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Morphogenesis: pattern formation, growth, and cell movement

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Morphogenesis: pattern formation, growth, and cell movement

"what about the horse part"

LAST TIME

Classical models of (pre)pattern formation
Themes: supervised modeling; general models vs specific implementation
evolutionary drift in mechanism/trajectory but conserved/converged outcome and at multiple levels of "conservation" "divergence" "convergence"

TODAY

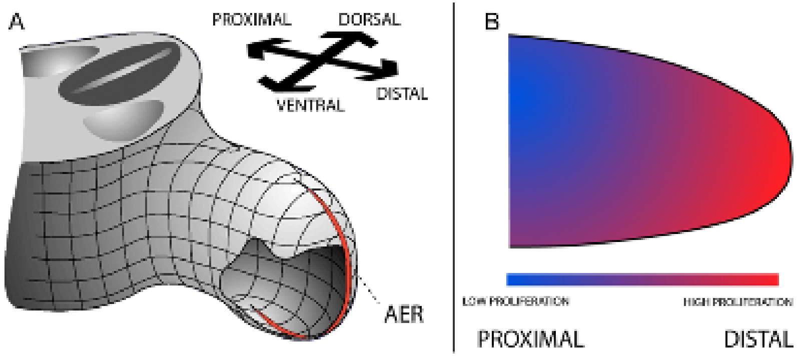
From pattern to morphogenesis, through growth, and cell movement

- Limbbud morphogenesis by differential cell growth rate possible/compatible with measurements?
- segmentation: pattern to shape
  -Elongation by segmentation
- single cell movement models
  - detailed model of keratocytes
  - mini model: keratocytes and amoeboids
- Multicellularity "by coming together"
  "from single cells to multicellular organism"
  through signaling, chemotaxis and differential adhesion

(from data intensive to behavior intensive models)

Making and fitting shape(1) measuring and modeling shape
limb bud development

Question: can limb bud MORPHOGENESIS be explained by gradient based differential cell proliferation?



The Role of Spatially Controlled Cell Proliferation in Limb Bud Morphogenesis Bernd Boehm1, Henrik Westerberg1, Gaja Lesnicar-Pucko1, Sahdia Raja1,2, Michael Rautschka1, James Cotterell1,2, Jim Swoger1, James Sharpe1,3* PLOS BIOL 2010

Measurement

Of 3D shape at 2 developmental stages

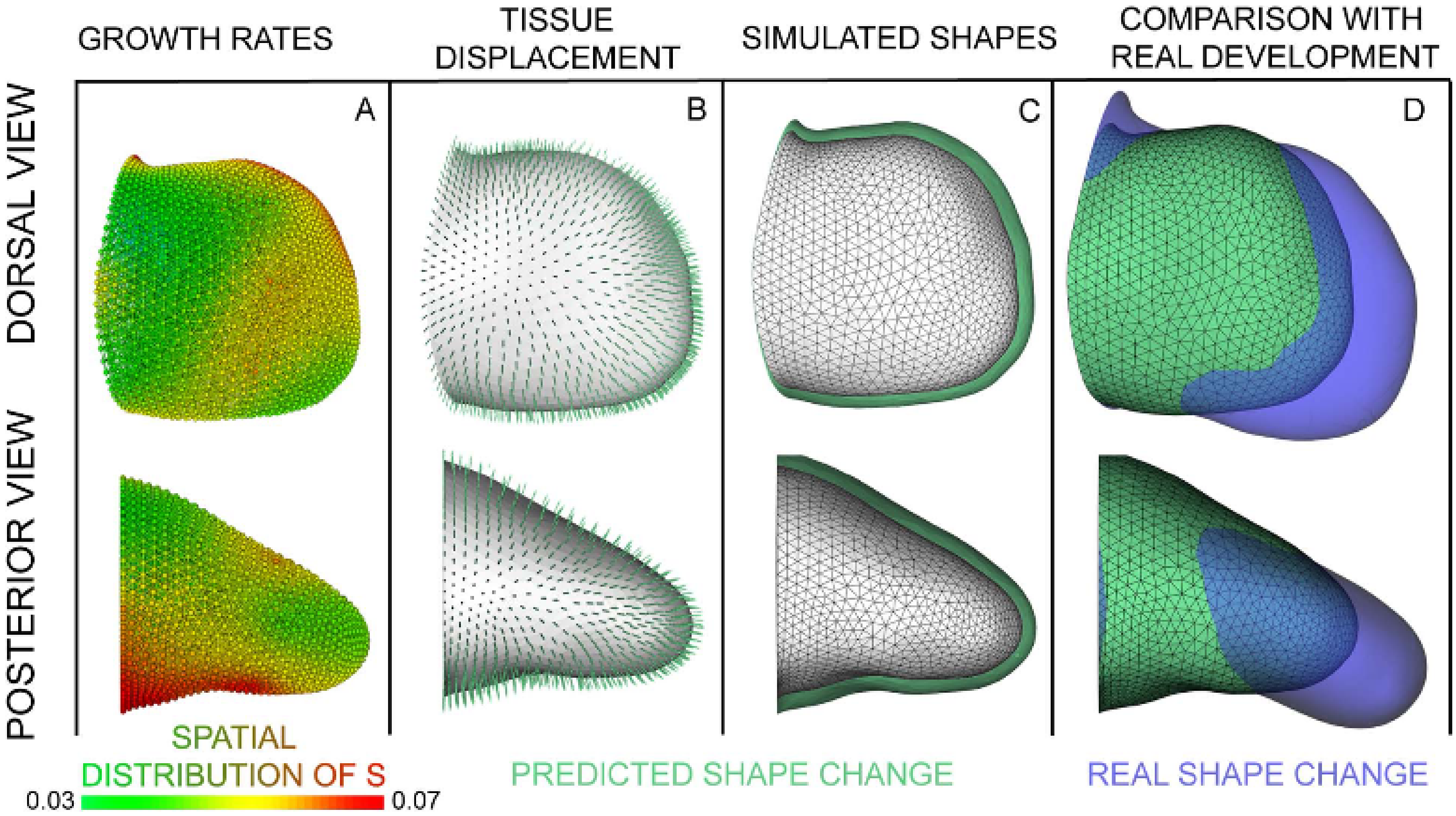
Of mitotic frequescies in different regions of the bud
colour cell cycle specific proteins
calculated cycle frequencies

DO these 2 measurement FIT?

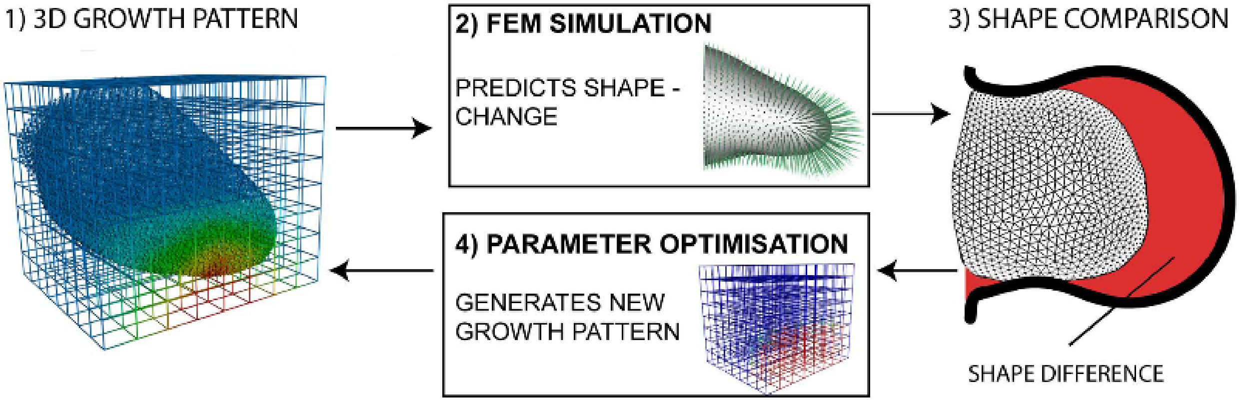
(Is differential proliferation sufficient to explain growth/morphoge

NO...

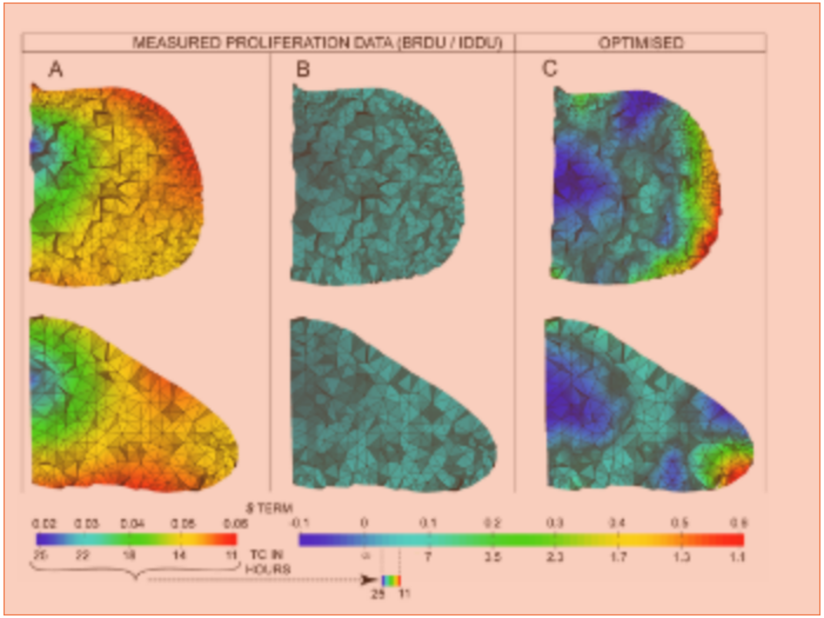
Finite element simulation of measured growth rates

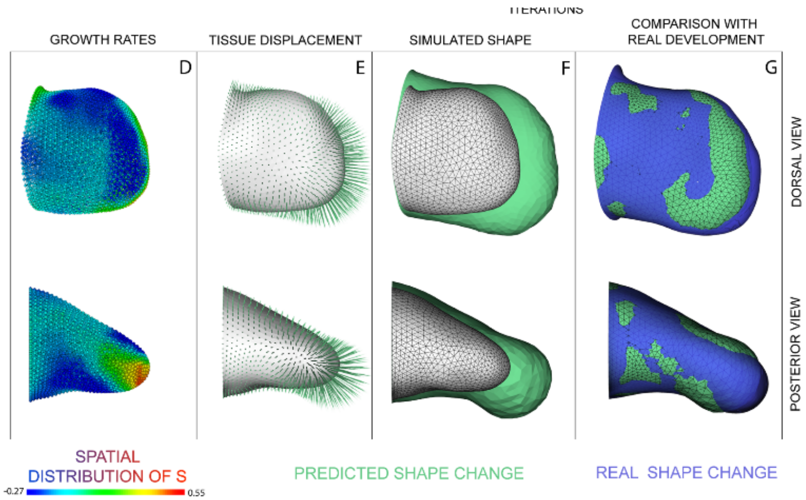


Failure due to mistakes in growthrates measurements?
Do growht rates exist such that shape emerges?



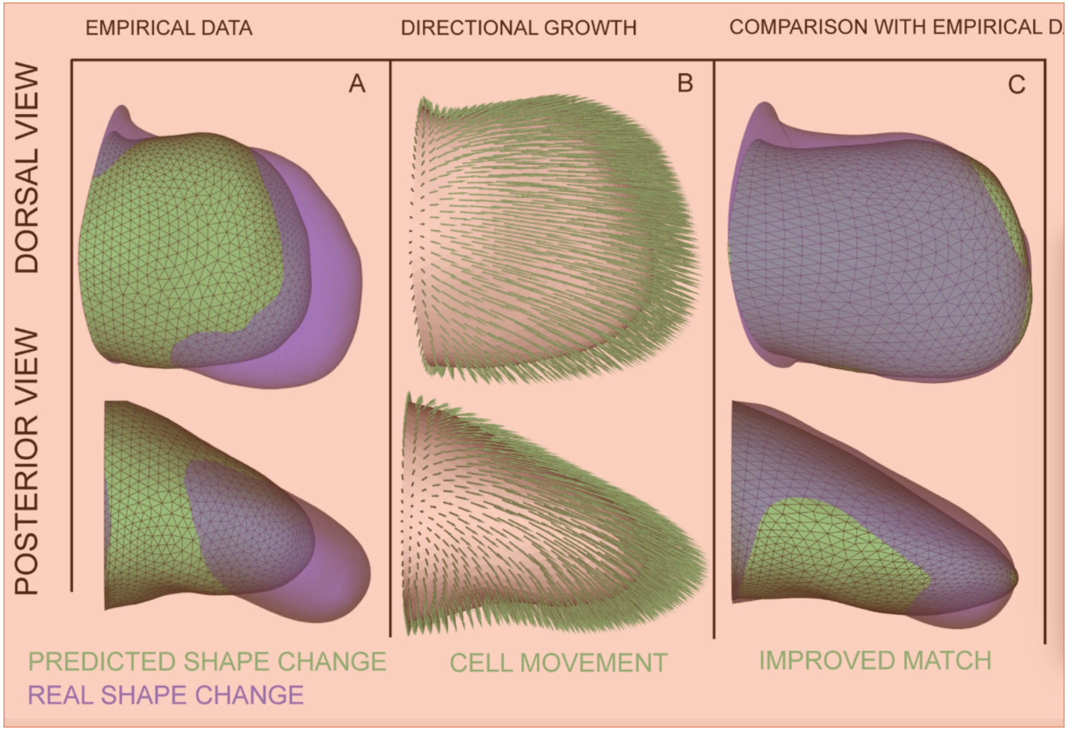
Yes differential growth CAN generate bud morphogenesis
BUT only for VERY different proliferation patterns (+ shrinkage)





conclusions

- Nice (because negative result!)
- Their hypothesis: directed cell movement plays a role



Use measured growth + fitted outward force (representing cell movement

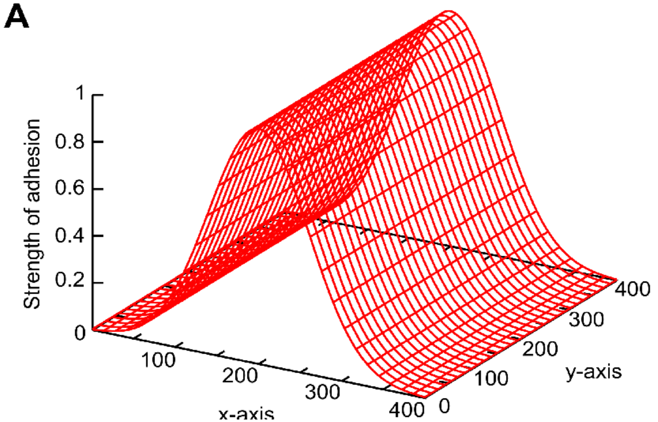
convergent extension, morphogenetic cell movement common to insects, fish, frogs,(mammals)

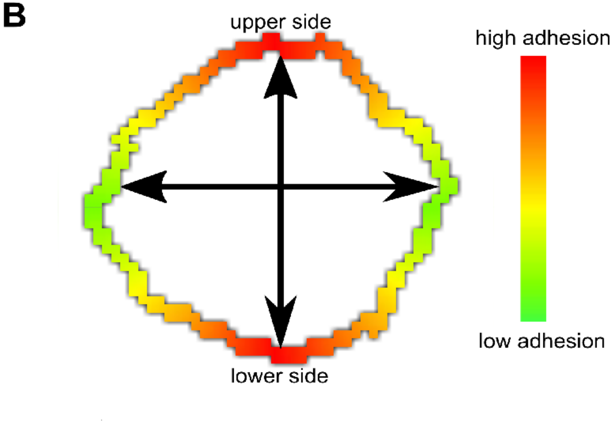
Elongation by intercalation but by different mechnisms, eg

- (Drosophila intercalation by contraction of those parts of the membrane that have a dorsal-ventral orientation)
- Xenopus: dorsal mesodermal cells polarize and change their adhesive properties; cells then crawl between each other in a zipper-like process (intercalation) *axial adhesion*
- Zebrafish: directed migration to the dorsal axis and intercalation follow a gradient in cadherin activity towards the central axis *graded adhesion*
- Xenopus and Drosophila: anterior-posterior patterning / segmentation crucial for cpnvergent extension

how is tissue patterning maintained during extensive cell movemennt?

adhesion based models; superimposed axis





graded adhesion

$$J' = J - w * e^{-\frac{(x-b)^2}{2*c^2}}$$

axial adhesion

$$J' = J - \beta^2 * |sin(\alpha)| * |sin(\alpha')|$$

adhesion:

$$\gamma_{i,j} = J_{i,j} - \frac{J_{i,i} + J_{j,j}}{2}$$

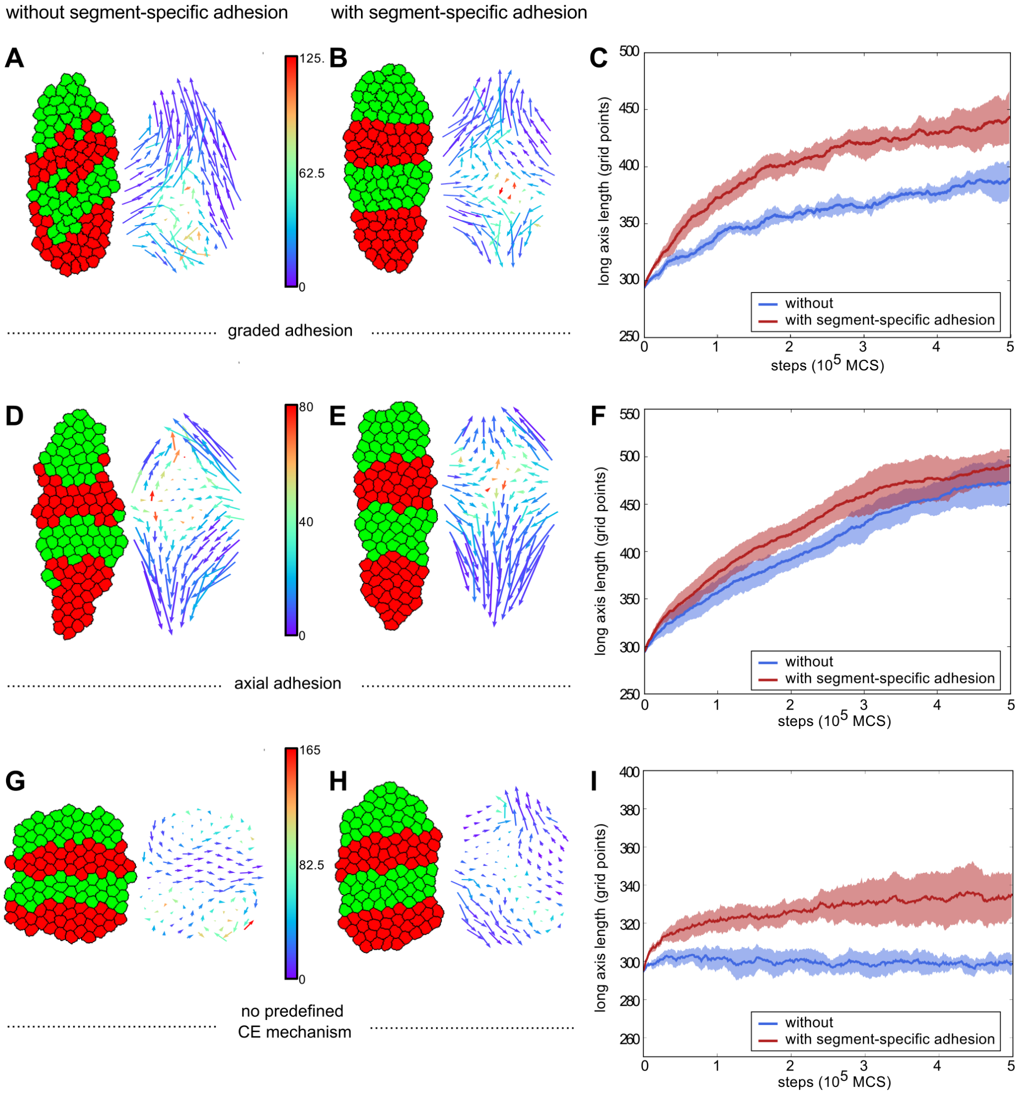
Segment-Specific Adhesion as a Driver of Convergent Extension Renske M. A. Vroomans et al 2015

Convergent extension (CE)

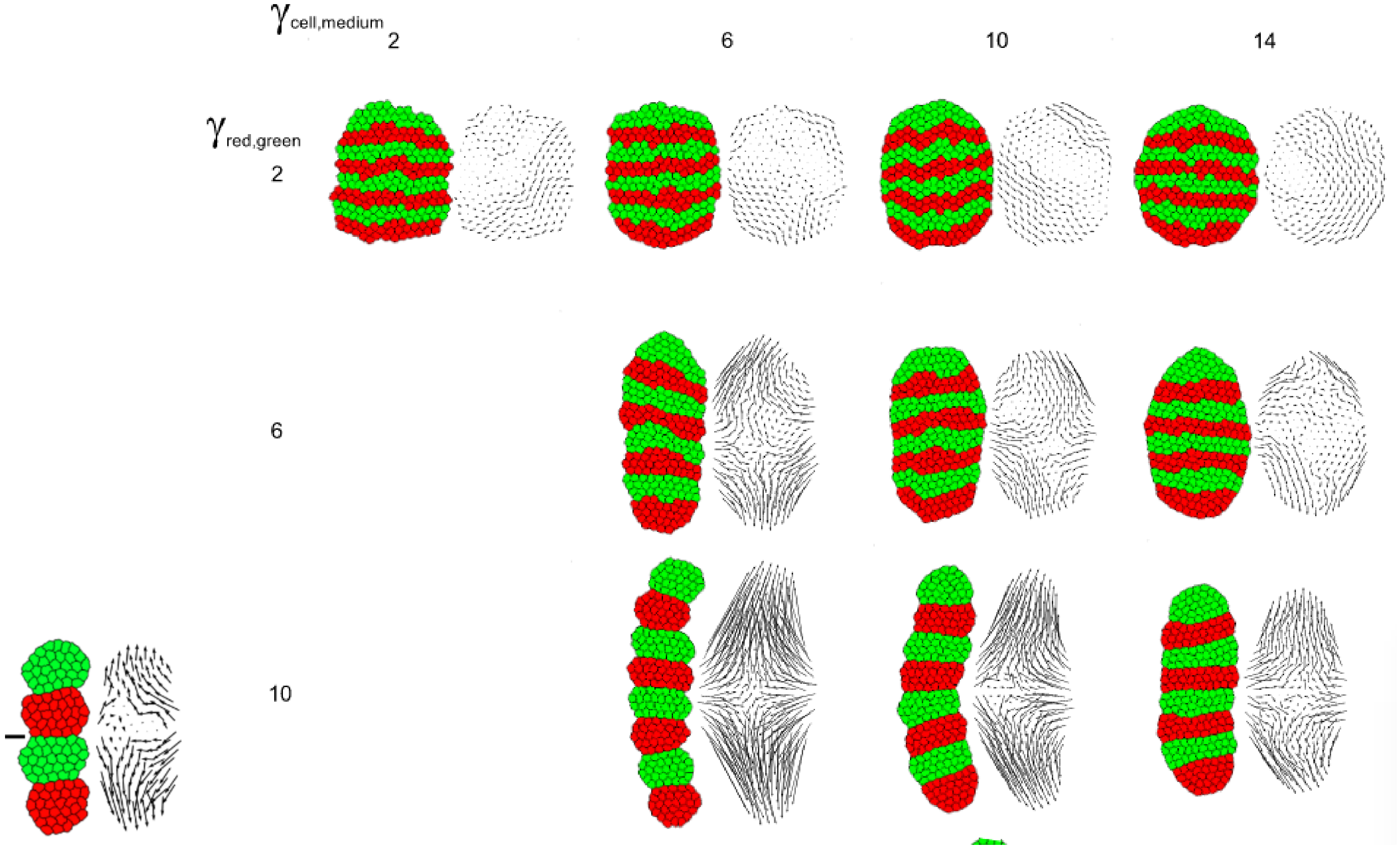
(often)
after segmentation;

How is segmentation conserved?

Segment specific adhesion
(here minimal)

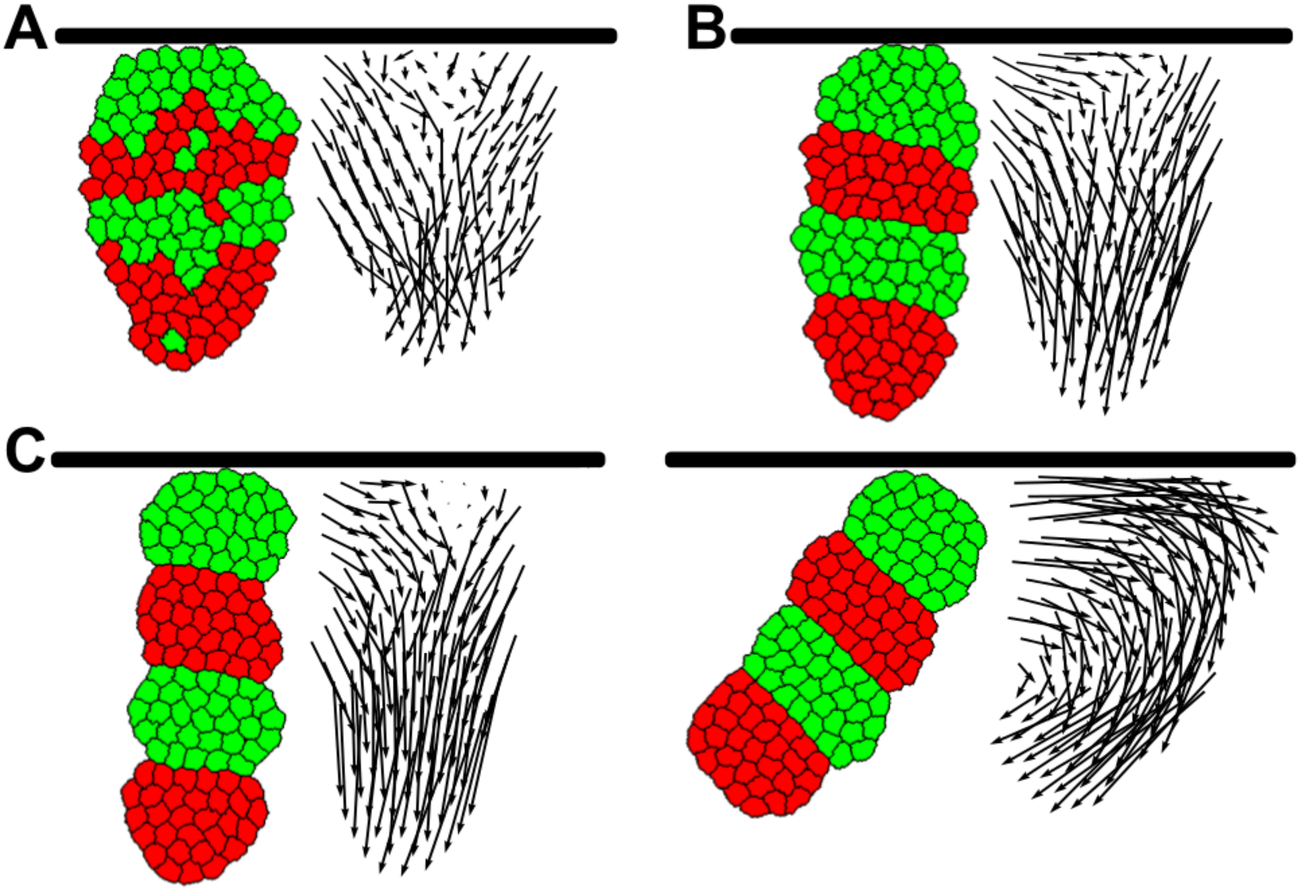


Segmentation by itself sufficient for CE (AND needed for CE (xenopus, drosophila)



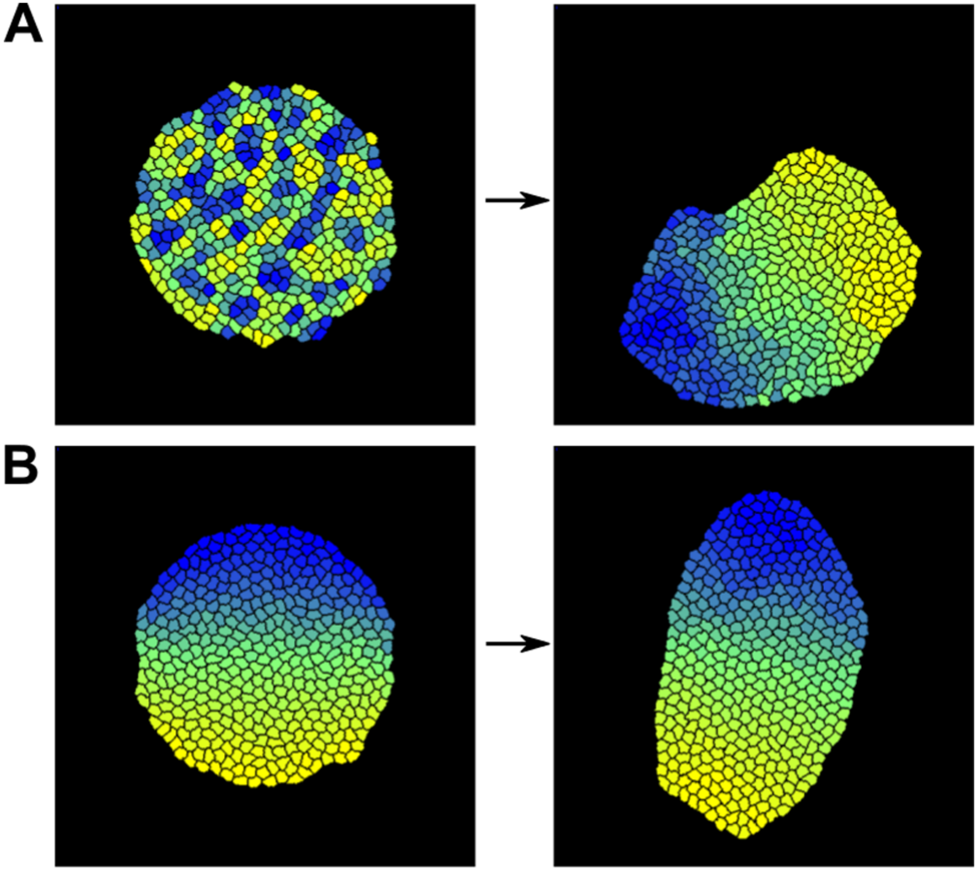
more “realistic”:extension to posterior only
same results

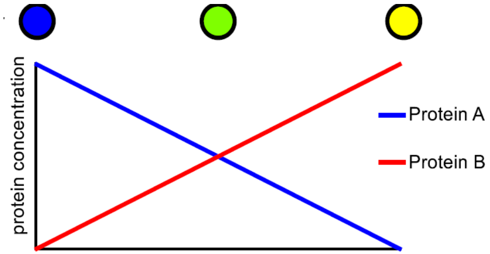
GRADED CE



ONLY SS adh.

Xenopus after mixing of cells: sorting AND CE





For sorting strong persistence is needed;
Weak persistence is sufficient in sorted tissue (WT)

chemotaxis: modeling internal dynamics at different levels of detail

In CPM model chemotaxis can be implemented as ’ extend phyllopodia preferentially in direction of gradient’

How does the cell do this?

Interaction of small g proteins and actin network

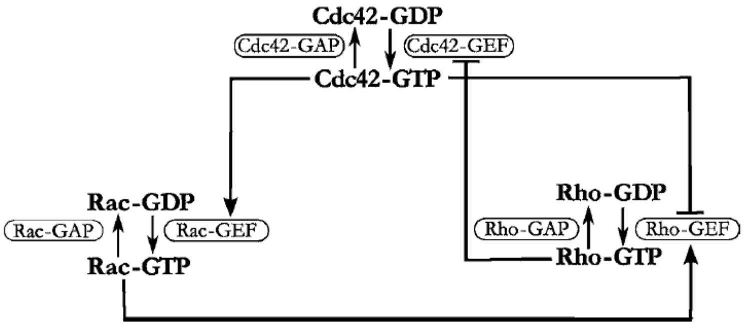
Well studied in Keratocytes

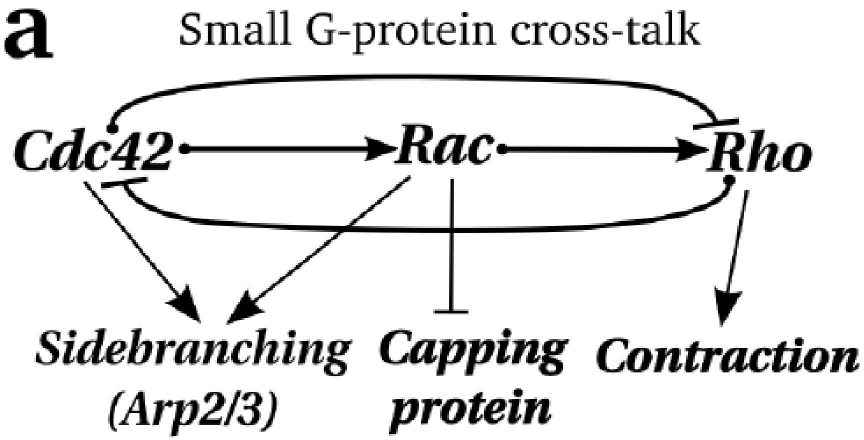
Modeled by Stan Maree et al (Bull Math Biol 2007 and Plos comp biol 2012)

importance of mutual feedback between cell shape and gene regulation

importance of biochemical detail ONLY apparent through this interaction

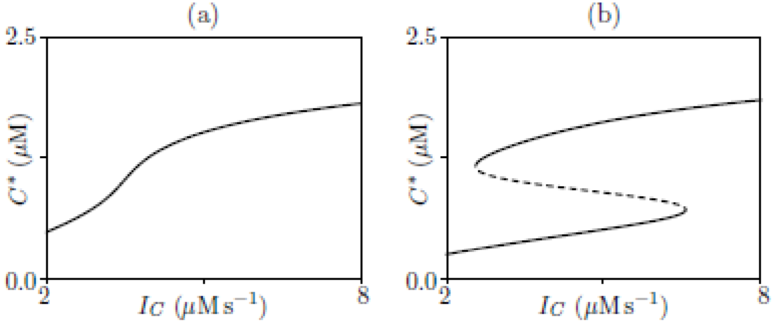
relevant small g protein interactions

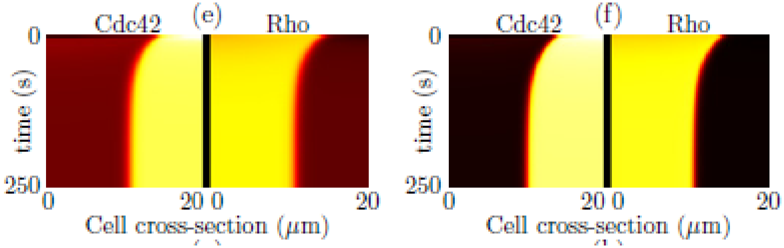




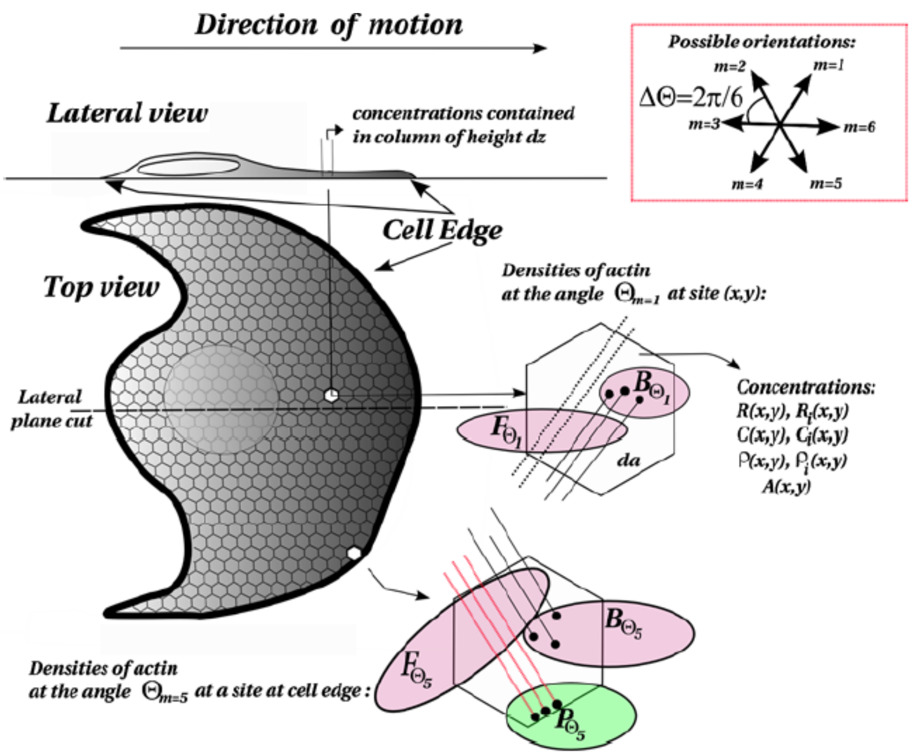
$$\frac{\partial G}{\partial t} = k_{\mathrm{G}}^{+}\mathrm{GEF}_{\mathrm{G}}G_i - k_{\mathrm{G}}^{-}\mathrm{GAP}_{\mathrm{G}}G + D_{\mathrm{m}}\Delta G \quad \text{with } G = C,\, R,\, \rho,$$

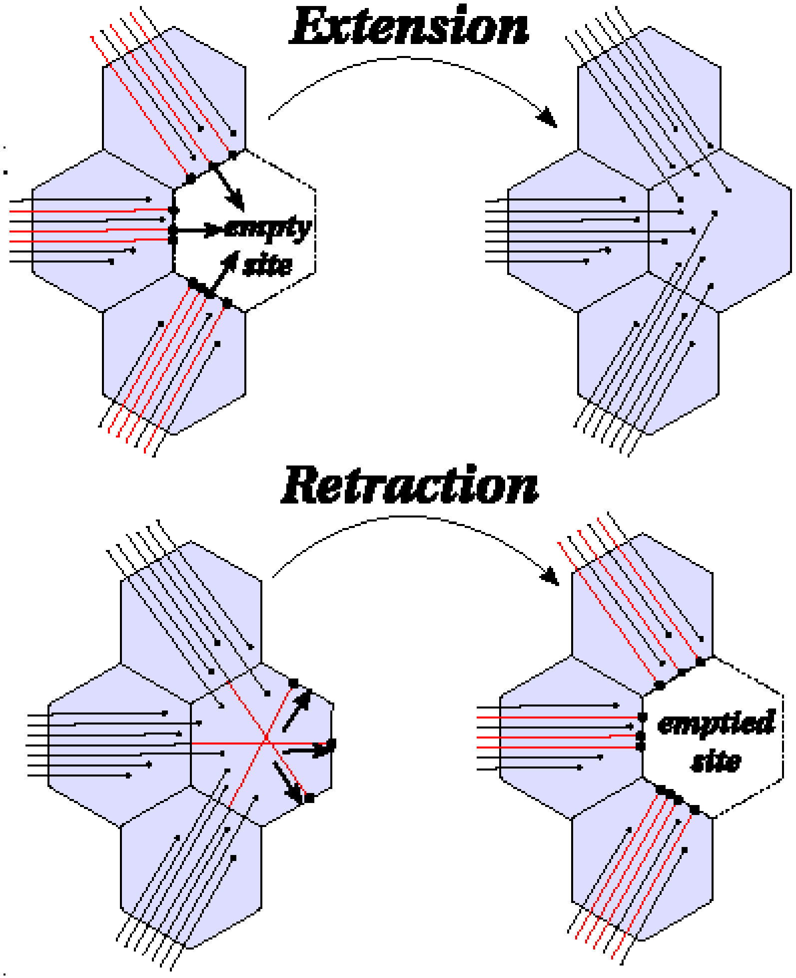
bistability in space due to fast diffusion inactive form





actin dynamics and cell wall dynamics





fully parametrized

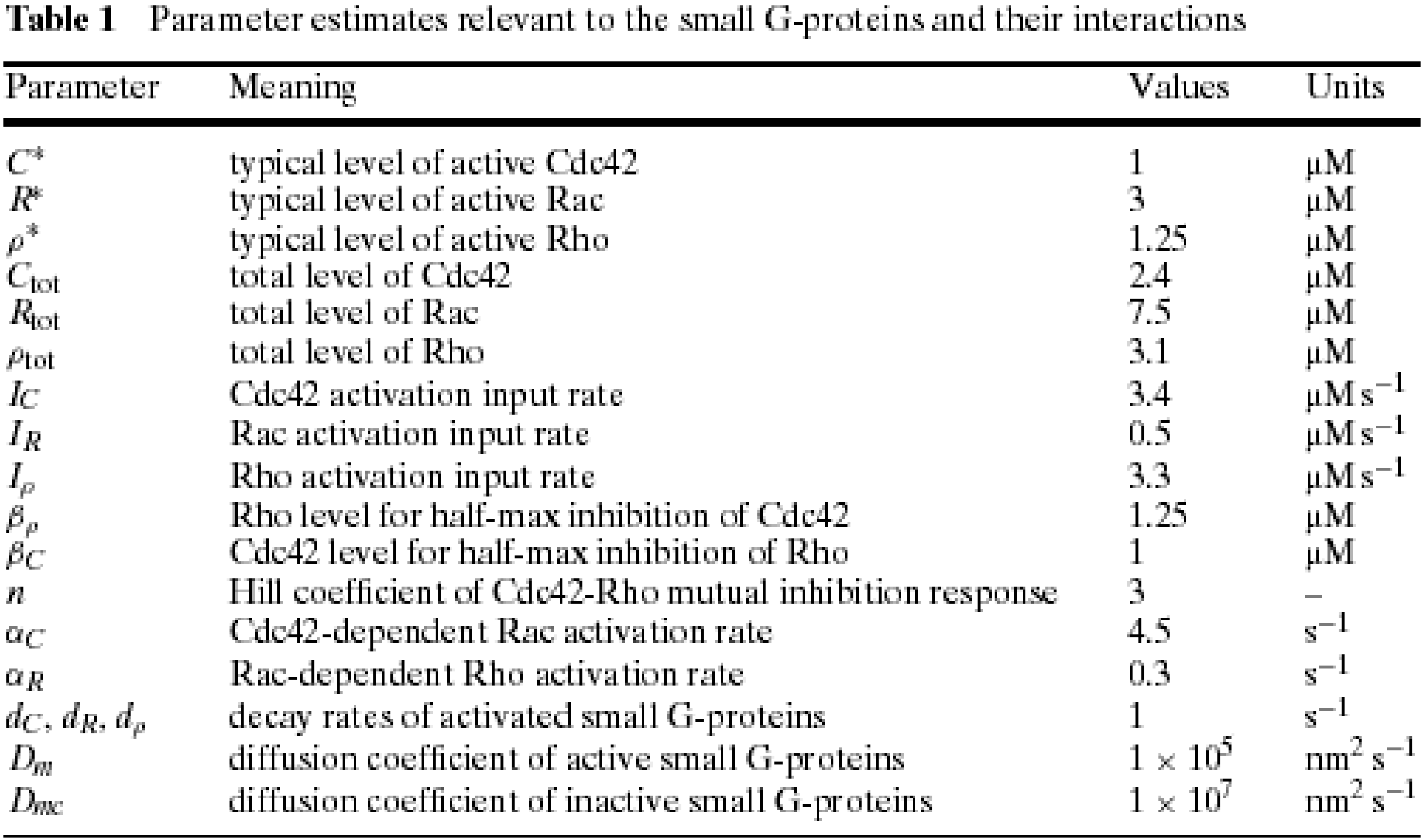


Table 1 Parameter estimates relevant to the small G-proteins and their interactions

Parameter	Meaning	Values	Units
C^*	typical level of active Cdc42	1	μM
R^*	typical level of active Rac	3	μM
ρ^*	typical level of active Rho	1.25	μM
C_{tot}	total level of Cdc42	2.4	μM
R_{tot}	total level of Rac	7.5	μM
ρ_{tot}	total level of Rho	3.1	μM
I_C	Cdc42 activation input rate	3.4	$\mu\mathrm{M\,s^{-1}}$
I_R	Rac activation input rate	0.5	$\mu\mathrm{M\,s^{-1}}$
I_ρ	Rho activation input rate	3.3	$\mu\mathrm{M\,s^{-1}}$
β_ρ	Rho level for half-max inhibition of Cdc42	1.25	μM
β_C	Cdc42 level for half-max inhibition of Rho	1	μM
n	Hill coefficient of Cdc42-Rho mutual inhibition response	3	–
α_C	Cdc42-dependent Rac activation rate	4.5	$\mathrm{s^{-1}}$
α_R	Rac-dependent Rho activation rate	0.3	$\mathrm{s^{-1}}$
d_C, d_R, d_ρ	decay rates of activated small G-proteins	1	$\mathrm{s^{-1}}$
D_m	diffusion coefficient of active small G-proteins	1×10^5	$\mathrm{nm^2\,s^{-1}}$
D_{mc}	diffusion coefficient of inactive small G-proteins	1×10^7	$\mathrm{nm^2\,s^{-1}}$

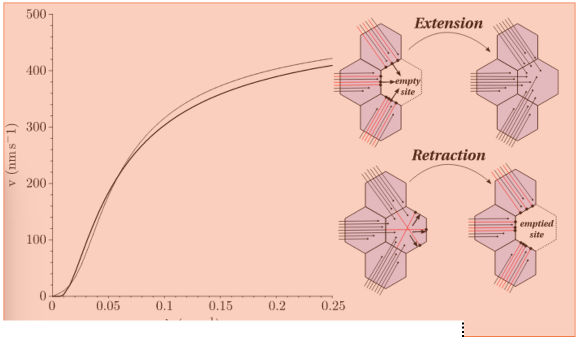
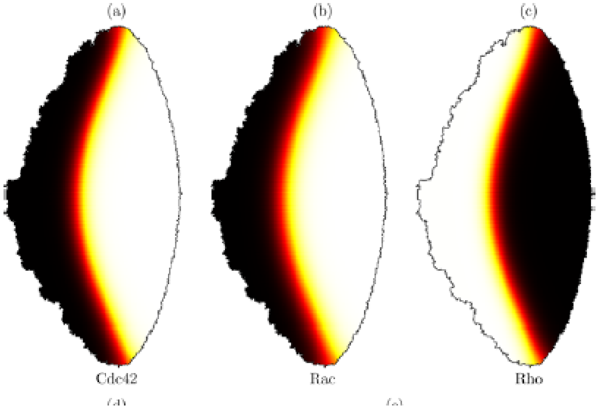
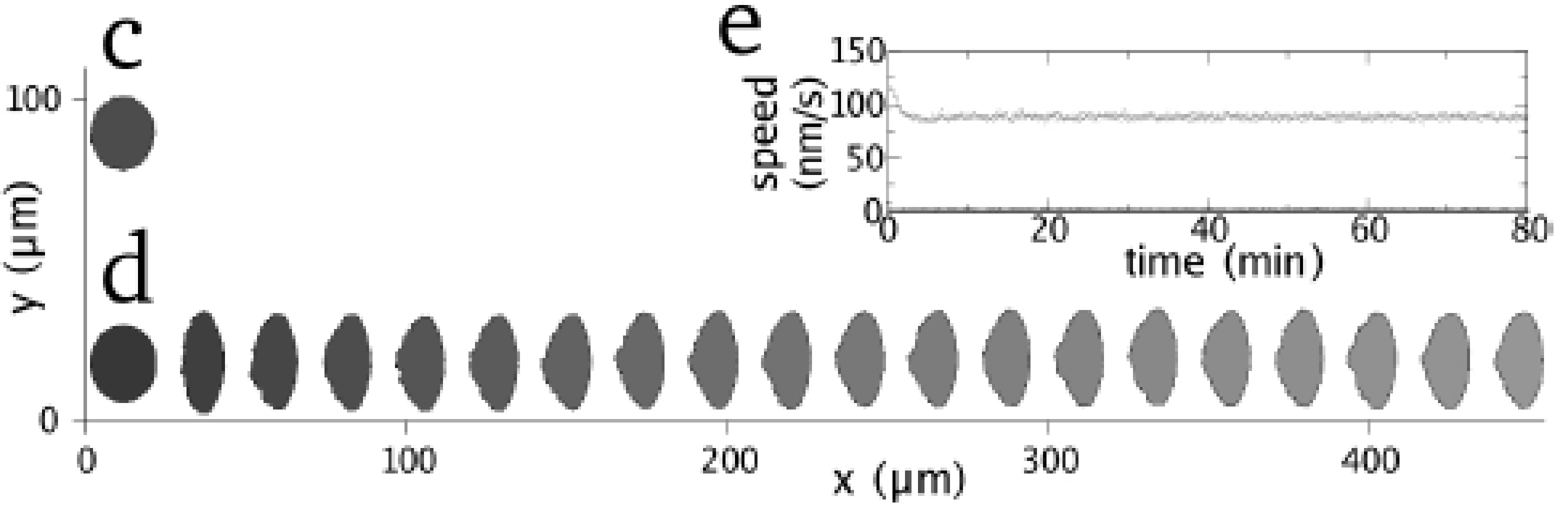


Table 2 Parameter estimates relevant to actin dynamics

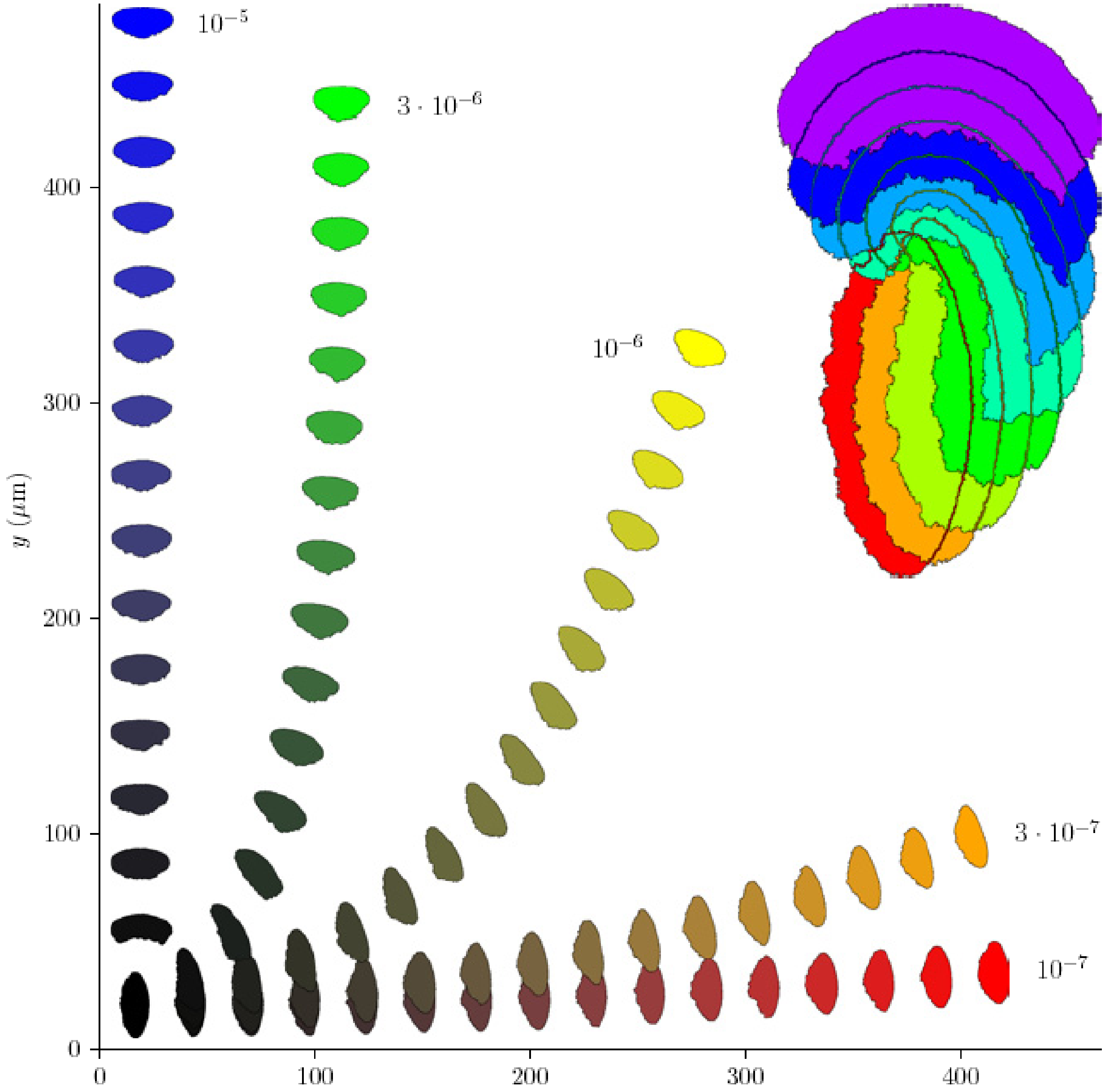
Parameter	Meaning	Values	Units
A^*	typical Arp2/3 concentration	2	μM
F^*	typical filament density	0.278	nm^{-1}
B^*	typical barbed end density	1.7×10^{-5}	nm^{-2}
P^*	typical edge density of barbed ends	0.05	nm^{-1}
μ_C, μ_R	Cdc42 and Rac-dependent Arp2/3 activation	0.16	s^{-1}
d_A	activated Arp2/3 decay rate	0.1	s^{-1}
D_A	diffusion coefficient of Arp2/3	1×10^6	nm^2 s^{-1}
η_0	Arp2/3 nucleation rate	60	μM nm s^{-}
K_m	saturation constant for Arp2/3 nucleation	2	μM
l	scale factor converting units of F to concentration	255	μM nm
k	scale factor converting concentration to units of B	1.06×10^{-4}	nm^{-2} μM
v_0	actin filament growth rate (free polymerization)	500	nm s^{-1}
d_F	actin filament turnover rate	0.03	s^{-1}
κ_{max}	barbed end capping rate	2.8	s^{-1}
κ_{rac}	max reduction of capping by Rac	2.1	s^{-1}
K_R	Rac level for half-max reduction of capping	3	μM
r	reduction of capping close to the edge	0.14	–

Shapes itself into a walking keratocyte and Walks! (and at the correct speed)



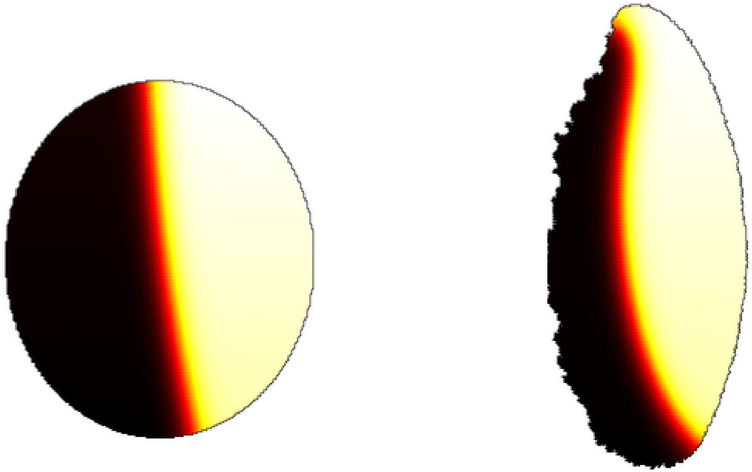


Can reorient itself:
polarity and/vs rotation and/vs shape

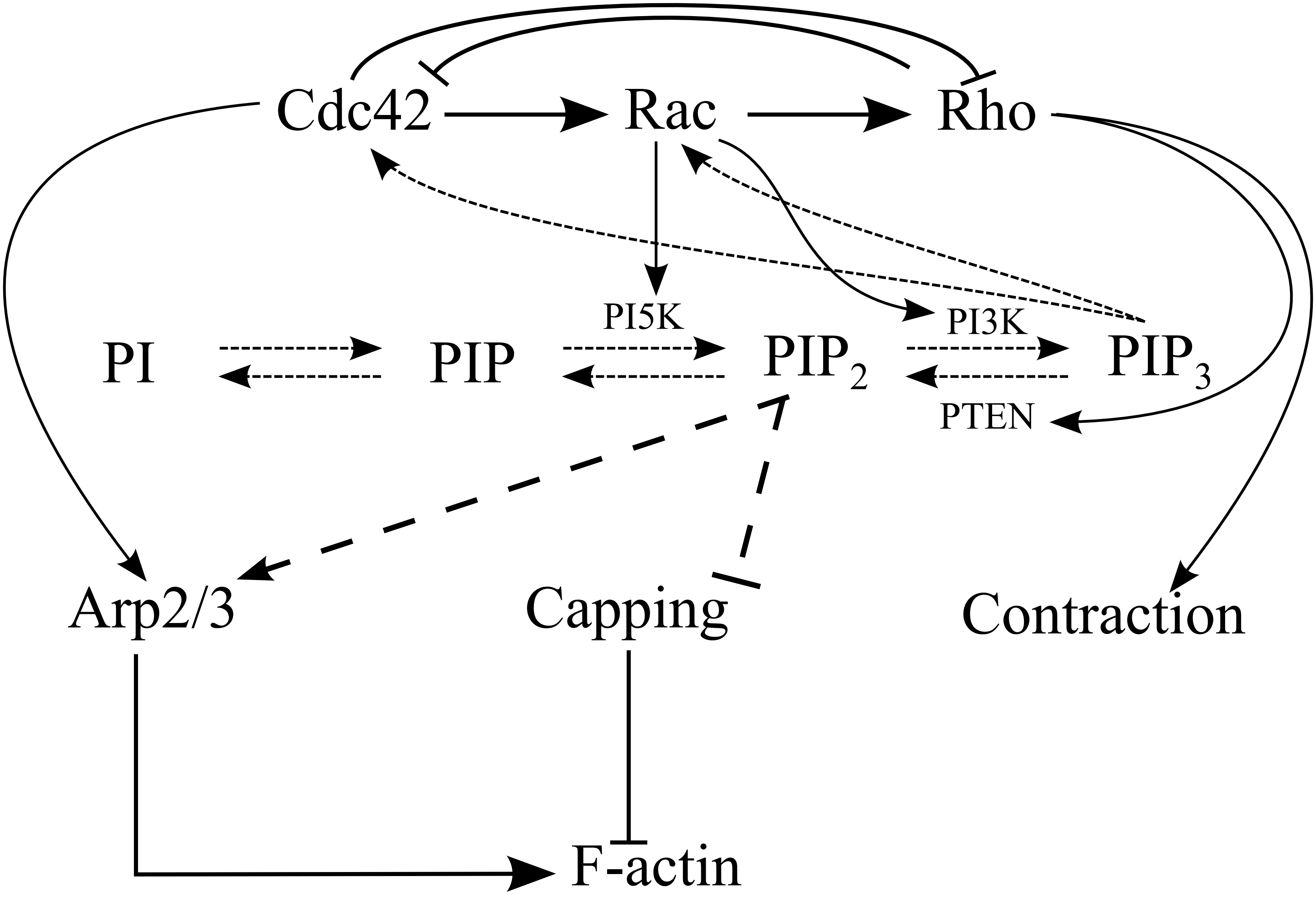


10^{-5}
$3 \cdot 10^{-6}$
10^{-6}
$3 \cdot 10^{-7}$
10^{-7}
y (μm)
0
100
200
300
400
0
100
200
300
400

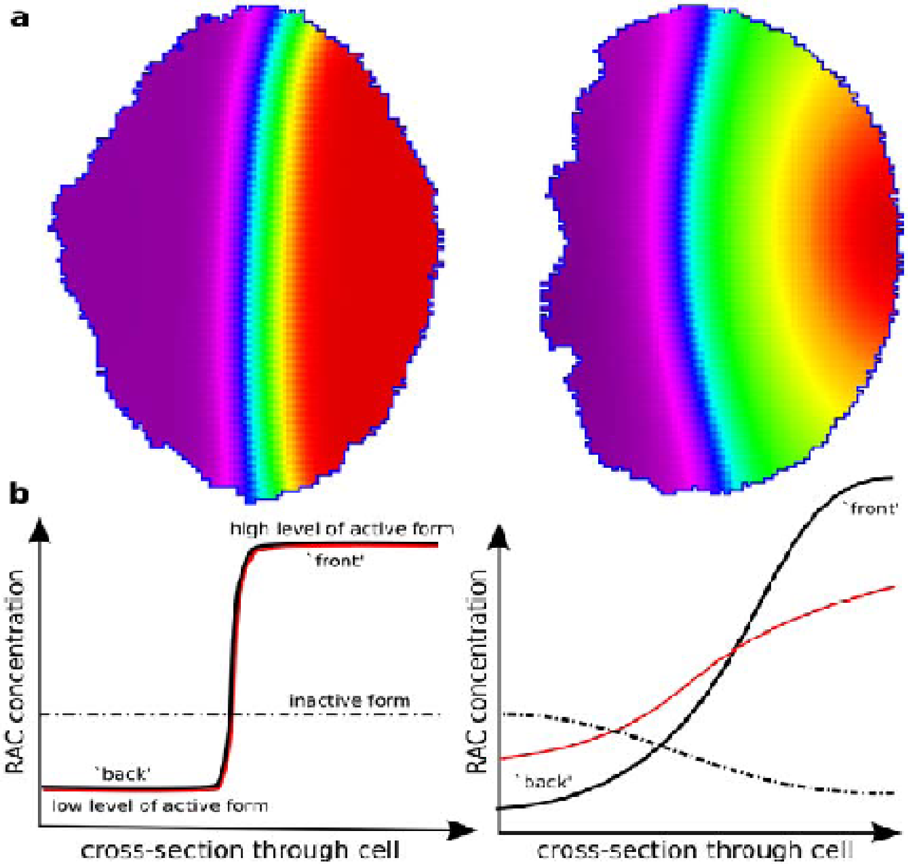
feedback internal dynamics and cell shape faster internal polarity change because of cell shape changes (which are caused by internal polarity change)



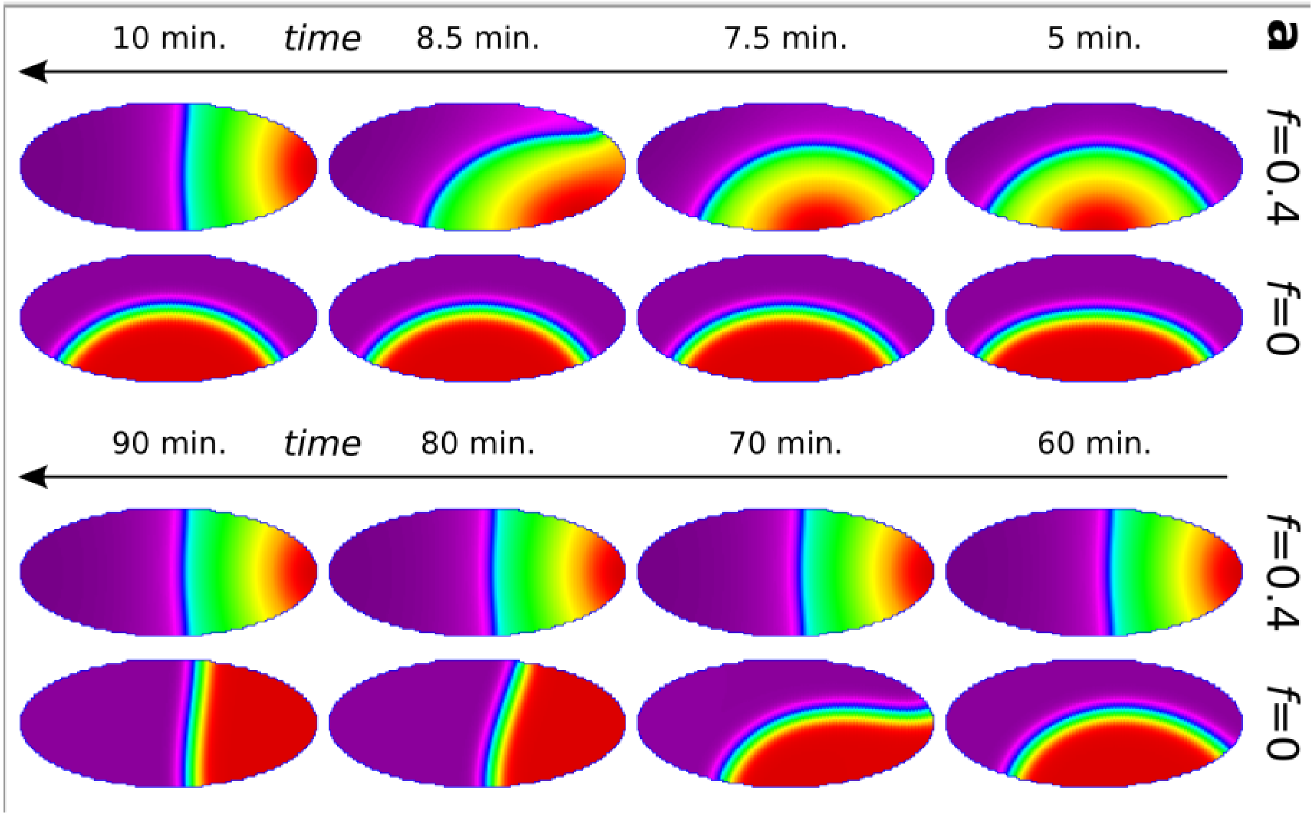
HOWEVER, internal dynamics more complex WHY?



Feedback through PIP network smoothes out gradient

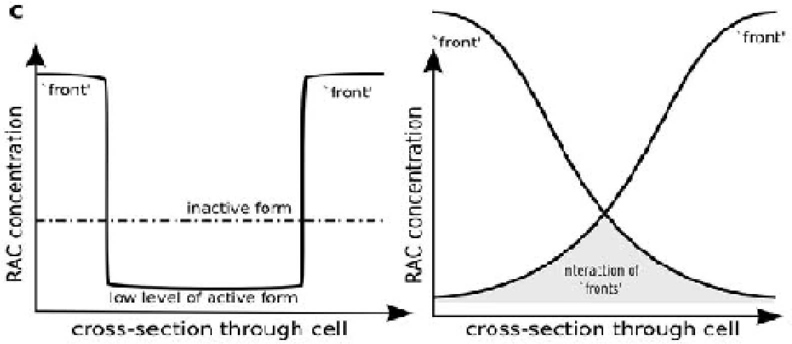


Feedback through PIP network causes faster adaptation

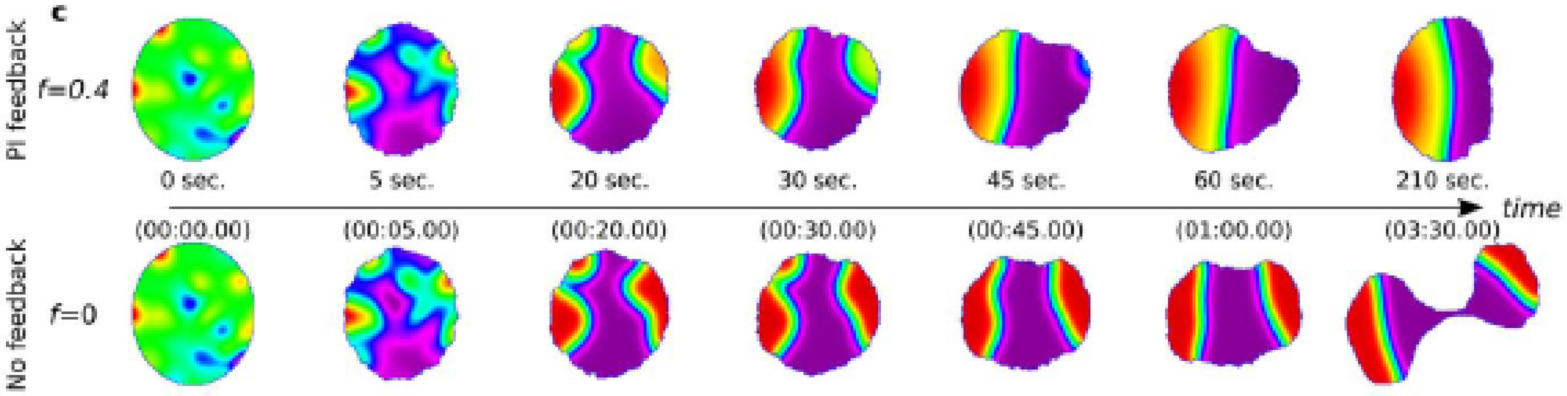


(HOWEVER: in round cell SLOWER reorientation to external signal!)

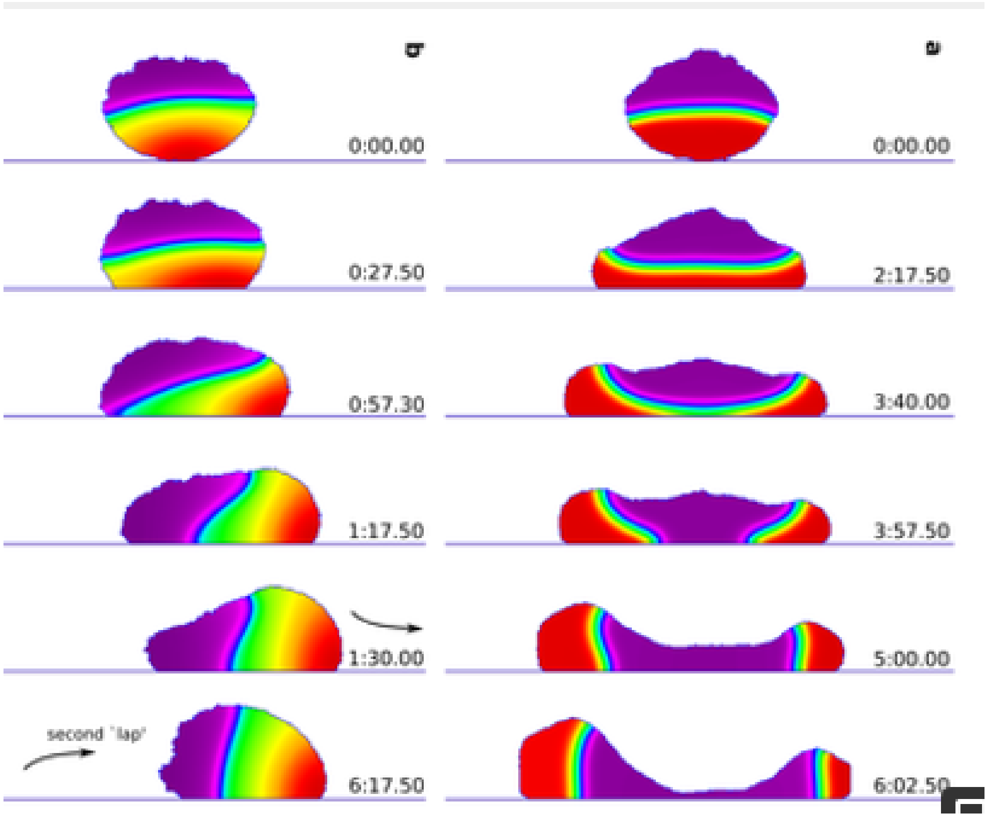
Feedback through PIP network enable resolving conflicting signals



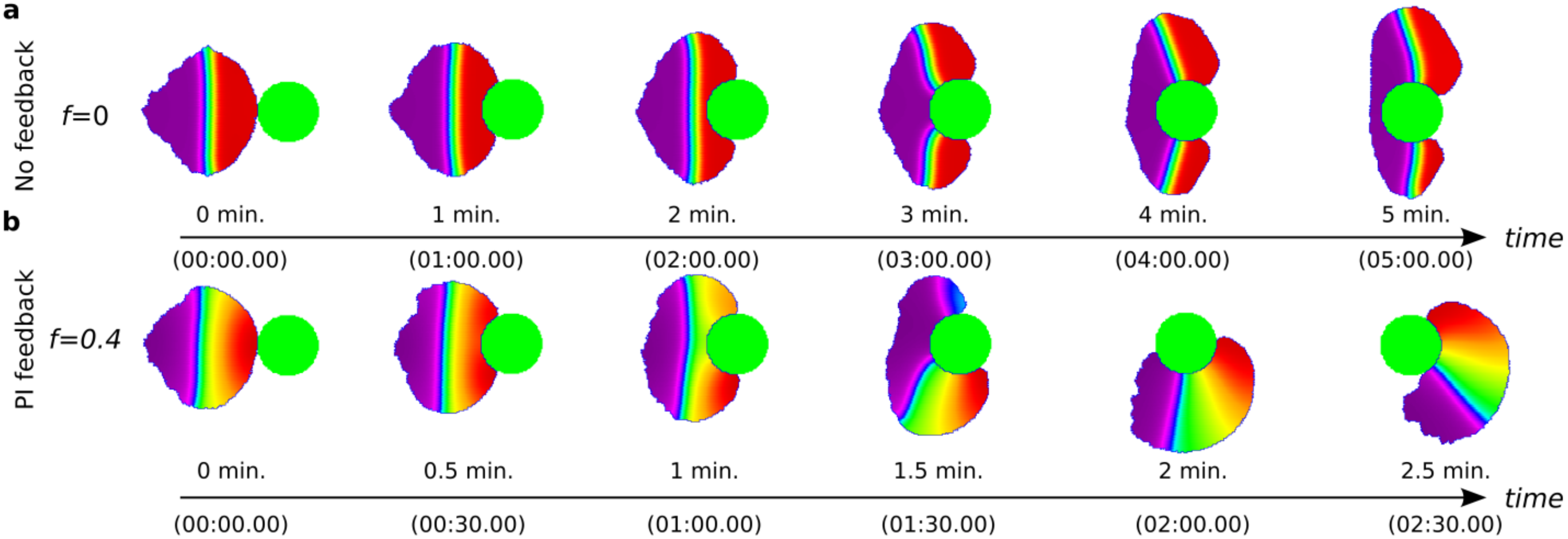
polarization through noise instead of gradient



Feedback through PIP network maintains cell integrity when bumping in wall



Feedback through PIP network maintains cell integrity when bumping in obstacle



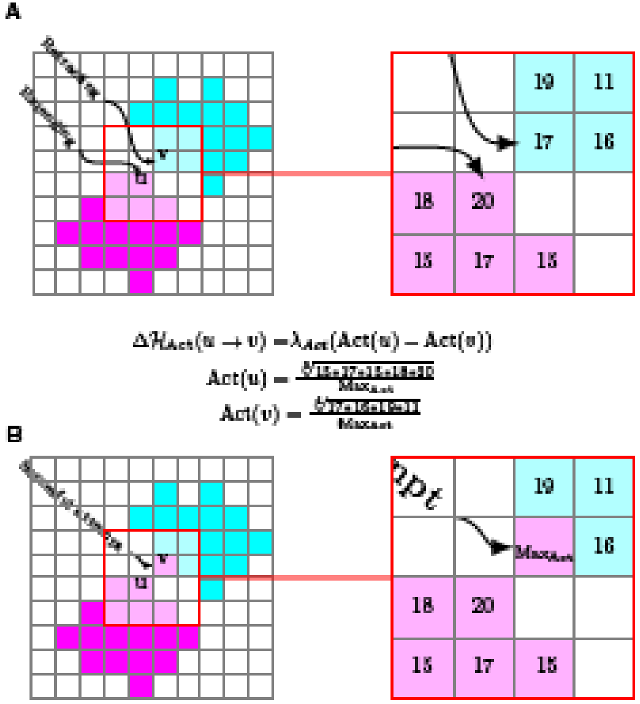
conclusions

Multilevel modeling makes things simpler!

Understanding of complexity at one level
needs understanding of multilevel interactions

speeds up response to cell shape
AND reorientation in flexible cell
AND Maintains cell integrity

Very simple model for Keratocyte AND Amoeboid movement duration of local, directional memory (== actin network persistence) Ioanna Niculescu and Rob de Boer Plos comp biol 2015

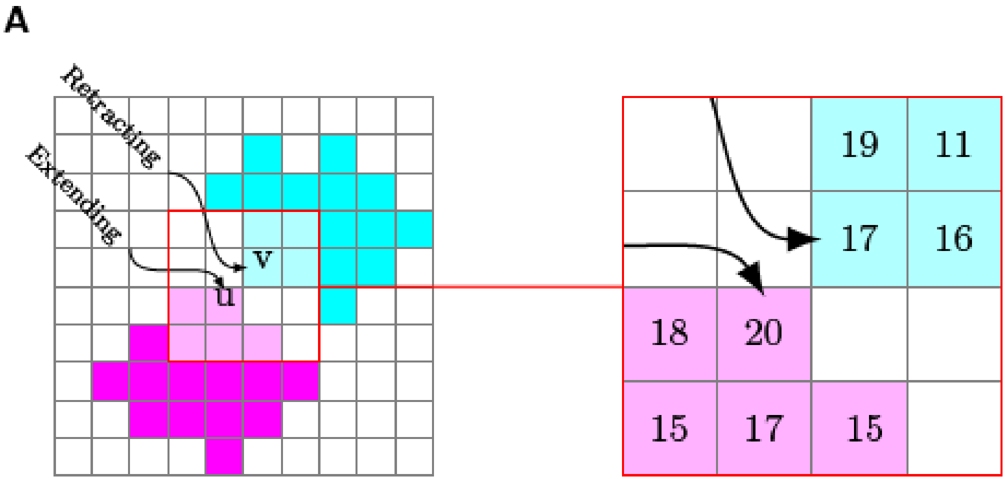


Simple extension of CPM model wit
No representation of internal dynam
Only memory of previous movement
builds up from spontaneous
membrane fluctuations

2 parameters: strength λ

and duration Max

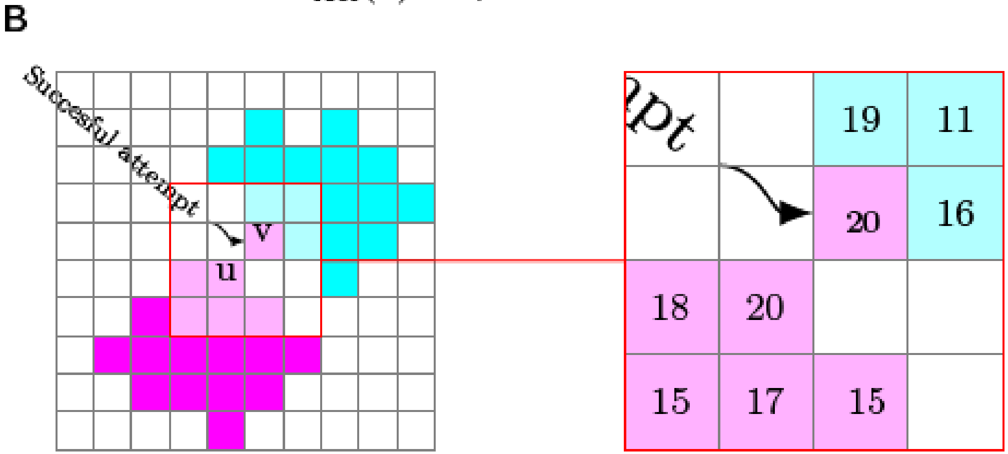
.



$$\Delta\mathcal{H}_{\text{Act}}(u \rightarrow v) = \frac{\lambda_{\text{Act}}}{\text{Max}_{\text{Act}}}(\text{GM}_{\text{Act}}(u) - \text{GM}_{\text{Act}}(v))$$

$$\text{GM}_{\text{Act}}(u) = \sqrt[5]{15 * 17 * 15 * 18 * 20}$$

$$\text{GM}_{\text{Act}}(v) = \sqrt[4]{17 * 16 * 19 * 11}$$

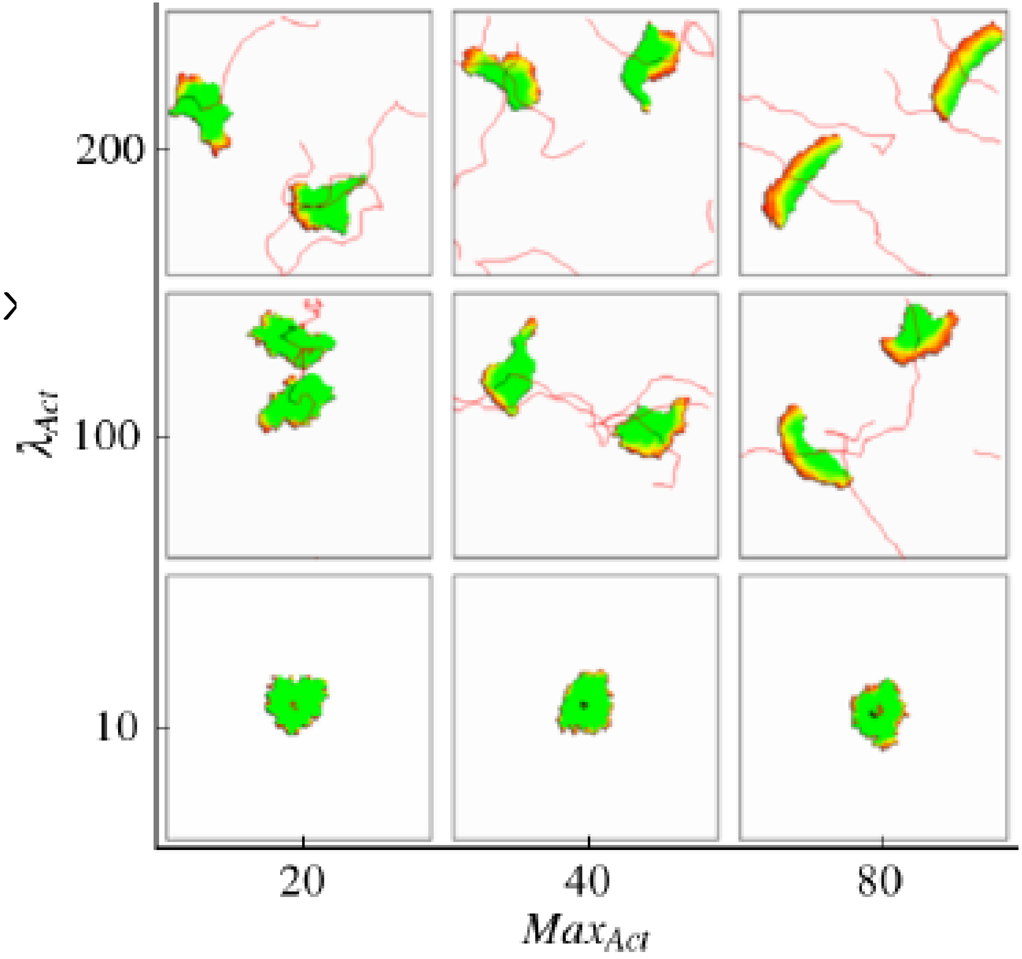


Duration (MAX) determines mode of movement

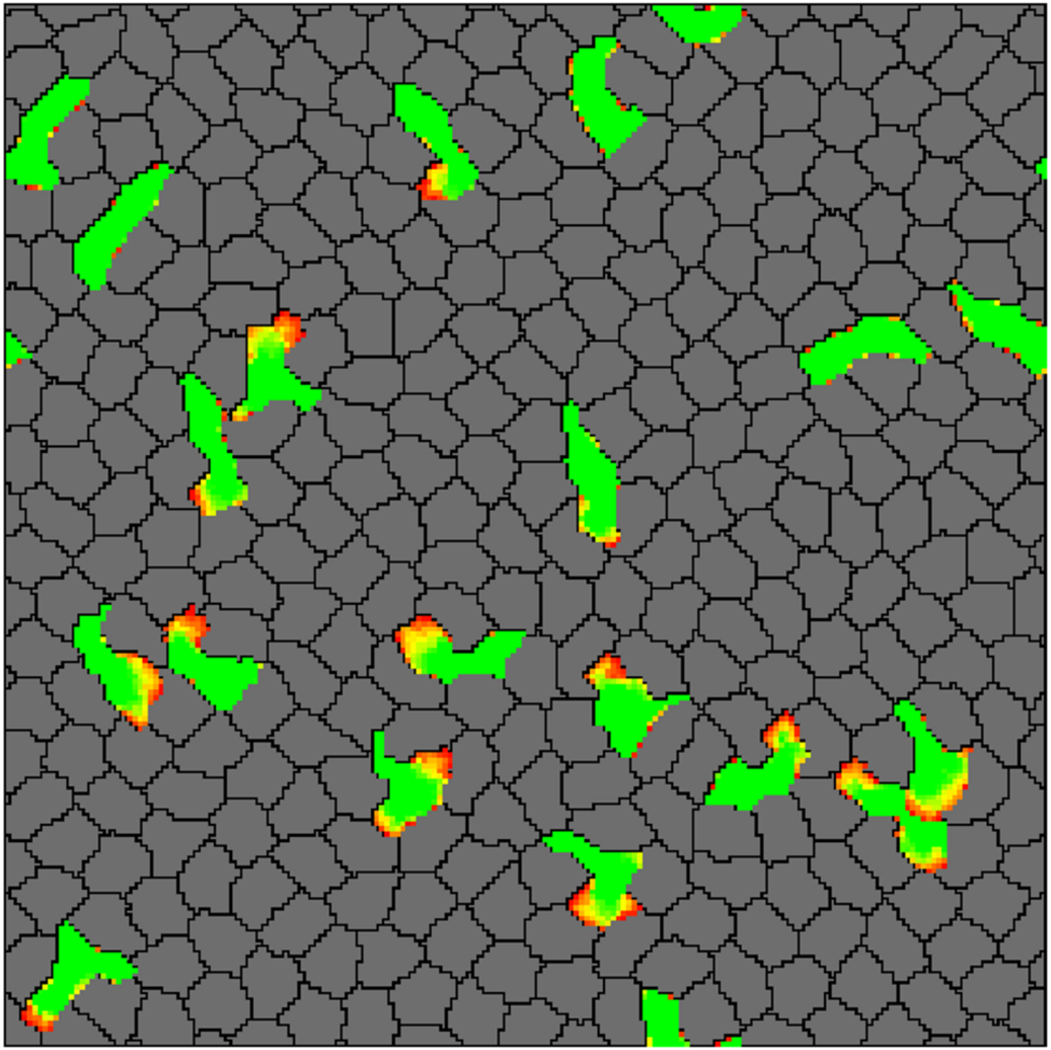
limited duration

long duration

sensitive to chemota



lymphocyte movement through skin



conclusions

Duration of local memory of protrusion sufficient to model difference between keratocyte and amoeboid movement

Keratocytes very robust (like extended model with PIP network)
Why?

Efficient Movement within tight tissue by small cell shape fluctuations

“How to compute an organism Multilevel modeling of Morphogenesis bridging levels of organization

Model premises

- Target morhogenesis ss (not only pattern formation)
- Cell basic unit (growth, division, movement, ...)
- Cell is NOT point, bead, homunculus
- Cells are deformable highly viscuous objects
- Genes act through cells ’with a dynamics of their own”

use CPM as simple but basically correct representation of a cell

Finding Sufficient Conditions for complex behavior using only (subset of) known processes allowing many (open set) different observations

---

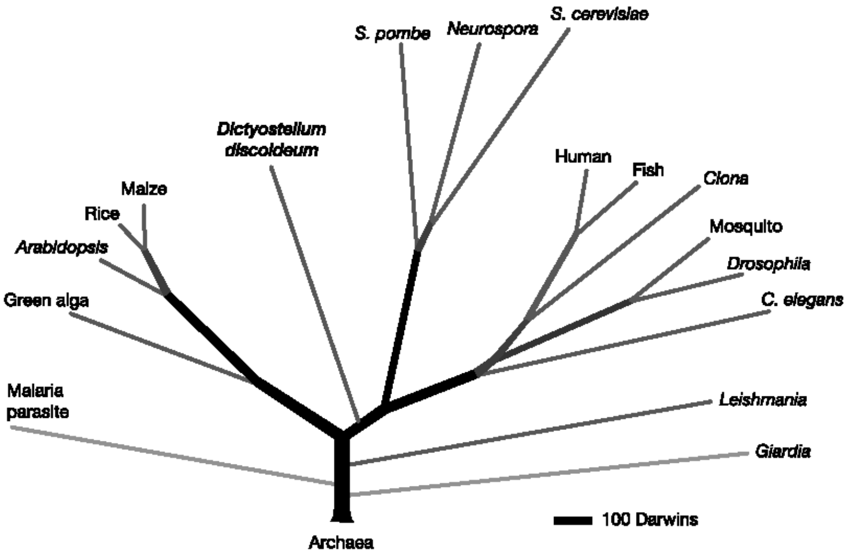
explicit 2-level model for implicit multilevel behavior

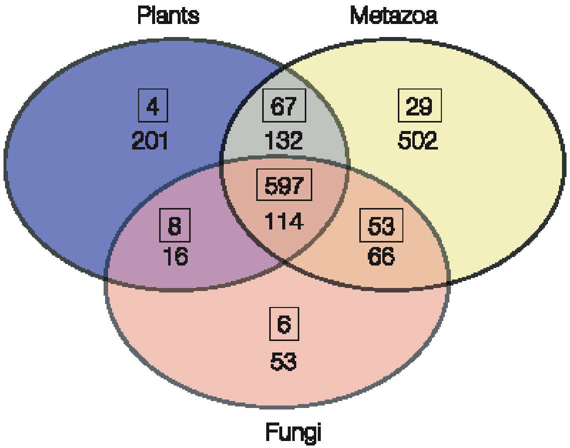
Dd morphodynamics:

From single cells (amoebae) to
multicellular 'individuals'
with 'new' ways of sensing
and metamorphosis
to groups of those

Savill et al 1997, Marée et al 1999a,b, 2001,2002

Dictyostelium phylogeny

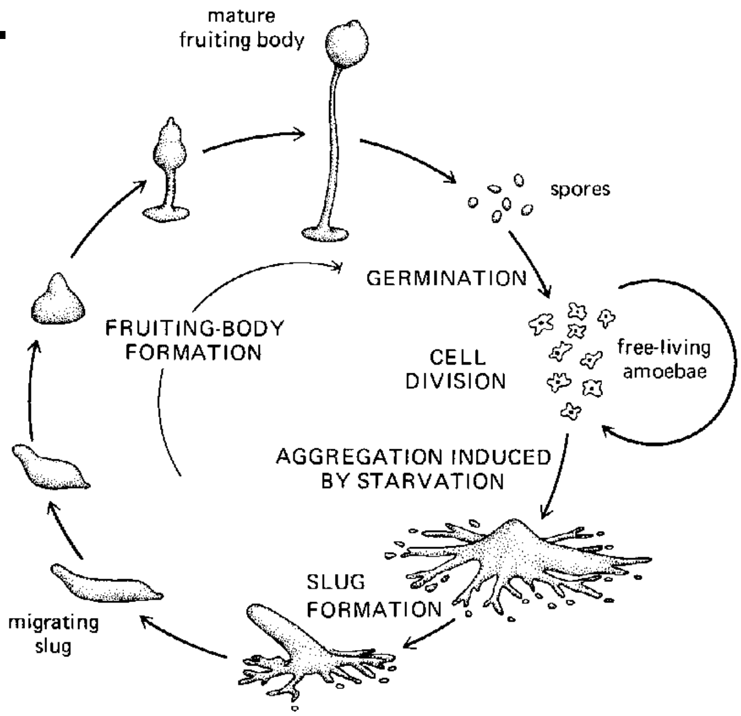




Early offshoot:

shares protein domains otherwise exclusive for plants, fungi, and animals

Lifecycle Dictyostelium discoideum



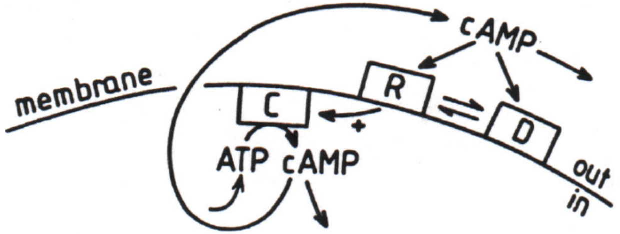
Question

Can the morphodynamics of Dd emerge by selforganization
from the behavior of the 2scale CA "cells" ?
when (a minimum of) known properties of Dd are added?

YES...(almost)

Goldbeter-Martel model of cAMP signaling

Models for cAMP signalling in Dictyostelium



equations

$$\frac{d\rho}{dt} = -f_1(\gamma)\rho + f_2(\gamma)(1-\rho), \tag{1}$$

$$\epsilon' \frac{d\beta}{dt} = s_1\Phi(\rho,\gamma) - \beta, \tag{2}$$

$$\epsilon \frac{d\gamma}{dt} = s_2\beta - \gamma, \tag{3}$$

where

ρ = fraction of receptor in active state,

$\beta = [\mathrm{cAMP}]_{\mathrm{intracellular}}/K_{\mathrm{R}}$,

$\gamma = [\mathrm{cAMP}]_{\mathrm{extracellular}}/K_{\mathrm{R}}$,

$t = k_1 \times$ time,

and

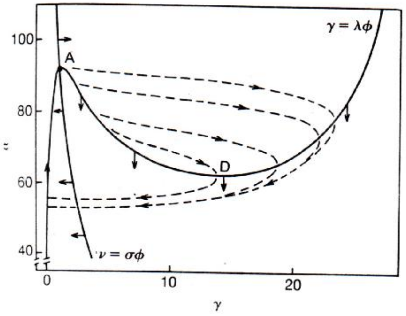
$$f_1(\gamma) = \frac{1+\kappa\gamma}{1+\gamma}, \quad f_2(\gamma) = \frac{L_1 + \kappa L_2 c\gamma}{1+c\gamma},$$

$$\Phi(\rho,\gamma) = \frac{\lambda_1 + Y^2}{\lambda_2 + Y^2}, \quad Y = \frac{\rho\gamma}{1+\gamma}.$$

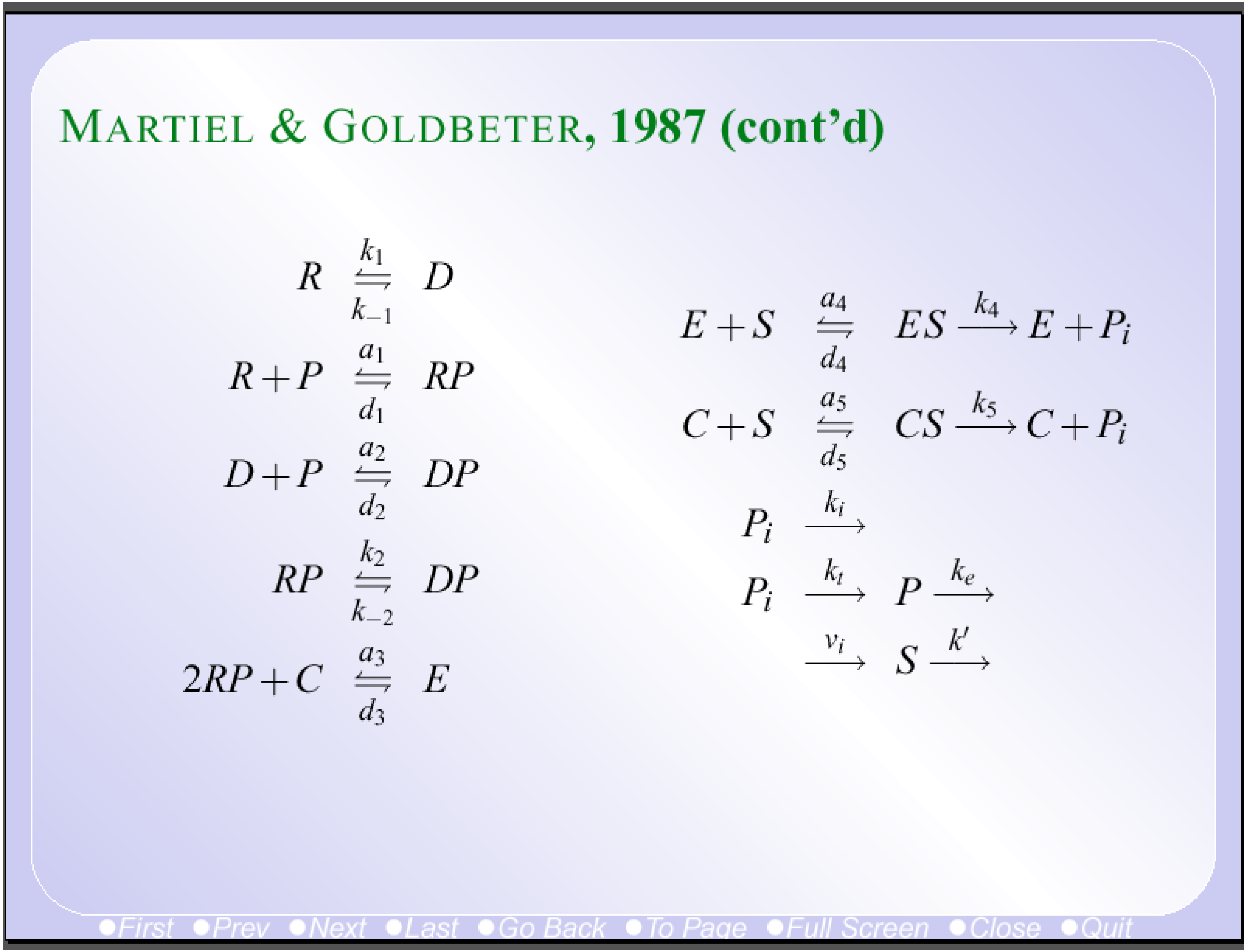
The parameters appearing in system (1)–(3) are explained and estimated in tables I and II; refer also to fig. 2.

Parameter set A in table II was used by Martiel and Goldbeter [16] to model autonomous oscillations of cAMP in stirred suspensions of *Dictyostelium* cells. The numerical solution of the

5.3 Relay and oscillations



chemical reactions Goldbeter model



MARTIEL & GOLDBETER, 1987 (cont’d)

$$R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} D$$

$$R+P \underset{d_1}{\overset{a_1}{\rightleftharpoons}} RP$$

$$D+P \underset{d_2}{\overset{a_2}{\rightleftharpoons}} DP$$

$$RP \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} DP$$

$$2RP+C \underset{d_3}{\overset{a_3}{\rightleftharpoons}} E$$

$$E+S \underset{d_4}{\overset{a_4}{\rightleftharpoons}} ES \xrightarrow{k_4} E+P_i$$

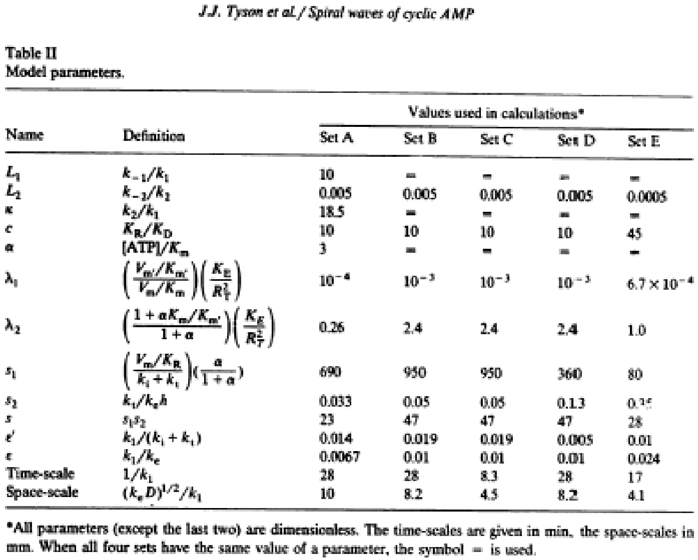
$$C+S \underset{d_5}{\overset{a_5}{\rightleftharpoons}} CS \xrightarrow{k_5} C+P_i$$

$$P_i \xrightarrow{k_i}$$

$$P_i \xrightarrow{k_t} P \xrightarrow{k_e}$$

$$\xrightarrow{v_i} S \xrightarrow{k'}$$

Parameter estimates of Goldbeter-model (Tyson 1989)



J.J. Tyson et al. / Spiral waves of cyclic AMP

Table II
Model parameters.

Name	Definition	Values used in calculations*				
		Set A	Set B	Set C	Set D	Set E
L_1	k_{-1}/k_1	10	=	=	=	=
L_2	k_{-2}/k_2	0.005	0.005	0.005	0.005	0.0005
κ	k_2/k_1	18.5	=	=	=	=
c	K_R/K_D	10	10	10	10	45
α	$[ATP]/K_m$	3	=	=	=	=
λ_1	$\left(\frac{V_{m'}/K_{m'}}{V_m/K_m}\right)\left(\frac{K_E}{R_T^2}\right)$	10^{-4}	10^{-3}	10^{-3}	10^{-3}	6.7×10^{-4}
λ_2	$\left(\frac{1+\alpha K_m/K_{m'}}{1+\alpha}\right)\left(\frac{K_E}{R_T^2}\right)$	0.26	2.4	2.4	2.4	1.0
s_1	$\left(\frac{V_m/K_R}{k_i+k_t}\right)\left(\frac{\alpha}{1+\alpha}\right)$	690	950	950	360	80
s_2	$k_t/k_e h$	0.033	0.05	0.05	0.13	0.35
s	$s_1 s_2$	23	47	47	47	28
ϵ'	$k_1/(k_i+k_t)$	0.014	0.019	0.019	0.005	0.01
ϵ	k_1/k_e	0.0067	0.01	0.01	0.01	0.024
Time-scale	$1/k_1$	28	28	8.3	28	17
Space-scale	$(k_e D)^{1/2}/k_1$	10	8.2	4.5	8.2	4.1

*All parameters (except the last two) are dimensionless. The time-scales are given in min, the space-scales in mm. When all four sets have the same value of a parameter, the symbol = is used.

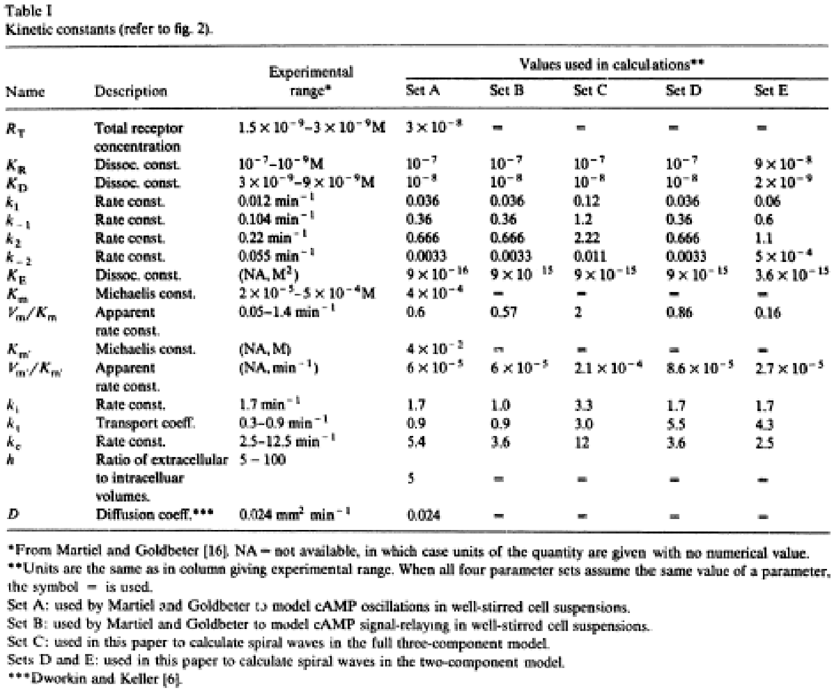


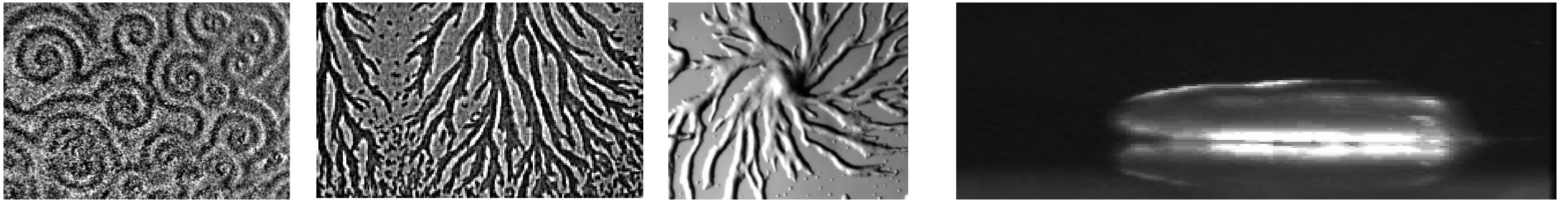
Table I
Kinetic constants (refer to fig. 2).

Name	Description	Experimental range*	Values used in calculations**				
			Set A	Set B	Set C	Set D	Set E
R_T	Total receptor concentration	1.5×10^{-9}–3×10^{-9}M	3×10^{-8}	=	=	=	=
K_R	Dissoc. const.	10^{-7}–10^{-9}M	10^{-7}	10^{-7}	10^{-7}	10^{-7}	9×10^{-8}
K_D	Dissoc. const.	3×10^{-9}–9×10^{-9}M	10^{-8}	10^{-8}	10^{-8}	10^{-8}	2×10^{-9}
k_1	Rate const.	0.012 min^{-1}	0.036	0.036	0.12	0.036	0.06
k_{-1}	Rate const.	0.104 min^{-1}	0.36	0.36	1.2	0.36	0.6
k_2	Rate const.	0.22 min^{-1}	0.666	0.666	2.22	0.666	1.1
k_{-2}	Rate const.	0.055 min^{-1}	0.0033	0.0033	0.011	0.0033	5×10^{-4}
K_E	Dissoc. const.	(NA, M^2)	9×10^{-16}	9×10^{-15}	9×10^{-15}	9×10^{-15}	3.6×10^{-15}
K_m	Michaelis const.	2×10^{-5}–5×10^{-4}M	4×10^{-4}	=	=	=	=
V_m/K_m	Apparent rate const.	0.05–1.4 min^{-1}	0.6	0.57	2	0.86	0.16
$K_{m'}$	Michaelis const.	(NA, M)	4×10^{-2}	=	=	=	=
$V_{m'}/K_{m'}$	Apparent rate const.	(NA, min^{-1})	6×10^{-5}	6×10^{-5}	2.1×10^{-4}	8.6×10^{-5}	2.7×10^{-5}
k_i	Rate const.	1.7 min^{-1}	1.7	1.0	3.3	1.7	1.7
k_t	Transport coeff.	0.3–0.9 min^{-1}	0.9	0.9	3.0	5.5	4.3
k_e	Rate const.	2.5–12.5 min^{-1}	5.4	3.6	12	3.6	2.5
h	Ratio of extracellular to intracelluar volumes.	5 – 100	5	=	=	=	=
D	Diffusion coeff.***	0.024 $\mathrm{mm}^2\ \mathrm{min}^{-1}$	0.024	=	=	=	=

*From Martiel and Goldbeter [16]. NA = not available, in which case units of the quantity are given with no numerical value.
**Units are the same as in column giving experimental range. When all four parameter sets assume the same value of a parameter, the symbol = is used.
Set A: used by Martiel and Goldbeter to model cAMP oscillations in well-stirred cell suspensions.
Set B: used by Martiel and Goldbeter to model cAMP signal-relaying in well-stirred cell suspensions.
Set C: used in this paper to calculate spiral waves in the full three-component model.
Sets D and E: used in this paper to calculate spiral waves in the two-component model.
***Dworkin and Keller [6].

$-- >$ spirals

from single cell to moving slug



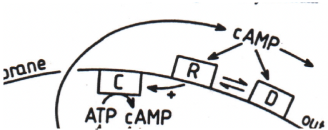
GG 2scale CA (CPM) + excitable mediun (PDE) + chemotaxis

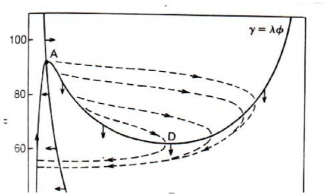
$J_{y,y} < J_{y,g} = J_{g,g} < J_{*,M}$

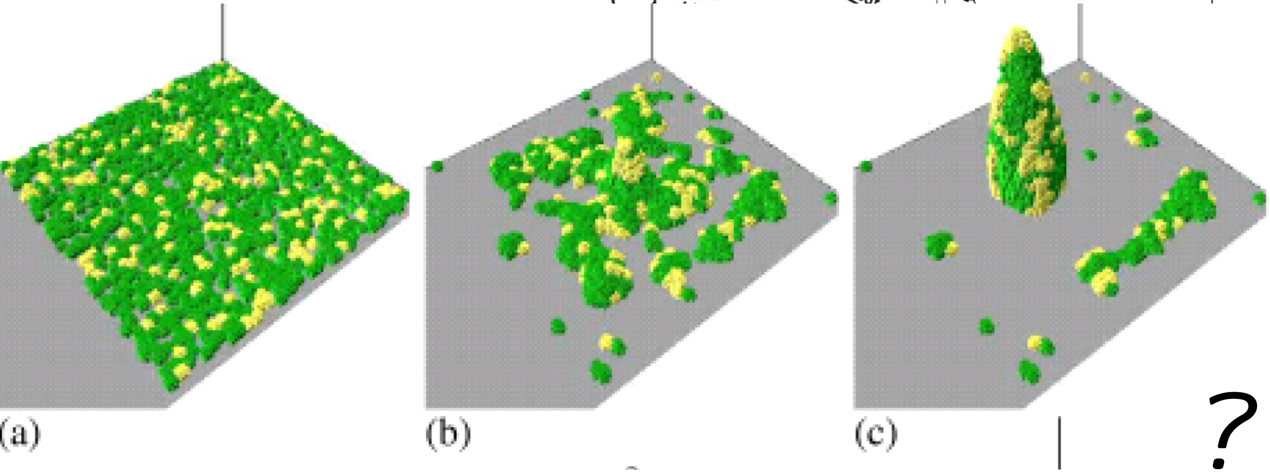
cAMP dynamics

towards cAMP

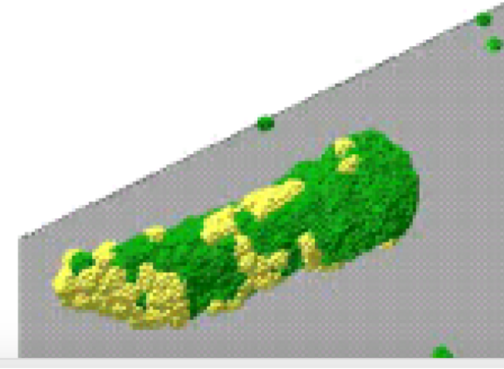
refractory period







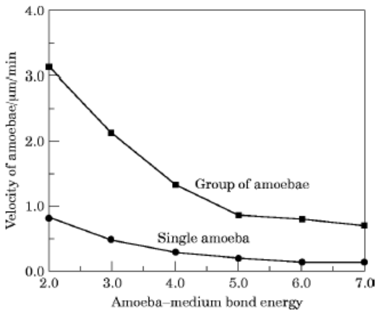
?

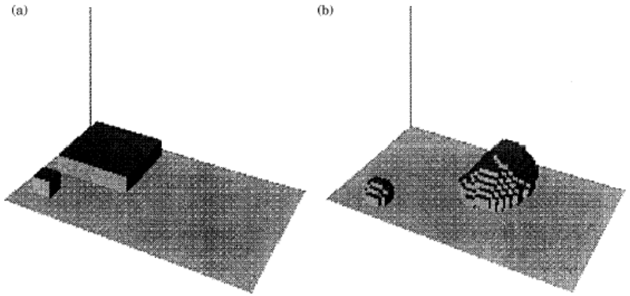


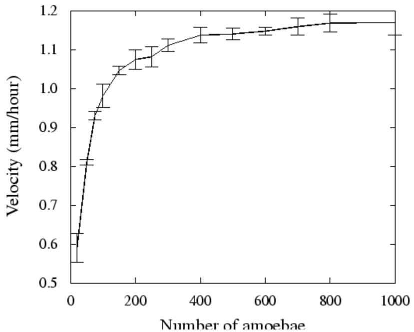
Savill & Hogeweg J Theor. Biol (1997)

aggregation and SLUG: behaviour ++

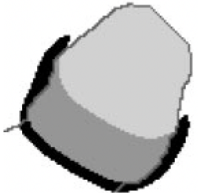
- Faster movement in streams & larger slug move faster then smaller ones



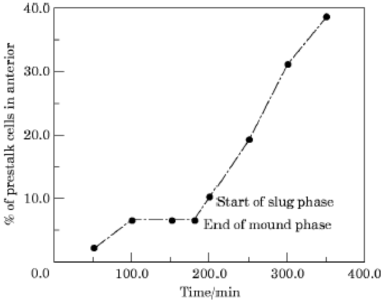




- Slug keeps elongated shape because of cAMP diffusion: curved wavefront



- Cell sorting during slug-phase:
  differential adhesion + equal chemotaxis + movement



Marée & al 1999, Savill & Hogeweg 1997

Emergent sensing of environment in slug
Thermotaxis (and phototaxis)

cAMP dynamics depends on temperature

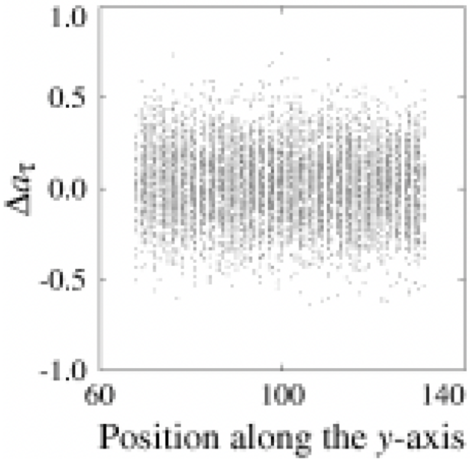
skews shape of wave front

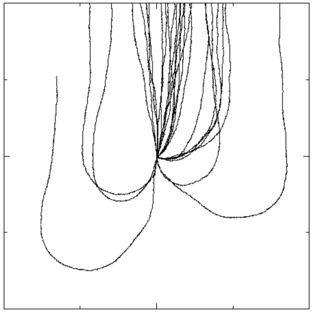
cell chemotaxis up gradient

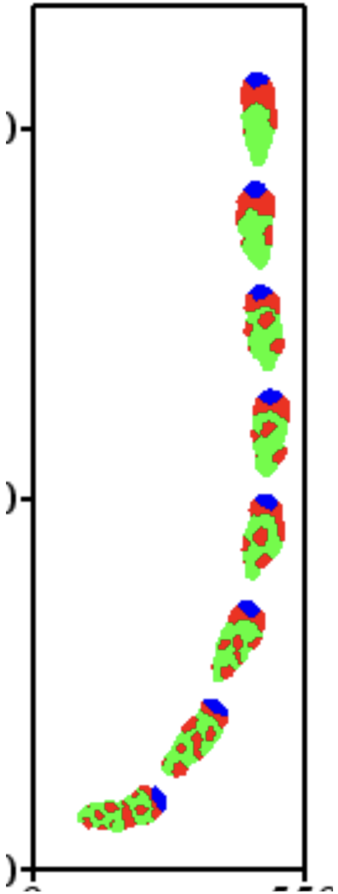
pushes slug towards higher temeratures

noise reduction!

Marée & al 1999







the culmination (fruiting body formation)

++Cell differentiation:

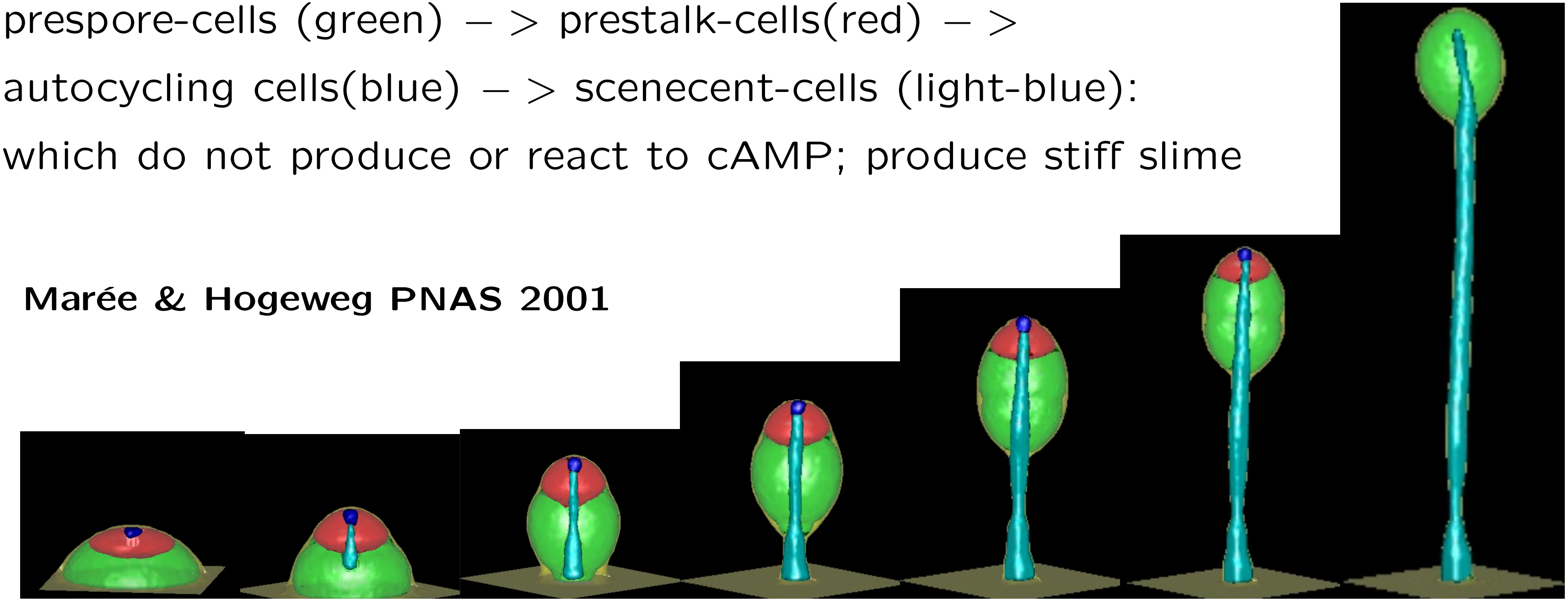
prespore-cells (green) −> prestalk-cells(red) −>

autocycling cells(blue) −> scenecent-cells (light-blue):

which do not produce or react to cAMP; produce stiff slime

Marée & Hogeweg PNAS 2001

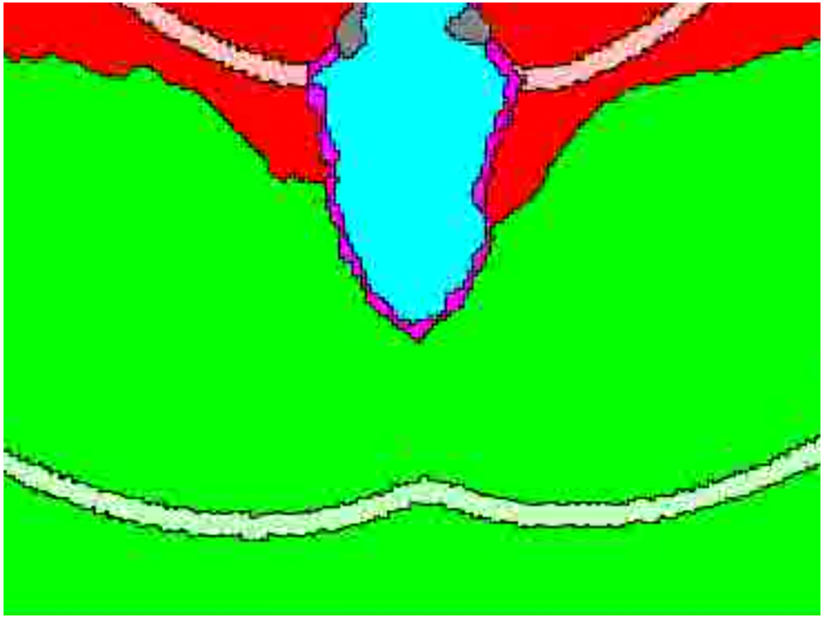
?



CPM mechanics of culmination

how does the stalk move down?

why does it stop when bottom is reached?



front of cAMP wave

cell sizes due to chemotaxis

Pressure waves in prespore cells push
the non-responding scenecent cells downwards
This stops when no prespore cells surround them
(i.e. when the prespore cells moved upwards toward the cAMP waves)

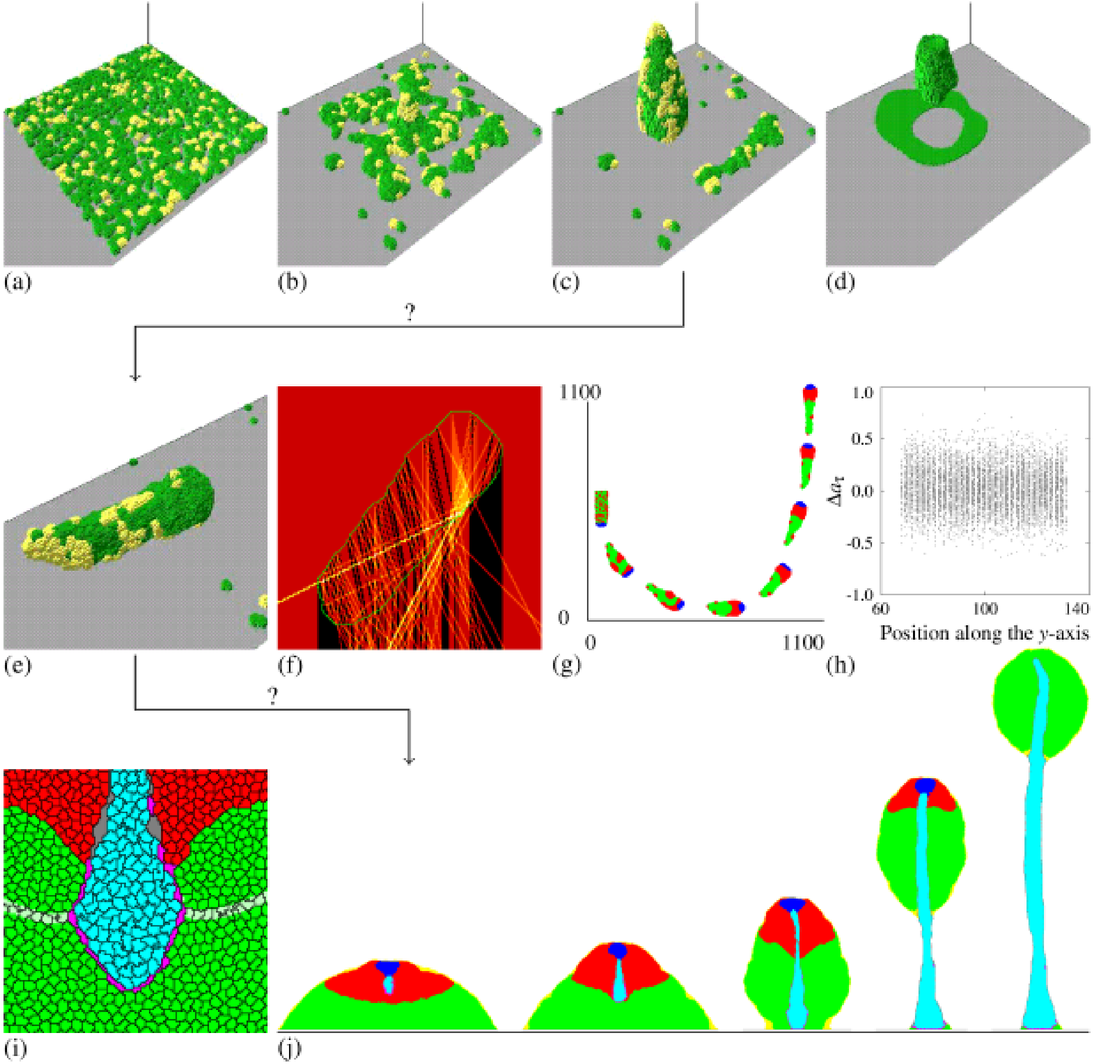
Marée & Hogeweg PNAS 2001

Lifecycle of Dd by chemotaxis and adhesion

aggregation
streams

orientation

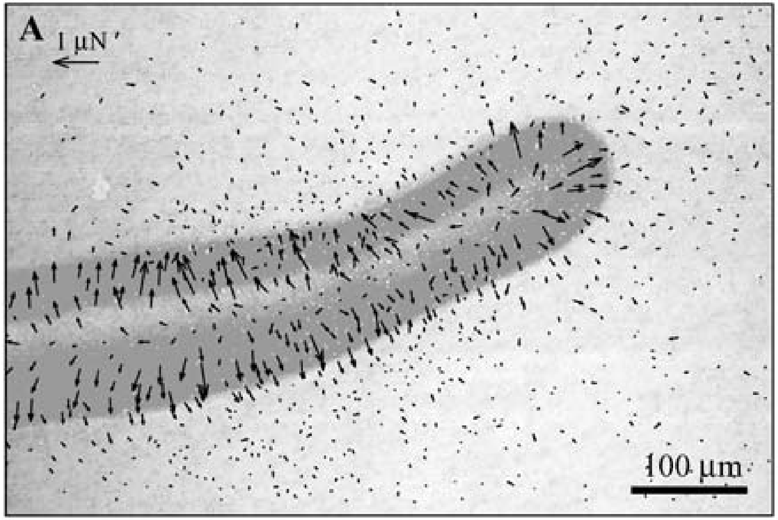
culmination

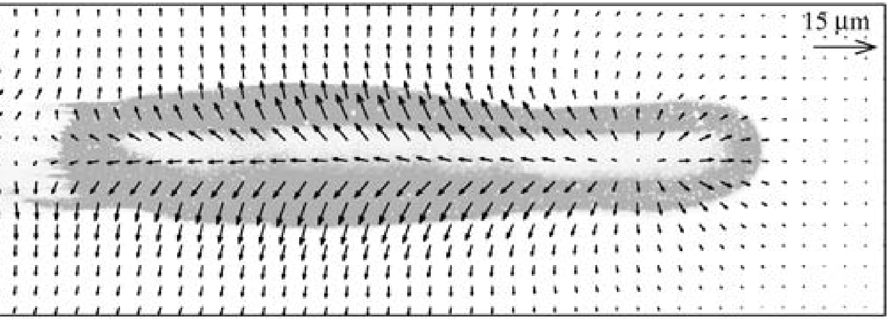


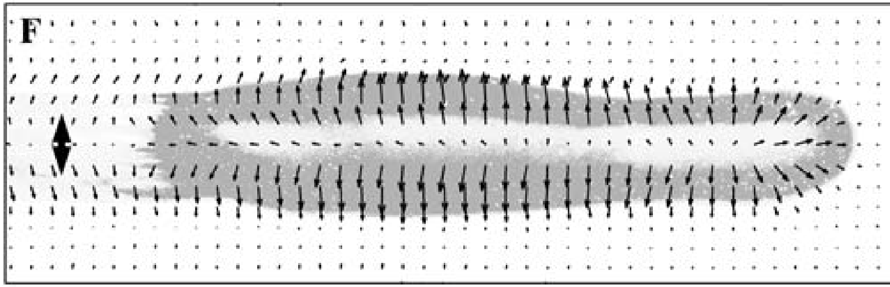
Dd morphodynamics: multiple causes and multiple effects

Aggregation	streams if wave propagation dep on density faster movement in streams
Mount/slug	cell sorting by differential adhesion AND chemotaxis
slug	slug shape attractor of energy minimization vs inward movement (wave shape) taxis (thermo- photo-taxis) via NH3 effect on excitability) slug shape and wave shape bi-directional mutant direction of movement vs momentum
culmination	needs dynamic cell differentiation downward movement of stalk cells caused by peristalsis caused by upward movement of spore cells pressure waves and wave shape self-correcting and self-terminating

Movement Dd slugs: measured bead displacement and calculated force fields cf Rieu, Baranth, Maeda and Sawada 2005







displacement field

stress field

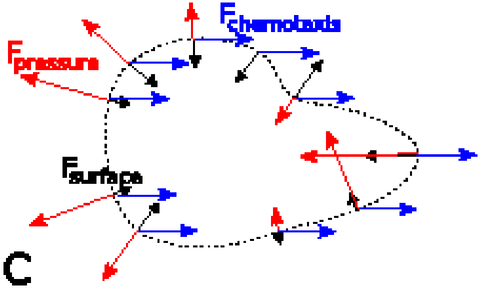
outward directed forces!

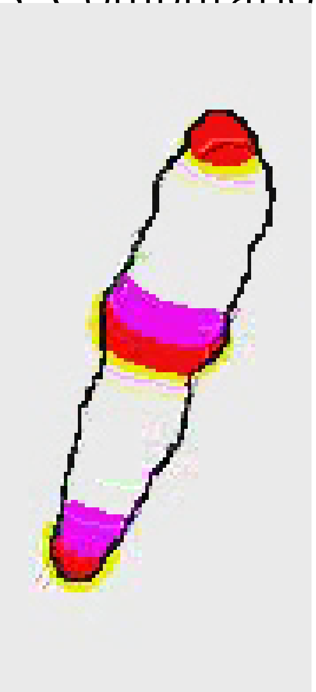
similar forces in model Dd slugs?

Note:
forces are (emergent) observables
instead of model ingredients!

Can be measured (like in experiments)
cf From energy to cellular forces in the Cellular Potts Model: An algorithmic approach EG Rens, L Edelstein-Keshet - PLoS Computational Biology, 2019

Perpendicular forces expected because:
- wave shape (most concave in middle of slug)
- sideward push because of pressure gradient

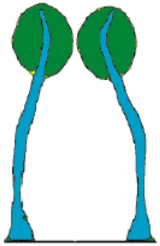




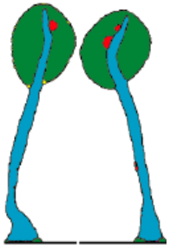
b

conclusions

- Using simplifications which allows multilevel modeling we *“can go for the horse part”*
- Development as trajectory of dynamical system
  model minimizes regulation within cells
- Assumption of CPM seem very suitable to describe biological cells
- Relatively few parameters need to be specified; large set of ’new’ observables
- Treating forces as observables rather than model assumption alllow close comparison with experimental measurements



BUT WHAT ABOUT THE GENES?



Evolutionary “testing” of the model

who wants to be a stalk?, cf Queller

how to come become another dictyosteloid?

multiple levels needed to understand complexity

Who want to become a stalk?

Evolution of cooperation and why cheaters do not take over

single gene greenbeard effect

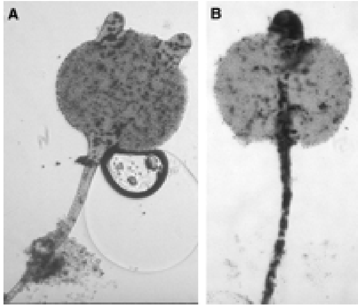
Who depends on phase in cell cycle

Cell adhesion gene csA binds to csA

on agar csA knockouts become spores because wildtye cells have more adhesion $->$ go to front - become stalk

BUT

in soil csA knockouts are left behind during aggreg. phase $->$ fruiting body 85% wildtype



Queller et al. Science 299:105-106 (2003)

conclusion: who wants to become a stalk

Simple optimality reasoning often flawed

Important role of non-inheritable behaviour

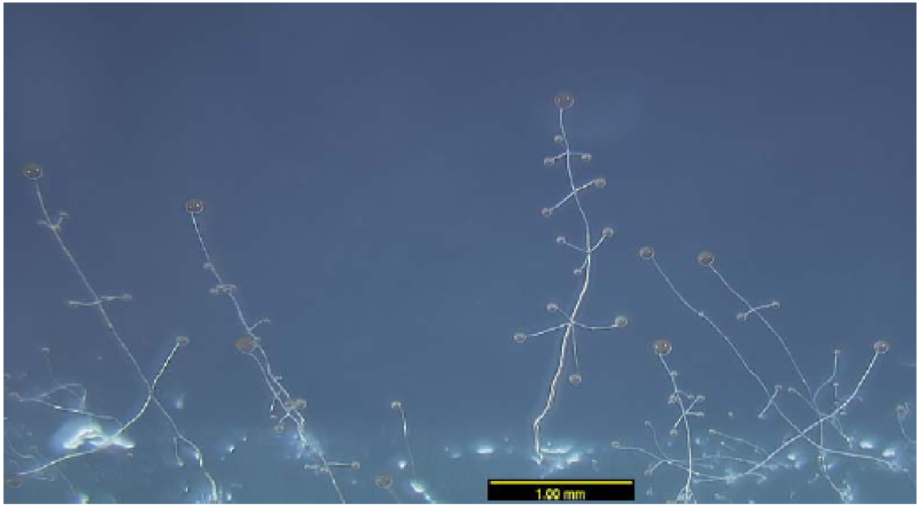
stochasticity

environmental heterogeneity

selforganization

from Dictyostelium to other discyosteliids
Polysphondinium

Polysphondylium violaceum



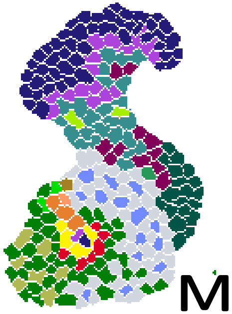
A.R. Swanson, A Guide to the Common Dictyostelid Slime Molds of Great Smoky Mountains National Park

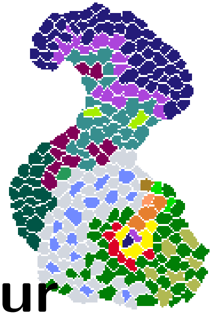
continuous redifferentiation prestalk-stalk

sidebranches (polyshondinium)

....so far - so good BUT
mostly unidirectional micro− >macro level causation
cell property changes only externally imposed
within CPM one can do better!

(include macro− >micro level causation)



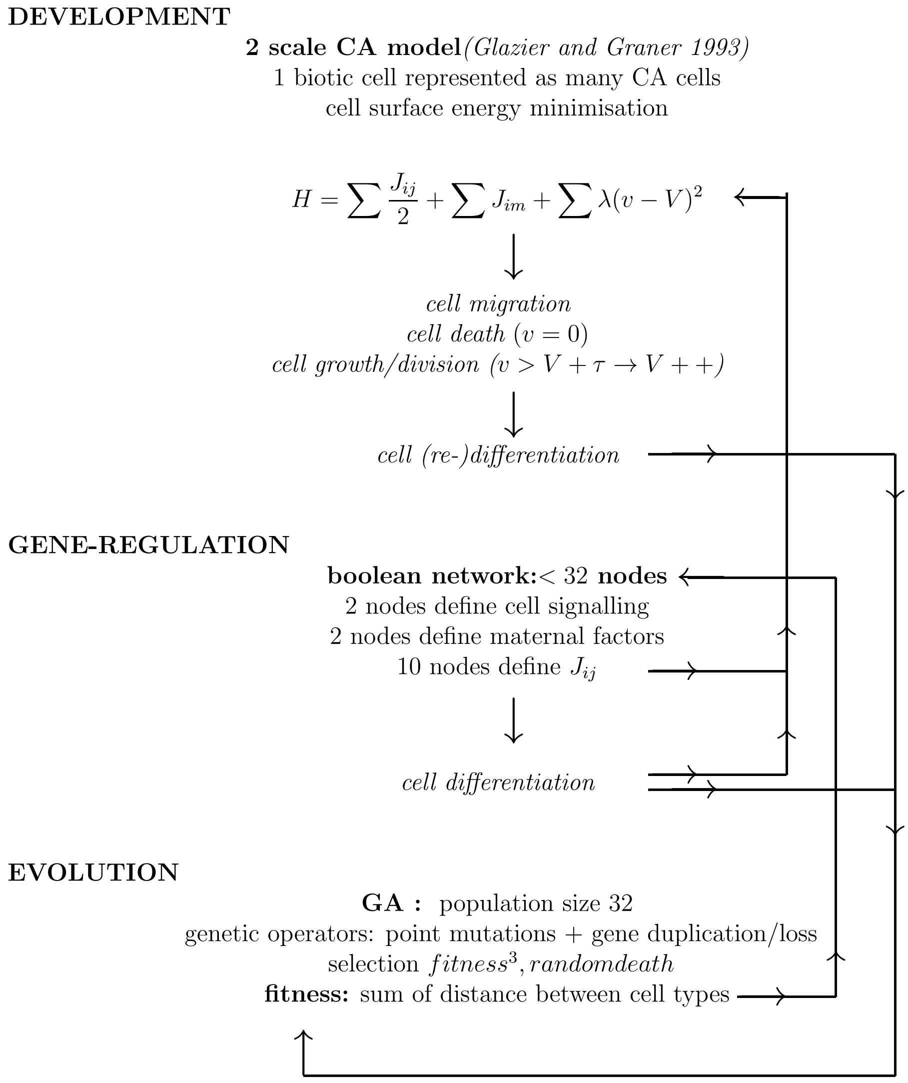


Multilevel modeling of multilevel behaviour
Morphogenesis as side effect of
cell differentiation and differential adhesion

combining
within cell dynamics (gene regulation)
between cell dynamics (signalling and adhesion)
cell growth and division
evolutionary dynamics (fitness cell differentiation)

physical processes + inherited information

Hogeweg 2000a,b



DEVELOPMENT

2 scale CA model*(Glazier and Graner 1993)*
1 biotic cell represented as many CA cells
cell surface energy minimisation

$$H = \sum \frac{J_{ij}}{2} + \sum J_{im} + \sum \lambda(v - V)^2$$

cell migration
cell death $(v = 0)$
cell growth/division $(v > V + \tau \rightarrow V{+}{+})$

cell (re-)differentiation

GENE-REGULATION

boolean network:$<$ **32 nodes**
2 nodes define cell signalling
2 nodes define maternal factors
10 nodes define J_{ij}

cell differentiation

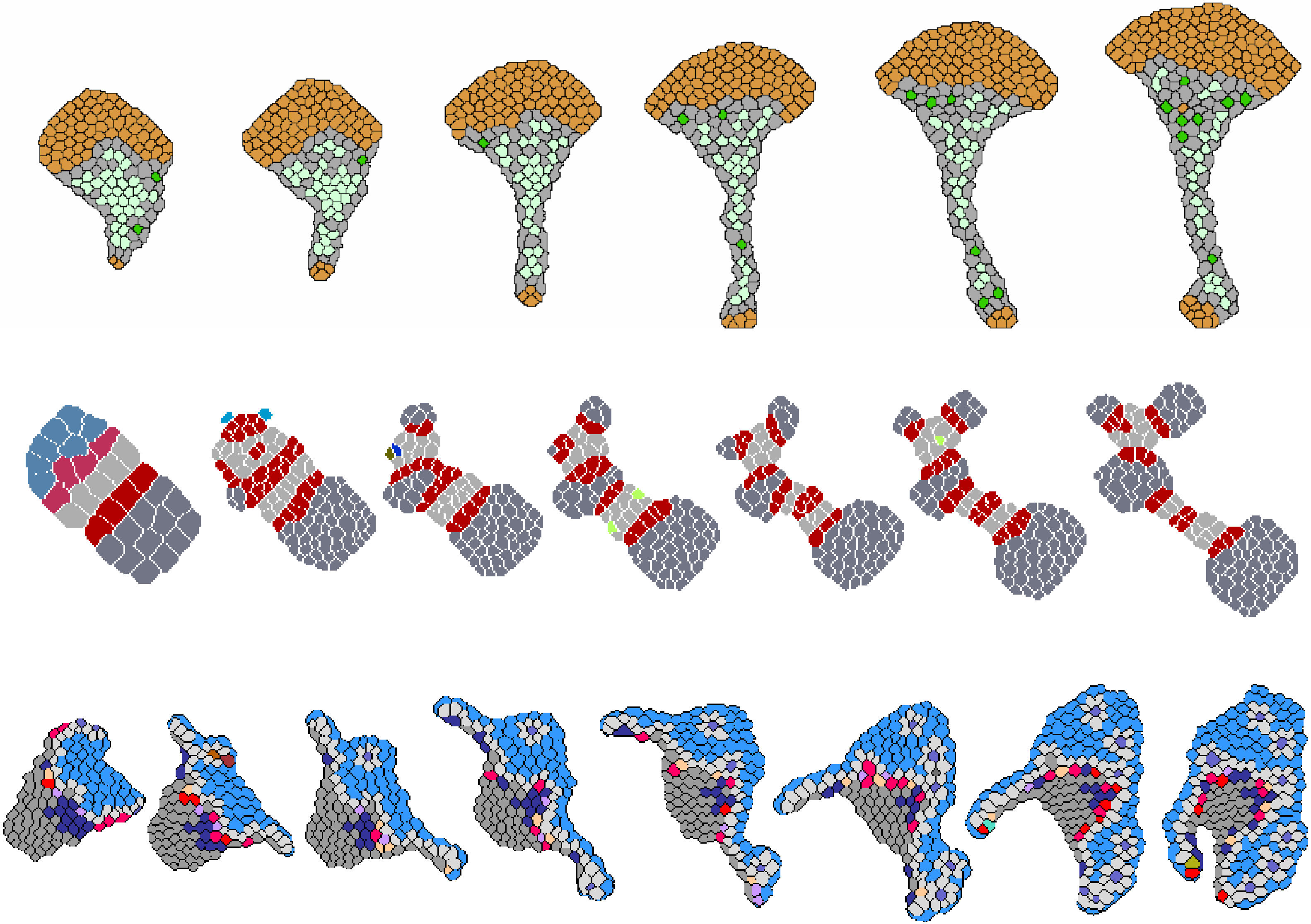
EVOLUTION

GA : population size 32
genetic operators: point mutations + gene duplication/loss
selection $fitness^3, randomdeath$
fitness: sum of distance between cell types

Modelling Morphogenesis:

Interplay between Gene regulation, Differential adhesion and Evolution

Morphogenesis by differential adhesion and cell differentiation

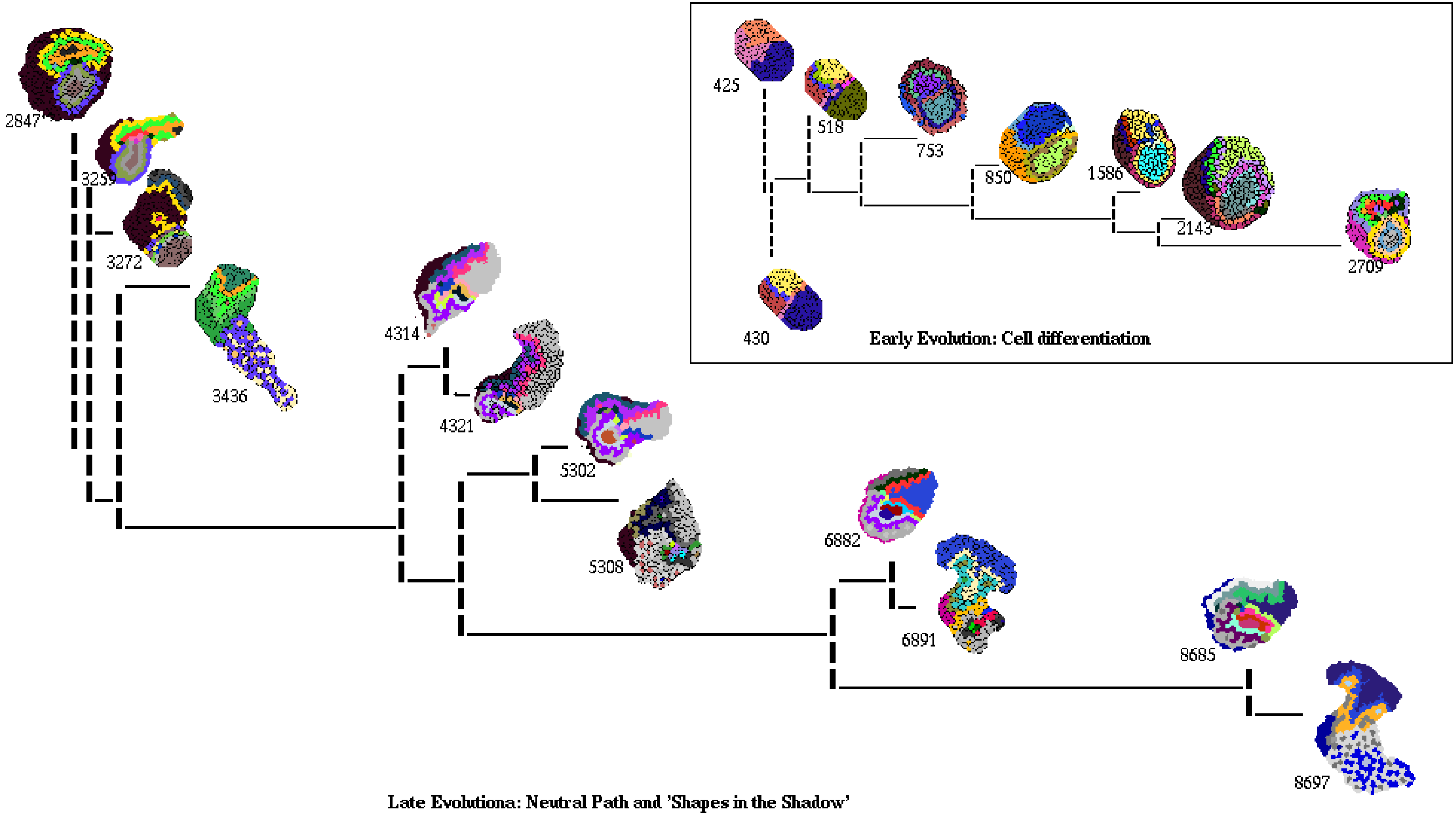


modes of cell differentiation and morphogenesis

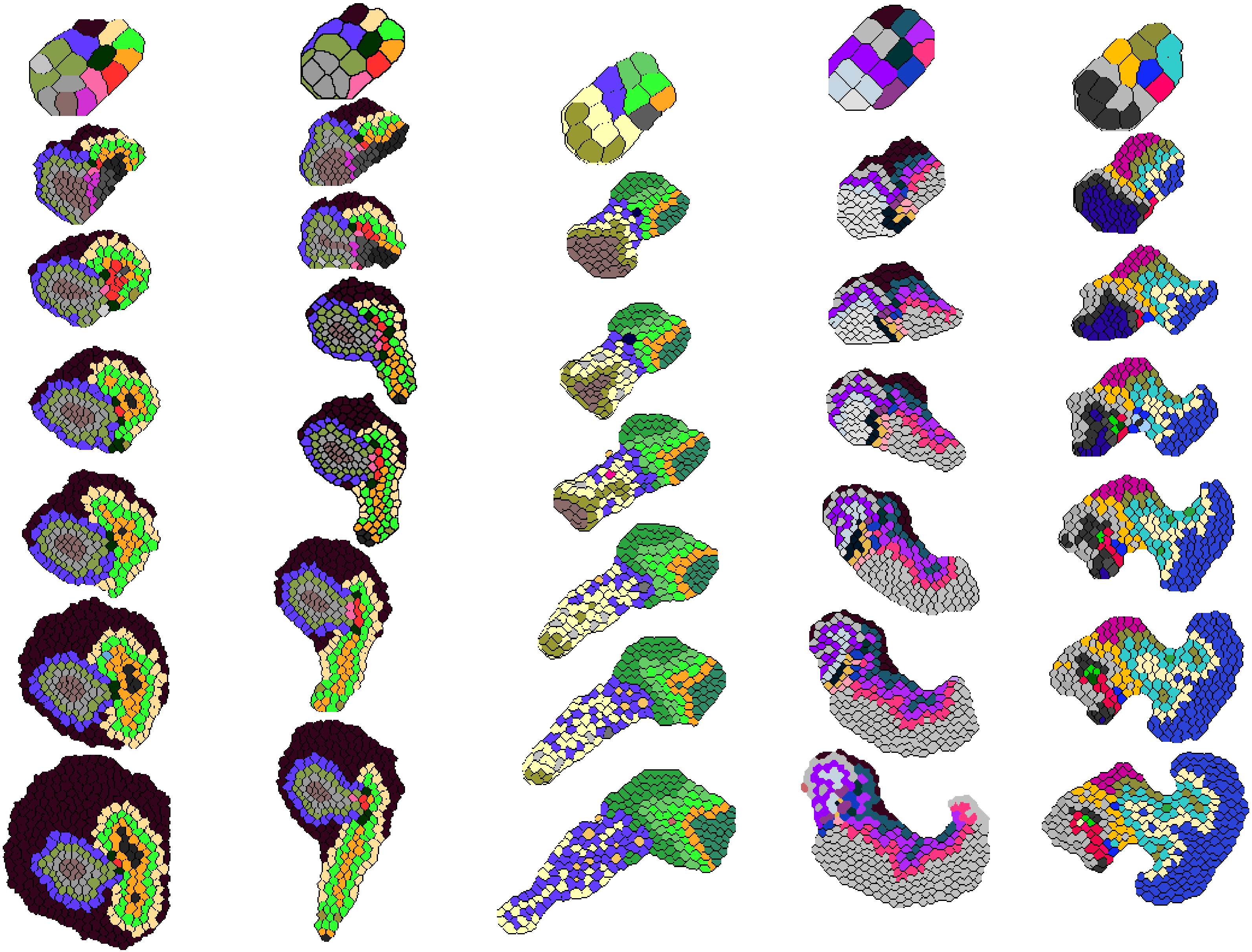
cell differentiation	**evolved morphogenesis**
alternative attractors of gene regulation network = *stable memory* signal dependent cell differentiation *re-differentiation*	many morphemes by few mechanisms - engulfing - intercalation - convergence extension - meristematic growth - budding automatic orchestration of adhesion,migration,differentiation cell growth - division and - death “pseudo-isomorphic outgrowth”

Morphogenesis as sustained transient of energy minimization
intrinsic conflict maintained by
cell growth cell division and cell differentiation

Evolutionary history: after cell differentiation diversity of shapes



"conserved" ZOOTYPE followed by differential outgrowth



conclusion
FUN!
showing beauty of CPM

Simplicity

easily extendable

"natural" flexible interface between levels

dynamic micro-macro and macro-micro interactions

emergence at multiple space and time scales

(and .... models "real" biological cells pretty well)