Eukaryotic Chemotaxis in Dictyostelium - Getting from the signal to the mechanics

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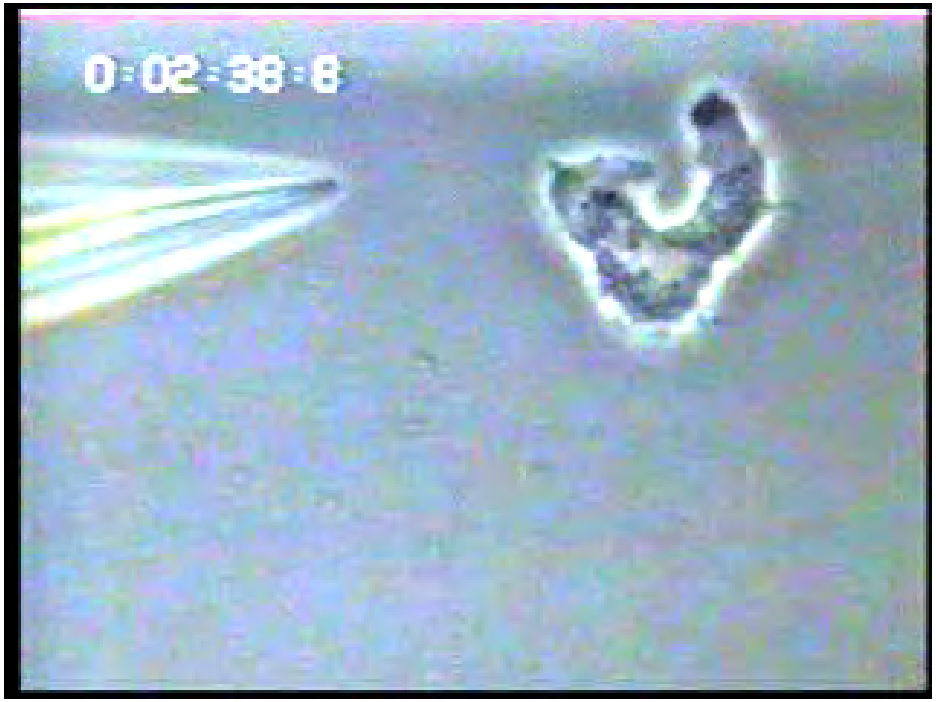
D. Kessler (Bar-Ilan); I. Hecht and E. Ben-Jacob (Tel-Aviv)

How molecules come to life?

Chemotaxis is an example of a living behavor

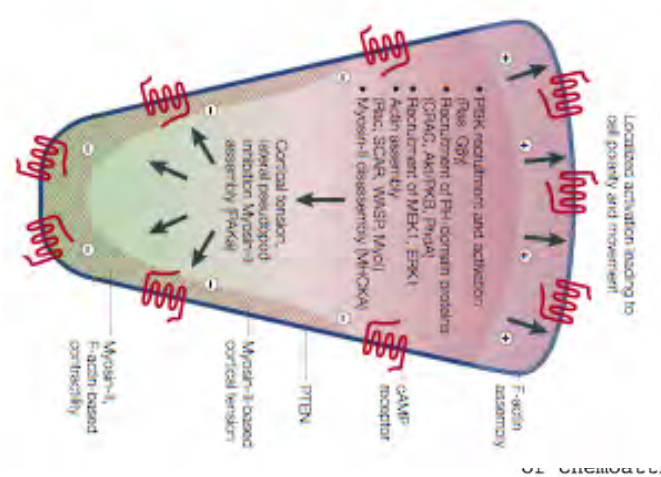


We work on chemotaxis in a model organism, the Dictyostelium amoeba

- simplified signaling
- availability of genetics tools
- ease of experiments
- chemotaxis is crucial for survival

Dictybase Website
http://dictybase.org/index.html

Cell moves about one cell diameter per minute
Decision-making maintains flexibility (limited hysteresis)



Sequence of subcellular events

Receptors

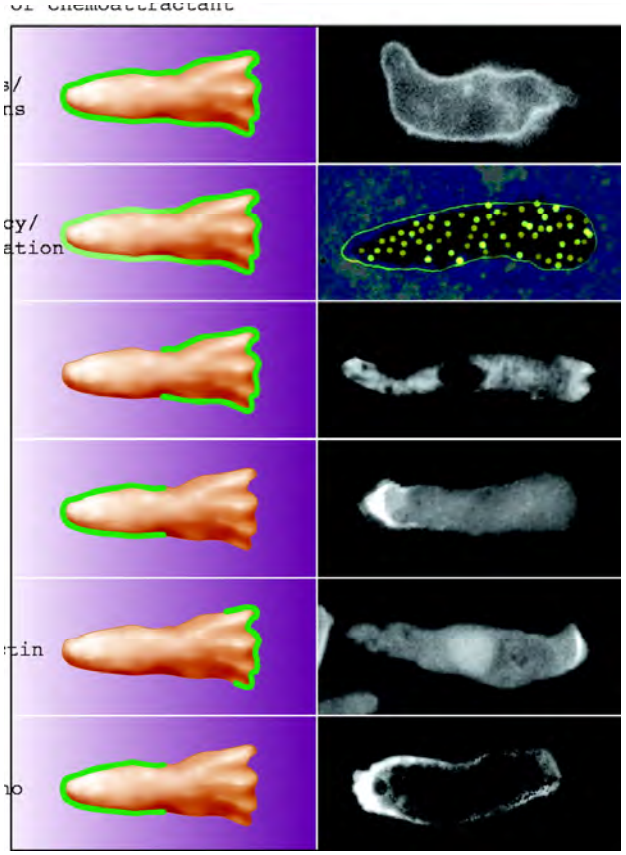
Occupancy

PI_3K

PTEN

RAC

Myosin



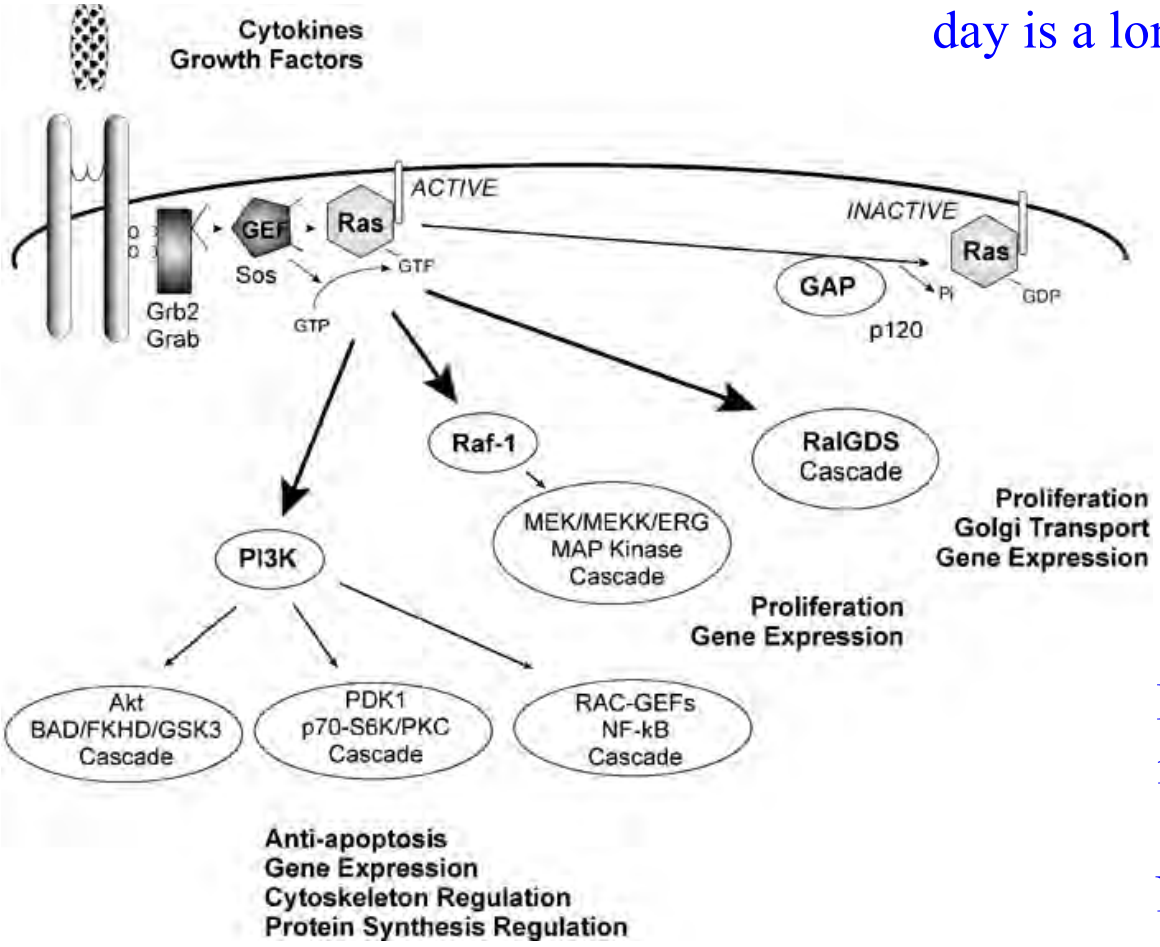
Starting with the work of Parent and Devreotes, response can be tracked by subcellular markers

- Uniform receptor
- Lipid modifications
- F-actin at the front
- Myosin at the rear

These allow for the measurement of the kinetics of the gradient sensing step

Focus on RAS

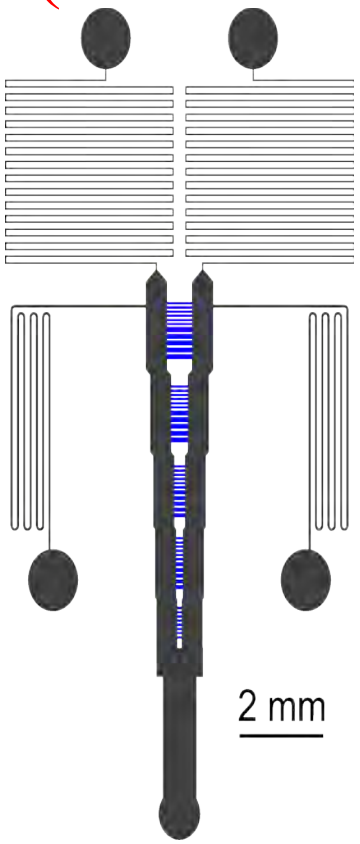
Maybe some day we will be able to deal with systems of this complexity, but that day is a long way off



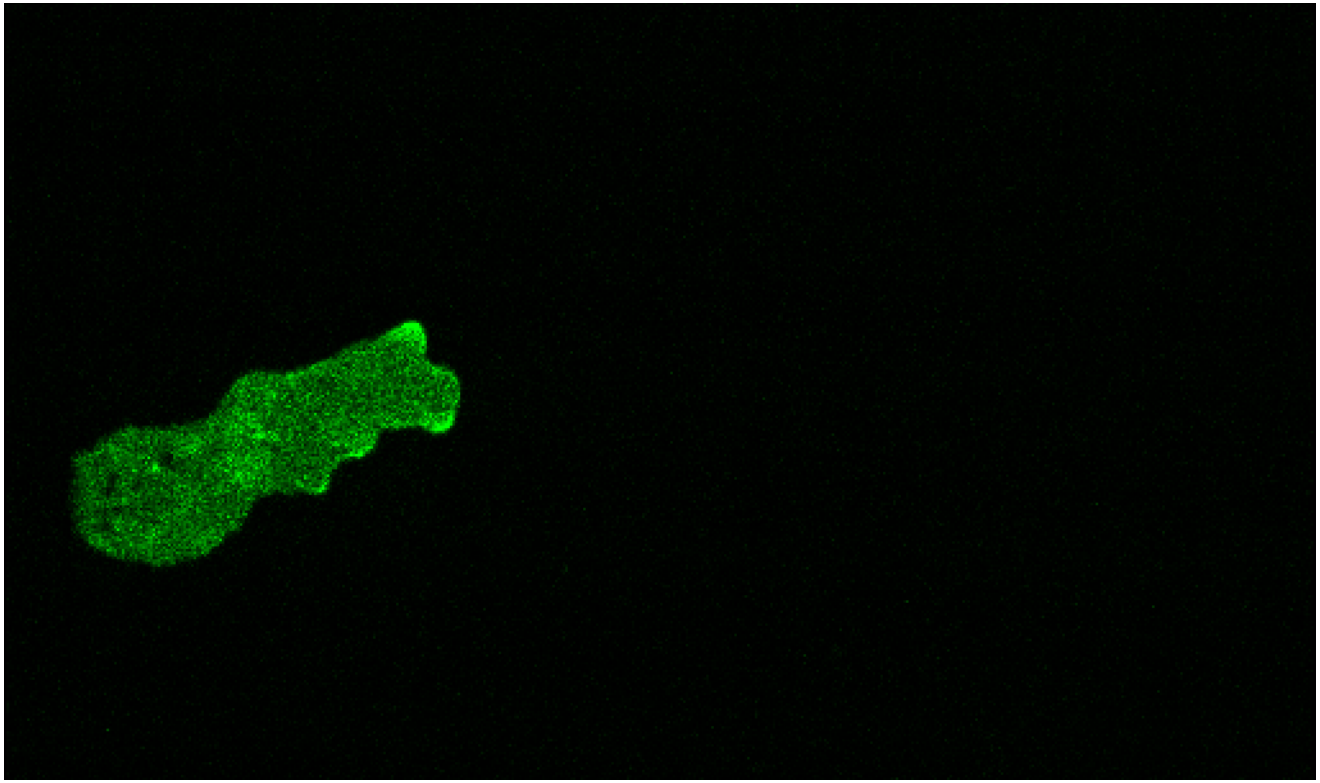
Ras is activated by a GEF; inactivated by a GAP

Note: Can attach fluorescent marker to RBD

Ras activations correlates with cell motility (Hecht et al)

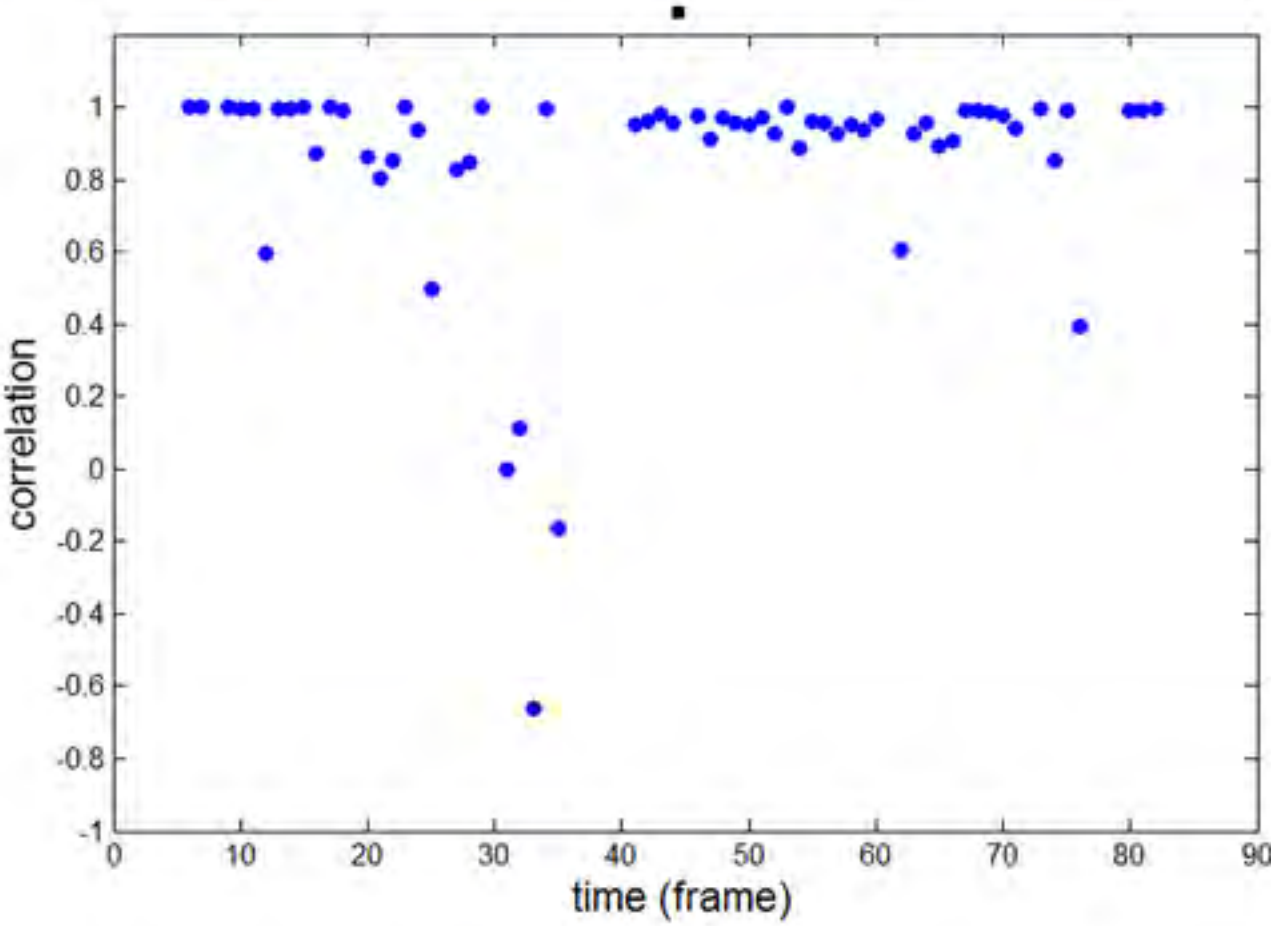


- Cell in a microfluidic device with chemoattractant gradient, variable vertical height.
- Most of the cell in focal plane: no bleaching issues
- Visualize Ras*, an upstream signaling component



Monica Skoge, Loomis lab

Detailed measure of correlation



Measure is the cosine of angle between patch and protrusion

Done both in under agar expts and in microfluidic chamber

From Hecht et al PLoS Comp. Bio (2011)

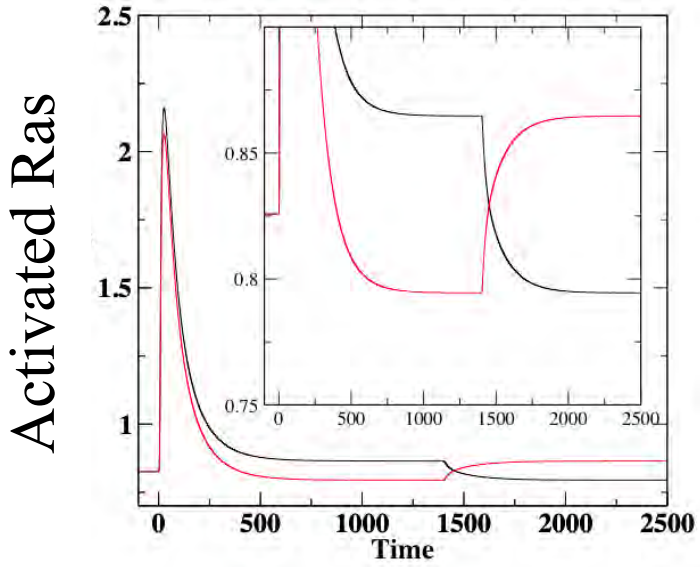
Cell Decision Model

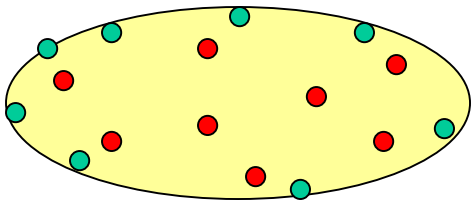
- Gradient sensing is hard because it cannot be done by local circuits in the cell
- Models must postulate an inhibitory mechanism (either direct or via depletion)
- We will focus on conceptual approaches, which try to explain how external signals can get amplified to the level of decisions

- If RAS is critical to this gradient sensing, theory can dictate how it must behave

LEGI

Local activation and global inhibition explains adaptation to global stimulus versus steady gradient response





- Membrane-bound activator
- Diffusing inhibitor

LEGI as applied to RAS activation

$$\begin{aligned}
\frac{\partial A}{\partial t} &= k_a S - k_{-a} A \\
\frac{\partial I}{\partial t} &= k_i S - k_{-i} I + D\nabla^2 I \\
\frac{\partial E}{\partial t} &= k_+ A(1-E) - k_- I E
\end{aligned}$$

Since A and I are both proportional to S in steady-state, uniform S results in a transient activation of E but eventual **perfect adaptation**. With a non-uniform S, I gives average value and A remains local - pattern in the effector E

Amplifying by Ultrasensitivity

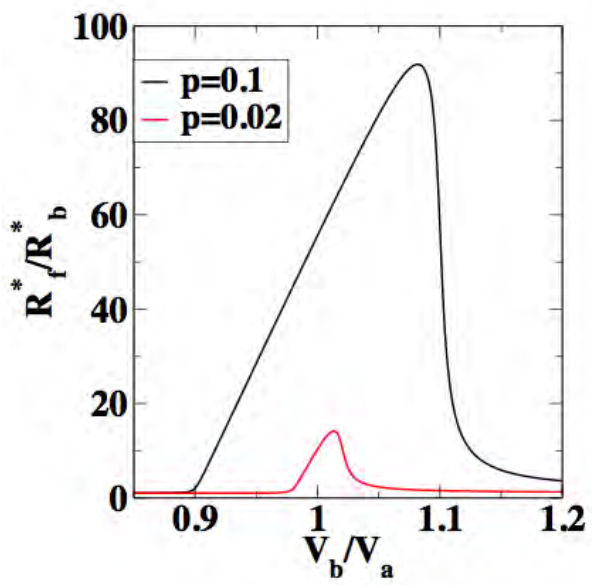
$$\frac{\partial E}{\partial t} = k_+ A \frac{1-E}{K_A + (1-E)} - k_- I \frac{E}{K_I + E}$$

Assume that the K's are very small and the baseline rates are balanced

$$\frac{k_+ k_a}{k_{-a}} = \frac{k_- k_i}{k_{-i}} \qquad E_0 = \frac{K_I}{K_I + K_A}$$

Then, we get a large amplification of the value of E in the versus the back; the price for this is the needed constraint

Loomis, Levine, Rappel (2009)



LEGI

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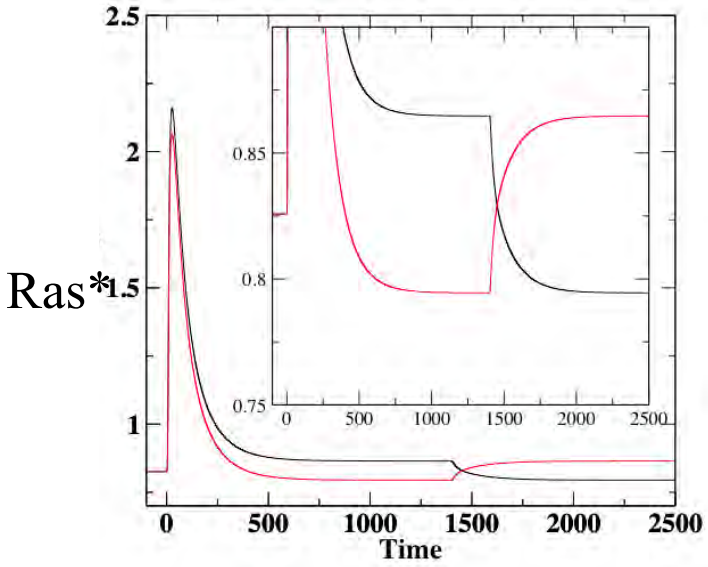
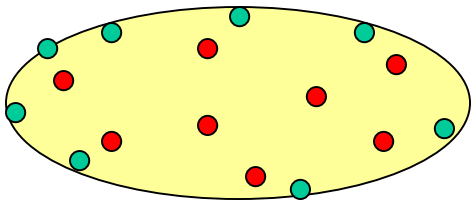


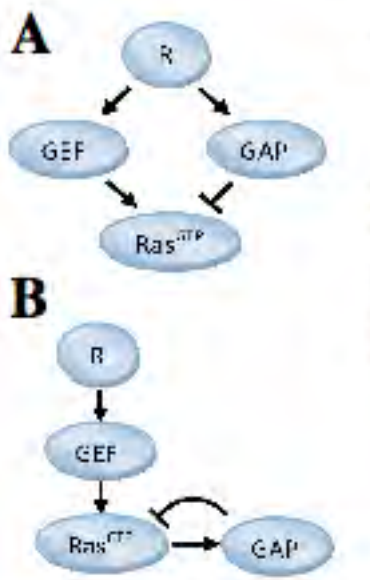


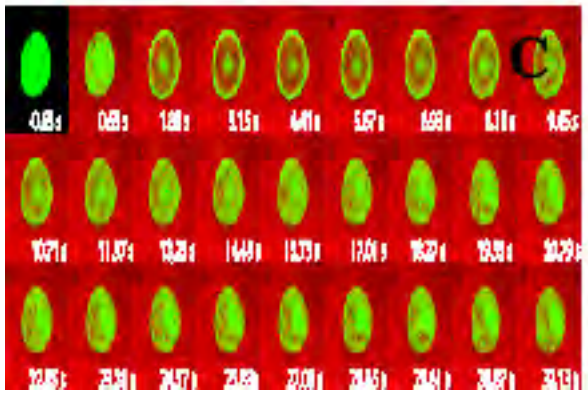
- Membrane-bound activator
- Diffusing inhibitor

We will assume that RAS is the effector molecule

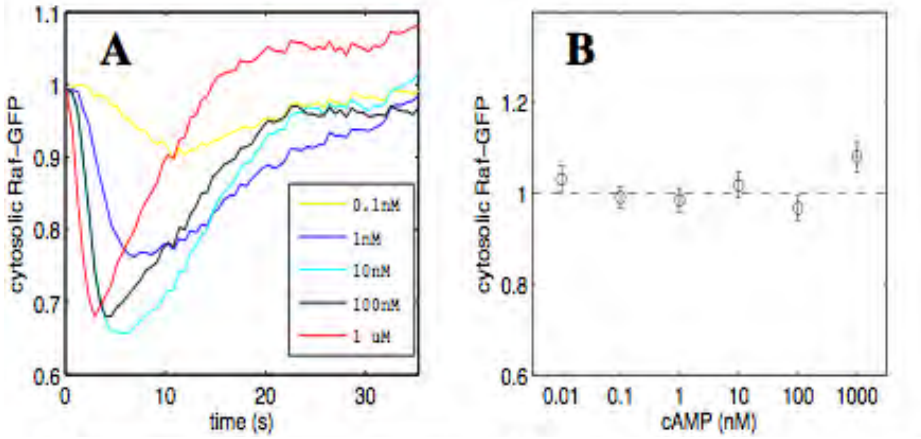
Perfect Adaptation

- Only two simple ways to obtain perfect adaptation in signaling; integral feedback (B) versus incoherent feed-forward (A)
- Integral feedback relevant for E. Coli chemotaxis; here, other strategy is used
- Can be directly investigated using microfluidic device (~ 1 sec switching time) and fluorescent marker with Ras binding domain

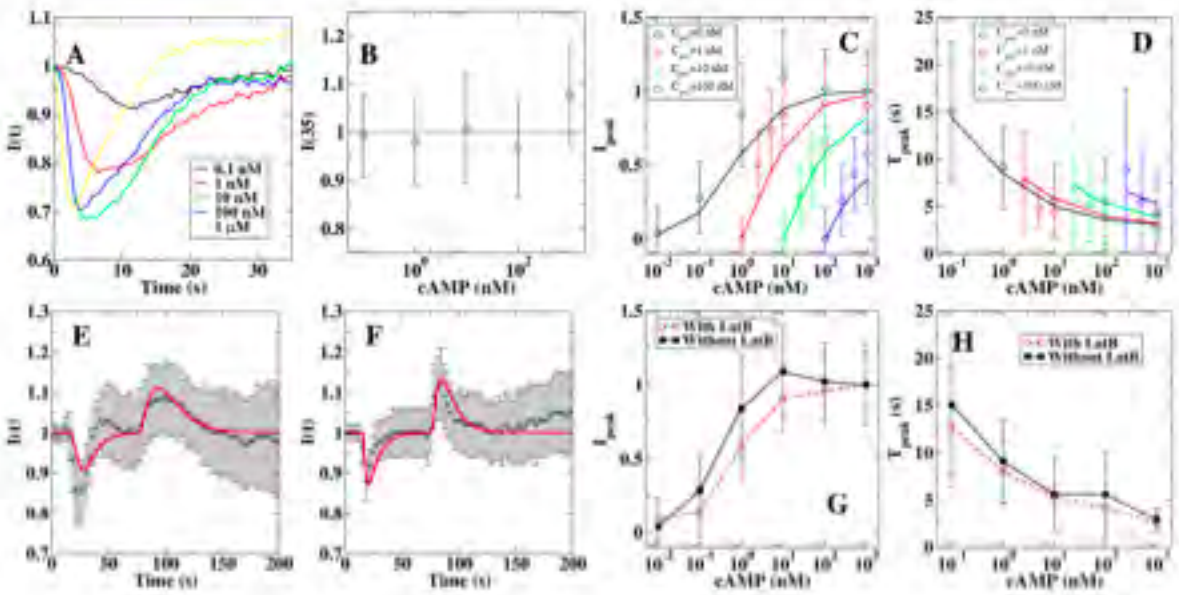




K. Takeda , Firtel lab



Adaptation kinetics



Takeda et al, under review

Back to the gradient problem

- In the presence of a gradient, activated Ras becomes localized to the front but in a stochastic, patchy fashion
  - May be due to feedback from the actin cytoskeleton
  - This can be described by models which couple "compass" systems such as LEGI to excitable ("actin") dynamics (see Hecht et al, PRL (2010); Xiong et al PNAS (2010)
  - Of course, there are many other possibilities (see Keshet, 2011)

- As we have seen, these patches are very highly correlated with sites of actin polymerization and membrane protrusion
  - Models for Ras patches can be used to create simple models of cell morphodynamics

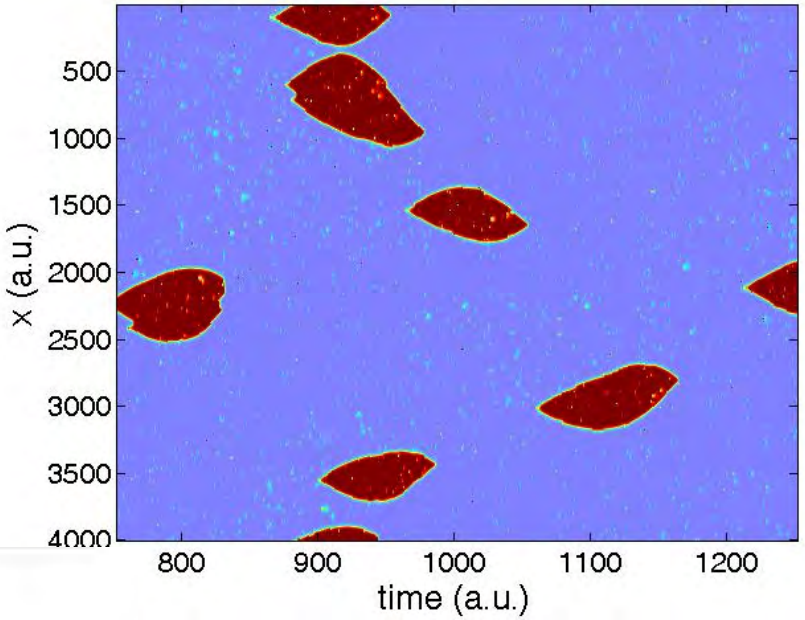
Conceptual model of motility

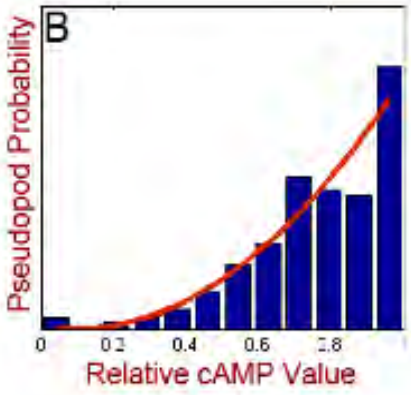
Actin-Myosin dynamics

SIMPLE PHENOMENOLOGICAL MODEL

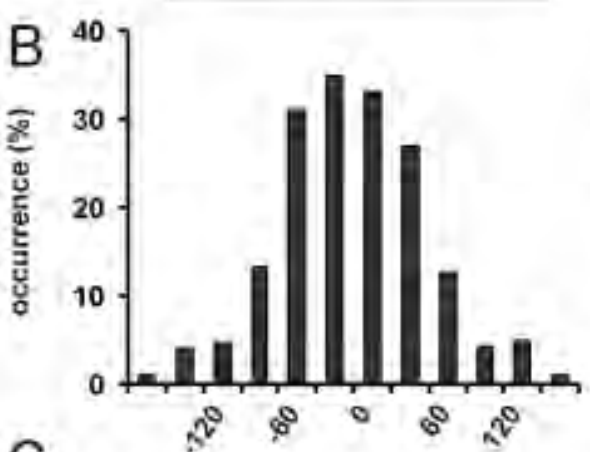
Model has two components:

- A "biochemical" model which is able to produce regions of elevated concentration of a component (patches); Here, noise is amplified by positive feedback from the cytoskeleton





M. Skoge, unpublished

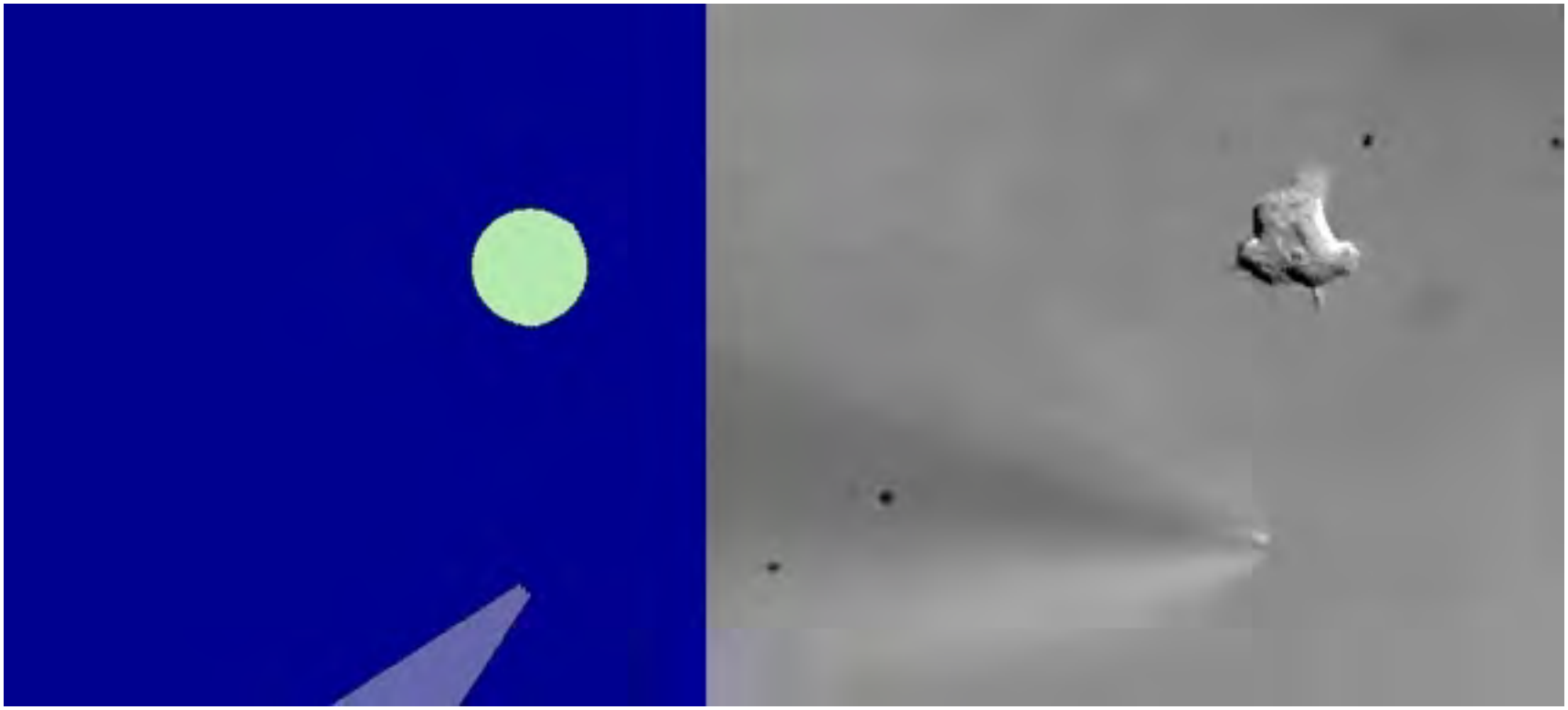


Bosgraef et al (2009)

-> Patches are only made in the front

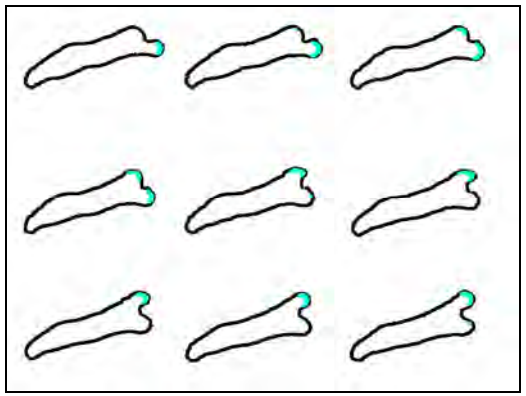
- A mechanical model which deforms the cell based on the coupling of patch concentration to actin-based protrusion
- NB (Correctly?) predicts that there are no RAS patches in Latrunculin treated cells

Fun and Games

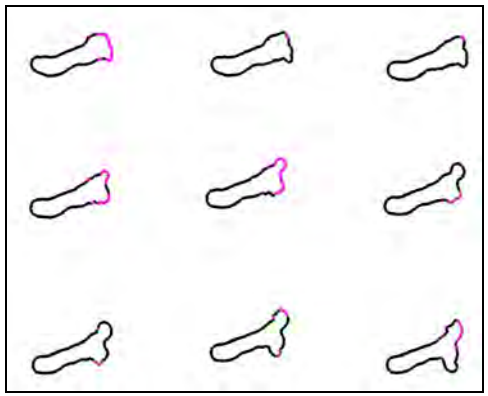


Note: Mechanical model contains just nodes connected by springs, together with an overall area constraint and patch forcing

Previous experiments have been interpreted as evidence for an explicit tip-splitting mechanism. Is such a physical mechanism necessary?

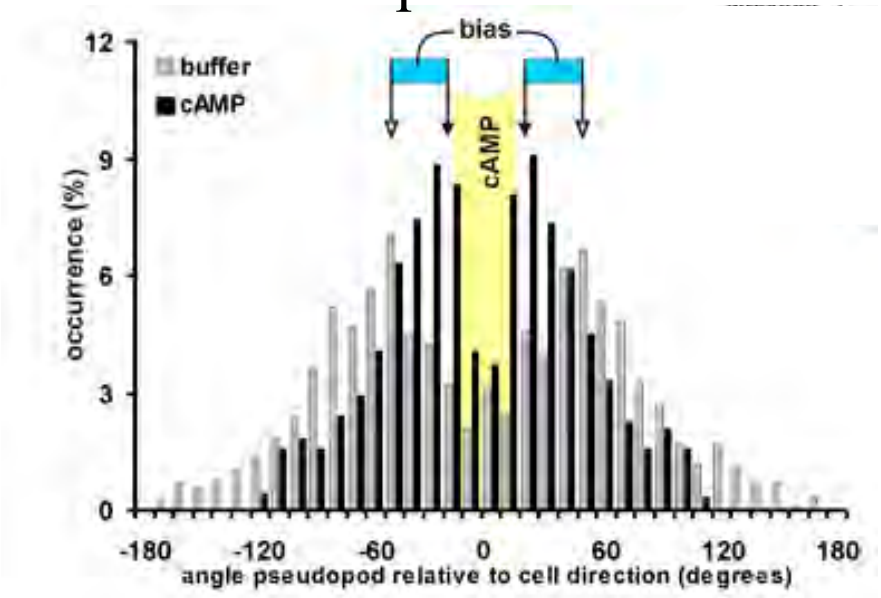


Tip-splitting; expt. versus simulation

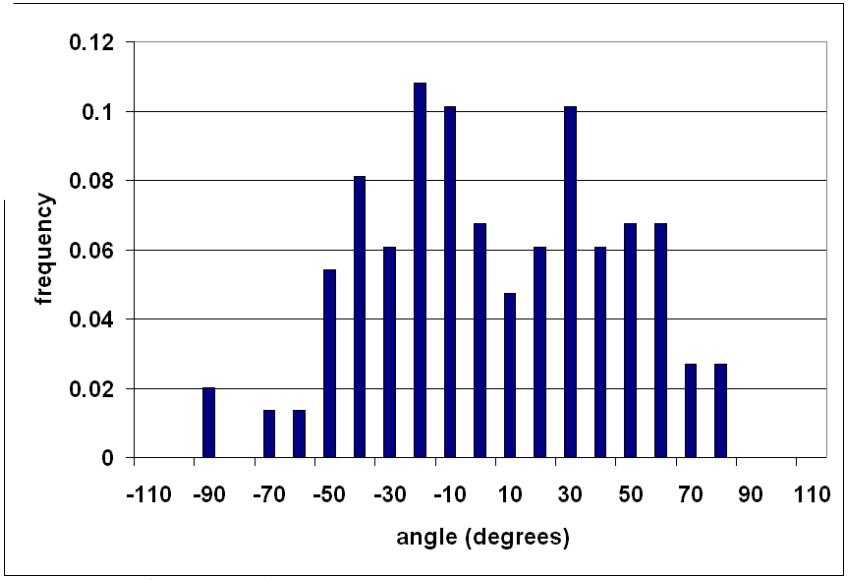


Experiments

Simulations



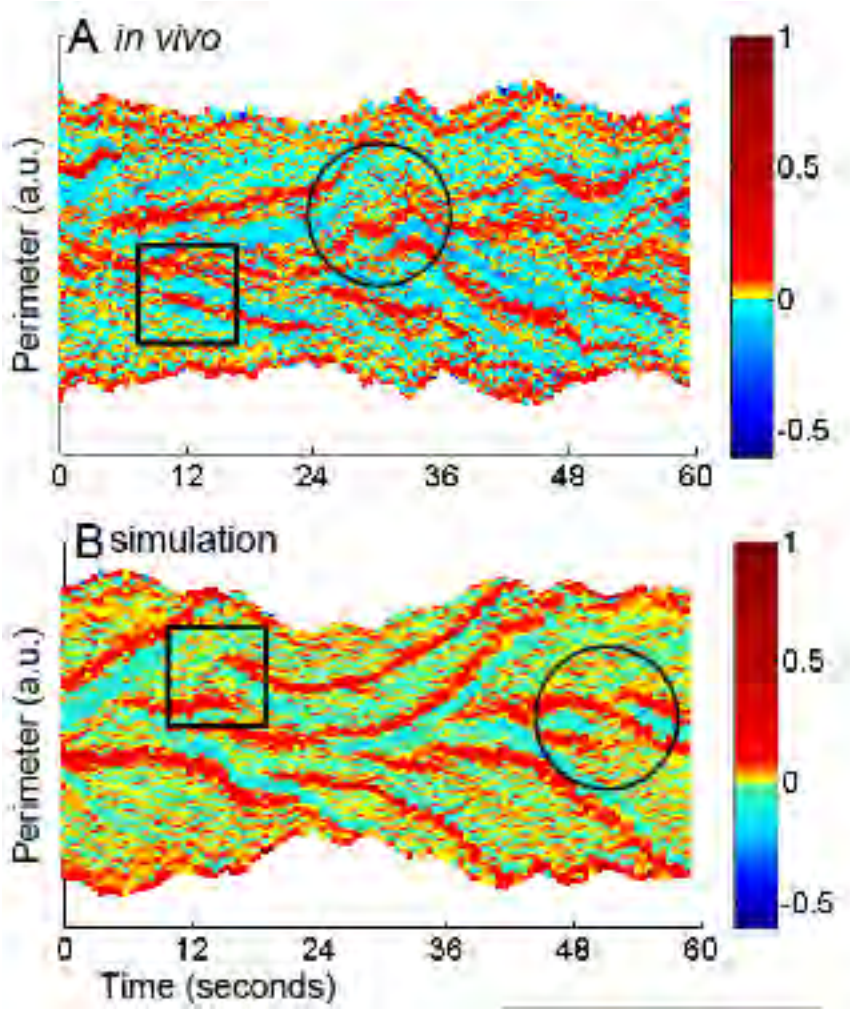
Bosgraaf et al., 2009

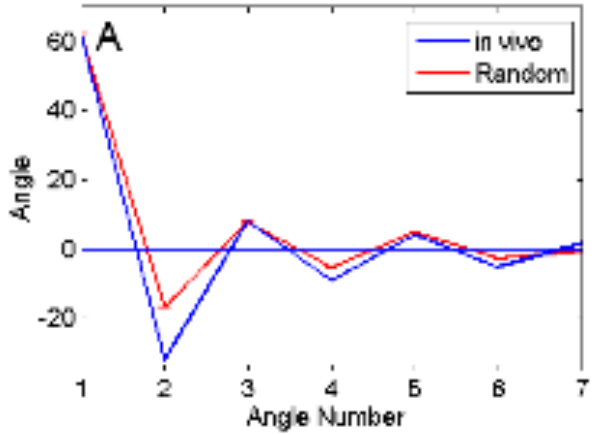


Hecht *et al.*, 2011

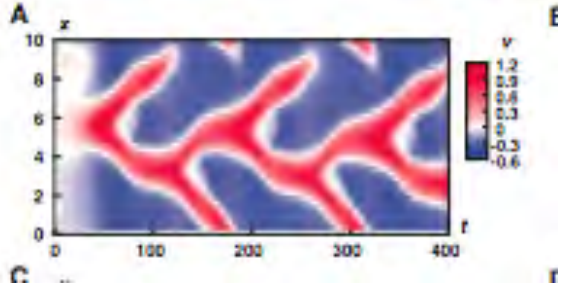
Simulation shows: no need for explicit tip-splitting mechanism

Left-right temporal ordering?





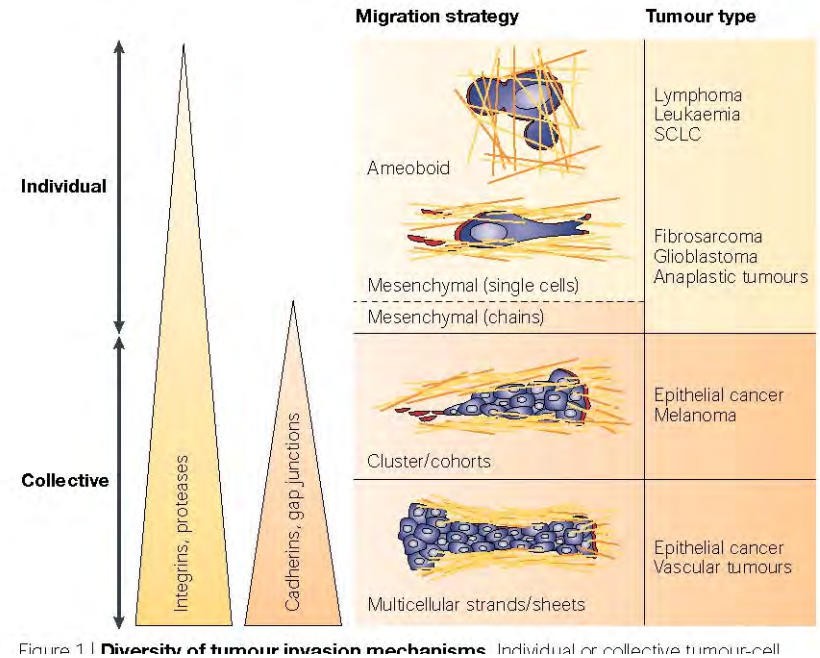
Even in a random pseudopod simulation, there is automatically left-right bias for chemotaxing cells



Alternative - Otsugi (2010)

A last word about applications

- Amoeboid motion is one of the ways that tumor cells can spread
- This capability limits current approaches to metastatic disease
- We have begun studying the interplay of noise with more complex 3d geometries for amoeboid motion



Friedl and Wolf, 2003

- Ex. "amazing" simulation

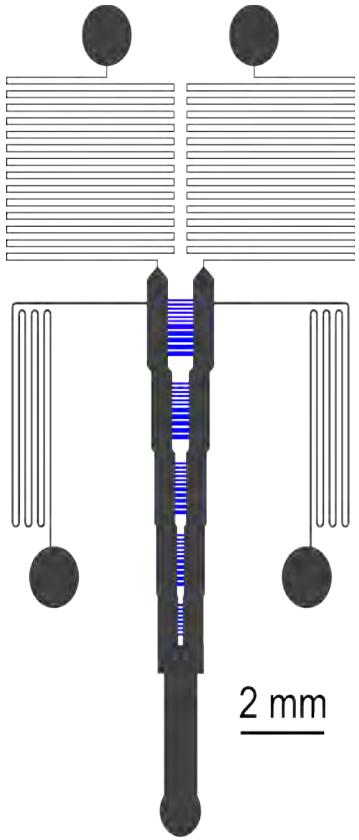
(PloS One - to appear)

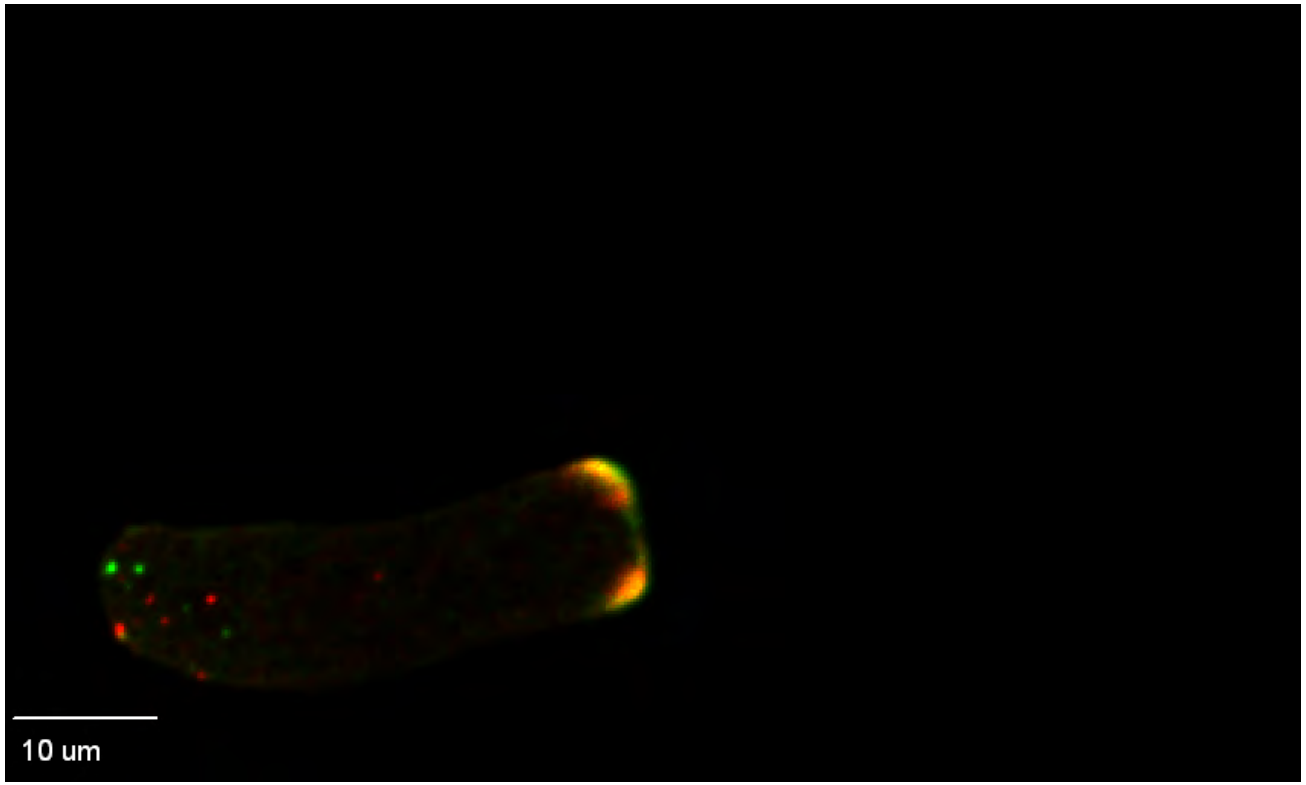
Towards more biophysical reality

- This class of model does not, of course, deal faithfully with the mechanics of cell motion

- This mechanics involves:
  - Stresses due to actin polymerization
  - Myosin-based contraction and actin network flow
  - Adhesion sites between cell and substratum
  - Forces exerted by the membrane (tension, bending)

Dictyostelium cells have (non-specific) adhesion sites





Vertically restricted Dictyostelium cell in gradient, with actin marker limE at the top (green) and at the bottom surface (red)

Deforming Cells

- We have begun the task of constructing a model of the mechanics of deformation
- Our approach is based on a phase-field formulation of the membrane energy coupled to actin-polymerization forces

$$E = \sigma \int ds + \frac{c}{2} \int \kappa^2 ds + \frac{M_A}{2} (A - A_0)^2$$

- Surface energy
- Bending energy
- Area constraint

$$\int d^2x \left(\frac{\varepsilon}{2} (\nabla \phi)^2 + G(\phi) \right) \quad \text{surface}$$

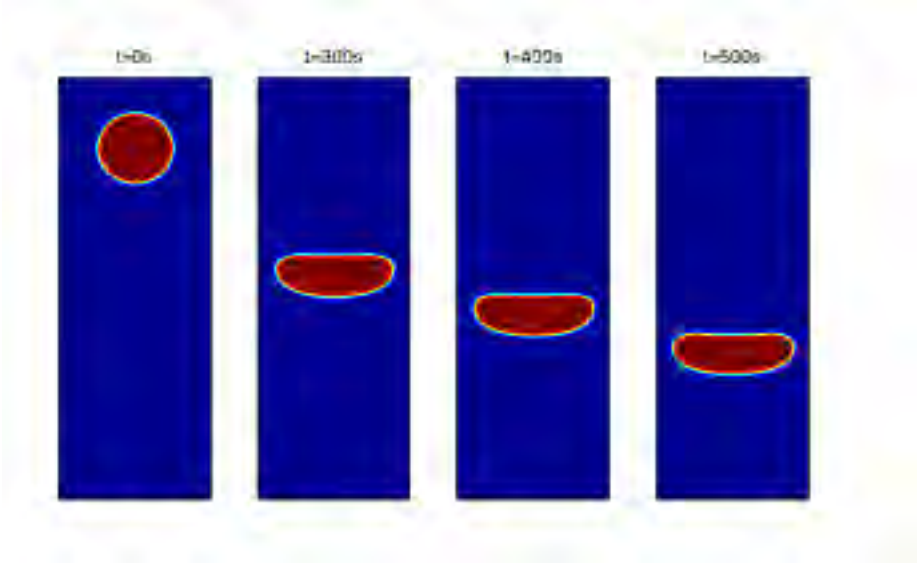
$$\int \frac{d^2x}{\varepsilon} \left(\varepsilon \nabla^2 \phi - \frac{G(\phi)}{\varepsilon} \right)^2 \quad \text{bending}$$

This formulation can allow us to reproduce results on the equilibrium shapes of vesicles etc

Shao et al, PRL (2011)

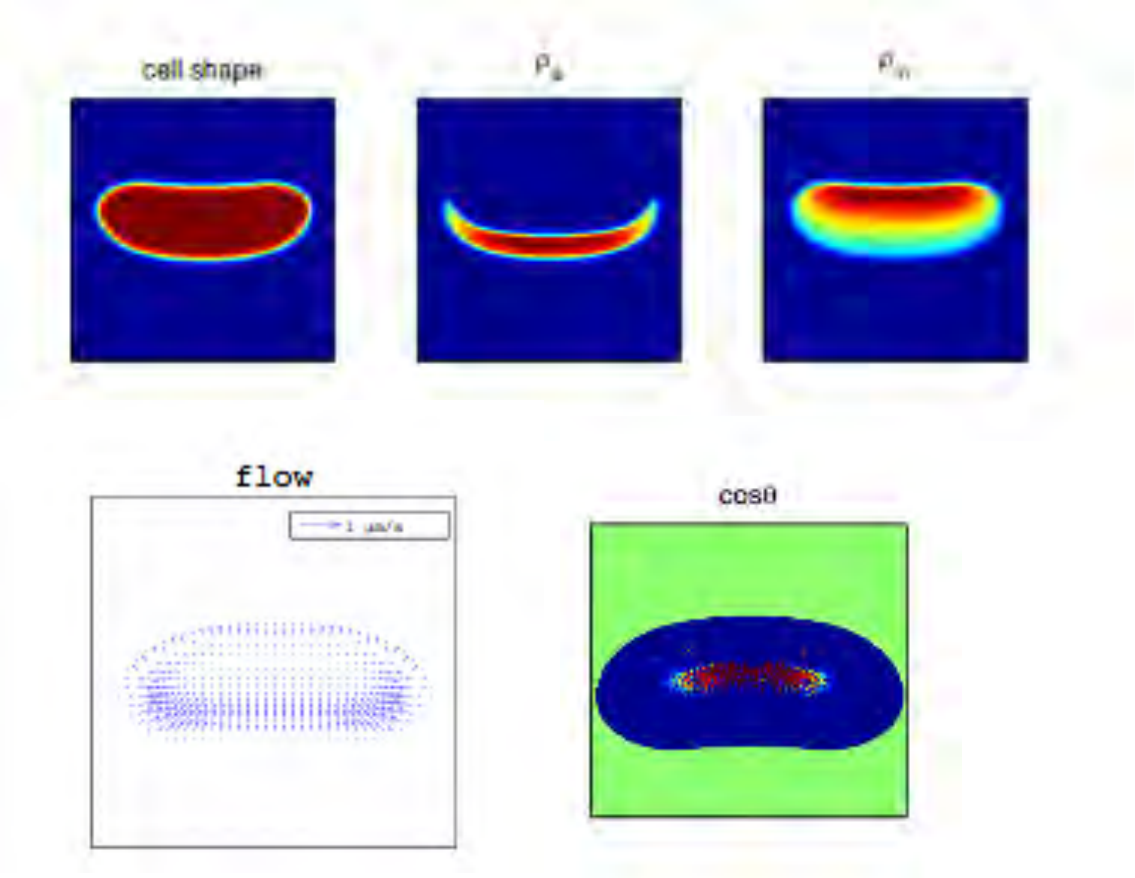
Preliminary results (keratocytes)

- We decided to try first to make a model for steady-state models of keratocyte motion
- Includes compressible flow equation for actin, discrete slipping/gripping sites …
- Results can be compared to traction microscopy data, actin flow measurements, myosin concentration …



D. Shao, unpublished

More details



These are in reasonable agreement with data from Fournier et al (2010)

Need to understand better how this model works and how robust it is

Afterwards: extend this to allow for actin excitability (rather than bistability) to move back towards Dicty

Note: we used force-induced transition from gripping to slipping

Summary

- Work on chemotaxis has reached the point where one can try to connect signaling ideas to mechanical consequences
- Dictyostelium remains a very useful model system and many groups are working on both theory and experiment
- Critical issues are:
  - Directional sensing models versus other approaches
  - Do pseudopods exhibit dynamical tip-splitting; is left-right alteration surprising? We have addressed these with phenomenological approaches
  - Can one move towards more biophysical models? We think one can create methods to do this, calibrated by traction/flow data