Dorsal closure in the fruit fly: what controls cell oscillation and tissue contraction?

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Collaborators

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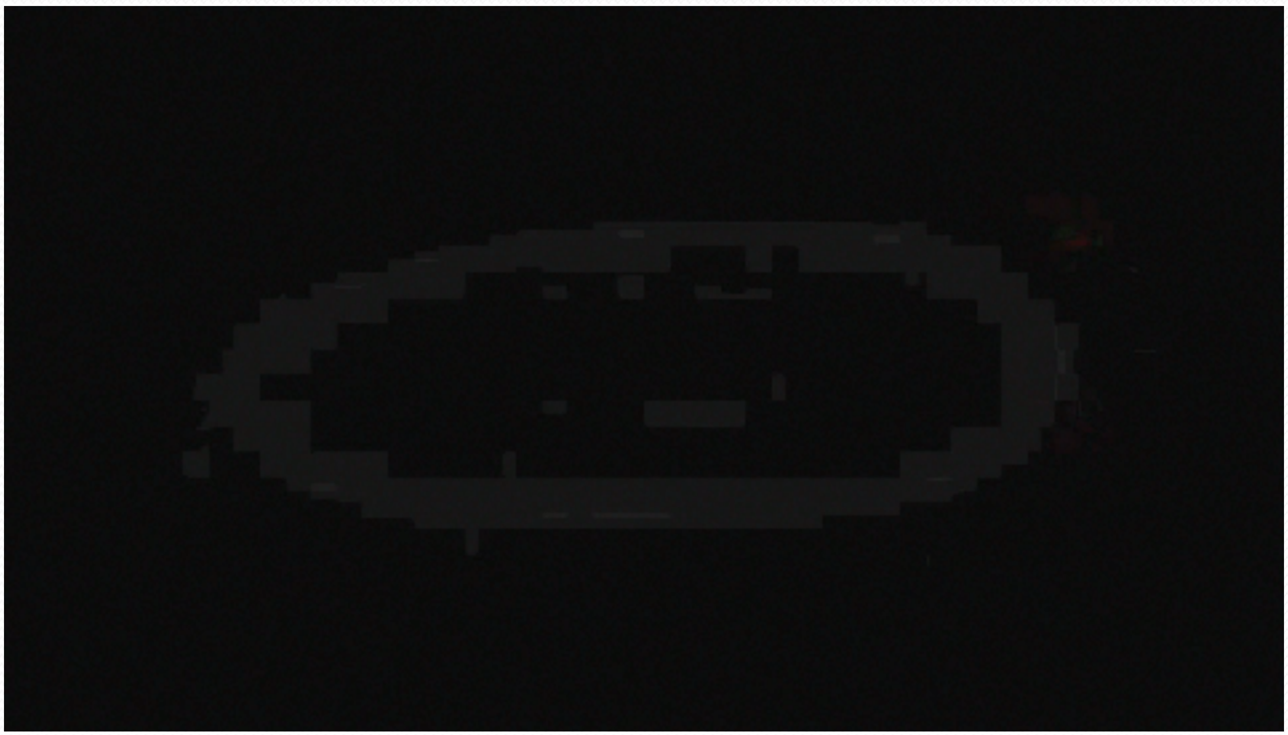
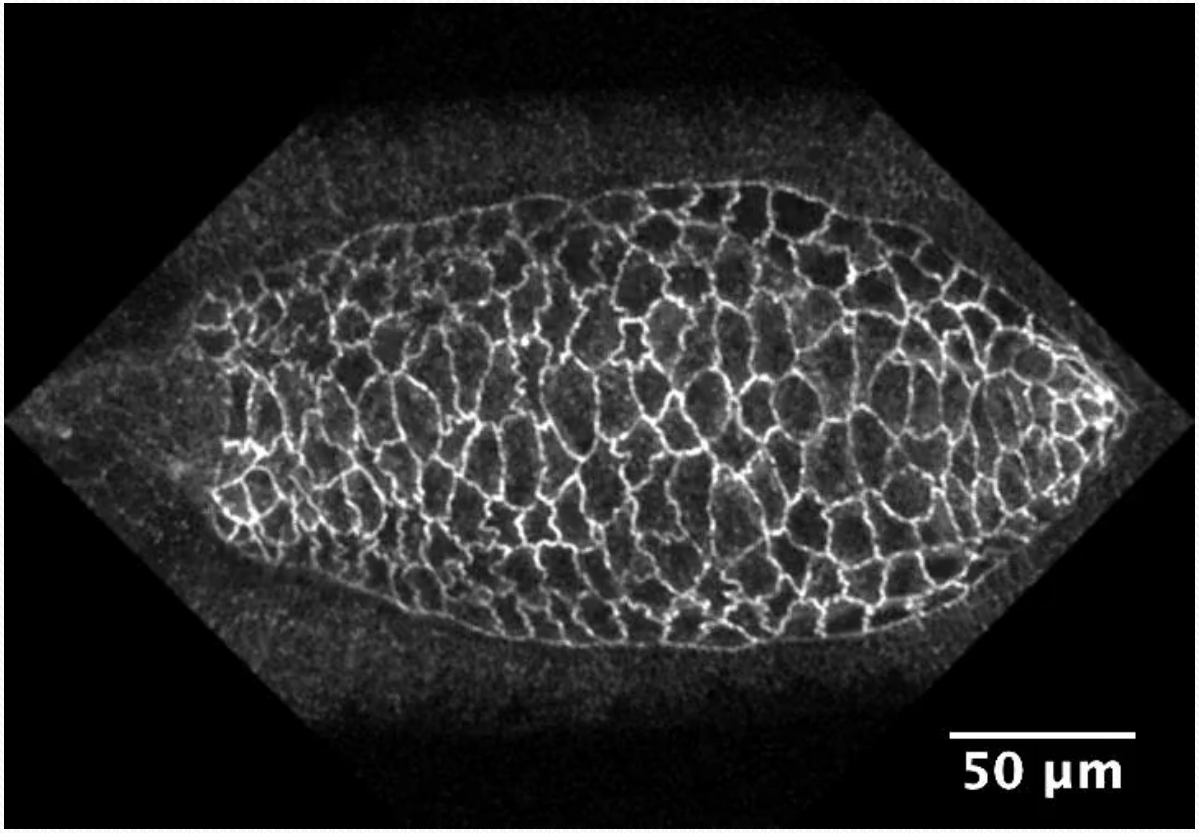
Morphogenesis of *Drosophila*: overview



Thomas C. Kaufman, Indiana University

A magnified view:



AS: amnioserosa
AC: actin cable

Mateus, et al. PLoS ONE 6(4): e18729, 2011

Outline

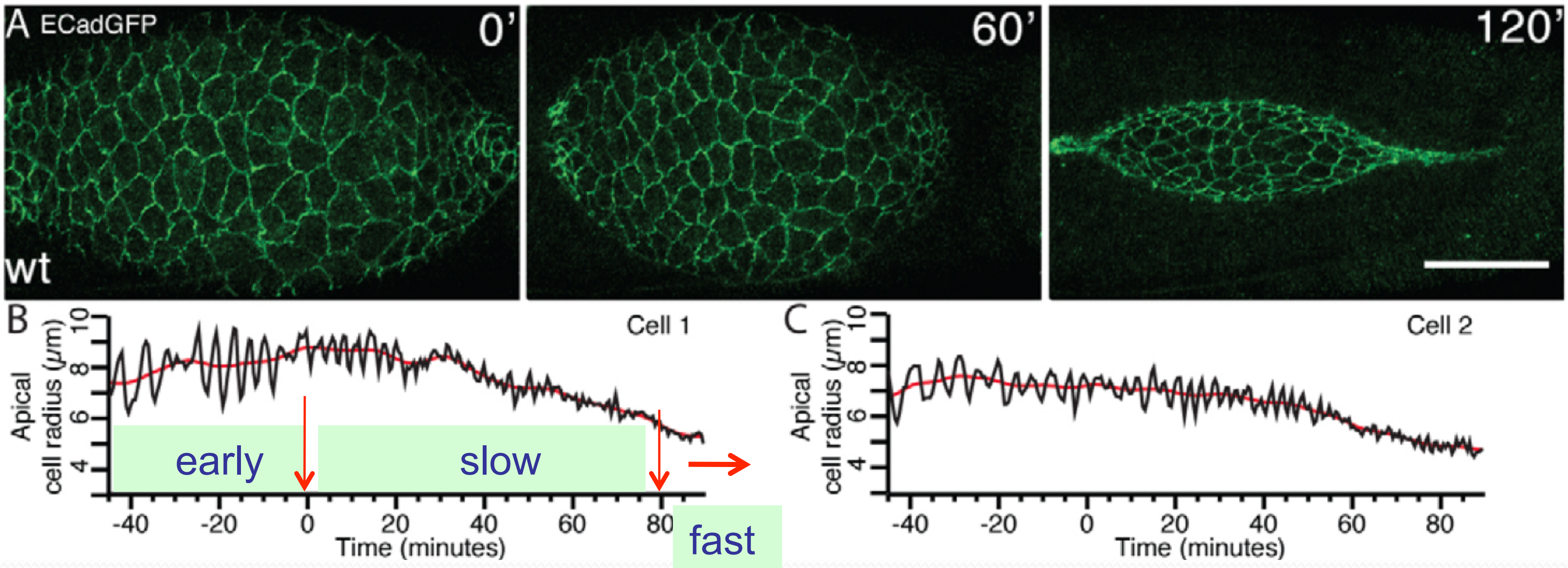
Drosophila dorsal closure (DC): cell and tissue oscillate and contract

I. Mechanical oscillation & contraction
   A. Experimental observations
   B. A *mechanical* model

II. Signaling and control
   A. Experimental observations
   B. A *chemical* model

I. Experimental observation of DC

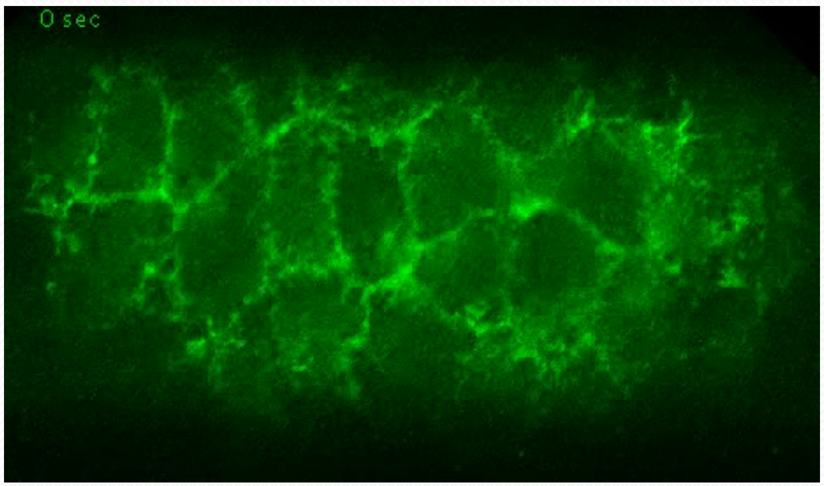
Three phases: early, slow and fast



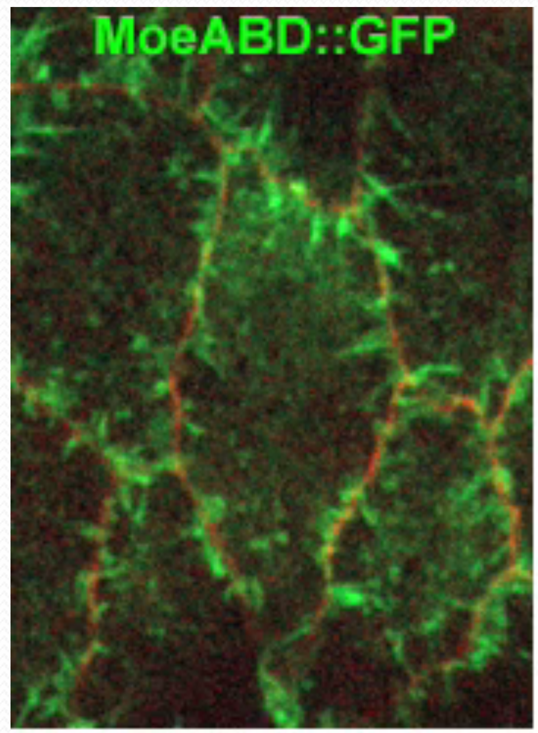
Gorfinkiel *et al.* Development (2009); PLoS One (2014)
Solon *et al*. Cell (2009); Blanchard *et al*. Development (2010)
Kiehart *et al.* Biophys. J. (2012); Hutson *et al*. Biophys. J. (2013)

1. Early phase: before DC starts …

Frank *et al*. (2005); David *et al*. (2010); He *et al*. (2010); Martin (2010); Blanchard *et al*. (2010); Gorfinkiel *et al*. (2011); Kiehart *et al*. (2012)

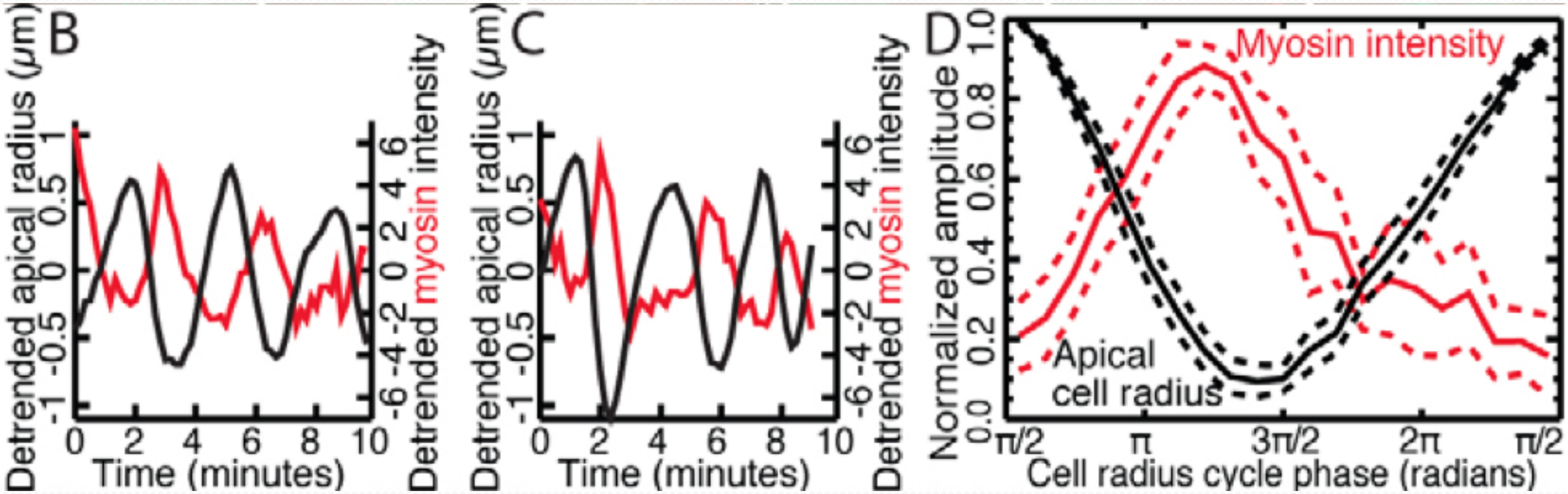


(Blanchard et al.: myosin)



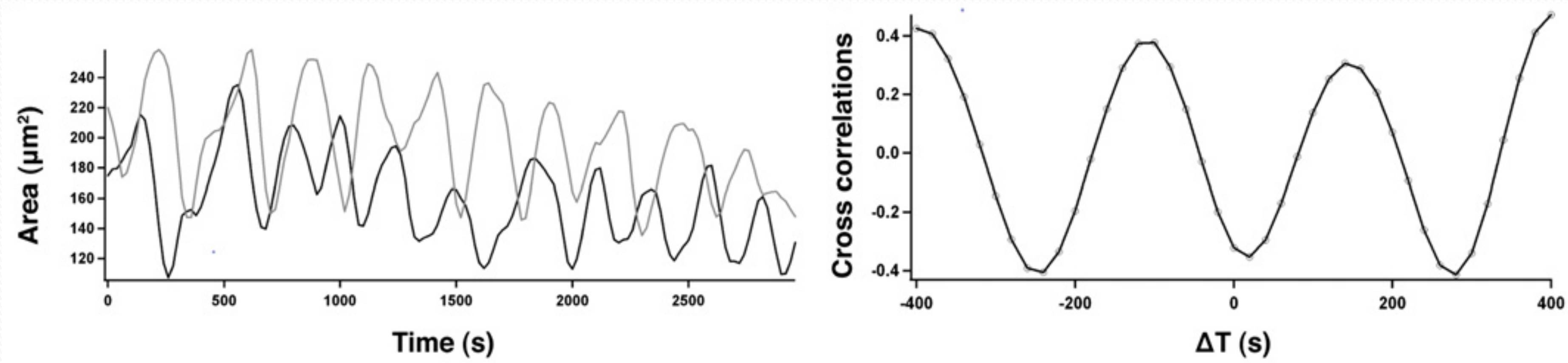
(David et al.: actin)

Blanchard *et al.* Development (2010):

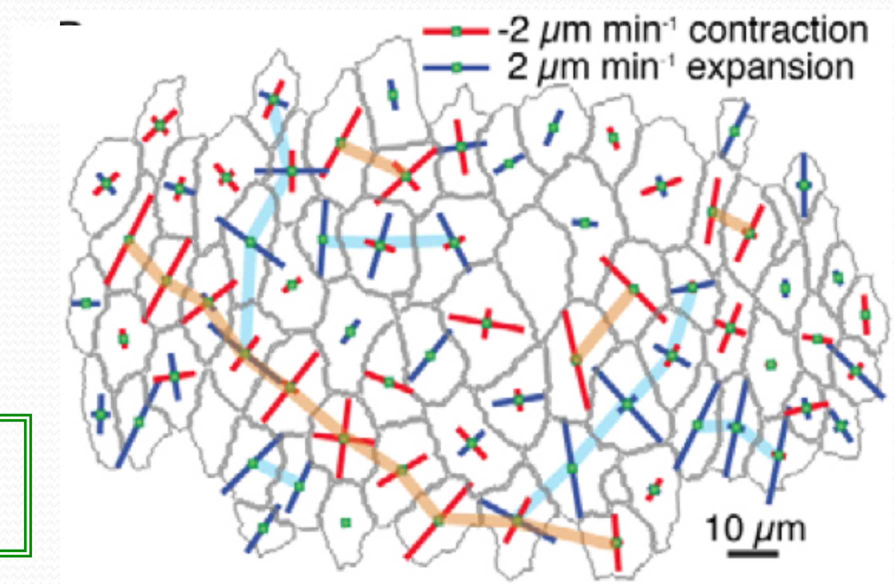


- Myosin concentration vs. cell area
- Myosin peak precedes area valley

Correlation between neighbors



Solon *et al.* Cell (2009)

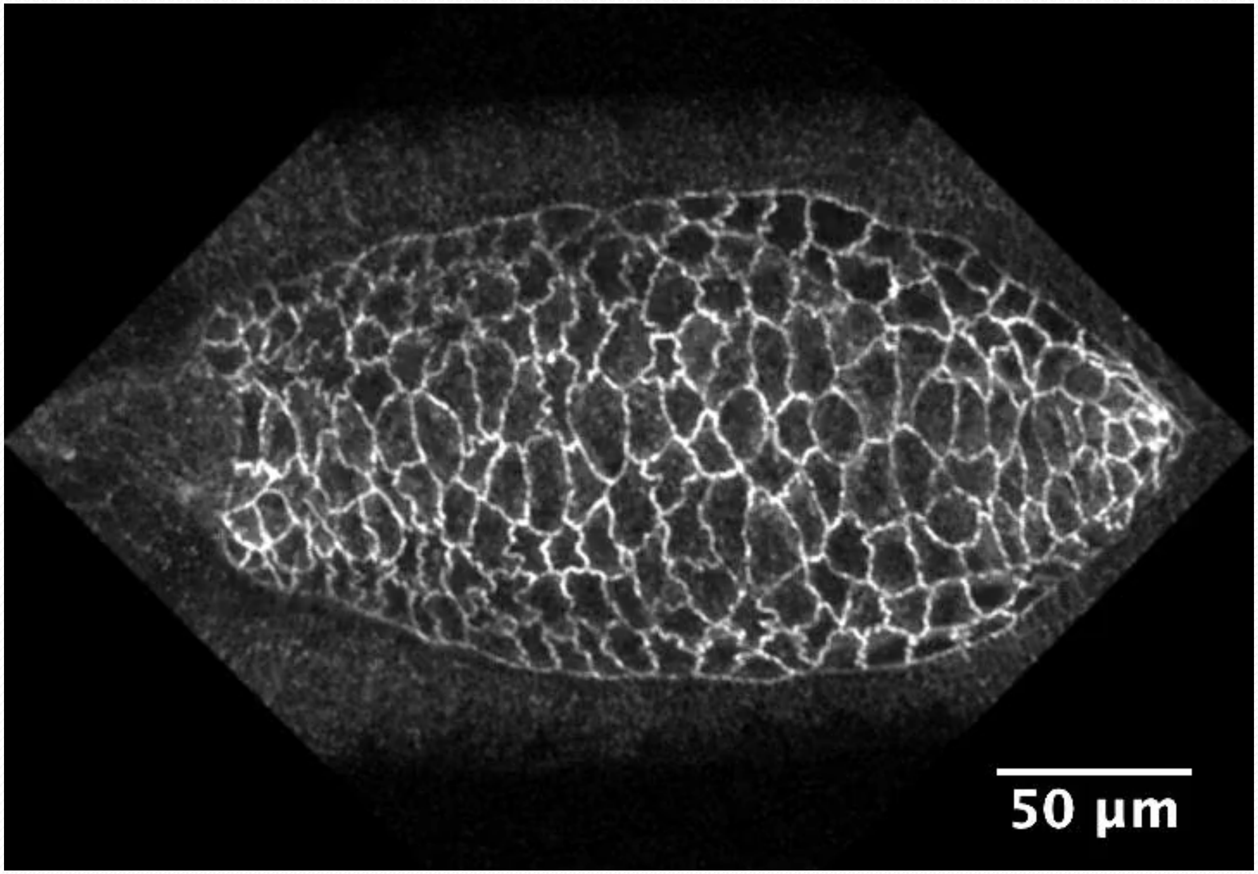


Blanchard *et al*. Development (2010)
Gorfinkiel *et al*. Genesis (2011)

Significant questions on early phase:

- What causes the quasi-periodic oscillation? Why not steady state?
- *Why oscillatory approach to constriction and closure?*

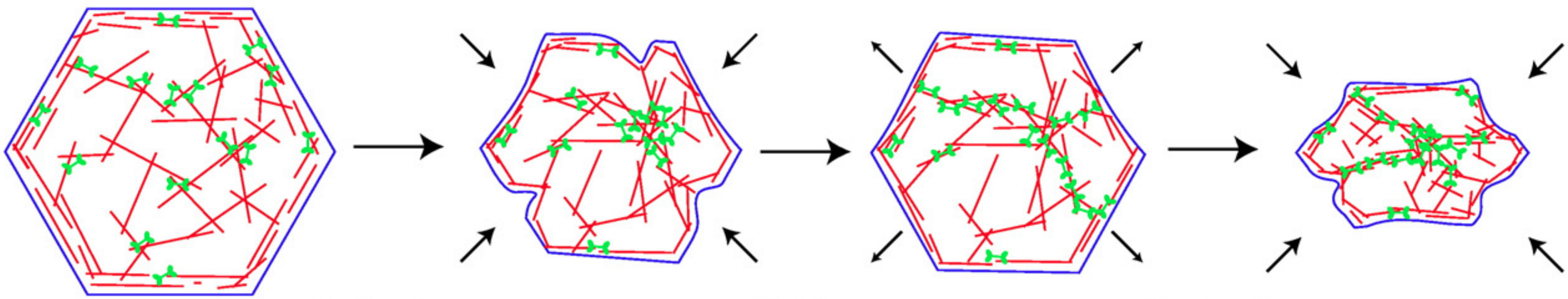
2. Slow phase: cell/tissue contraction



Mateus, et al. PLoS ONE 6(4): e18729, 2011

Ratcheting mechanisms: two proposals

- *Intracellular* or *internal* ratchet:

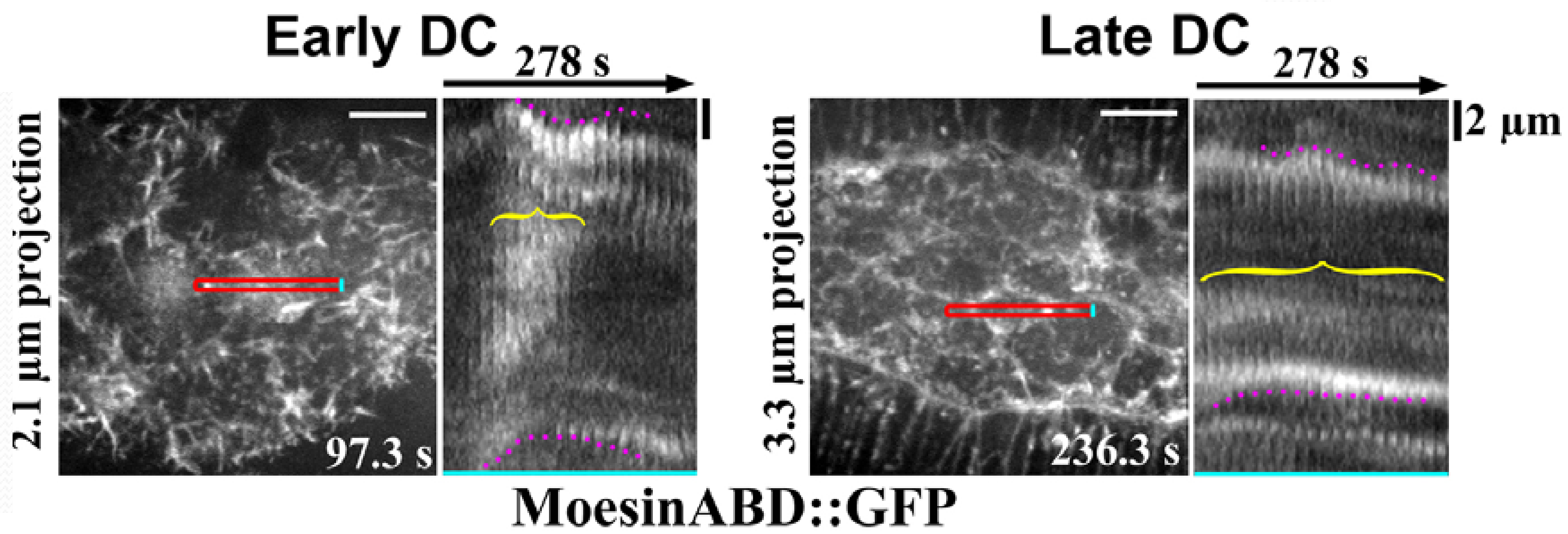


Martin *et al.* (2009); Blanchard *et al*. (2010); David *et al.* (2013)

- Medial-apical actomyosin network → contraction
- Stabilization due to
  - Remodeling of cell borders to restrict expansion *and/or*
  - Apicomedial cytoskeleton strengthening over each cycle

Evidence for *internal* ratchet:

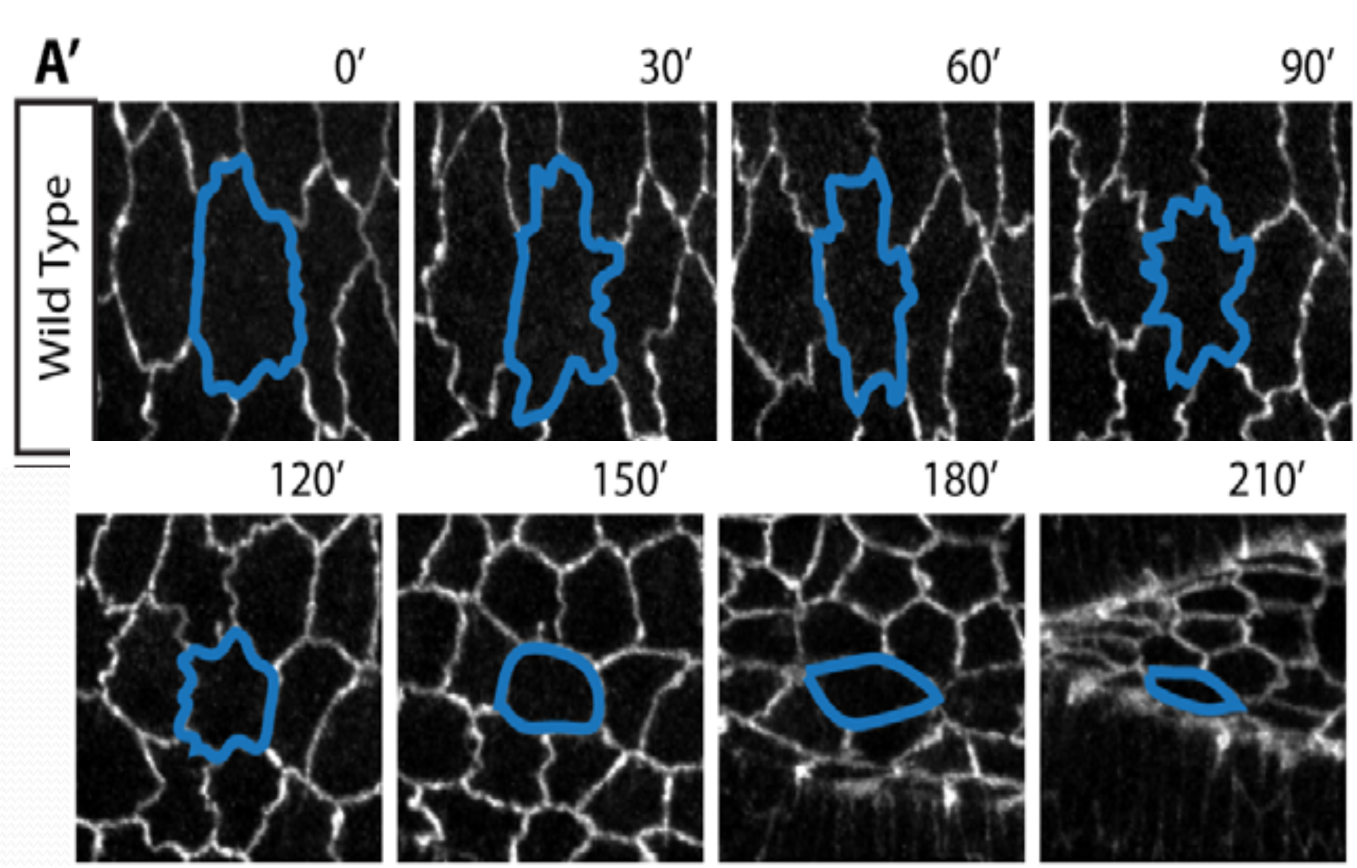
- Apicomedial actomyosin network grows over cycles



David *et al.* Development (2013)

Evidence for *internal* ratchet:

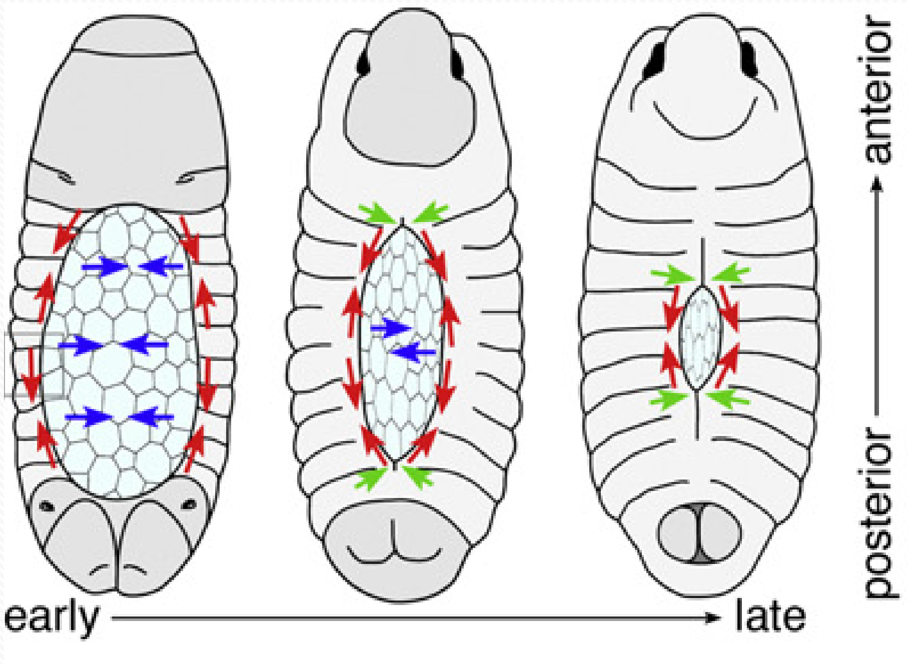
- Cell borders: wiggly → straight in slow and fast phases



Mateus, et al. PLoS ONE 6(4): e18729, 2011

Ratcheting mechanisms: two proposals

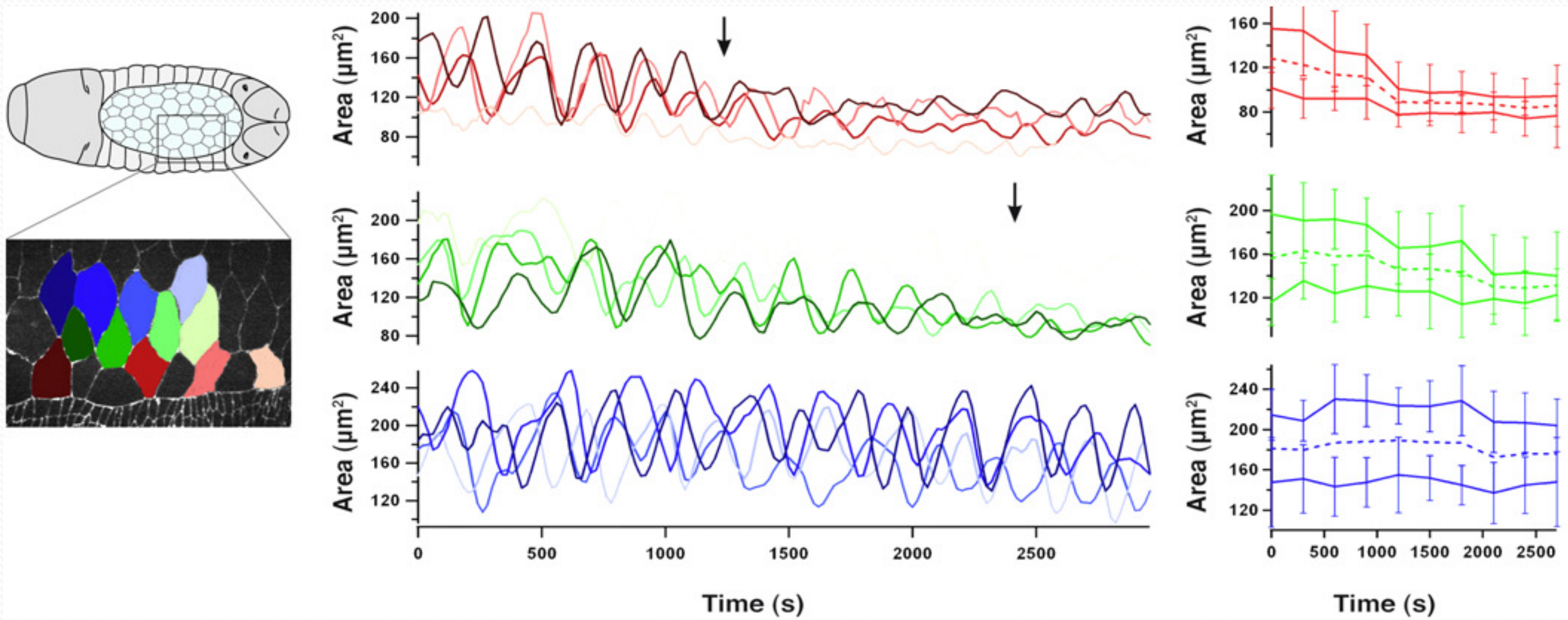
- *Actin-cable* (AC) ratchet:



Solon *et al.* Cell (2009)

Ratcheting mechanisms: two proposals

- *Actin-cable* (AC) ratchet:
  - AC cable forms at start of slow phase
  - Cells next to cable get arrested first (disputed!)



Significant questions on slow phase:

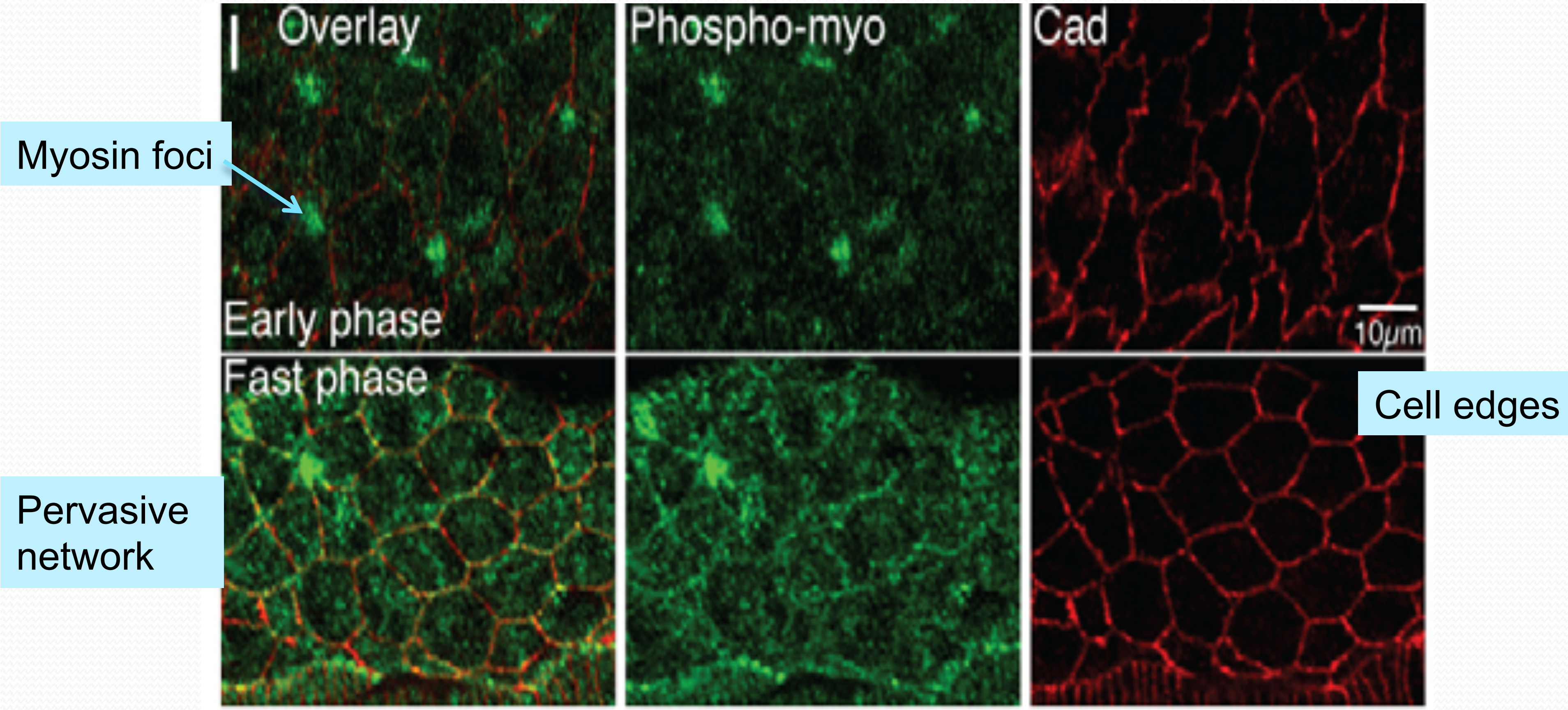
- *Internal* or *AC* ratchet?

  Solon *et al*. (2009): mutants with defective AC cannot realize DC
  Blanchard *et al*. (2010): mutants can still have complete AS contraction

- Perhaps both are corporative or redundant mechanisms.
- Chemo-mechanical trigger for the ratchet?

3. Fast phase: fast contraction of amnioserosa

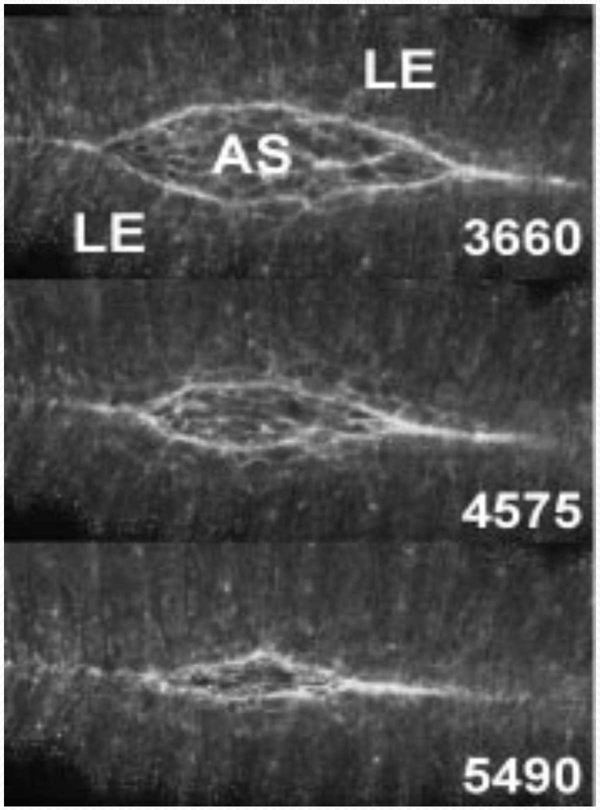
- Apicomedial myosin network: covers entire apical surface, sustained in time



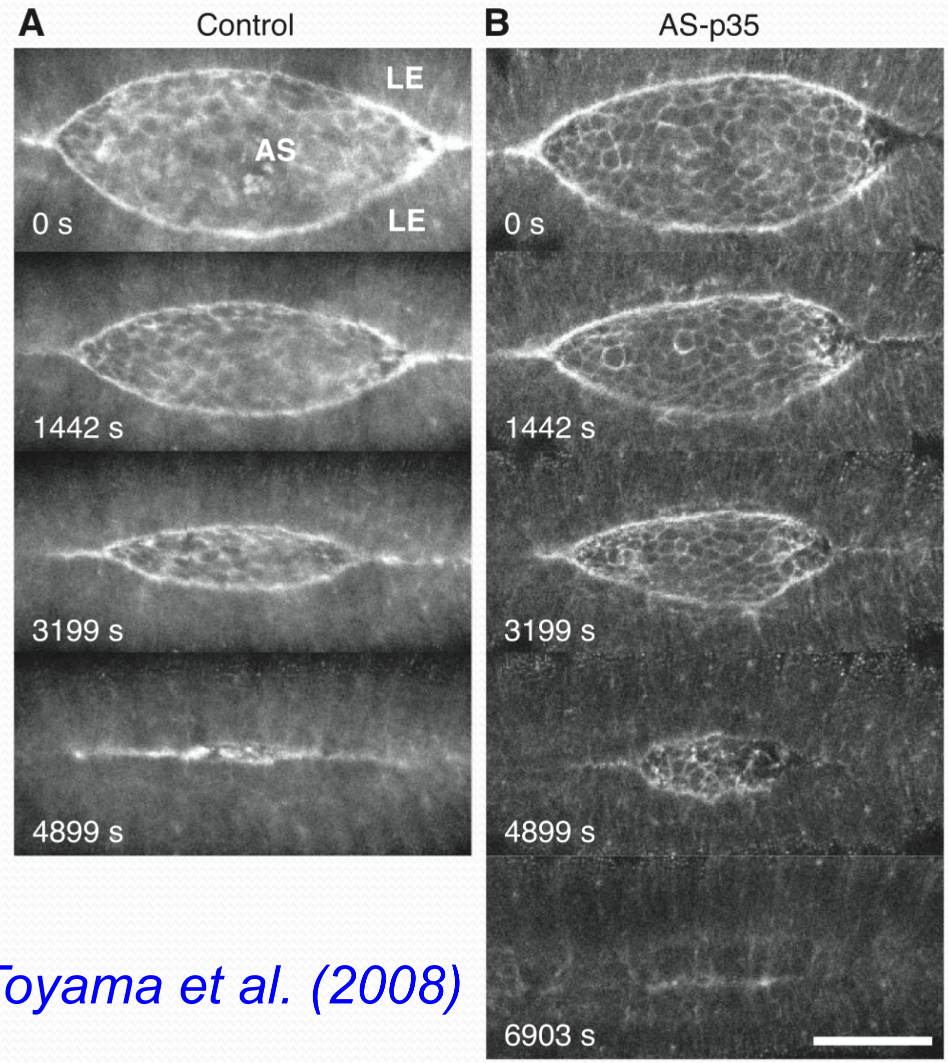
Blanchard *et al.* (2010)

Fast phase: other effects

- Zippering, cell apoptosis and epidermis expansion

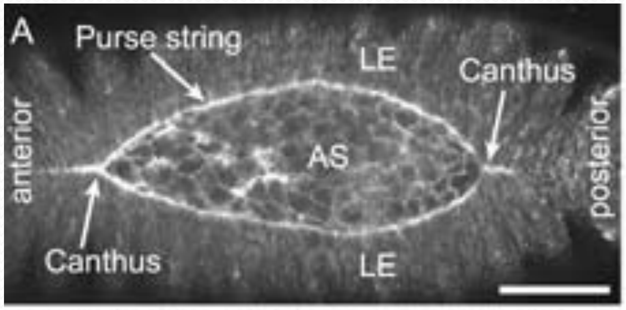


Hutson et al. (2003)



Toyama et al. (2008)

A mathematician/engineer's summary of biological observations:



- Amnioserosa cell contraction (Hutson et al. 2003, 2013, Solon et al. 2009, Gorfinkiel et al. 2009, 2014)
- Supracellular actin cable (Kiehart et al. 2000, Franke et al. 2005, Solon et al. 2009)
- Zippering from canthi (Hutson et al 2003)
- Amnioserosa cell apoptosis (Toyama et al. 2008)
- Active epidermis elongation (Gorfinkiel et al. 2011)

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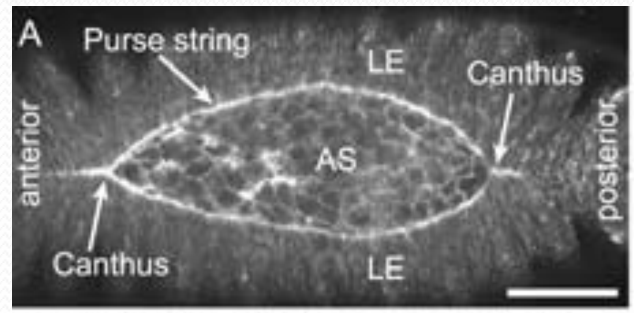
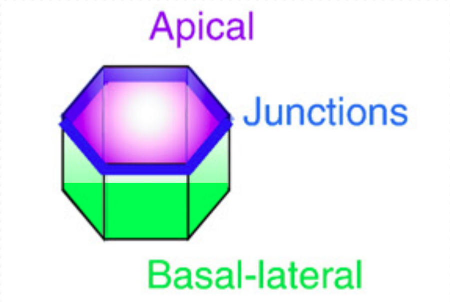


- Amnioserosa cell contraction (Hutson et al. 2003, 2013, Solon et al. 2009, Gorfinkiel et al. 2009, 2014)
- Supracellular actin cable (Kiehart et al. 2000, Franke et al. 2005, Solon et al. 2009)
- ~~Zippering from canthi~~ (Hutson et al 2003)
- ~~Amnioserosa cell apoptosis~~ (Toyama et al. 2008)
- ~~Active epidermis elongation~~ (Gorfinkiel et al. 2011)

Our objective: model with mechano-chemical coupling to capture the main features

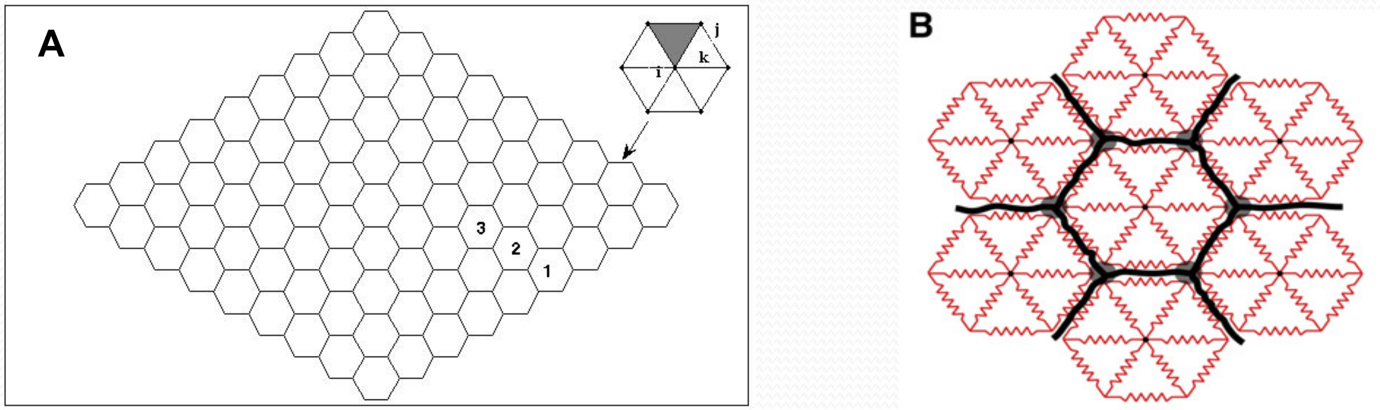
I.B. Building a 2D mechanical model

Features to be captured:



- Why 2D? Squamous cells in epithelium; all action on apical surface
- Myosin: concentrates in medial network and cell cortex
- Cyclic contraction: medial network pulling on the adherens junctions
- Actin cable surrounding the entire AS tissue

2D mechanical model:



Edges and spokes: cortical and medial actin filaments onto which myosin attaches; *ignore actin turnover*

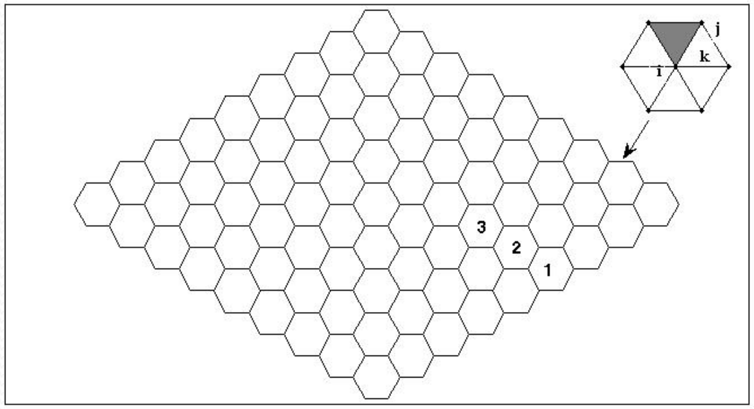
How to model the forces?

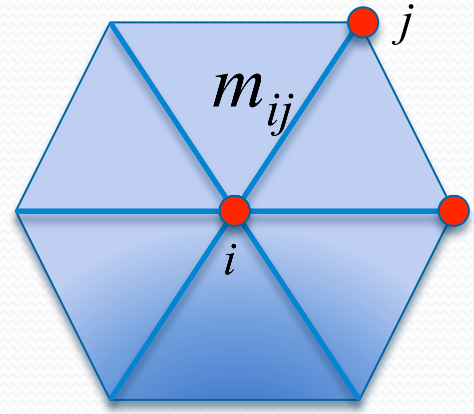
(a) Spokes: passive elasticity + myosin contraction

(b) Edges: passive elasticity only (no active myosin-based contraction)

(c) Actin cable: 2 elastic “rubber bands” along the outline of AS tissue

Mathematical formulations





Nodal motion:

$$\eta \frac{d\mathbf{x}_i}{dt} = \mathbf{f}_i, \qquad \mathbf{f}_i = \sum_j f_{ij} \frac{\mathbf{x}_j - \mathbf{x}_i}{|\mathbf{x}_j - \mathbf{x}_i|},$$

$$f_{ij} = \mu(l_{ij} - l_{0ij}) + \beta m_{ij}$$

Myosin & “signal” dynamics (hypothesized):

$$\dot{m}_{ij} = k^+ h_{ij} s_i - k^- m_{ij}, \quad \dot{s}_i = q - k_0 \sum m_{ij}; \qquad k^- = k_1 e^{-k_2 f_{ij}}$$

Model Parameters

Mechanical parameter:

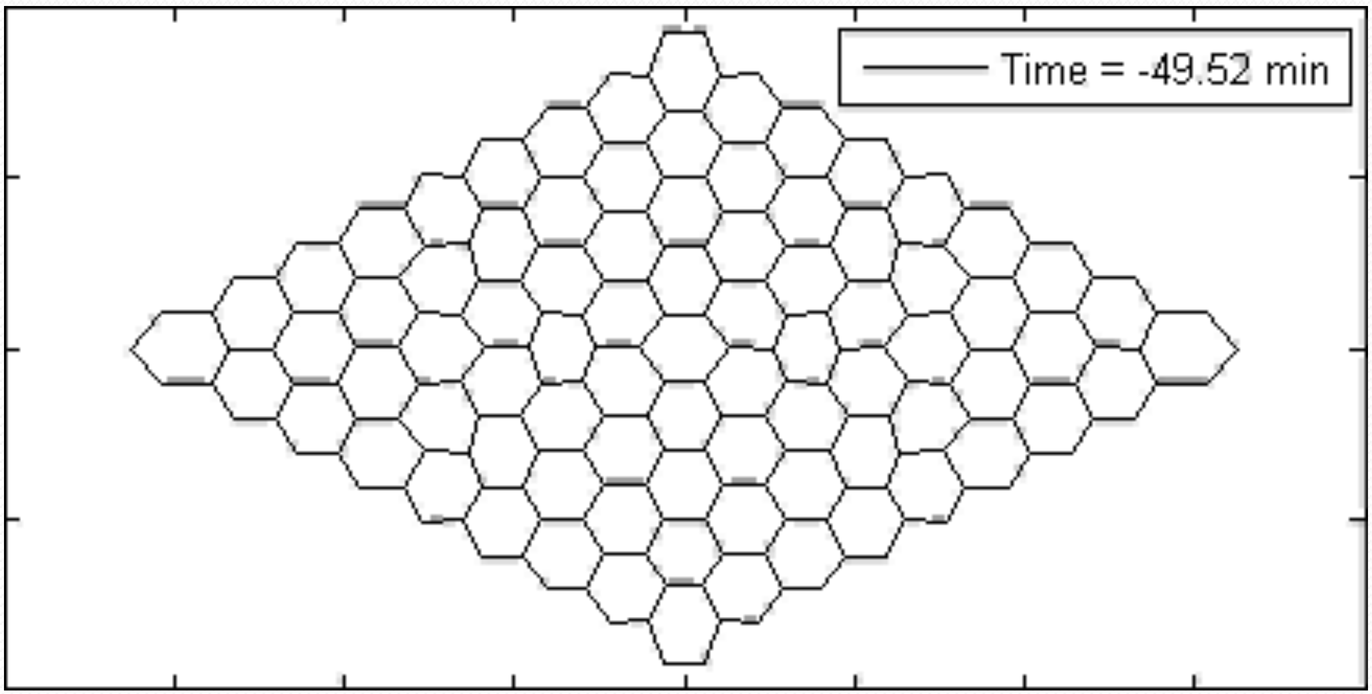
η = 100 nN·s/μm, μ = 1 nN/μm (following Solon *et al.*)
β = 0.75 nN/μM

Kinetic parameters:

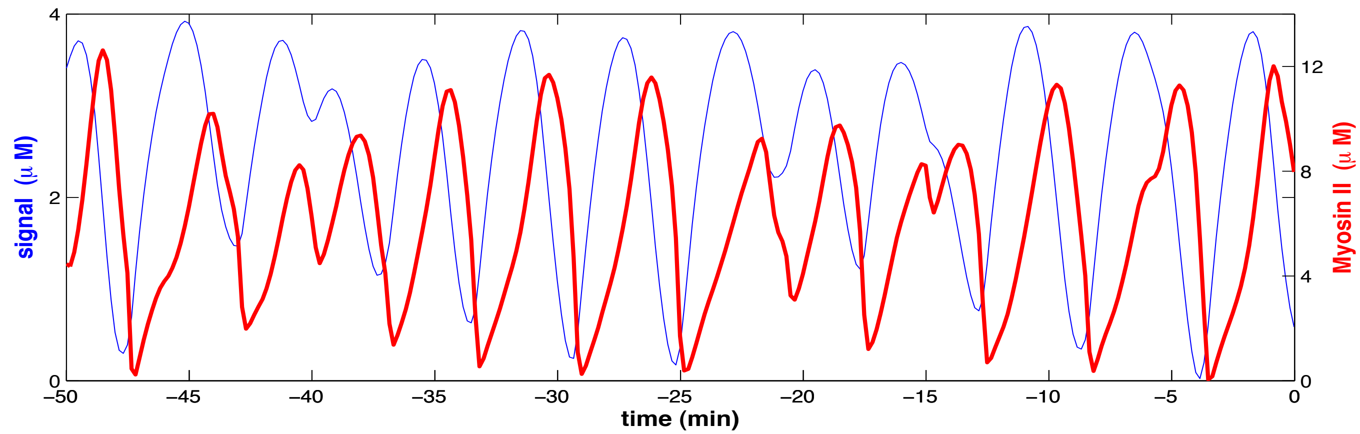
k_1=0.25 s^{-1} (Kovacs *et al.*)
k_2=1.33 nN^{-1}
k^+=0.25 s^{-1}
q=0.05 μM/s
k_0=0.0083 s^{-1}

Model predictions

1. Early phase: AS tissue and cells fluctuate

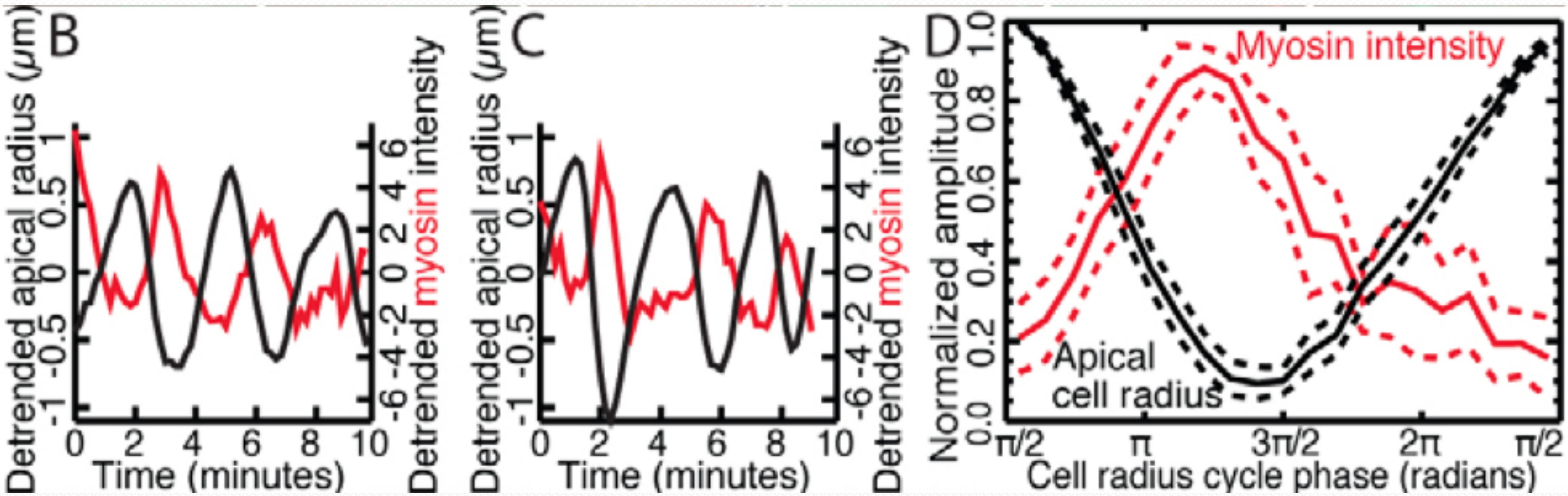


Model predictions: early phase

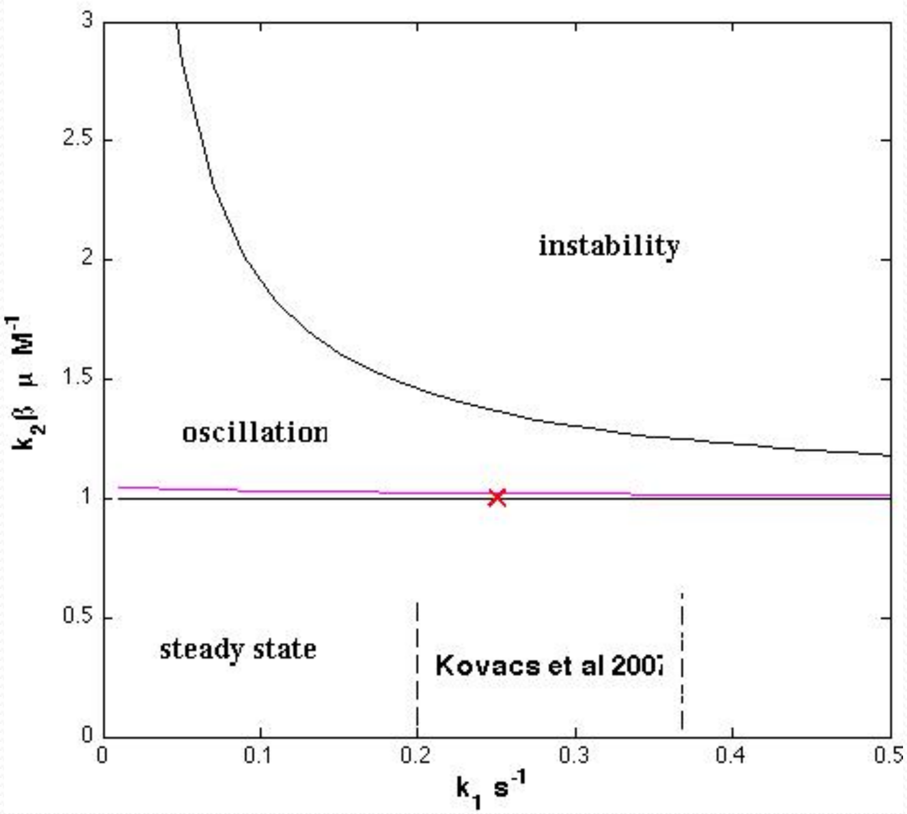


(a) Cell area oscillates nearly anti-phase with myosin intensity
(b) Period T = 4 ~ 5 min (controlled by myosin attachment rate!)
(c) Myosin peaks precede area valleys
(d) Myosin peaks track signal peaks

Recall Blanchard *et al.* (2010):



Cause of oscillation: delayed negative feedback



Analysis for a single cell:

$$\dot{m}_{ij} = k^{+} h s_i - k^{-} m_{ij}$$

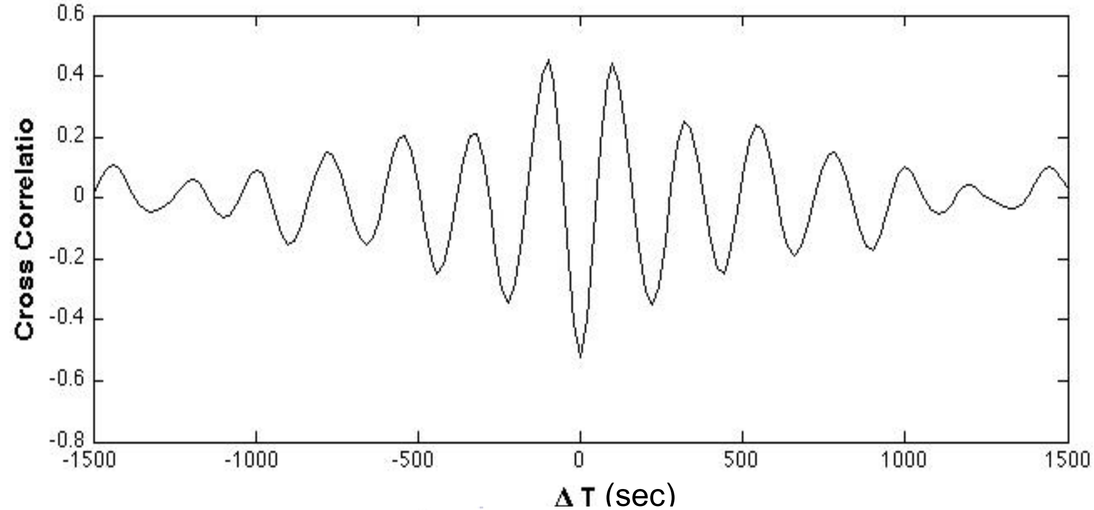
$$\dot{s}_k = q - k_0 \sum m_{ij}$$

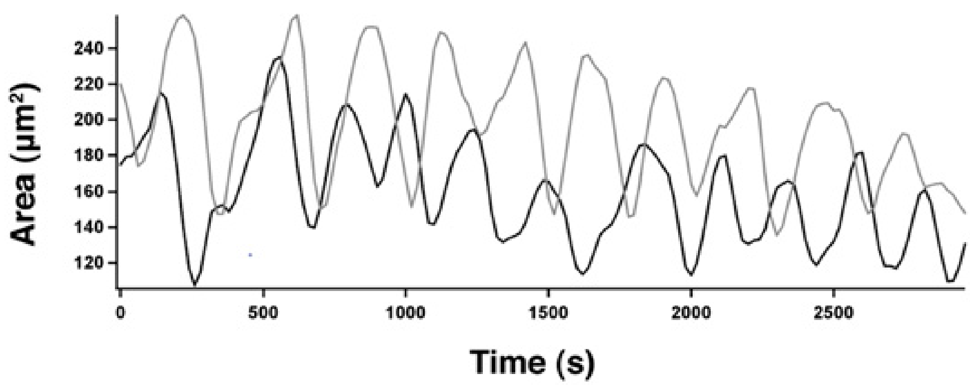
$$k^{-} = k_1 e^{-k_2 f_{ij}}$$

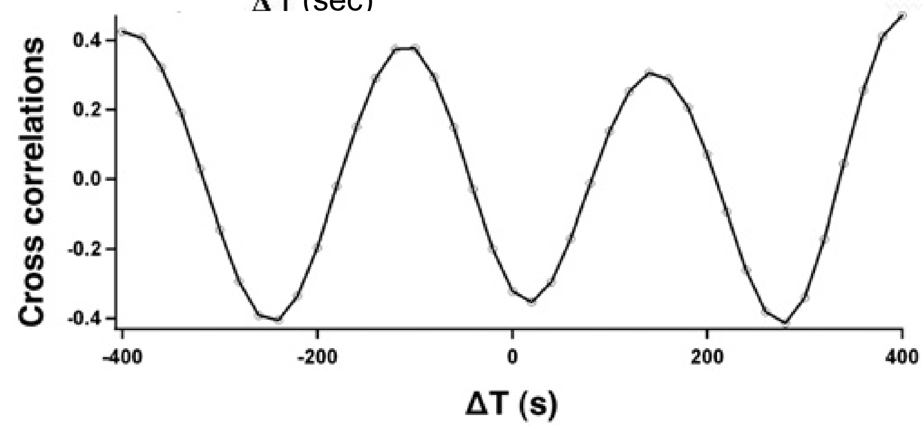
- Purely chemical oscillation: due to negative feedback
- Mechanical contraction: affect oscillation through tension, which affects myosin off-rate
- Stability boundary: sensitive to myosin off rate

Neighbor-neighbor correlation of oscillation:

Cross correlation with negative peak at zero time lag:

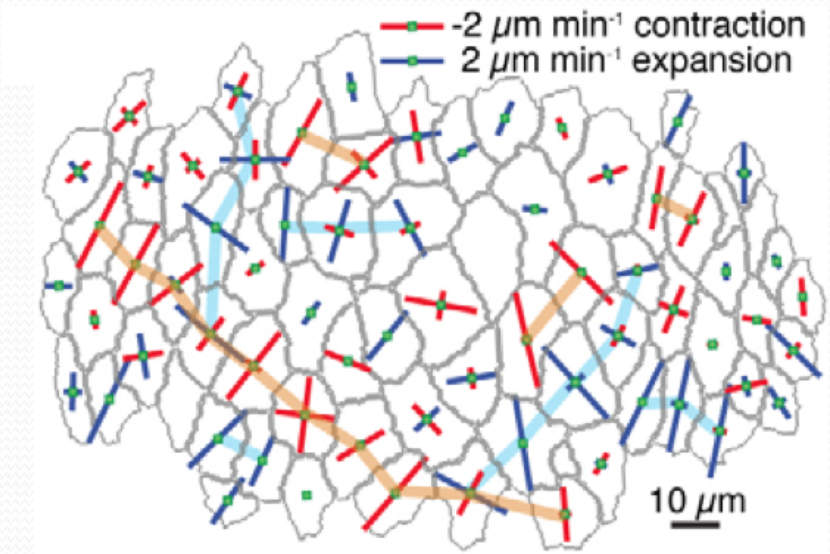




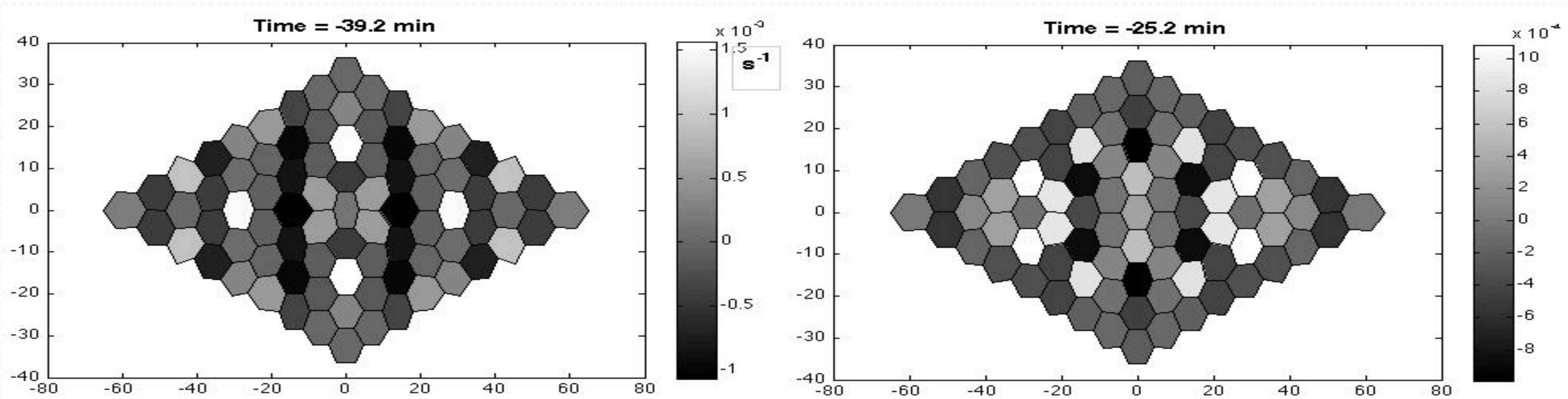


Neighbors predominantly oscillate in anti-phase, in agreement with data of Solon et al. (2009)

Neighbor-neighbor correlation: ribbons

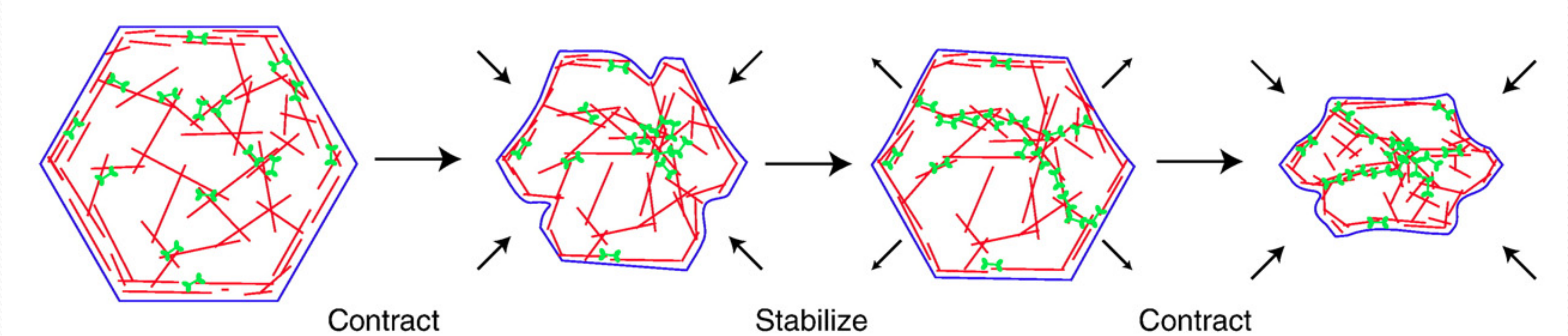


Blanchard et al. 2010
Gorfinkiel et al. 2011



2. Slow phase: internal and AC ratchets

Recall Martin et al.'s cartoon of internal ratcheting:



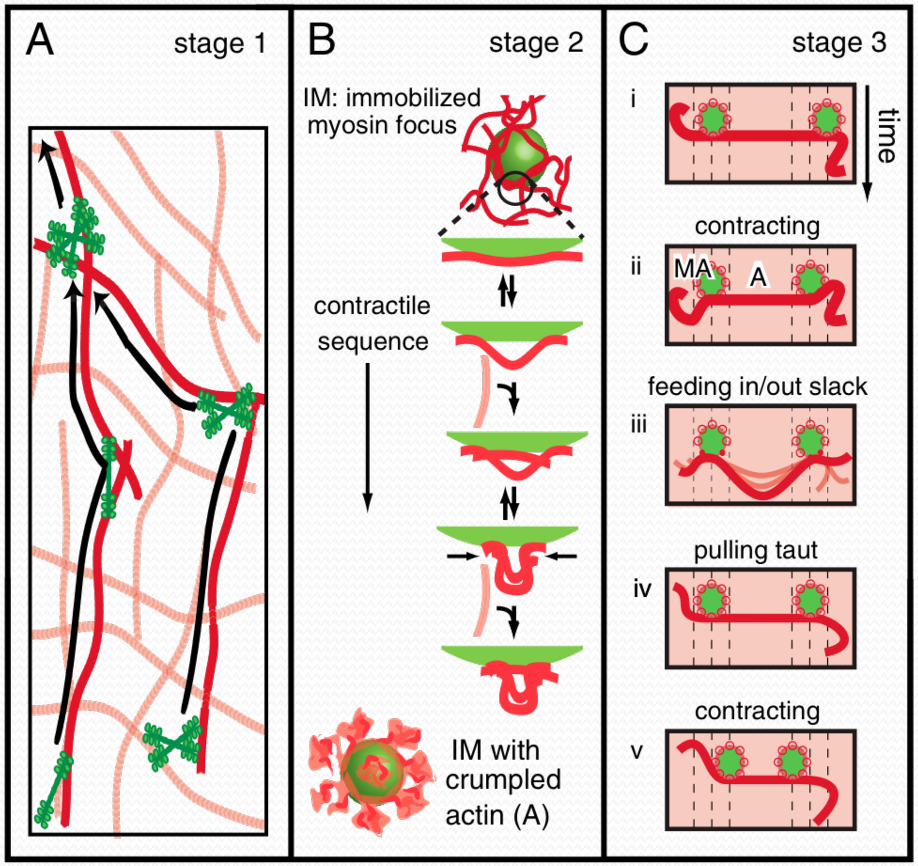
➔ Remodeling of cortical actomyosin and/or strengthening of medial myosin to inhibit recovery

➔ How to represent this "remodeling" in our mechanical model?

Slow phase: modeling the ratchets

Formation and aggregation of actomyosin foci (Soares e Silva *et al*, 2011):

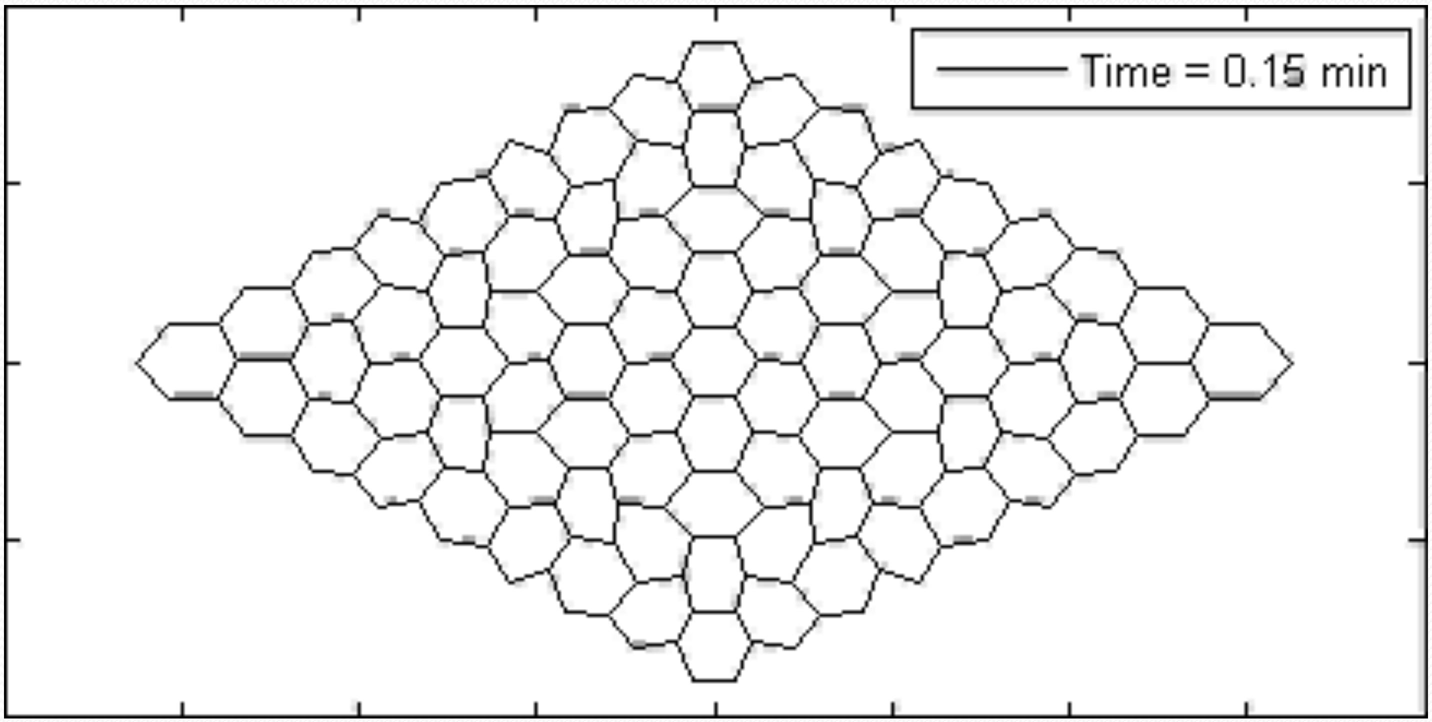
- Buckling under compression
- Taking up slacks
- Borrow the picture for ratchet



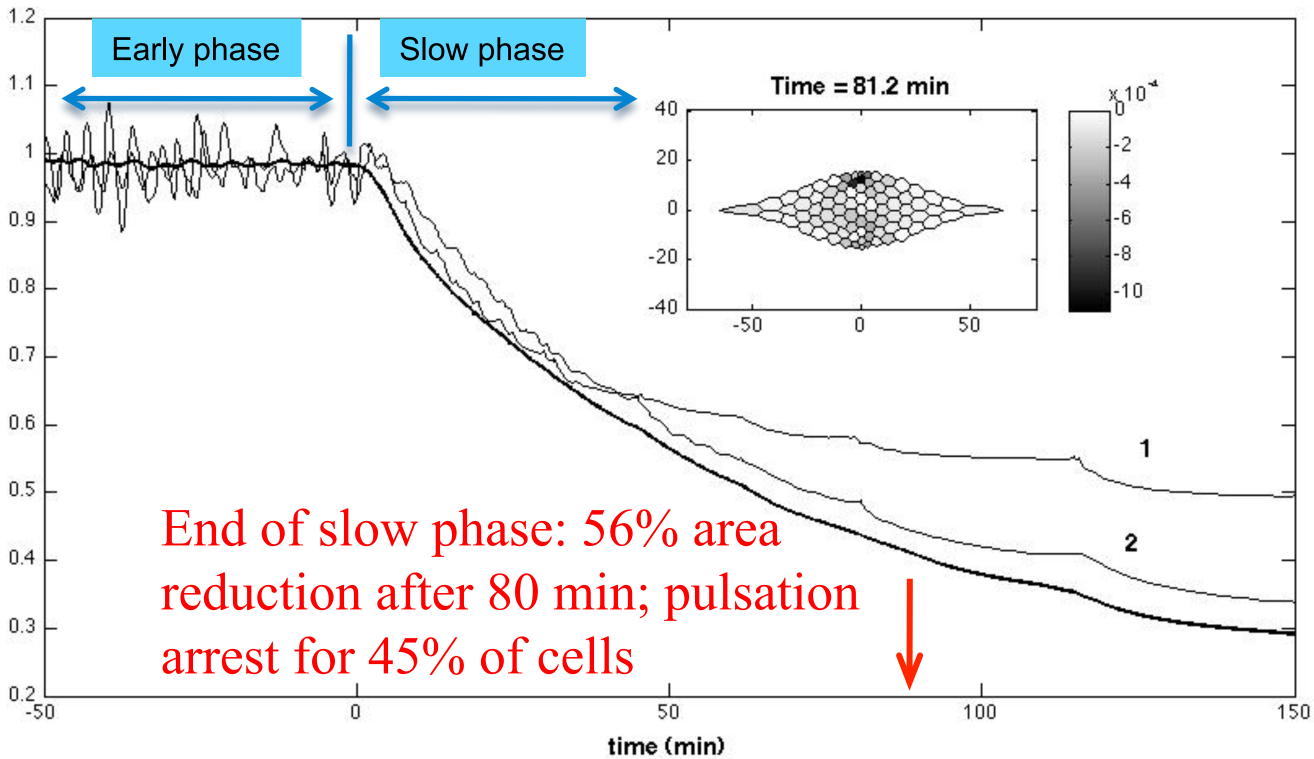
➔ We shrink the rest length of edges and spokes in each cycle of oscillation
➔ We model AC by supracellular "rubber band", with shrinking rest length each cycle

Numerical prediction of ***internal ratcheting***:

(3% reduction in l_0 in each cycle)

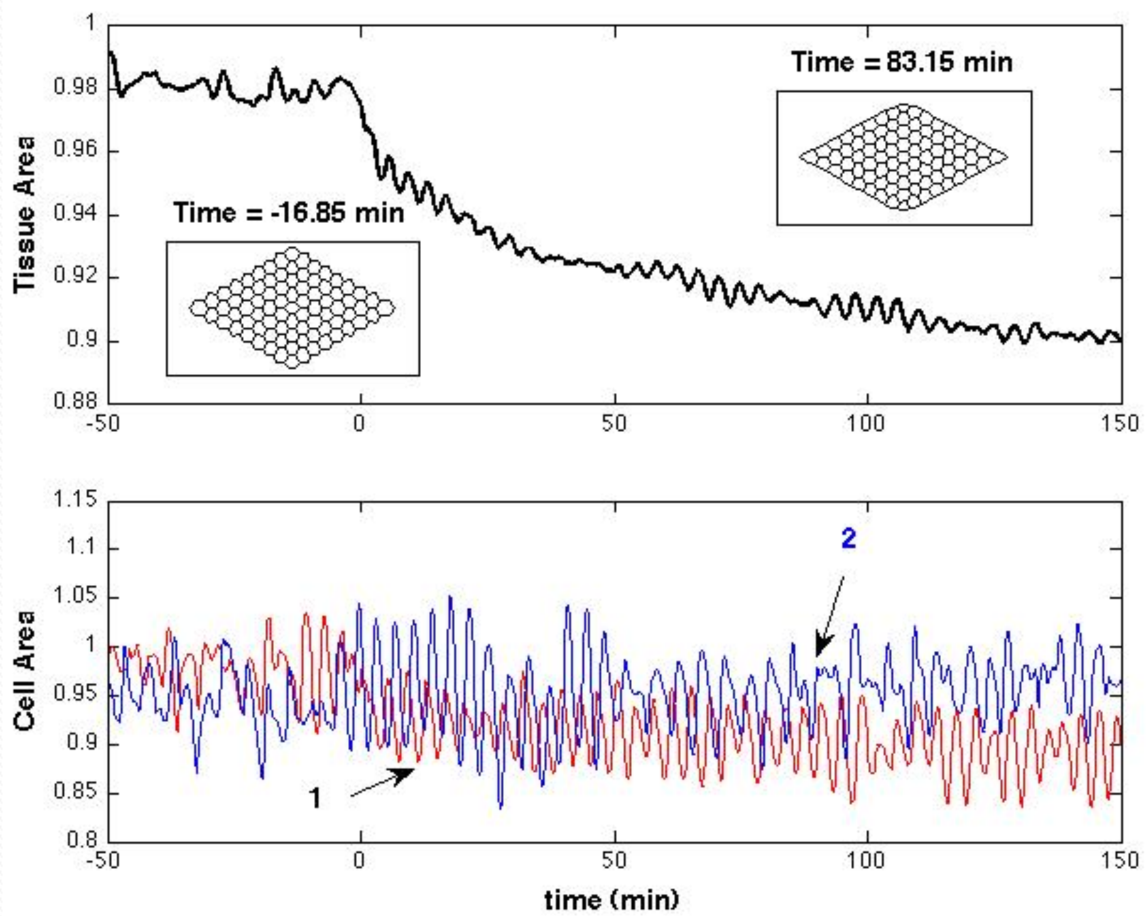


Slow phase: internal ratcheting



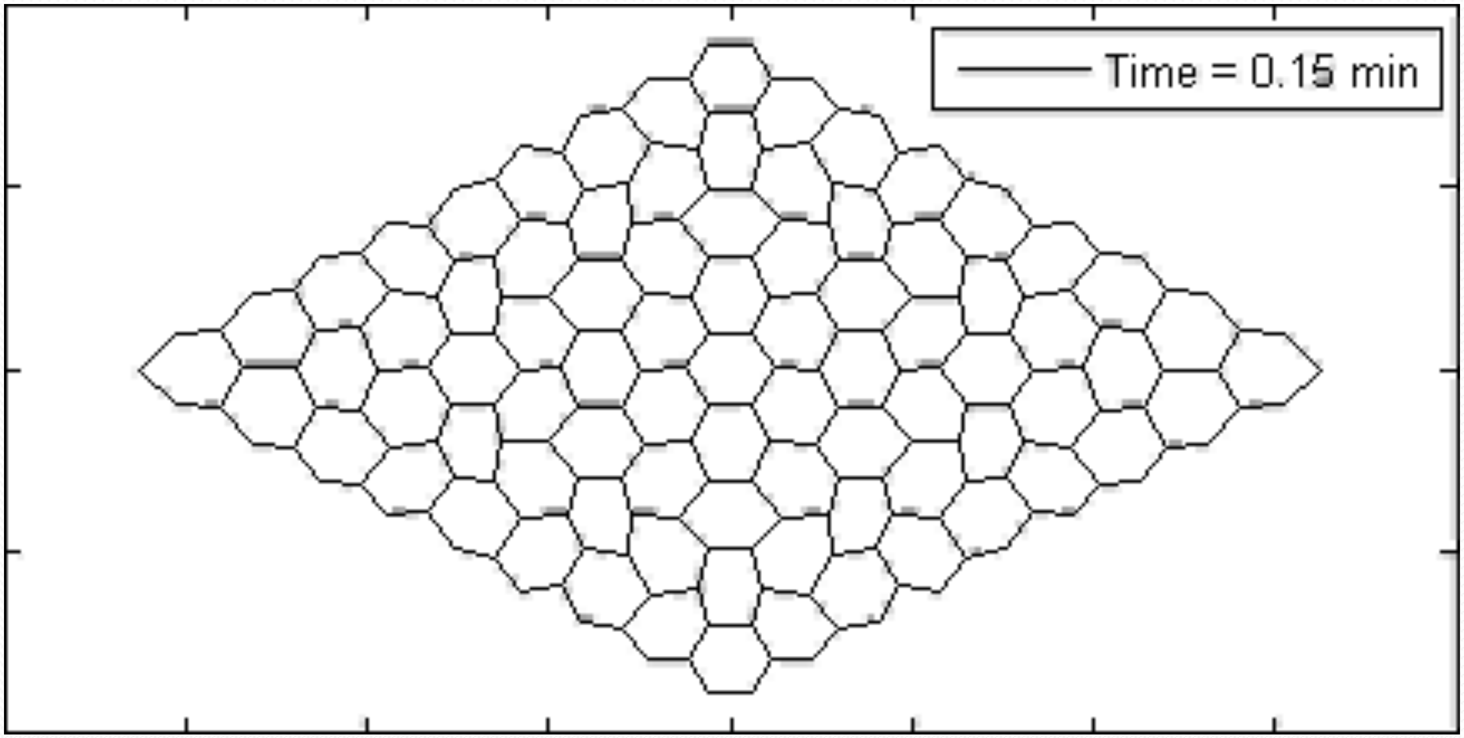
(a) Cell and tissue contraction
(b) Arrest of cell pulsation
(c) Cells closer to canthi stop oscillating first (cf. experiment)
(d) End of slow phase: controlled by δl_0 (3% each cycle)

<u>Slow phase</u>: AC ratcheting (1% reduction in L_0)



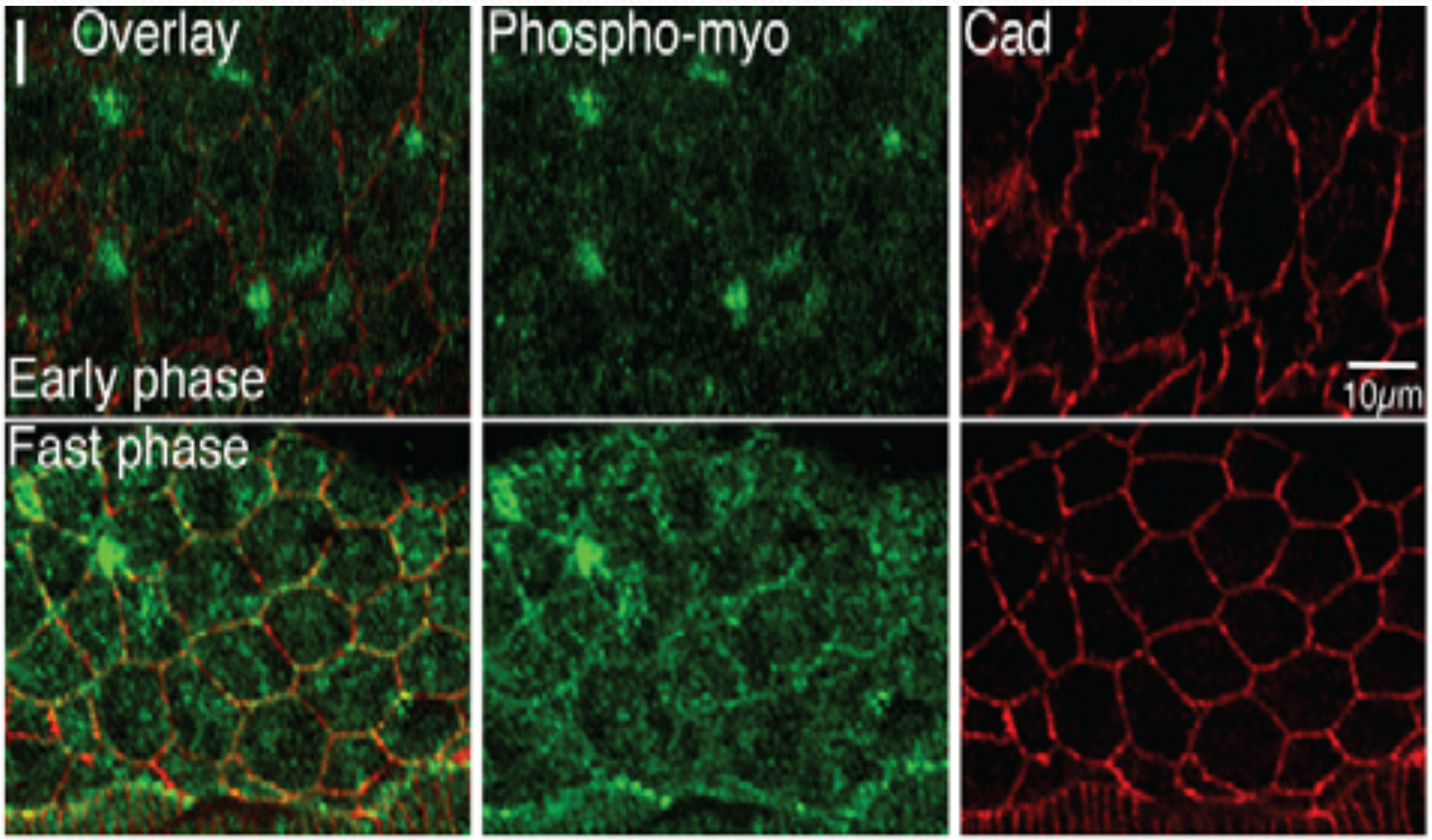
(a) Tissue area reduction small (~ 6% in 150 min)
(b) Does not arrest AS cells (peripheral ones more affected)
(c) AC ratcheting ineffective; but smoothes tissue edges

With both *internal and AC ratchets*:



3. Fast phase: how to model?

- Experiment: sustained increase in myosin density and network coverage (Blanchard et al. 2010):

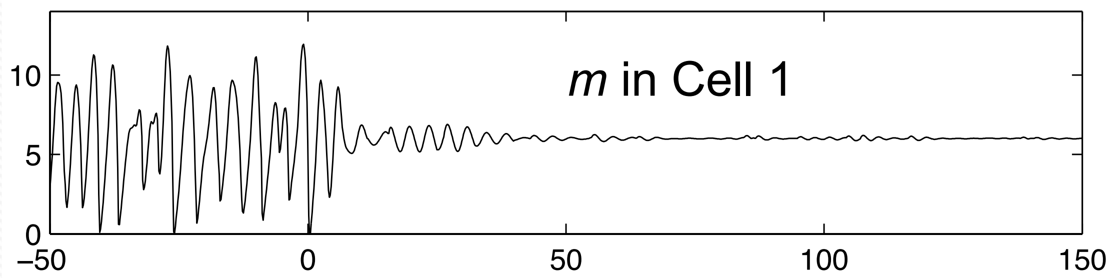


Fast phase: how to model?

- Our signaling/myosin model too simple: *s, m* stabilize upon arrest of oscillation:

$$\dot{s}_k = q - k_0 \sum m_{ij}$$

$$\dot{m}_{ij} = k^+ h s_i - k^- m_{ij}$$



- More sophisticated chemical model needed

II.A. Control of oscillation & contraction: molecular signals

Limitations of mechanical model:

- What triggers transition from oscillation to contraction (early → slow)?
- Ratcheting through passive elasticity, not actomyosin itself
  - *How does the apicomedial network strengthen?*
- What triggers arrest of oscillation (slow → fast)?

Experimental evidence

- David, Tishkina & Harris, The PAR complex regulates pulsed actomyosin contractions during amnioserosa apical constriction in *Drosophila*. Development **137**, 1645-1655 (2010)

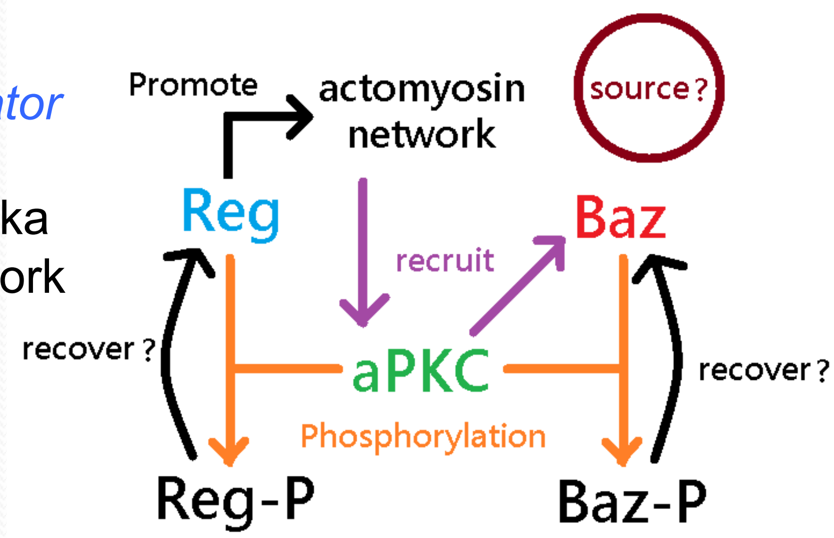
- Prominent role of *Par-family* of proteins: Par-6, aPKC, Bazooka
- Bazooka: prolongs actomyosin network; suppresses disassembly
- Par-6/aPKC: suppresses assembly, prolongs lull
- Apparent antagonism between aPKC and Bazooka

Experimental evidence (cont'd):

- David, Wang, Feng & Harris, Bazooka inhibits aPKC to limit antagonism of actomyosin networks during amnioserosa apical constriction. *Development* **140**, 4719-4729 (2013)

- Spatial relationship: colocalization
  - actomyosin network recruits aPKC from cortex to apicomedial surface
  - aPKC recruits Bazooka from cortex to apicomedial surface

- Chemical relationship:
  - *aPKC phosphorylates network regulator*
  - *Causes network disintegration*
  - aPKC binds & phosphorylates Bazooka
  - aPKC-Baz compound stabilizes network
  - *Competitive inhibition: Bazooka promotes network*

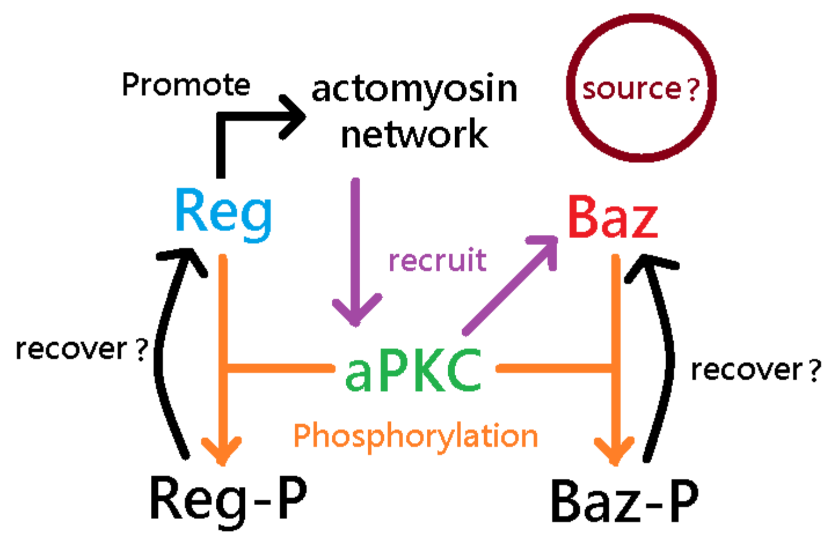


Experimental evidence (cont'd):

- Temporal relationship:
  - Cyclic variations of aPKC and Baz
  - Baz gradually builds up over cycles
  - Apicomedial actomyosin network becomes more persistent ("stabilization")
  - Cytoskeletal stabilization accompanies damped cell oscillation
  - *Baz eventually overwhelms aPKC; cell oscillation arrested*

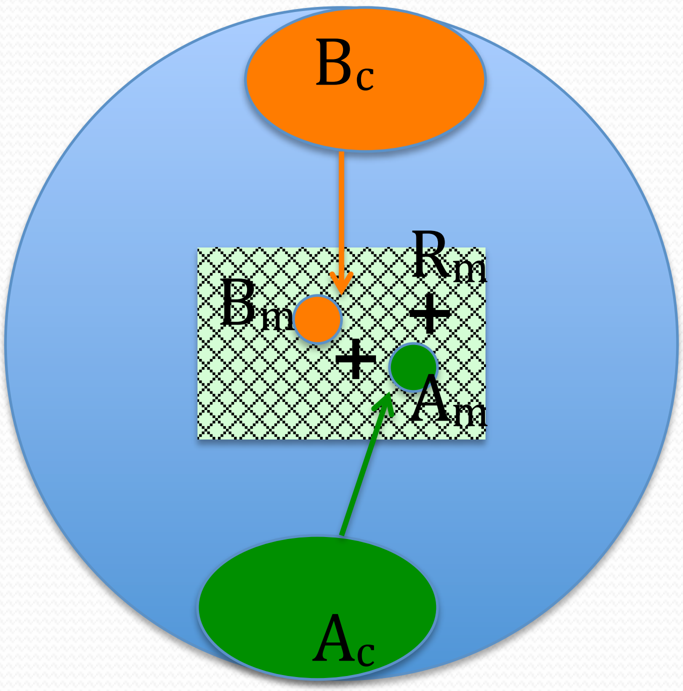
- Natural transition to slow phase → activating "internal ratchet"?

- Complete suppression of oscillation → onset of fast phase?



II.B. Chemical model for signaling proteins

	Cortical	Medial
aPKC	A_c	A_m
Bazooka	B_c	B_m
Actomyosin regulator	--	R_m
aPKC-Baz compound	--	AB
aPKC-Reg compound	--	AR



Recruitment and reactions

- Recruitment from cortex to medial surface:

$$A_c \xrightarrow[k_1]{R_m} A_m$$ with delay τ_1

$$B_c \xrightarrow[k_4]{A_m} B_m$$ with delay τ_2

- Phosphorylation:

$$A_m + R_m \underset{k_2}{\overset{k_2}{\longleftrightarrow}} AR$$

$$A_m + B_m \underset{k_3}{\overset{k_2}{\longleftrightarrow}} AB$$

AB decomposed through multiple steps; rate being 10 ~ 1000 times smaller

Delayed ODE model

$$\begin{cases} \dfrac{dA_c}{dt} = -k_1 A_c R_m; \qquad \dfrac{dB_c}{dt} = -k_4 A_m B_c \\ \dfrac{dA_m}{dt} = k_1 A_c(t-\tau_1) R_m(t-\tau_1) - k_2 A_m R_m - k_2 A_m B_m + k_3 AB + k_2 AR \\ \dfrac{dB_m}{dt} = k_4 A_m(t-\tau_2) B_c(t-\tau_2) - k_2 A_m B_m + k_3 AB \\ \dfrac{dR_m}{dt} = q_R - k_2 A_m R_m + k_2 AR \\ \dfrac{dAB}{dt} = k_2 A_m B_m - k_3 AB \\ \dfrac{dAR}{dt} = k_2 A_m R_m - k_2 AR \end{cases}$$

Initial conditions: $A_{\mathrm{c}} = A_0$; $B_{\mathrm{c}} = B_0$; $R_{\mathrm{m}} = R_0$; all others = 0.

Parameter values

Parameters	Values	Parameters	Values
$k_1(s^{-1})$	0.000003	$q_R(s^{-1})$	8
$k_2(s^{-1})$	0.15	$\tau_1(s)$	75
$k_3(s^{-1})$	0.009	$\tau_2(s)$	80
$k_4(s^{-1})$	0.000003	A_0	60000
R_0	500	B_0	4000

[1] McGill MA, McKinley RF, Harris TJ, *J. Cell Biol*. **185**, 787-796 (2009). [B_0]

[2] Fjeld CC, Denu JM, *J. Biol. Chem*. **274**, 20336–20343 (1999). [k_2, k_3]

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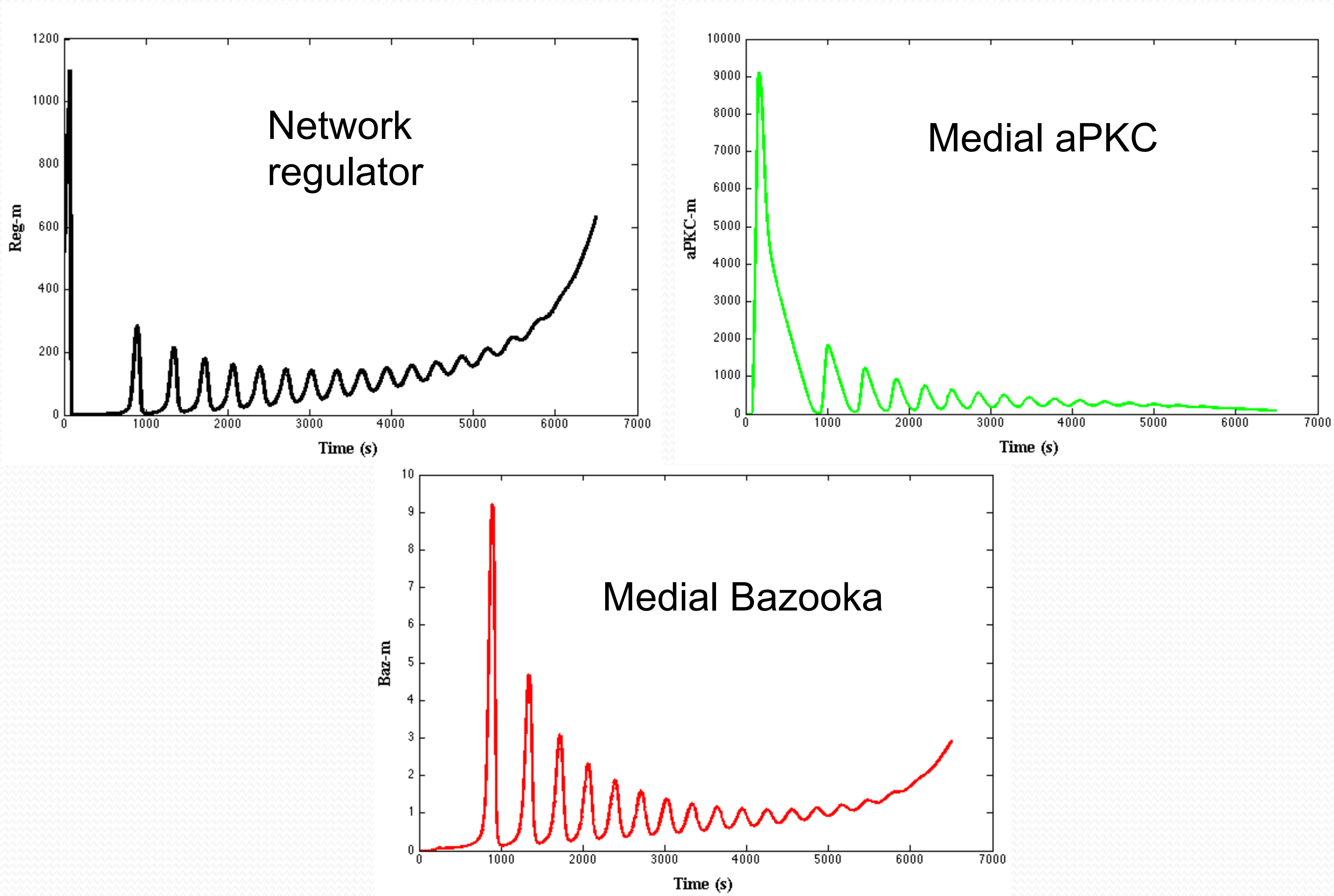
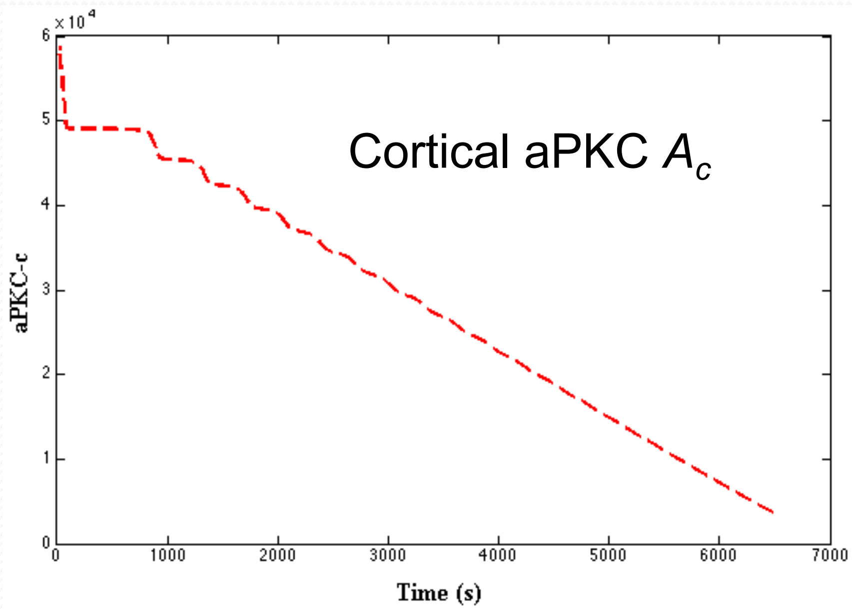
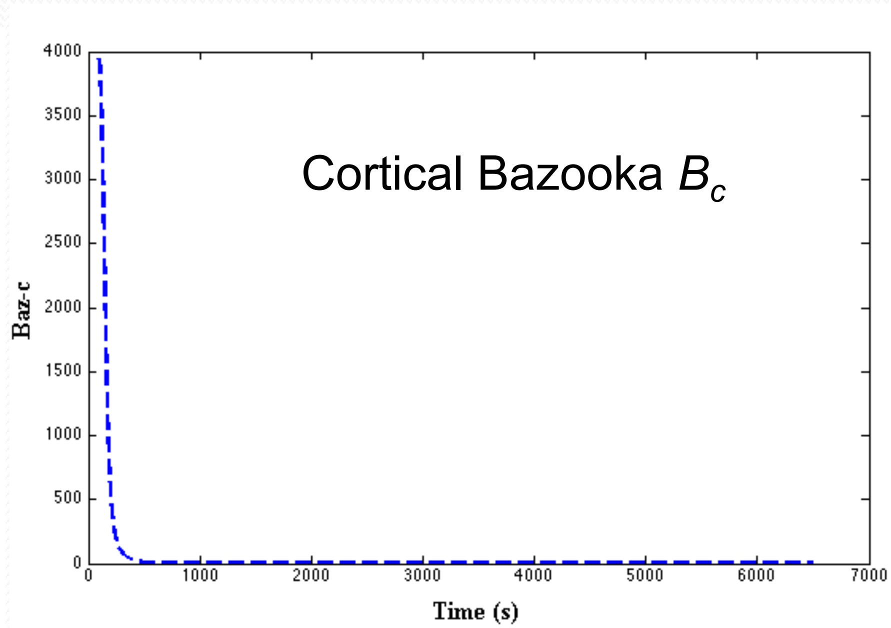
Results: evolution of the signaling molecules



Evolution of the cortical proteins



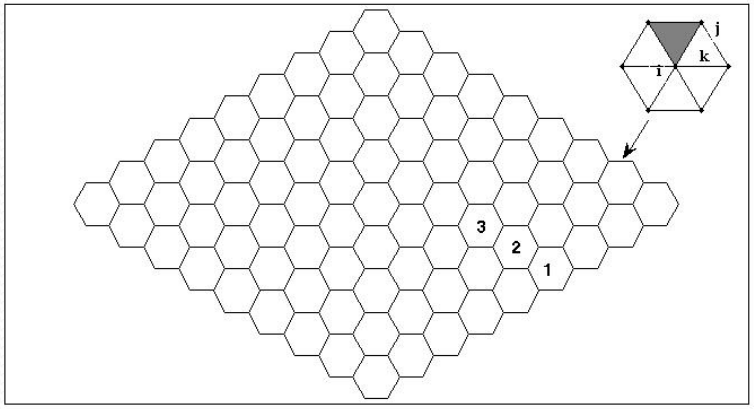


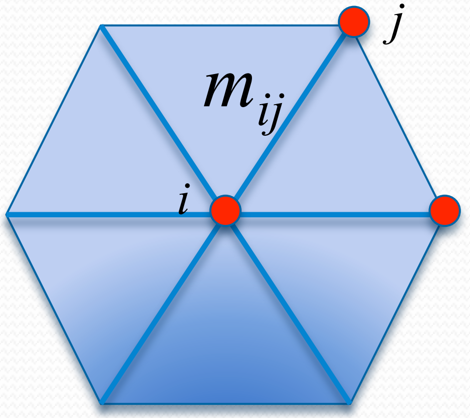
Desirable features of model predictions

- Cortical-to-medial transport of *aPKC* and *Bazooka*
- Initial oscillation of medial proteins: delayed negative feedback
- *Bazooka* grows relative to *aPKC*, damping oscillation
  - Chemical "trigger" for "internal ratchet"?
- Arrest of oscillation; then persistent growth of *cytoskeleton regulator*
  - Chemical "trigger" for onset of fast phase of dorsal closure?

Transitions effected naturally by signaling proteins, no longer triggered "by hand"

Chemical model + mechanical model:



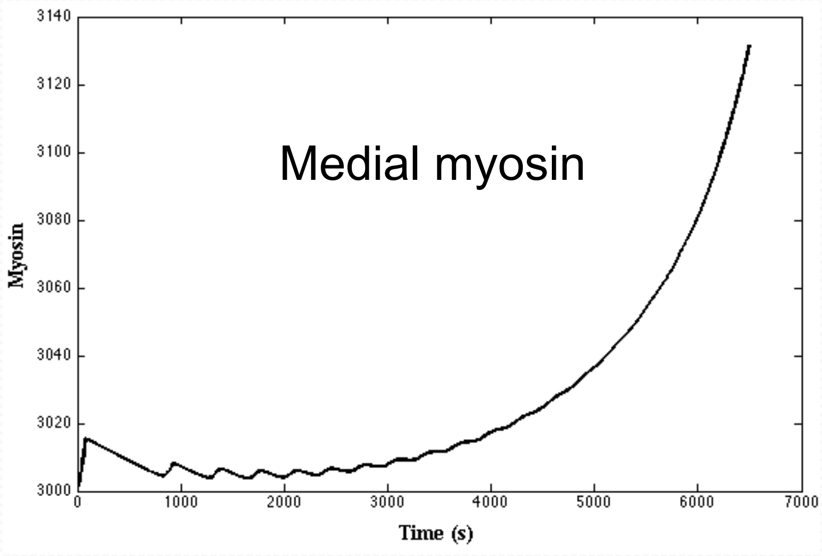


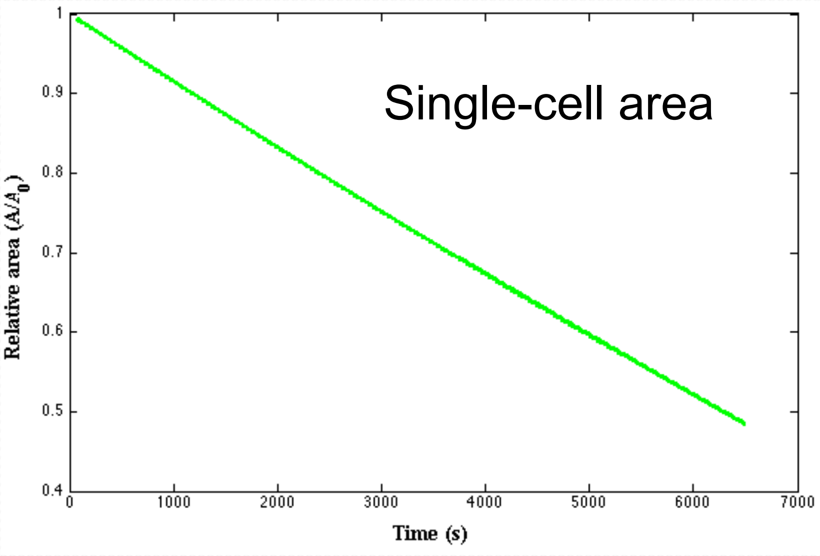
Nodal motion: $$\eta \frac{d\mathbf{x}_i}{dt} = \mathbf{f}_i, \qquad \mathbf{f}_i = \sum_j f_{ij} \frac{\mathbf{x}_j - \mathbf{x}_i}{|\mathbf{x}_j - \mathbf{x}_i|},$$

$$f_{ij} = \beta m_{ij}$$ (Get rid of "passive elasticity")

Myosin dynamics: $$\frac{dm_{ij}}{dt} = k^+ h_{ij} R_i - k^- m_{ij}, \quad k^- = c_1 e^{-c_2 f_{ij}}$$

Preliminary result for an isolated cell





Summary:

- Dorsal closure: coupling myosin kinetics, chemical signaling and cell/tissue deformation
- Key features captured by model:
  - Cell/tissue oscillation & contraction: *ratcheting* by passive elasticity
  - Chemical oscillation: *delayed feedback* among Par-proteins and medial actomyosin network
- Future tasks: integrating chem/mech models for DC
- Need more quantitative data

- Wang, Feng & Pismen, *Biophys. J.* **103**, 2265-2274 (2012).
- Gorfinkiel, New and Notable commentary, *Biophys. J.*, **104**, pp. 1-3 (2013).
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