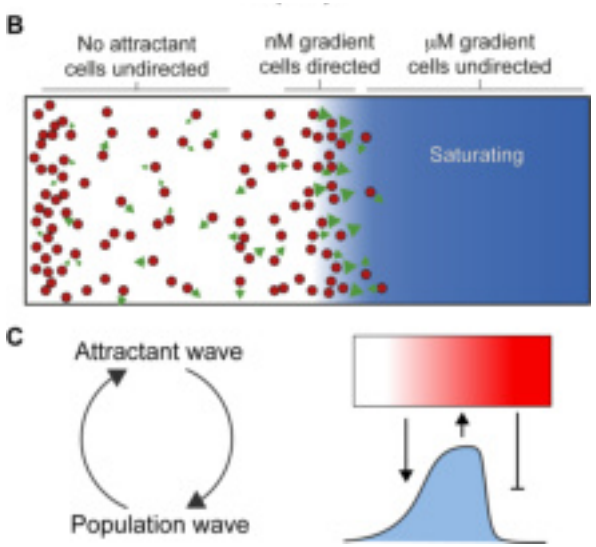
L. Tweedy, O. Susanto and R. Insall. *Current Opinion in Cell Biology* 42:46–51 (2016)

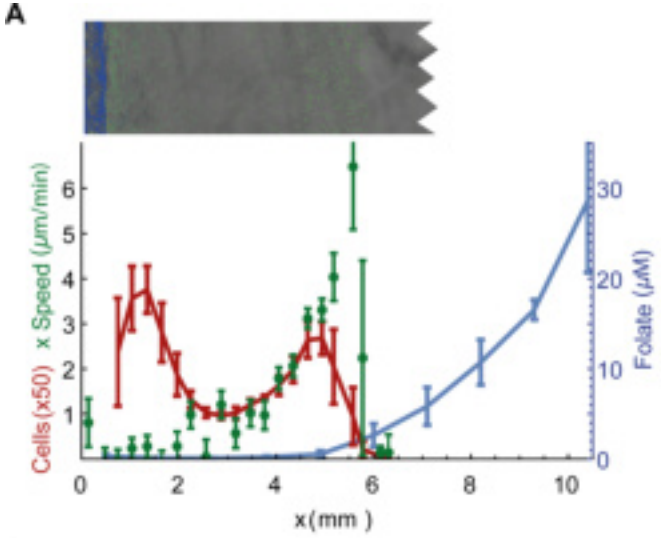
Tweedy L, Knecht DA, Mackay G, Insall RH: *PLOS Biol* 14:e1002404. (2016)

Self-generated gradients of chemotaxis

- Cells produce an activity that degrades the chemoattractant
- A **gradient of attractant is formed at the edge of cell cluster**, that steers cells forward leaving behind no attractant where cells have random motility.
- A front wave emerges that self-propagates
- **Self-reinforcing process:** if a few cells go pass the front, they will adopt random motility because they can't produce a new gradient of chemoattractant which is also saturating. If attractant diffuses behind the front, it will attract more cells







Tweedy L, Knecht DA, Mackay GM, Insall RH (2016) *PLOS Biology* | DOI:10.1371/journal.pbio.1002404

Self-generated versus passive chemotactic gradients

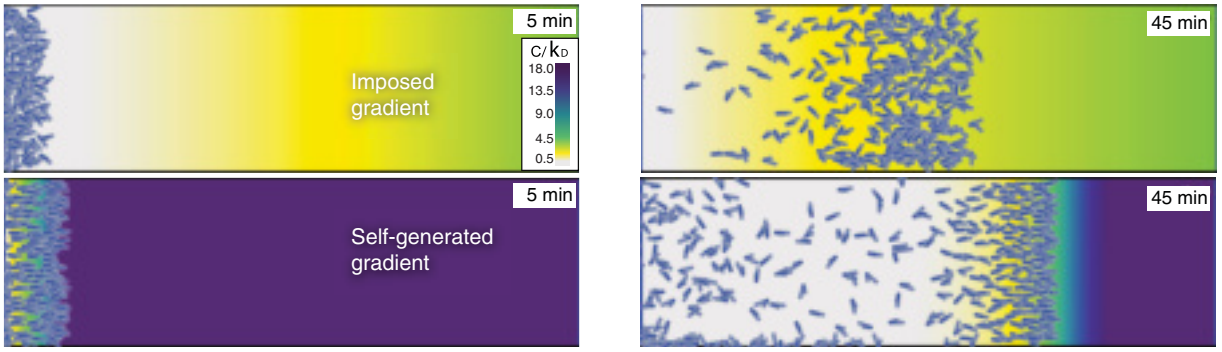
More robust migration in self-generated gradients

- **Simulations**

Imposed gradient:
— chemotaxis is not efficient because it is too shallow

Self-generated gradient:
— chemotaxis works best at near saturation of receptor binding, allowing formation of a steep local gradient

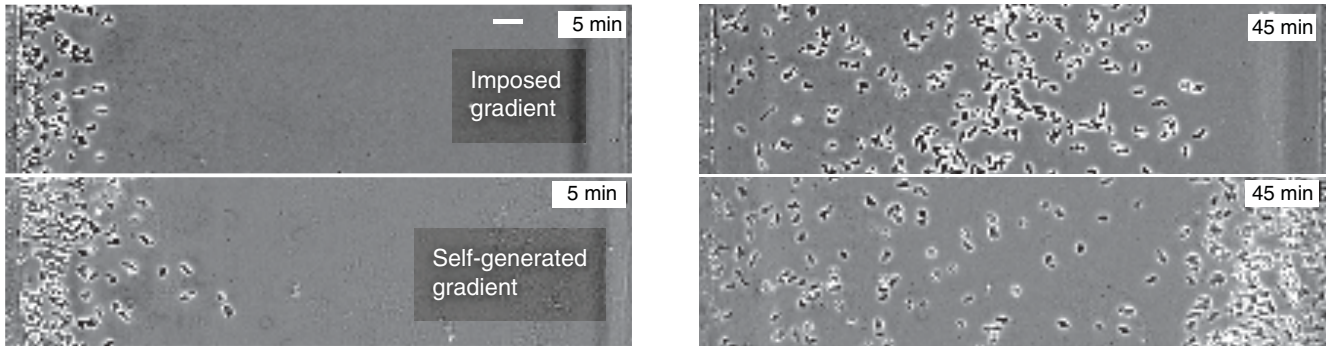
Persistent, biased random walk of cells in a bath with diffusion of attractant



- **Experiments**

Dictyostelium cells in a non degradable attractant Sp-cAMPS

cAMP gradient



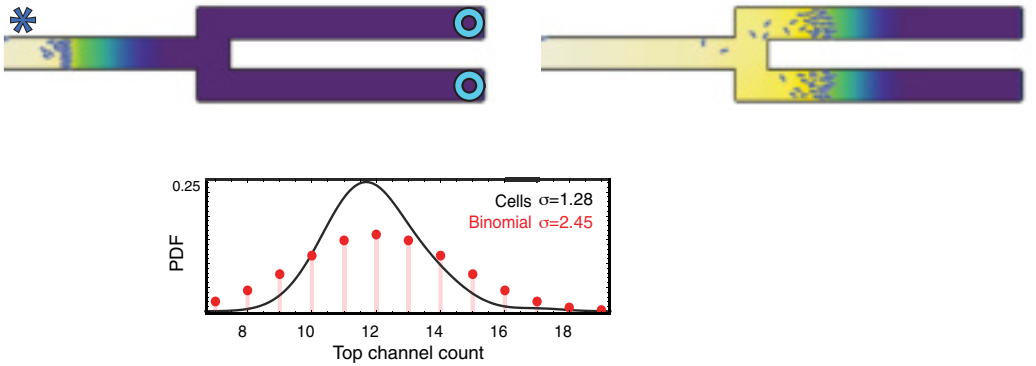
2.5 μM cAMP

L. Tweedy et al., and R. Insall. *Science* 369, 1075 (2020)
https://doi.org/10.1126/science.aay9792

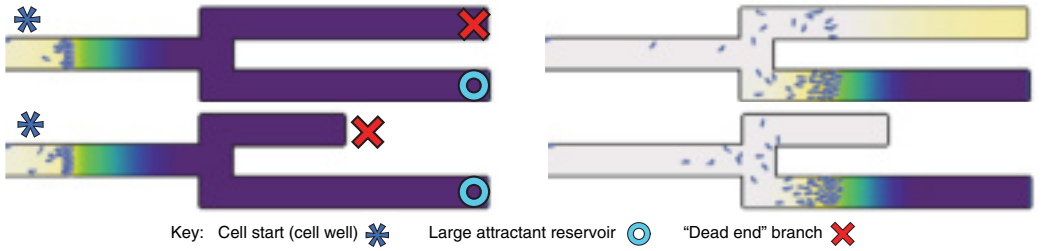
Pathfinding in a maze using self-generated gradients

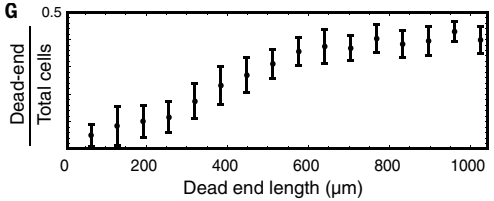
Cells detect and avoid dead ends

- When cells split between two free ends at a T-junction, they partition between the two channels with greater precision than expected by chance.
  If a cell enters a channel, the attractant will be degraded and this will feedback on the cells behind. So any imbalance in cell distribution will be corrected.
  In other words cells influence their followers



- Cells enter T-junctions and are given a choice between entering a Free or a Dead end.
- In a dead end, the chemoattractant is rapidly degraded and follower cells avoid the dead end.
  This results in a statistical bias in the distribution.
  This bias increases at the dead end is shorter



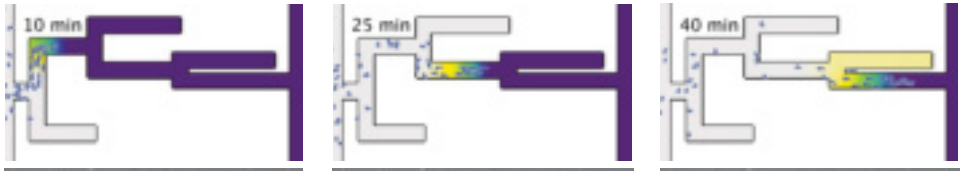


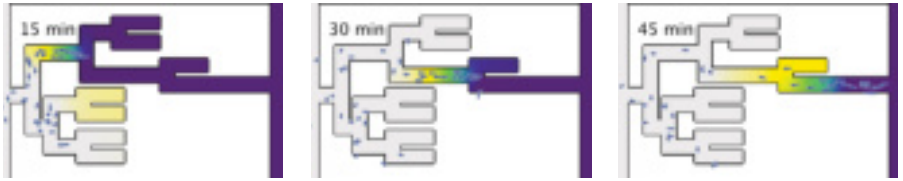
L. Tweedy et al., and R. Insall. *Science* 369, 1075 (2020)

Pathfinding in a maze using self-generated gradients

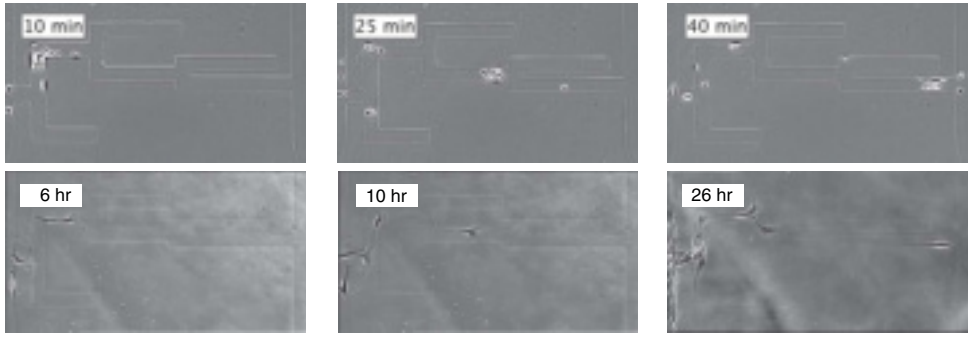
Cells detect and avoid dead ends

Simulations



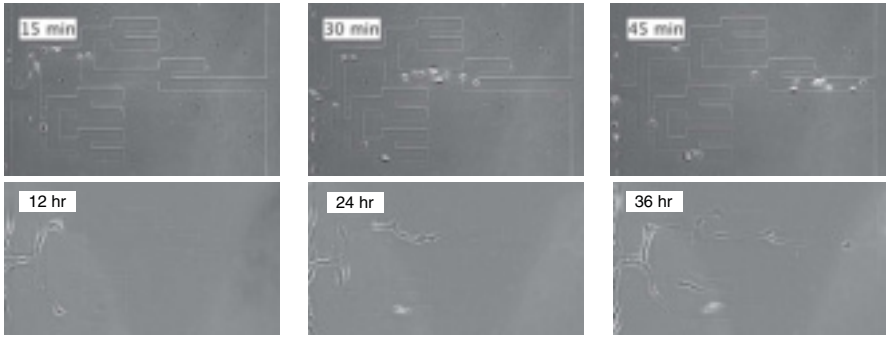


Dictyostelium cells



Pancreatic cancer cells

Dictyostelium cells



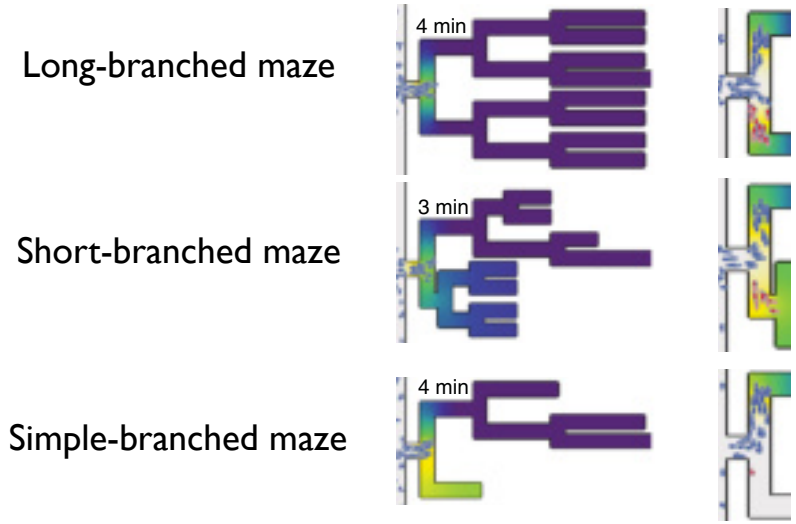
Pancreatic cancer cells

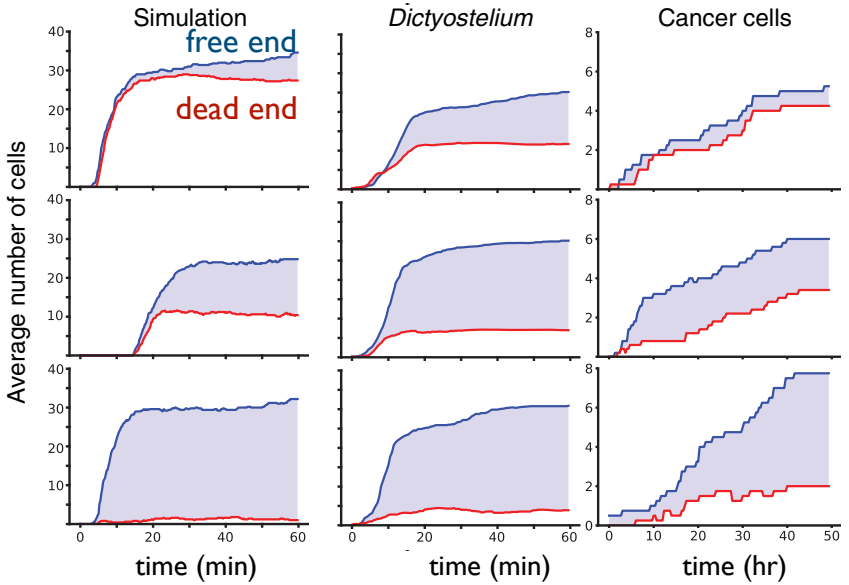
L. Tweedy et al., and R. Insall. *Science* 369, 1075 (2020)

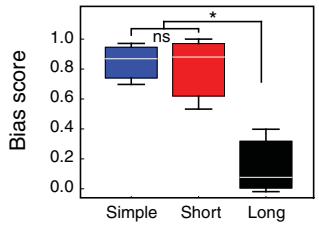
Pathfinding in a maze using self-generated gradients

Limits to the detection of dead ends

- More distant dead ends increase the error rate because the clearance of attractant is less efficient





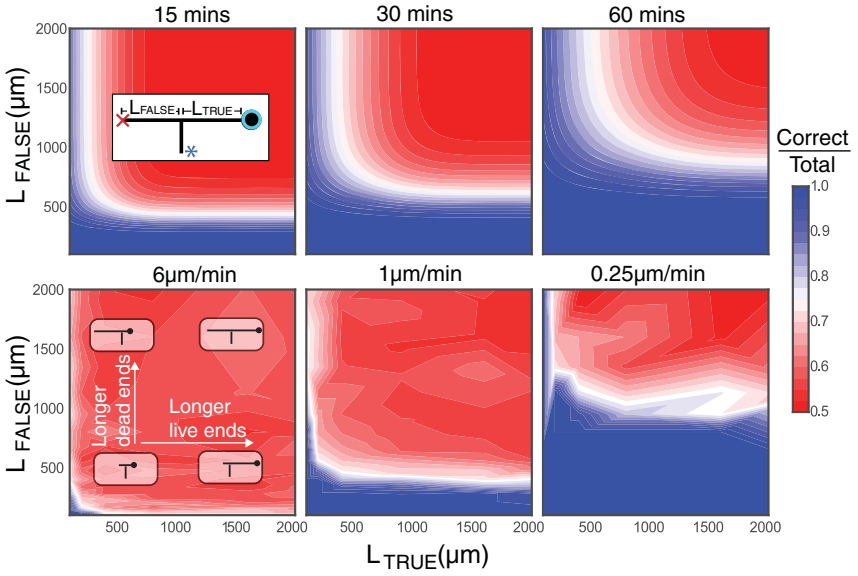


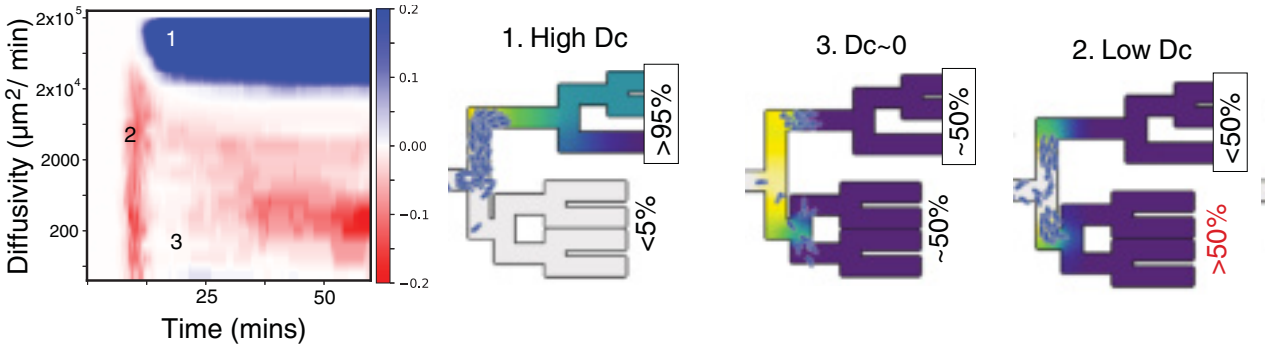
L. Tweedy et al., and R. Insall. *Science* 369, 1075 (2020)

Pathfinding in a maze using self-generated gradients

Limits to the detection of dead ends: cell speed and diffusion

- Longer dead ends leads to less accurate decision
- **Speed:** lower speed of cells leads to more accurate decision: cells have more time to clear attractant from a dead end
- **Diffusion**: At higher diffusion constant of chemoattractant, cells can more efficiently lower/ deplete attractant from a dead end
- A zero diffusion, there is no information to decode (cells cannot decode what is ahead of them)
- A low diffusion, short, parallel dead ends lure cells in the wrong direction.

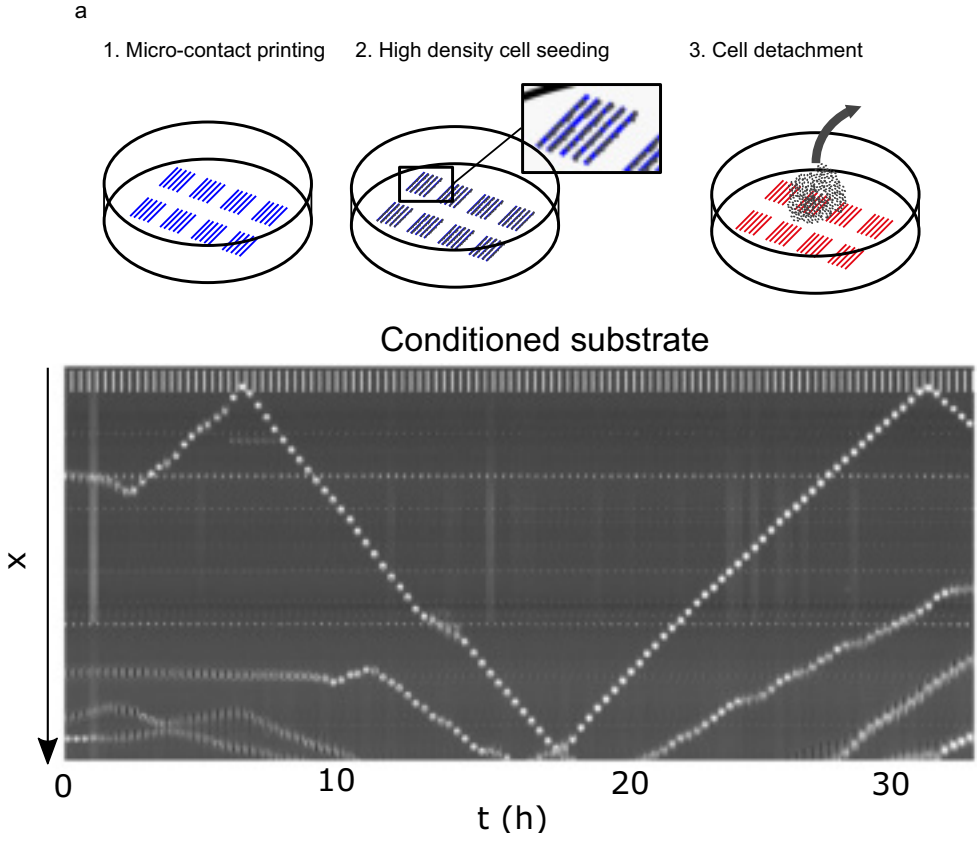




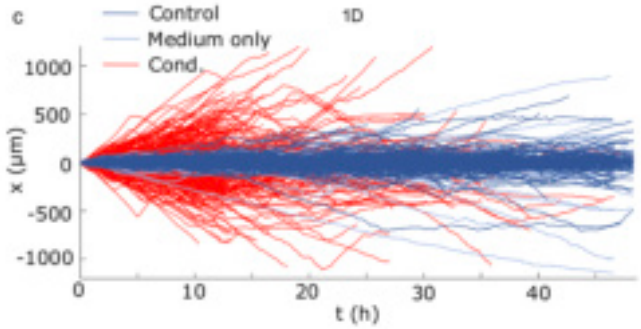
L. Tweedy et al., and R. Insall. *Science* 369, 1075 (2020)

Self-organised guidance

Reinforcement of guidance landscape by cells: spatial memory akin to stigmergia



- Conditioned substrate enhances cell motility
- Oscillatory migration in 1D channels



d'Alessandro et al. RM Mège R. Voituriez and B. Ladoux *Nature Communications* 12:4118 (2021) https://doi.org/10.1038/s41467-021-24249-8

General Conclusion

1. Biased random walk characterizes chemotaxis across scales

2. Two different mechanisms of gradient sensing:

- Spatial mechanism: comparison of chemoattractant concentration along cell length. This requires often (always?) self-generated gradients by depletion of activity. More robust and long range.

- Temporal mechanism: comparison of chemoattractant at different positions and requires memory.

3. Adaptation and memory manifest in different ways across scales

- Temporal gradient sensing in prokaryotes
- Persistence of motility in eukaryotes

Mechanical guidance

1. Substrate interactions in 2D and 3D are inherently mechanical
2. The stiffness, topography, etc of the environment can affect motility
3. Cells also decode the mechanical properties of their environment