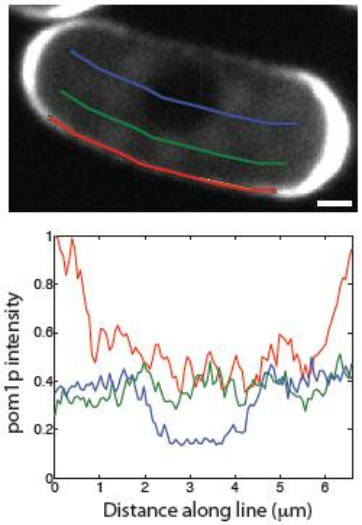
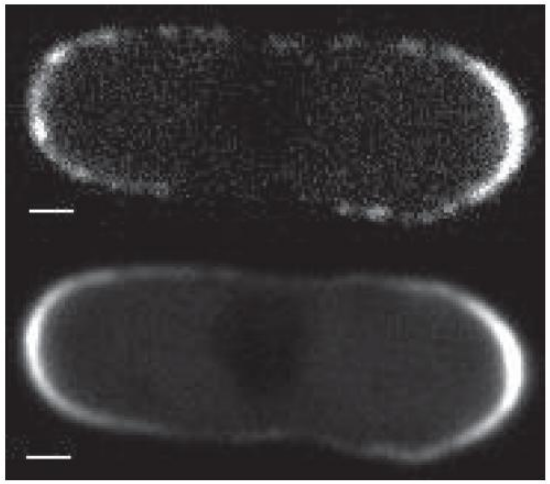
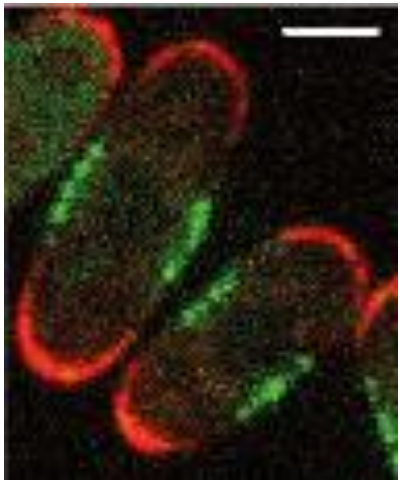
Dynamics of intracellular gradients and cell size control in fission yeast







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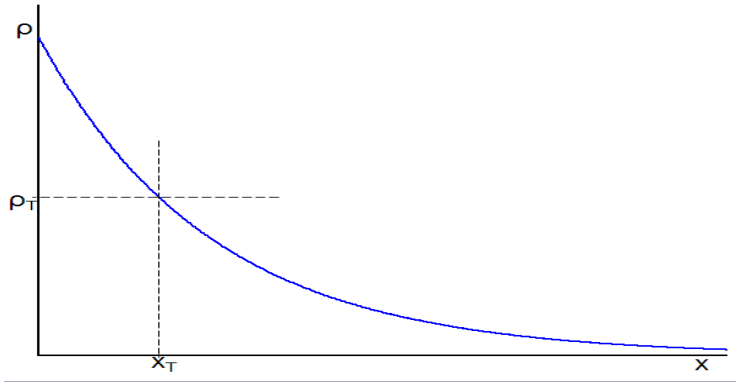
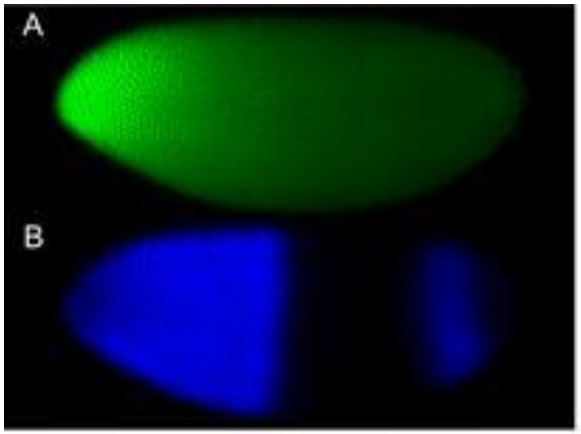
£££:

Concentration gradients in biology

- How to measure position in biological systems?
- Use gradients of protein concentration …





Bcd/*hb* in *Drosophila*

- Use local concentration to drive a switch
- Familiar in developmental biology
- Importance increasingly understood in subcellular context

A simple model of a concentration gradient

- Source: production at x=0 at rate J
- Diffusion: Diffusion constant D
- Disassociation/Degradation: at rate μ

$$\frac{\partial \rho}{\partial t} = D\nabla^2 \rho - \mu\rho + J\delta(x)$$

- At steady-state:

$$\rho(x) = \frac{J\lambda}{D}\exp\left(-\frac{x}{\lambda}\right) \quad \text{where} \quad \lambda = \sqrt{D/\mu}$$

- Very simple model, but is it biologically relevant in single cells?

Can gradients exist inside single cells?

- Need $\lambda = \sqrt{D/\mu}$ to be a few *μm*

- Suppose gradient is on membrane
- With D ~ 0.1 $\mu m^2 s^{-1}$
- For $\mu \sim 0.1\ s^{-1} \Rightarrow \lambda \sim 1\ \mu m$
- Short dwell time ~ 10 *s*

Brown & Kholodenko
FEBS Lett. (1999)

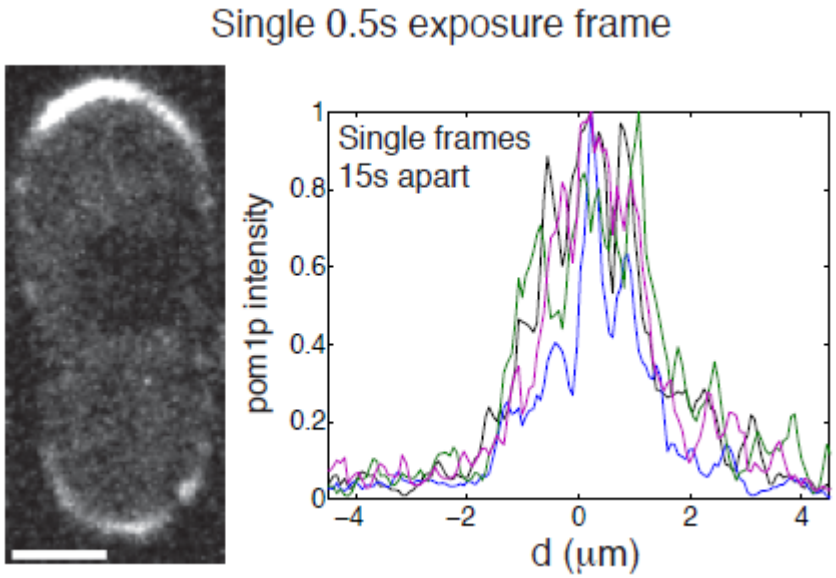
- But this isn't protein lifetime!
- Protein can be recycled from cytoplasm back to membrane

- Subcellular gradients are likely to be plausible/ubiquitous

Examples: intracellular gradients

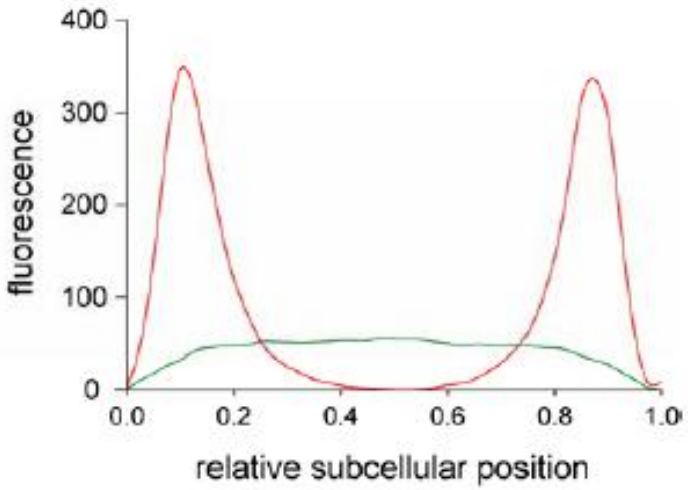
- Cell division:

Pom1p in fission yeast



Single Cell

MipZ in *Caulobacter*

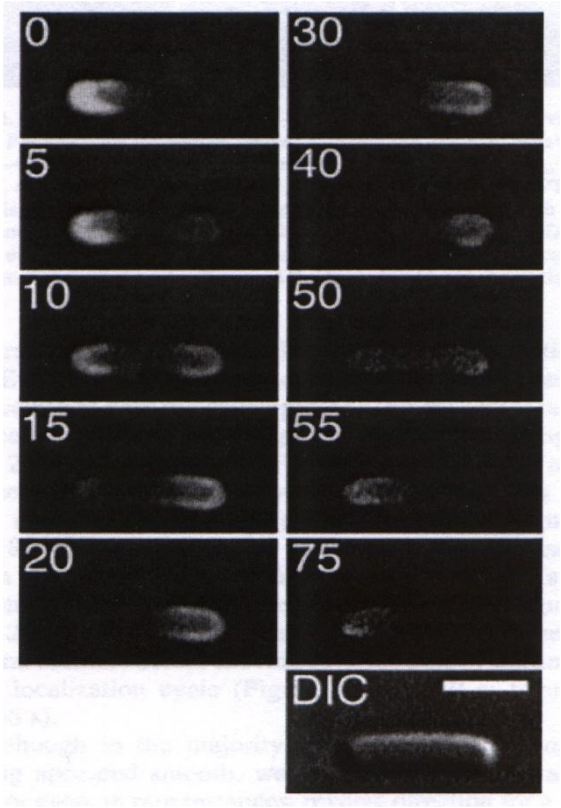


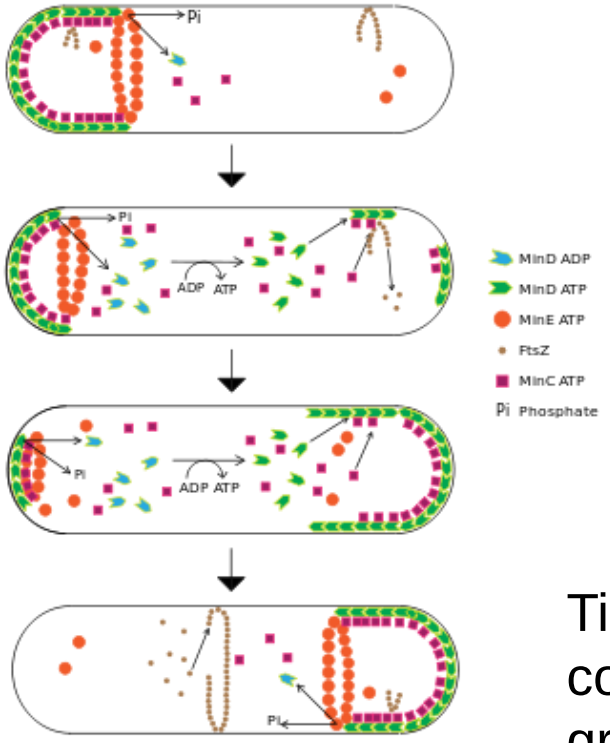
Population average

Thanbichler & Shapiro, Cell (2006)

Examples: intracellular gradients

- MinCDE system in *E. coli*:



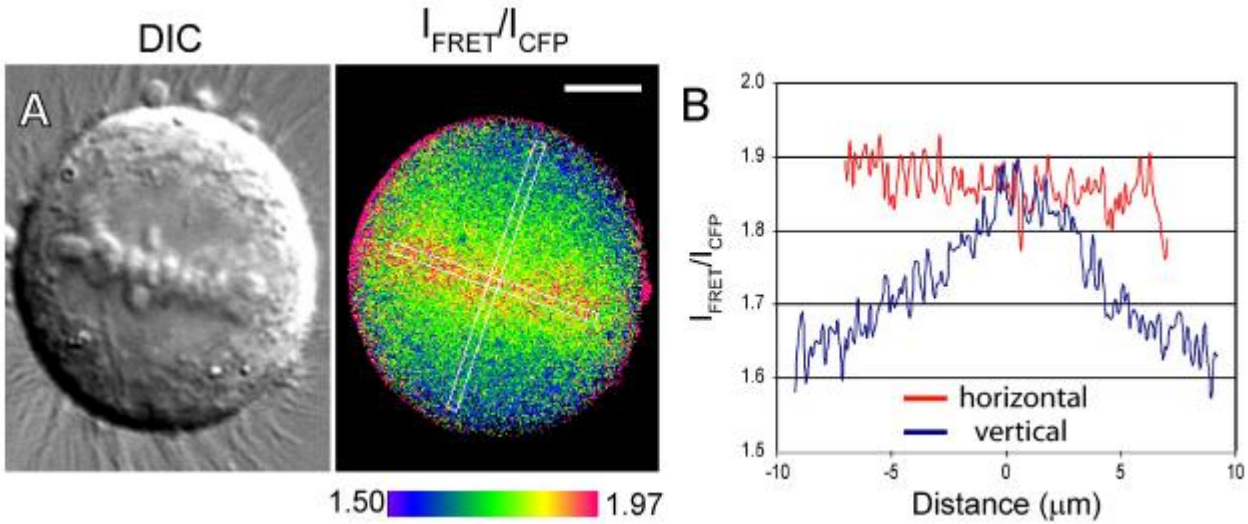


Time-averaged concentration gradient!

de Boer et al. PNAS (1999)
Howard et al. PRL (2001) also K. Kruse

Examples: intracellular gradients

- RanGTP system:

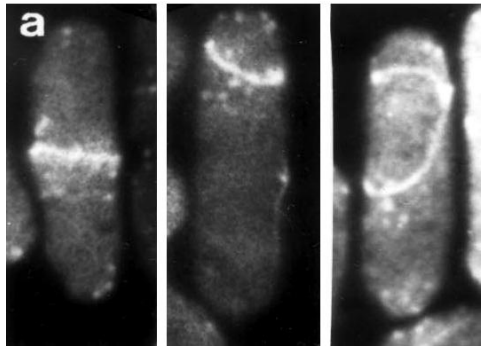


- RanGTP promotes microtubule nucleation/stabilization, aiding in spindle assembly

O'Connell et al JCB (2009)

Cell division positioning in *S. pombe*

- Nucleus pushed to cell centre via microtubules
- Nucleus then signals to cell cortex via Mid1p
- Mid1p localizes to broad band overlying the nucleus, shuttles in and out of nucleus and ring
- Mid1p then controls location of cytokinetic machinery



F-actin rings in wt and *mid1* mutant

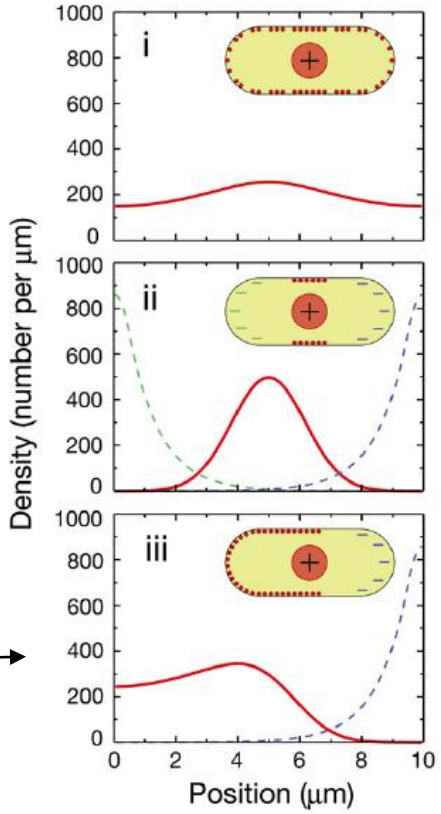
- *mid1* mutant forms rings at random sites not linked to nucleus

Pom1p is an inhibitory gradient

- Rapid cytoplasmic diffusion ⇒ nearly homogeneous Mid1p distribution

- Assume existence of polar inhibitor gradients

- If one polar inhibitor is removed, expect Mid1p to spread to that end of cell

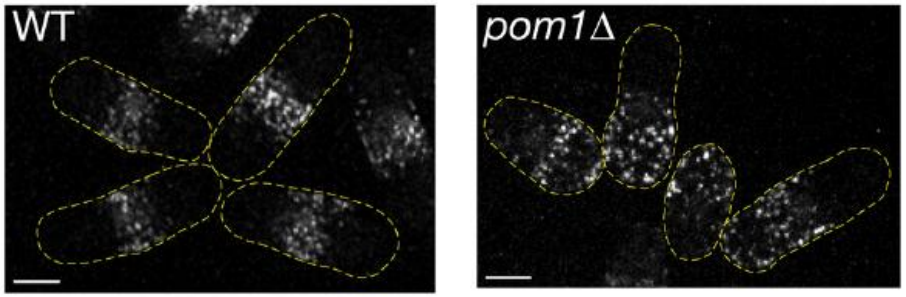


Padte et al. Curr. Biol. (2006)

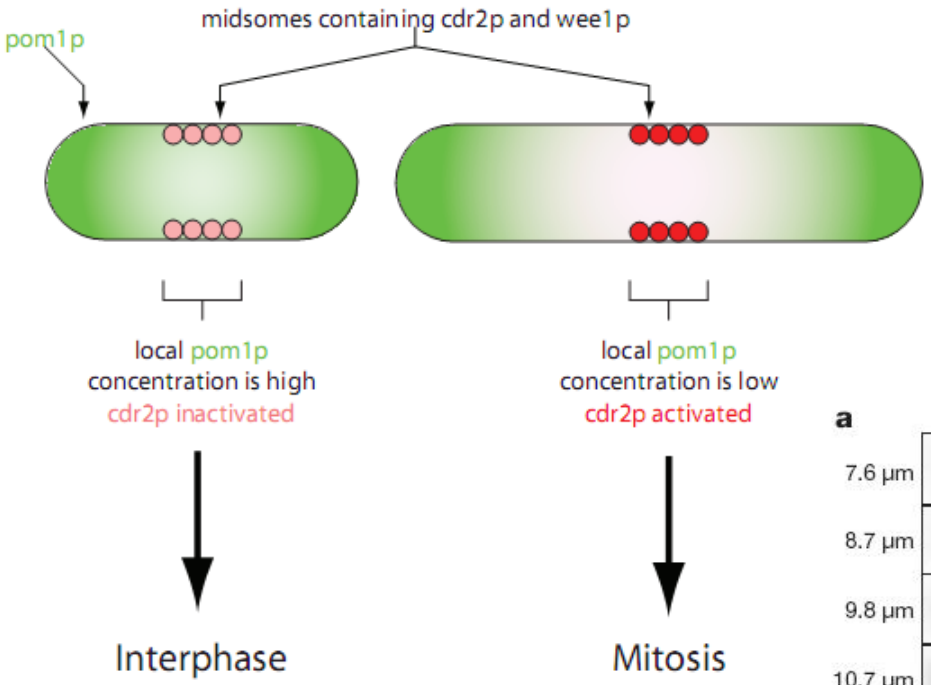
Also Paoletti lab (2006)

- *pom1* mutant exhibits cell division defect

- Predict that one of polar "morphogens" is Pom1p



Pom1p gradient as a cell-size sensor?



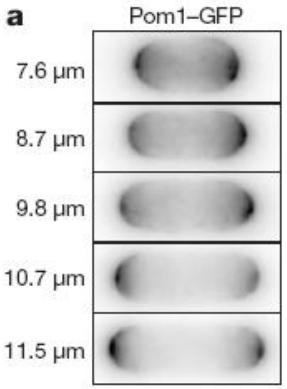
Pom1p

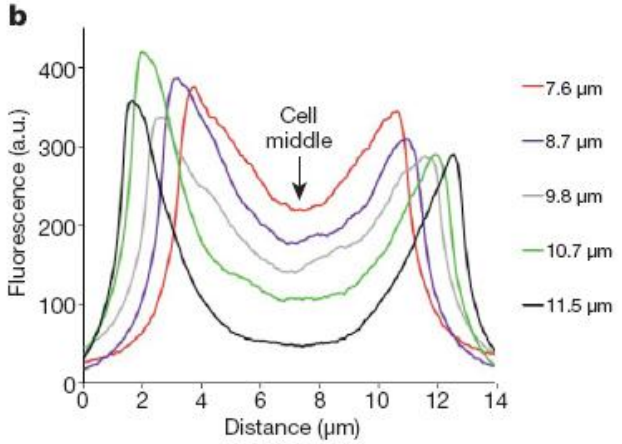
⊥

Mid1p, Cdr2p

↓

Wee1p





Moseley et al, Nature (2009)
Martin & Berthelot-Grosjean, Nature (2009)

Two important questions!

- What is the mechanism of gradient formation?

  - Simple source, diffusion, disassociation leading to exponential decay with distance?

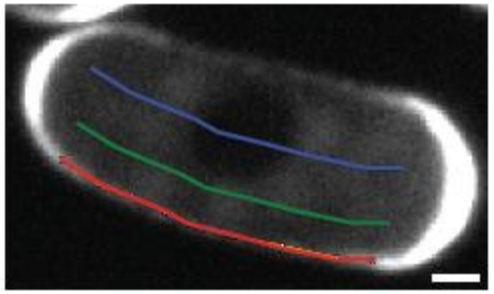
- How are fluctuations reduced?

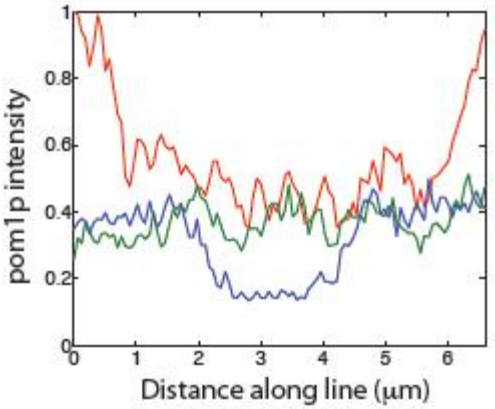
  - Gradient imparts positional/size information, so noise must be filtered out

- To answer these questions we need reliable measurements of the gradient profile…

Saunders et al Dev. Cell (2012)
Howard, Trends Cell Biol. (2012)

Extracting the pom1p profile





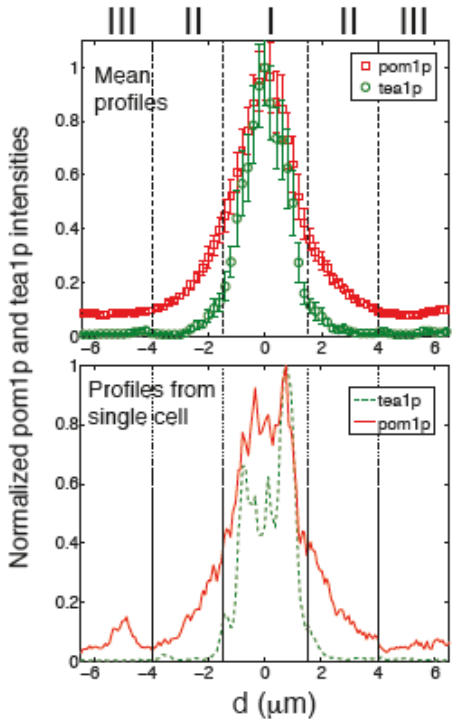
- Pom1p forms a cortical gradient
- Also cytoplasmic population, but excluded from nucleus

Blue contour is final mask

- Computer generates initial mask, using phase image
- Then contour/pixels adjusted to maximise overlap with fluorescent signal
- Finally, human intervention needed to finalise mask

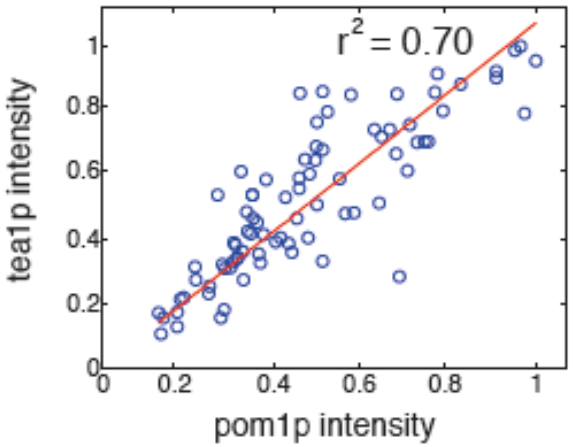
Making the gradient: tea1p dependent injection

• Pom1p homogeneously distributed in *tea1* mutant

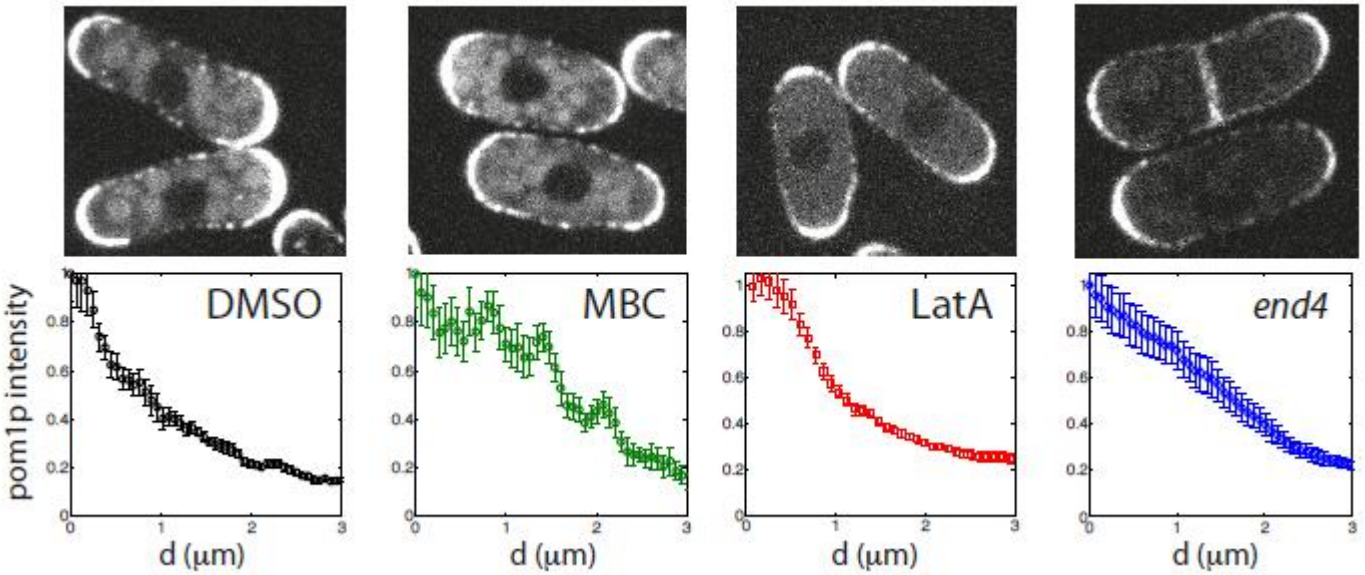


• Pom1p injected at poles and spreads along membrane

• Maximum Pom1p levels proportional to Tea1p consistent with insertion role at tips

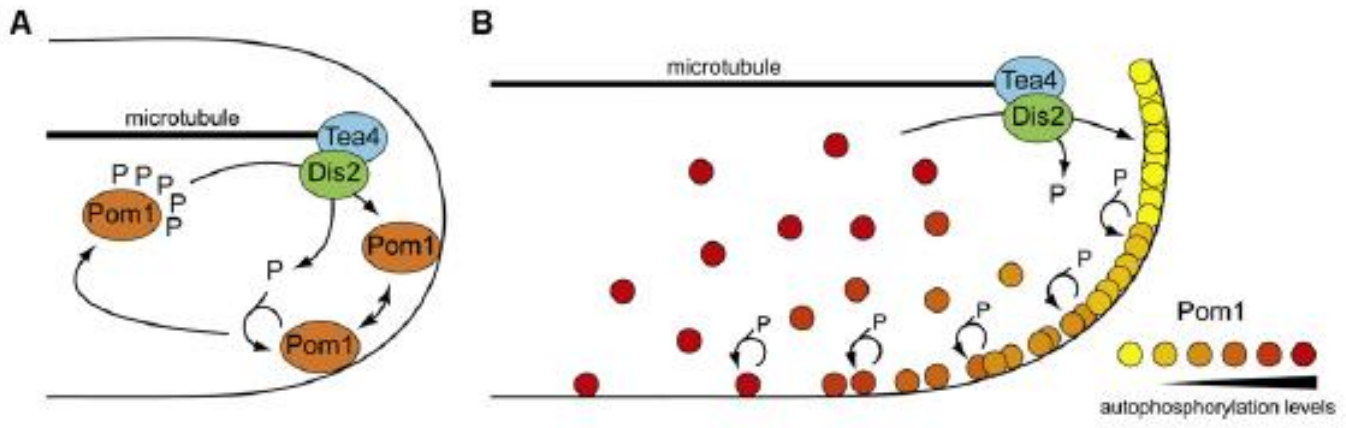


Gradient does not depend on cytoskeleton/endocytosis



- Gradient unaffected by cytoskeletal/endocytic perturbation

Gradient formation involves autophosphorylation



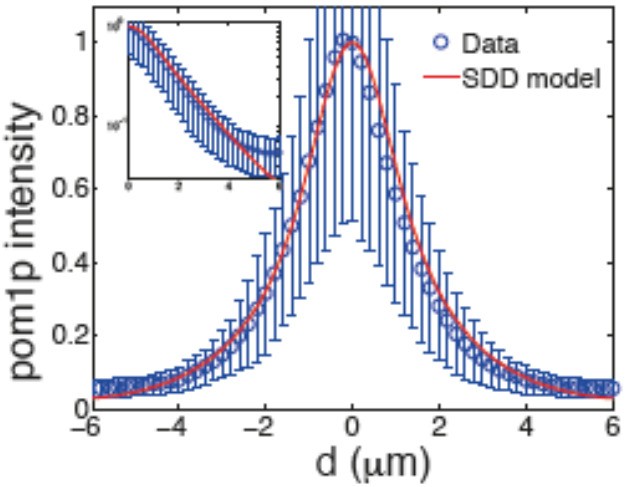
Hachet et al Cell (2011)

- Pom1p dephosphorylated by Dis2p at microtubule tips, allowing incorporation into membrane

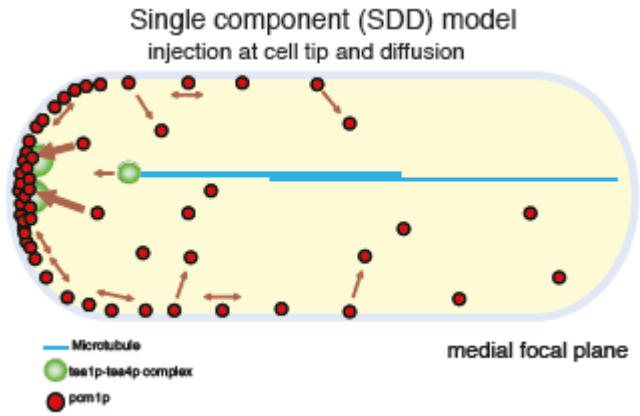
- Subsequently autophosphorylates, losing affinity for membrane

Gradient profile

- Away from tips, profile mostly consistent with exponential decay

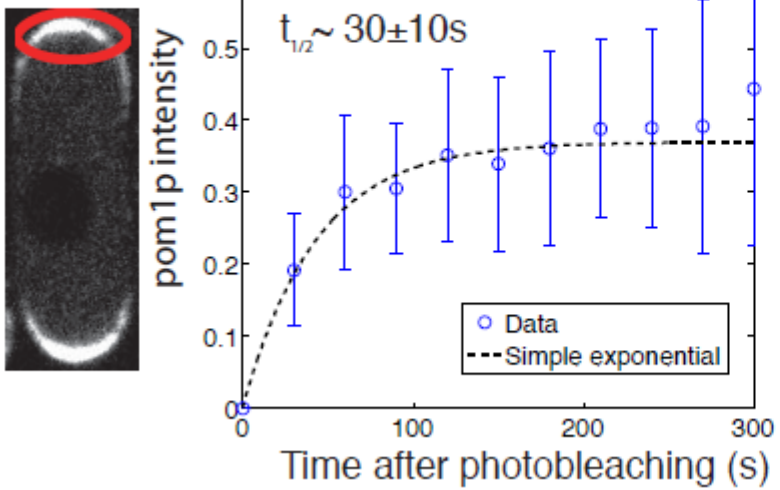


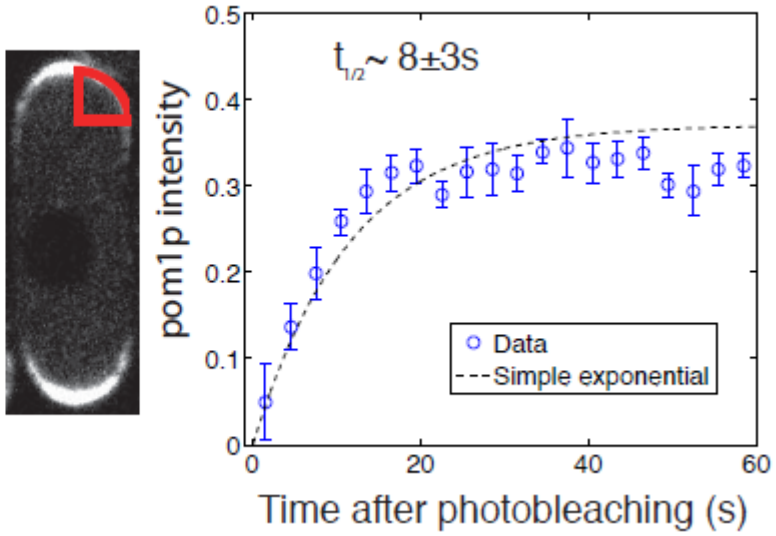
- Consistent with SDD model →

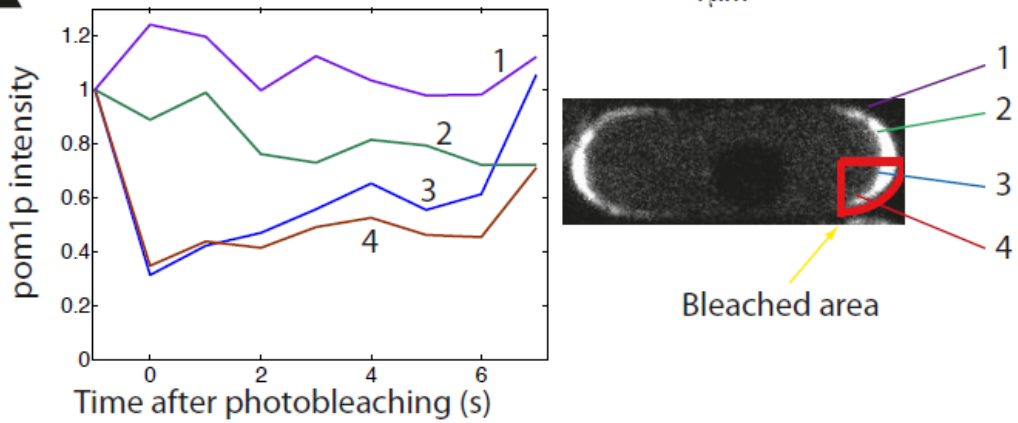


- So it's all very simple ...

FRAP experiments

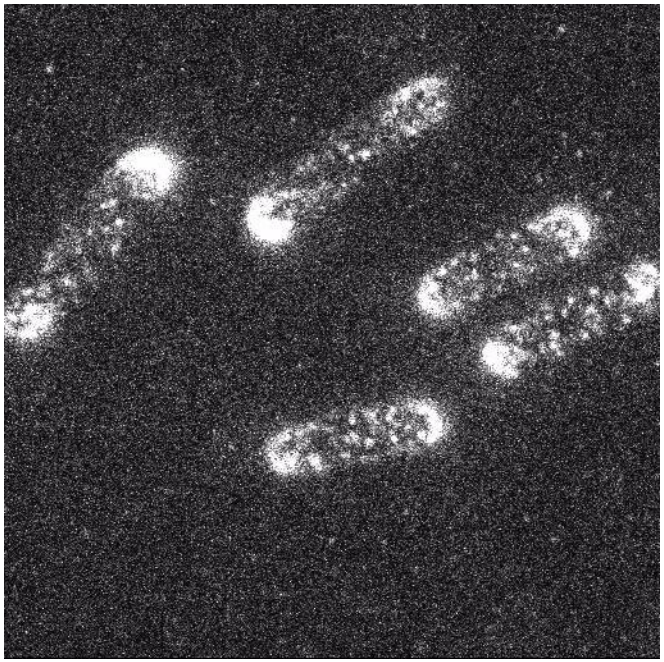






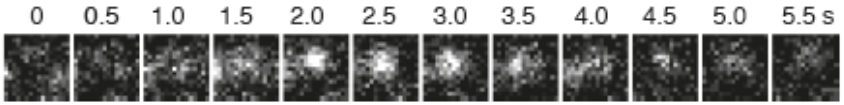
- Consistent with recovery through diffusion from unbleached half-tip
- lifetime ~ 30s
- diffusion ~ 0.1µm^2 s^{-1}

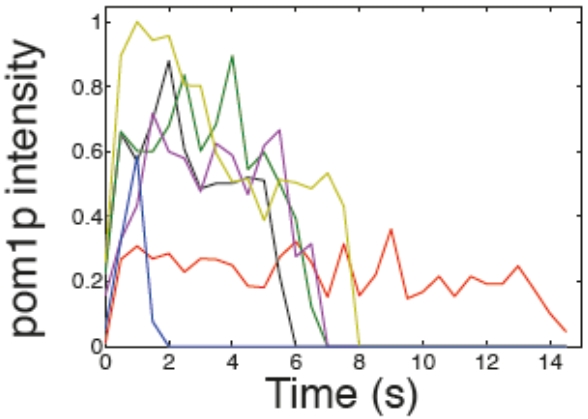
Imaging reveals a different timescale



Pom1p forms clusters!

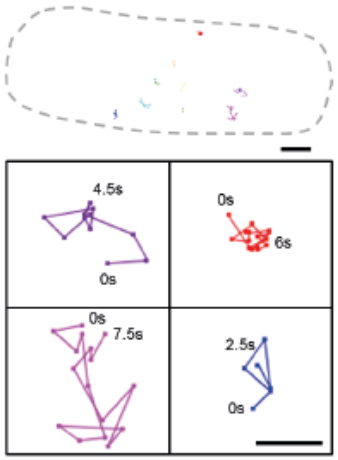
- Pom1p appears to concentrate into blobs that move around cortex …
- Movie from slice through upper cortex of cell





Cluster tracking:

- Lifetime ~ *4 ± 2 s*
- Diffusive tracks
- D ~ 0.01 μm^2 s^{-1}
- Problem!



SDD model fails!

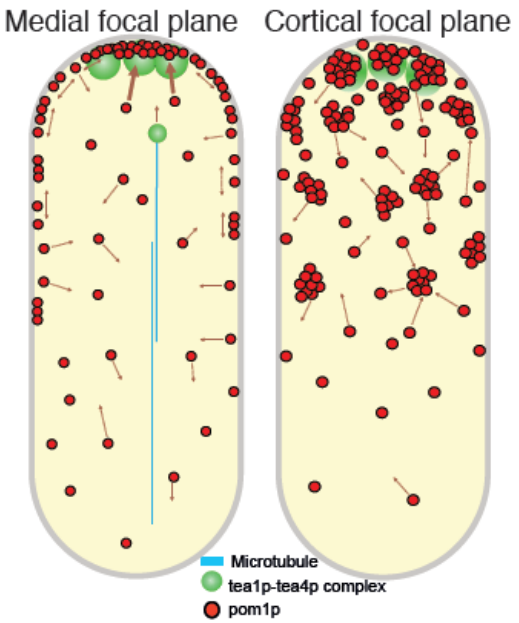
- SDD model only has a single lifetime (membrane dwell time)
- Cannot explain two timescales, order of magnitude apart
- Cannot different diffusion constants, factor of 5 different

- Hypothesis: more than one “species” of Pom1p on membrane

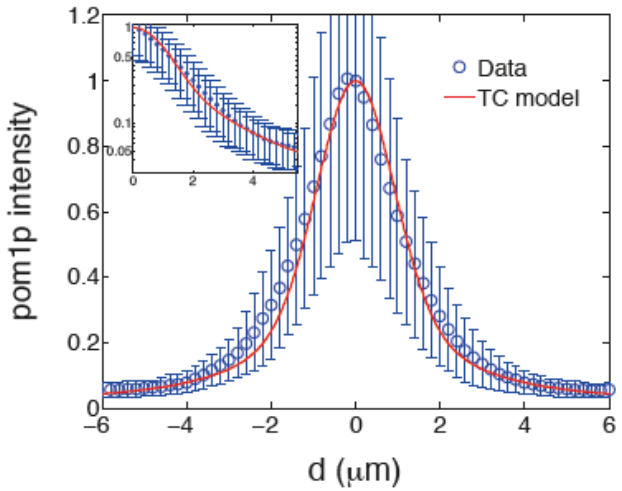
Two state (TS) model for pom1p dynamics

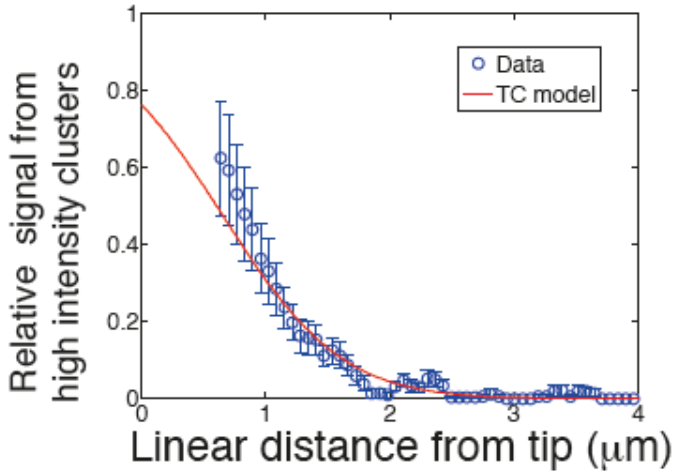
- Slow-diffusing, clustered form (S) and fast-diffusing form (F)

$$S + F \rightarrow S + S$$
$$S \rightarrow F$$
$$F \rightarrow \varnothing$$



- Fits well to mean intensity, FRAP data, aggregate lifetime, fraction of high intensity clusters

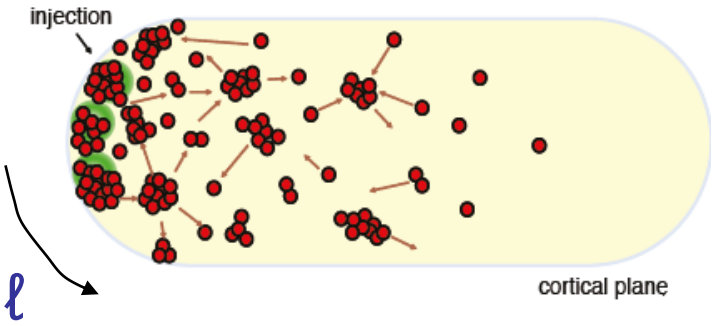




TS model technicalities

- Assume symmetry about long axis of cell
- Use arc-length coordinate ℓ:

$$\nabla^2 = \partial_\ell^2 + 1/(R\tan(\ell/R))\partial_\ell$$

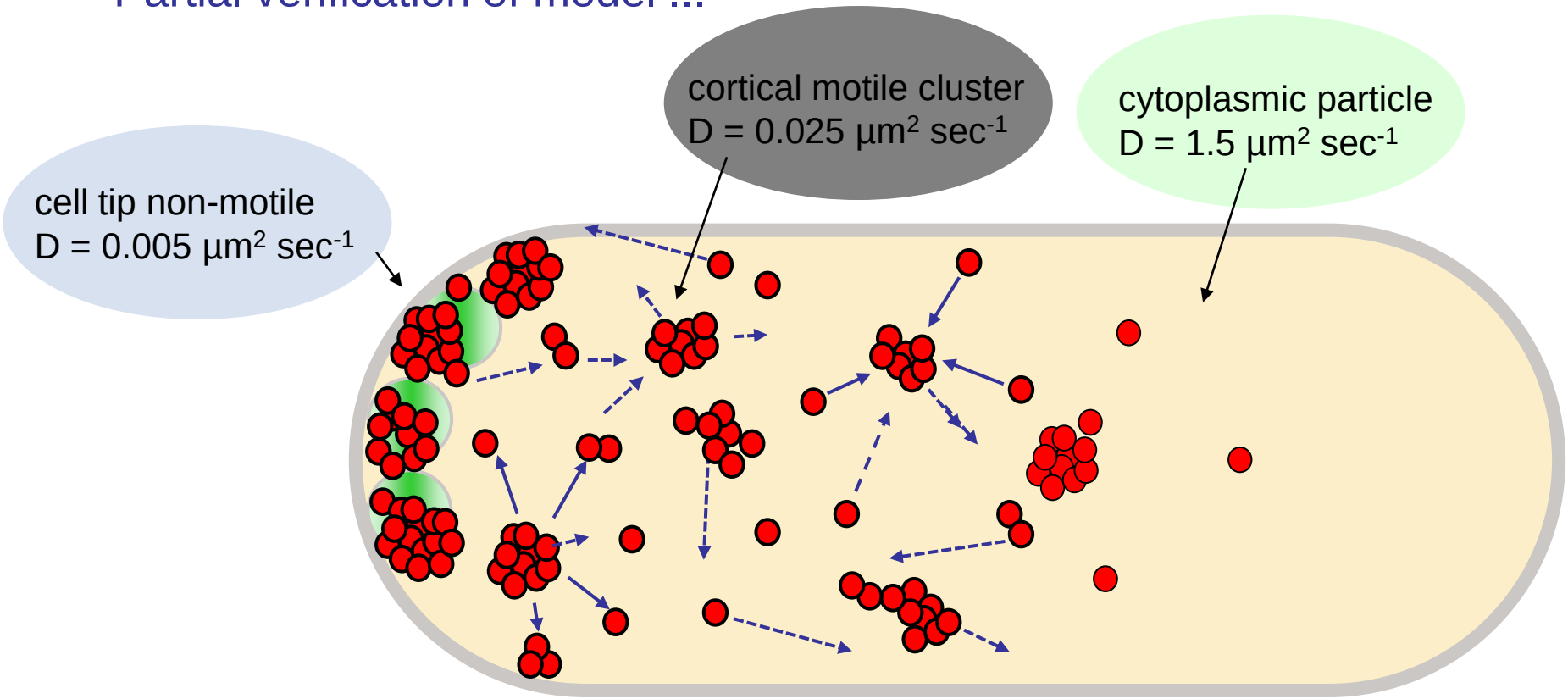


- Simplified equations, for slow and fast-diffusing components:

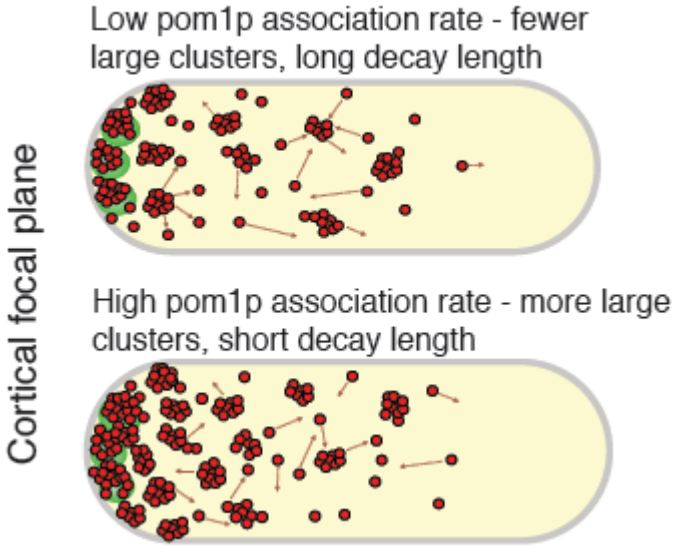
$$\partial_t \rho_s = D_s \nabla^2 \rho_s - \alpha \rho_s + \beta \rho_f \rho_s + (1-\epsilon) J f(d/\sigma)\,,$$
$$\partial_t \rho_f = D_f \nabla^2 \rho_f + \alpha \rho_s - \beta \rho_f \rho_s - \mu \rho_f + \epsilon J f(d/\sigma)\,.$$

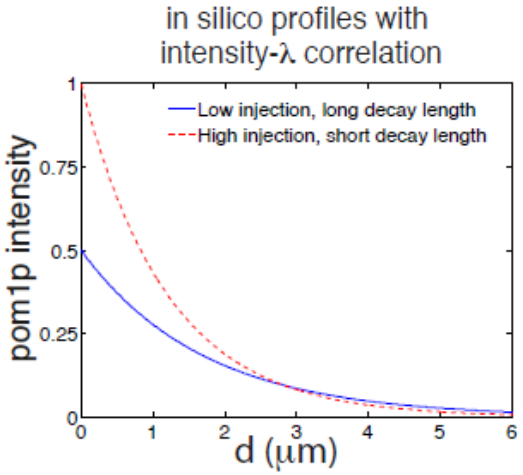
Measuring Pom1p membrane mobility

- Model predicts (at least) two membrane diffusion constants
- Use FCS to measure Pom1p membrane diffusion
- Find wide range of Pom1p diffusion constants
- Partial verification of model ...

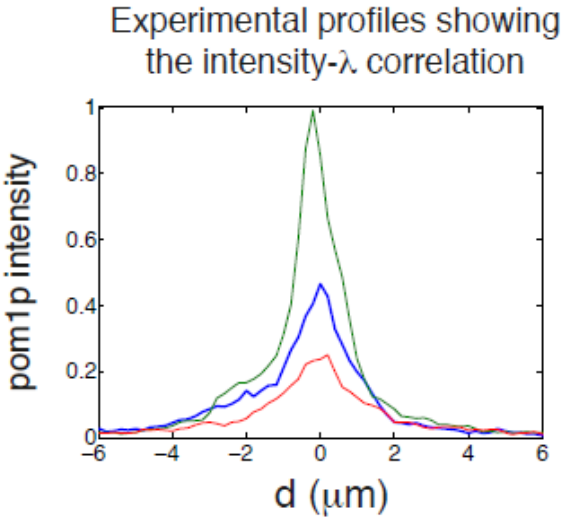


Model prediction: anticorrelation between amplitude and decay length





- Anticorrelation seems to be present in experiments



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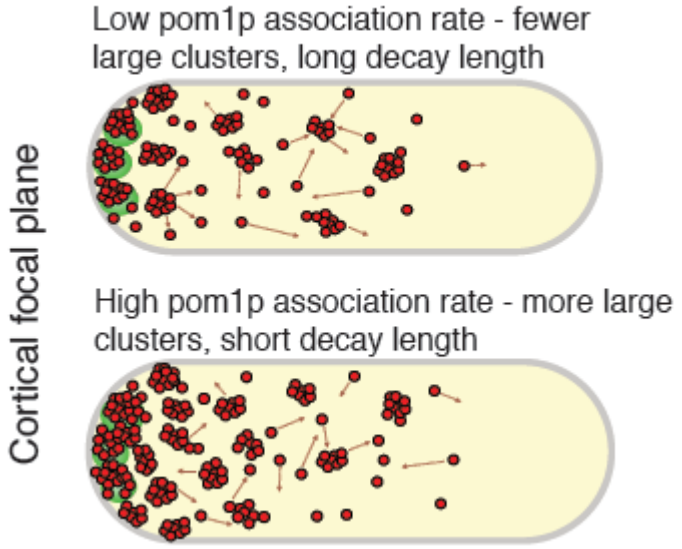
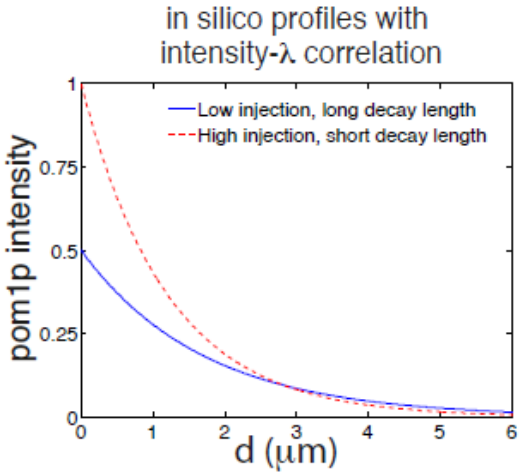
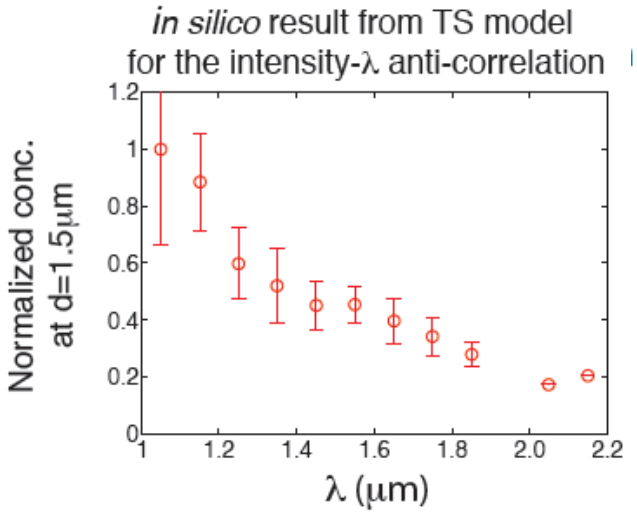
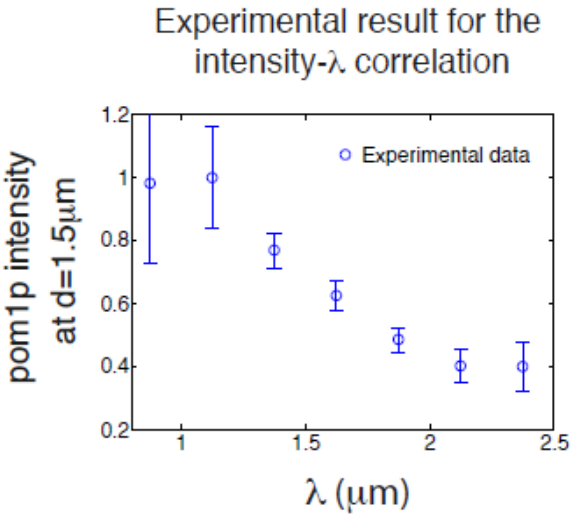


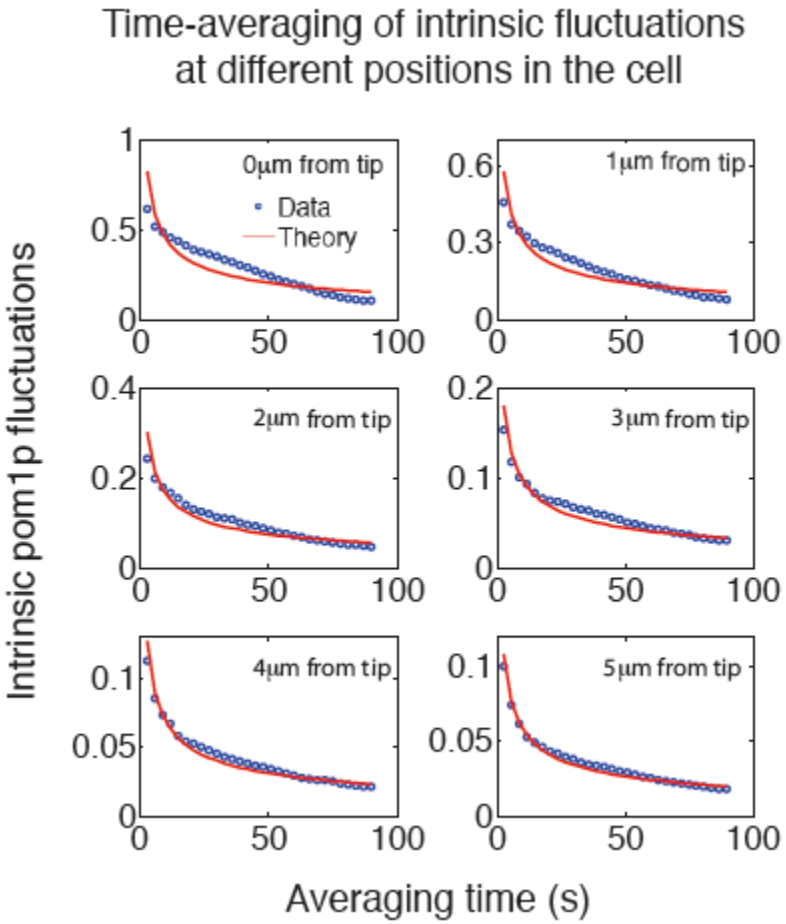






Reducing intrinsic fluctuations

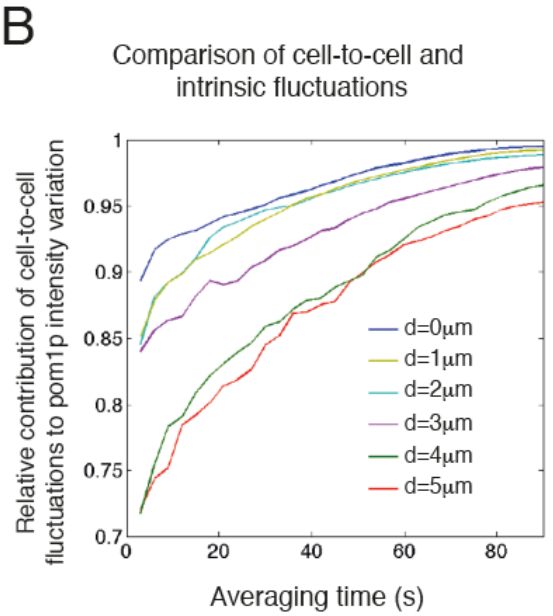
- Time averaging can reduce intrinsic fluctuations: **Tostevin, ten Wolde, Howard PLoS Comput Biol (2007)**



- Error reduced by $1/\sqrt{t}$

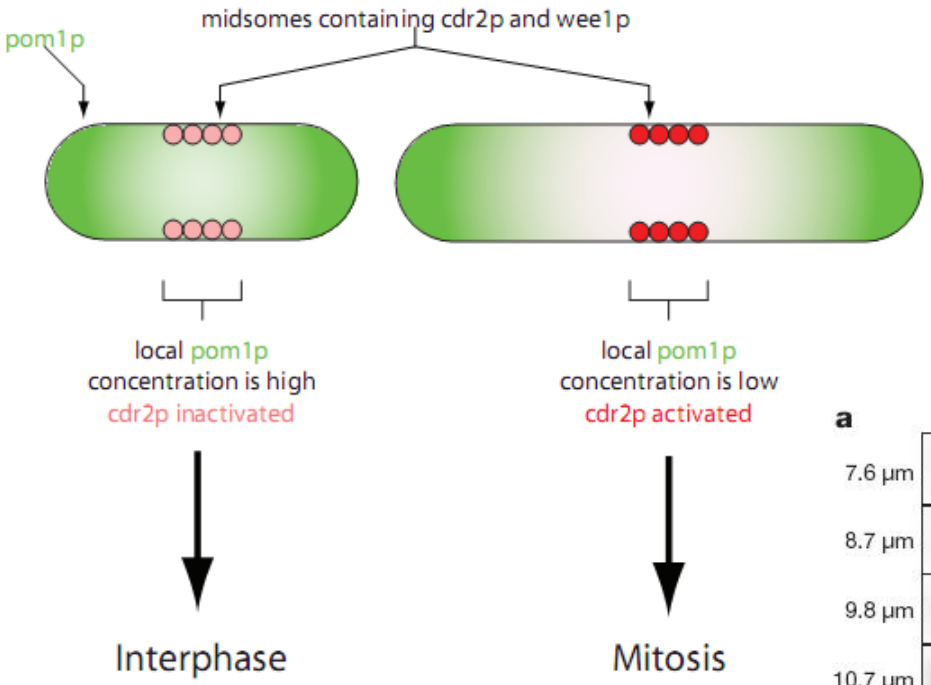
- Cell-to-cell variations dominate after about 10-20s of averaging

- First quantification of intrinsic noise



More general discussion of noisy gradients
Extrinsic & intrinsic noise: Saunders & Howard PRE (2009)
Pre-steady-state gradients: Saunders & Howard Phys. Biol. (2009)

Pom1p gradient as a cell-size sensor?



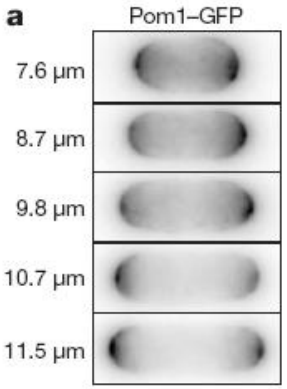
Pom1p

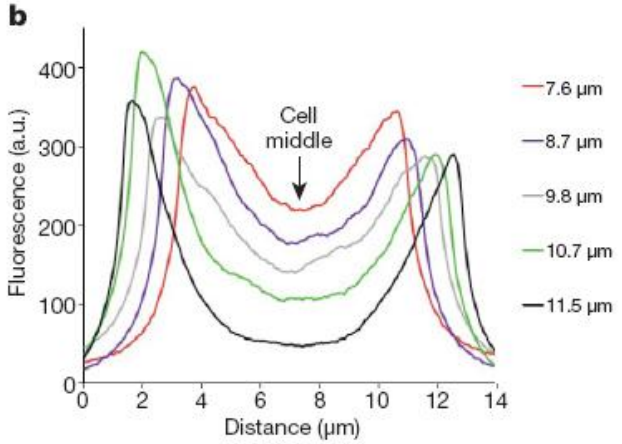
⊥

Mid1p, Cdr2p

↓

Wee1p

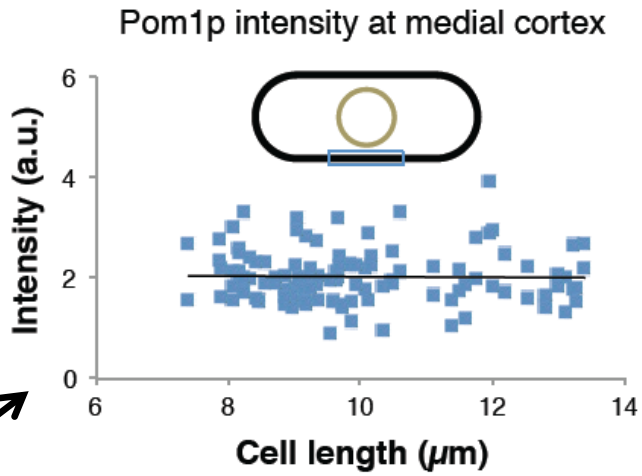


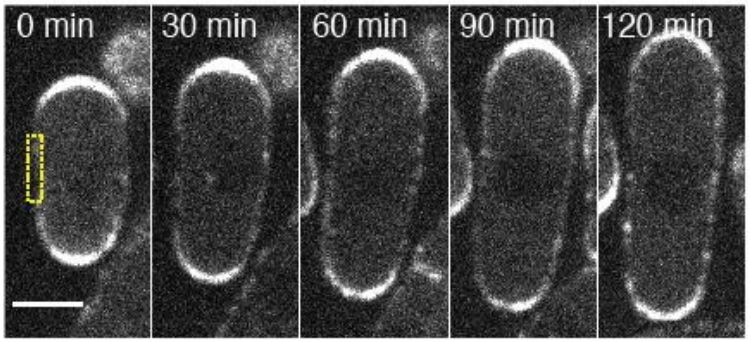


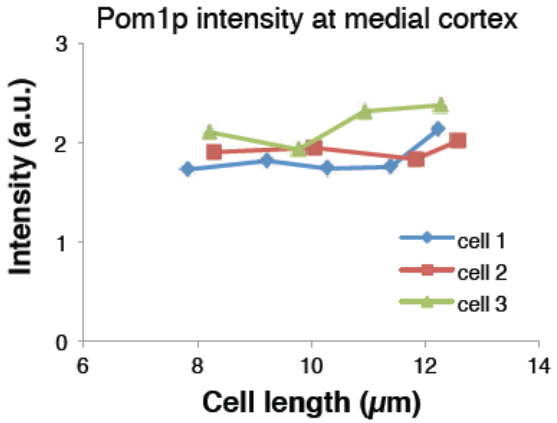
Moseley et al, Nature (2009)
Martin & Berthelot-Grosjean, Nature (2009)

Pom1p gradient as a cell-size sensor?

- Our data does not support this hypothesis
- Pom1p levels at medial cortex almost constant for different cell lengths in population of cells
- Same results holds when single cells are monitored

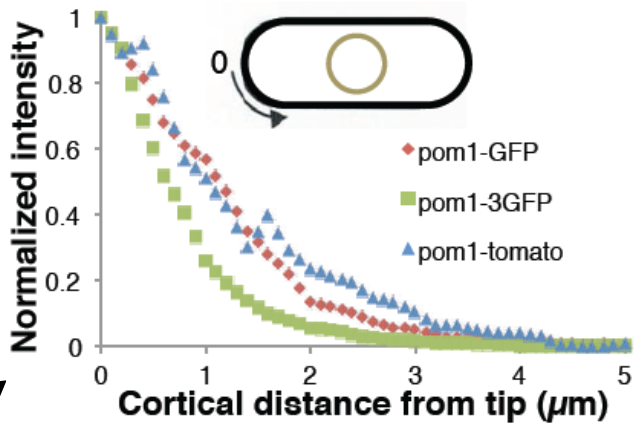


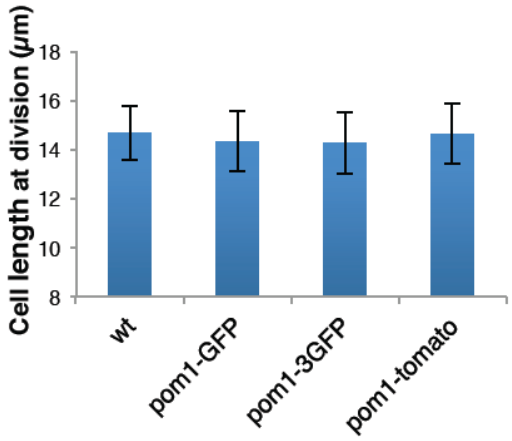


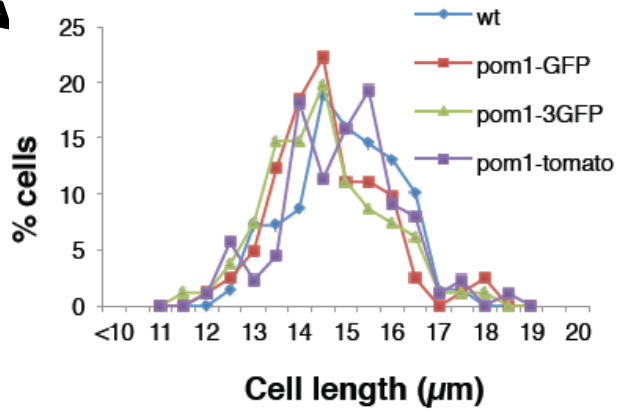


Pom1p gradient as a cell-size sensor?

- Our data does not support this hypothesis
- Manipulating gradient length scale via different fluorescent tags does not perturb size control
- Role of Pom1p more consistent as tip inhibitor

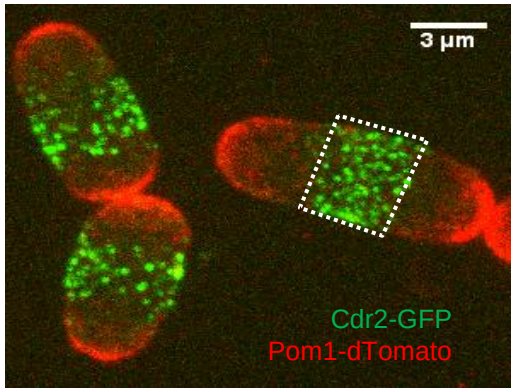


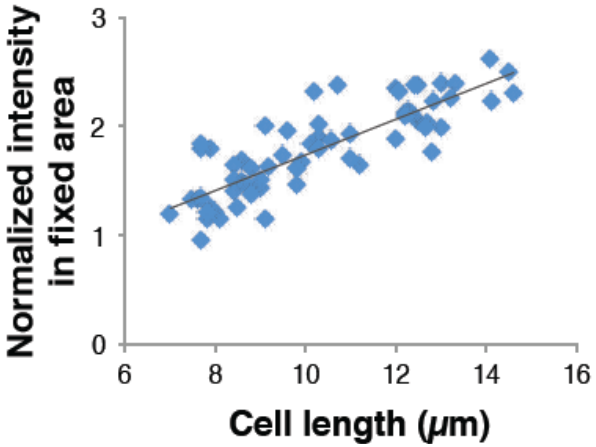


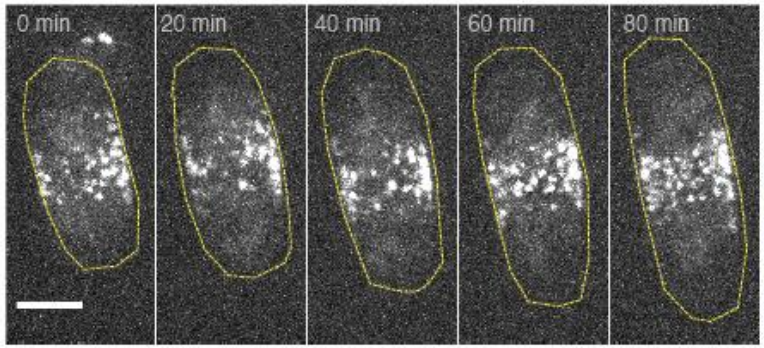


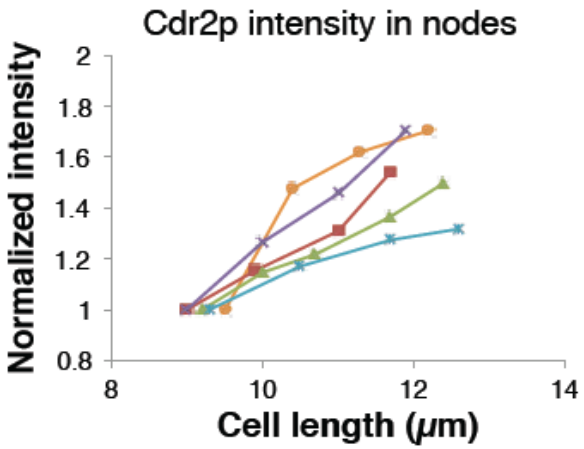
What is sensing cell size?!

- Could it be related to medial accumulation of Cdr2p?
- Medial density of Cdr2p, rises with cell length in cell population and in individual cells









Conclusions

- "Dissection" of Pom1p gradient
  - more complex mechanism of gradient formation!
  - could clustering be a general feature?

- Mechanism of cell size control
  - surprisingly does not centrally involve Pom1p
  - instead involves Cdr2p accumulation in medial zone of fixed area