E. coli's division decision: modeling Min-protein oscillations

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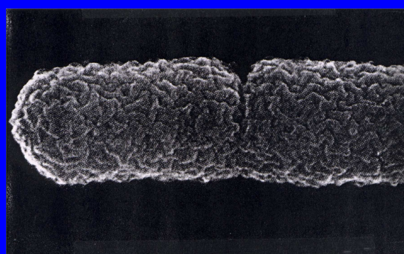
Outline

- Introduction to *E. coli*
- Two systems regulate division site placement
  - Nucleoid occlusion
  - Min proteins
- Min proteins oscillate from pole to pole!
- Modeling Min-protein oscillations
- Why does *E. coli* need an oscillator?

Introduction to *E. coli*



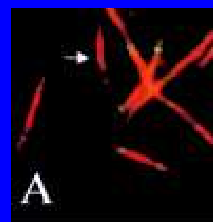
- Model prokaryote (no nucleus)
- Gram-negative bacterium
- Inhabits soil, water, and GI tracts of animals and humans
- Some pathogenic strains
- Genome is known (4×10^6 base pairs)

E. Coli cell division

- Rod-shaped bacterium
- Grows longer and divides in two
- Division is very accurate (.50 +/- .02)
- First step in division-site selection is formation of FtsZ ring, also very accurate (.50 +/- .01)

Two systems regulate placement of FtsZ ring

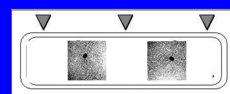
- Nucleoid occlusion
  - FtsZ ring formation is inhibited by nucleoid (condensed chromosome).



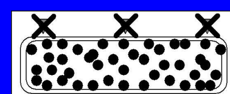
Sun *et al.* (1998)

FtsZ-GFP + DAPI

- Min-protein system
  - In absence of Min proteins, get minicelling phenotype (Min^-)

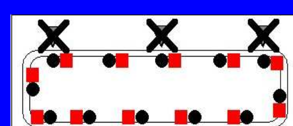


  - If MinC is overexpressed, get filamentous growth (Sep^-)

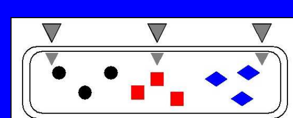


Min proteins

- MinC
  - Inhibits FtsZ ring formation
- MinD
  - MinD:ATP recruits MinC to membrane (MinD without ATP is cytoplasmic)
  - ~1000/micron



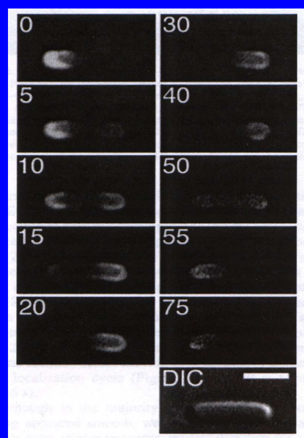
- MinE
  - Binds to MinD:ATP in membrane and induces ATP hydrolysis
  - ~700/micron



What happens when all three Min proteins are present?

Min proteins oscillate from pole to pole

Hale *et al.* (2001)



MinD-GFP

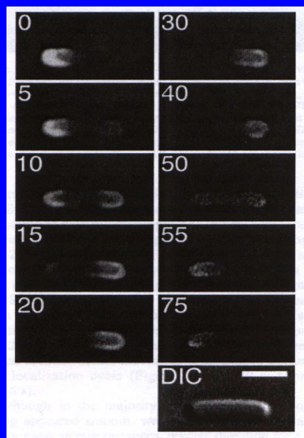
- *In vivo* oscillations require MinD and MinE but not MinC.

Phenomenology of Min oscillations

- MinD polar regions grow as end caps.

MinD polar regions grow as end caps

Hale *et al.* (2001)



MinD-GFP

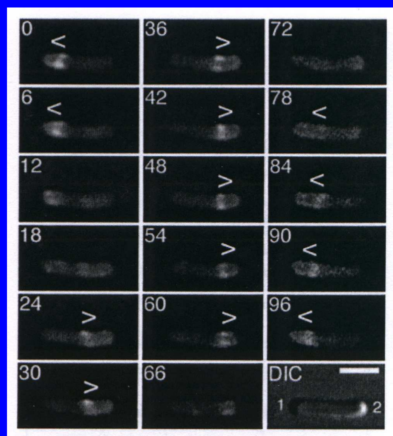
- MinD is primarily membrane bound.
- MinD concentration is always low near center of cell.

Phenomenology of Min oscillations

- MinD polar regions grow as end caps.
- MinE ring caps MinD polar region.

MinE ring caps MinD polar region

Hale *et al.* (2001)



MinE-GFP

- MinE ring is membrane bound.
- Ring appears near cell center and moves toward pole.
- Some MinE is also found in polar region.

Phenomenology of Min oscillations

- MinD polar regions grow as end caps.
- MinE ring caps MinD polar region.
- Oscillation frequency:
  – [MinE] ↑ ⇨ frequency ↑,
  – [MinD] ↑ ⇨ frequency ↓.

Oscillation frequency vs. MinD/E concentrations

Raskin and de Boer (1999)

Biological activity, cellular distribution, and oscillation parameters of Gfp-MinD

			Phenotype					
Exp.	Strain	Genotype	−IPTG	+IPTG	Dist.	Dwell (range)	Shift (range)	Cycle
1	PB103(λDR119)	*wt*(P_{lac}::*gfp-minD*)	WT	WT	O	33 (19-69)	15 (7-21)	96
2	PB103(λDR122)	*wt*(P_{lac}::*gfp-minDE*)	WT	WT	O	9 (5-14)	8 (6-12)	34
3	PB103/pDR119	*wt*/P_{lac}::*gfp-min*D	WT	Min−	O	93 (27-290)	22 (13-45)	230
4	PB103/pDR122	*wt*/P_{lac}::*gfp-minDE*	WT	WT	O	9 (5-16)	10 (6-20)	38
5	PB114(λDR119)	[Δ*minCDE*(P_{lac}::*gfp-minD*)]	Min−	Min−	M	NA	NA	NA
6	PB114(λDR122)	[Δ*minCDE*(P_{lac}::*gfp-minDE*)]	Min−	Min−	O	10 (5-17)	10 (6-14)	40
7	DR104(λDR119)	*minD*1 *recA*::Tn10(P_{lac}::*gfp-minD*)	Min−	WT	O	35 (17-68)	27 (13-49)	124
8	DR104(λDR122)	*minD*1 *recA*::Tn10(P_{lac}::*gfp-minDE*)	Min−	WT	O	10 (6-16)	10 (5-17)	40
9	DR104(λDR122)	As exp. 8, but treated with CAM	Min−	WT	O	10 (7-15)	10 (6-13)	40

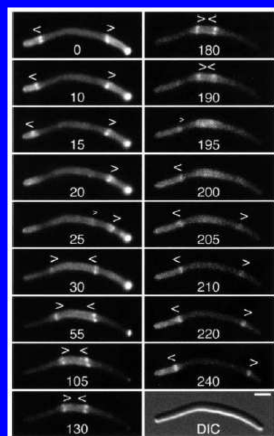
Oscillation frequency ~ [MinE]/[MinD]

Phenomenology of Min oscillations

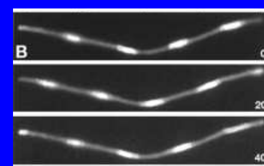
- MinD polar regions grow as end caps.
- MinE ring caps MinD polar region.
- Oscillation frequency:
  - [MinE] ↑ ⇨ frequency ↑,
  - [MinD] ↑ ⇨ frequency ↓.
- Filamentous cell has “zebra stripe” pattern of oscillations.

Filamentous cell has "zebra stripe" pattern of oscillations

Hale *et al.* (2001)



MinE-GFP



MinD-GFP

Raskin and de Boer (1999)

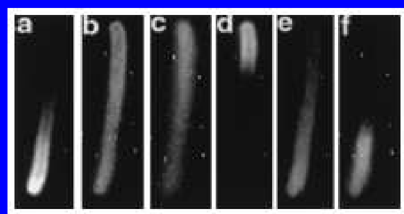
- Wavelength of oscillations is ~10 microns.

Phenomenology of Min oscillations

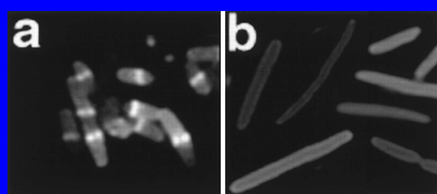
- MinD polar regions grow as end caps.
- MinE ring caps MinD polar region.
- Oscillation frequency:
  - [MinE] ↑ ⇨ frequency ↑,
  - [MinD] ↑ ⇨ frequency ↓.
- Filamentous cell has "zebra stripe" pattern of oscillations.
- MinE mutants may have no MinE ring.

MinE mutants may have no MinE ring

Rowland *et al.* (2000)



MinD



MinE MinE$^{1-53}$

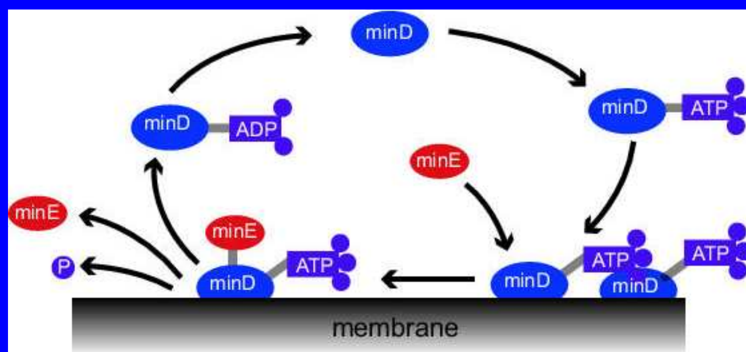
- MinE$^{1-53}$ mutant has slow oscillations ~10 minutes.
- No MinE ring for MinE$^{1-53}$-GFP.

  (Caveat – lack of MinE ring could be artifact of GFP fusion.)

Previous models fail to reproduce observed behavior

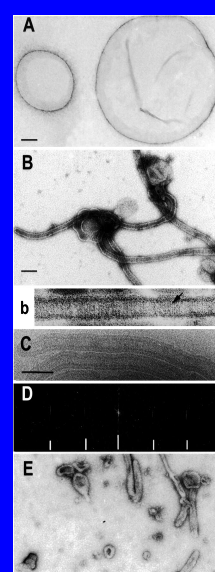
- Howard *et al.* (2001)
  - MinD polar region fails to reform at poles.
- Meinhardt and de Boer (2001)
  - Requires new protein synthesis.
- Kruse (2002)
  - No MinE ring, requires fast membrane diffusion.

A model with only known interactions reproduces observed behavior



Evidence from *in vitro* studies

A. Phospholipid vesicles
B. MinD:ATP binds to vesicles and deforms them into tubes
C. MinD:ATP polymerizes on vesicles
D. Diffraction pattern indicates well-ordered lattice of MinD:ATP
E. MinE induces hydrolysis of MinD:ATP and disassembly of tubes



Hu *et al.* (2002)

Equations for model

$$\frac{d\rho_{D:ADP}}{dt} = \mathcal{D}_D \nabla^2 \rho_{D:ADP} + \sigma_{de}\rho_{de} - \frac{\rho_{D:ADP}}{\tau_{D:ADP \to D}}$$

$$\frac{d\rho_D}{dt} = \mathcal{D}_D \nabla^2 \rho_D + \frac{\rho_{D:ADP}}{\tau_{D:ADP \to D}} - \frac{\rho_D}{\tau_{D \to D:ATP}}$$

$$\frac{d\rho_{D:ATP}}{dt} = \mathcal{D}_D \nabla^2 \rho_{D:ATP} + \frac{\rho_D}{\tau_{D \to D:ATP}} - \sigma_{dE}[\sigma_D + \sigma_{dD}(\rho_d + \rho_{de})]\rho_{D:ATP}$$

$$\frac{d\rho_E}{dt} = \mathcal{D}_E \nabla^2 \rho_E - \sigma_E \rho_d \rho_E + \sigma_{de}\rho_{de}$$

$$\frac{d\rho_{de}}{dt} = \sigma_E \rho_d \rho_E - \sigma_{de}\rho_{de}$$

$$\frac{d\rho_d}{dt} = -\sigma_E \rho_d \rho_E + \sigma_{dE}[\sigma_D + \sigma_{dD}(\rho_d + \rho_{de})]\rho_{D:ATP}$$

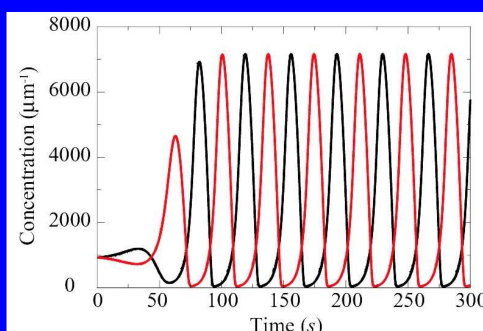
ρ_D = MinD in cytoplasm
ρ_E = MinE in cytoplasm
ρ_d = MinD:ATP in membrane
ρ_{de} = MinE:MinD:ATP in membrane

$\sigma_D = 0.025\left(\frac{\mu m}{s}\right)$; $\sigma_{dD} = 0.001\left(\frac{\mu m^3}{s}\right)$

$\sigma_E = 0.16\left(\frac{\mu m^3}{s}\right)$; $\sigma_{de} = 0.8\left(\frac{1}{s}\right)$

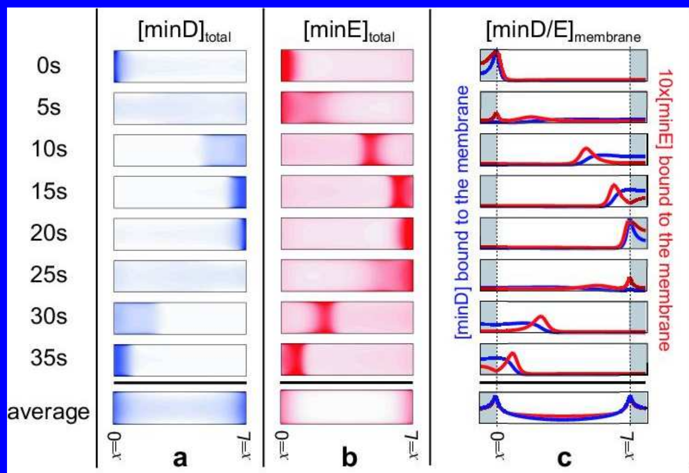
$\mathcal{D}_D = \mathcal{D}_E = 2.5\left(\frac{\mu m^2}{s}\right)$

Limit cycle oscillator



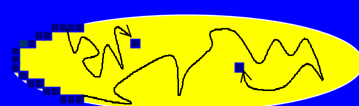
[MinD:ATP] in membrane at ends of cell

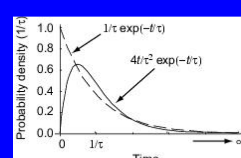
MinD end caps and MinE ring

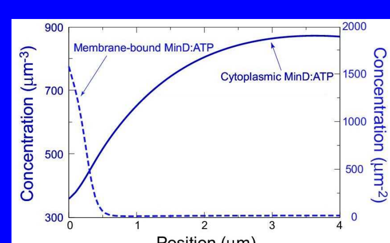


Mechanism for growth of MinD polar regions

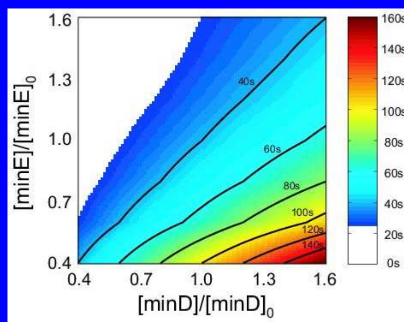
- MinD ejected from old end cap diffuses in cytoplasm.
- “Clocklike” delay implies uniform reappearance of MinD:ATP.
- Capture of MinD:ATP by old end cap leads to maximum of cytoplasmic MinD:ATP at opposite pole.







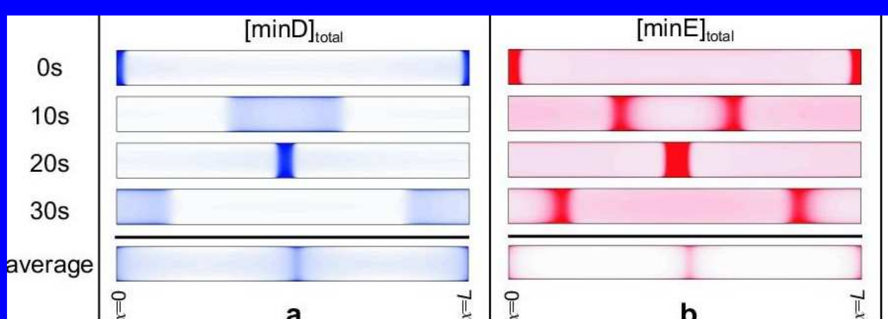
Frequency of oscillations ~ [MinE]/[MinD]



4 micron cell

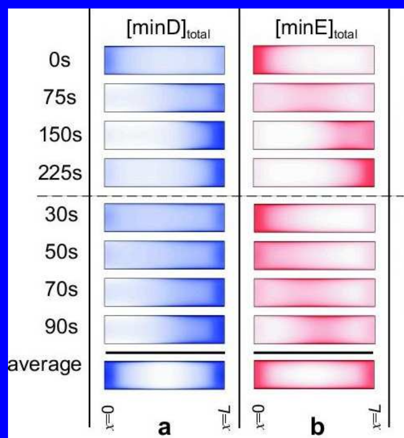
- No oscillations for [MinE] too high, or for [MinD] too low.
- Minimum oscillation period 25s.

"Zebra stripe" oscillations in long cells



- Stripes form with wavelength of ~10 microns

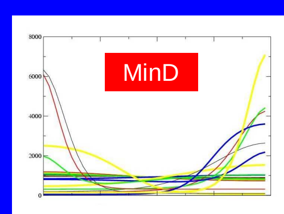
Fast diffusion of MinE eliminates MinE ring



- Oscillations persist, but MinE is diffuse.
- Possible relevance to $MinE^{1\text{-}53}$ mutant?

Robustness of oscillations

- Oscillations are a limit cycle, *i.e.* uniform solution is unstable to oscillations.
- Oscillations occur for a wide range of MinD and MinE concentrations.

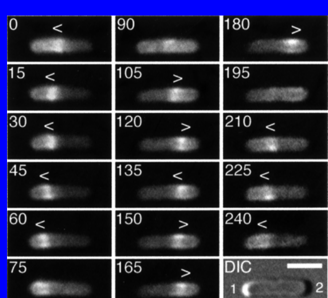


MinD in membrane

Predictions of model

- MinD:ATP recovery is a molecular clock.
- Rate of hydrolysis of MinD:ATP by MinE sets oscillation frequency.
- Diffusion length of MinD before rebinding to membrane sets oscillation wavelength.

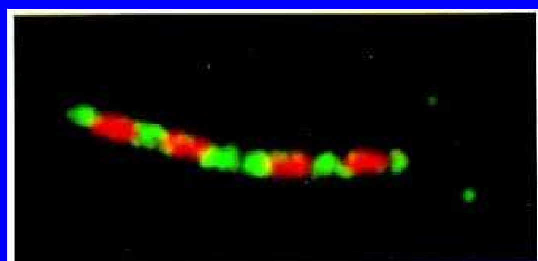
Open questions regarding Min oscillations



- MinE ring moving in reverse.
- Role of MinE dimerization.

Why does *E. coli* need an oscillator?

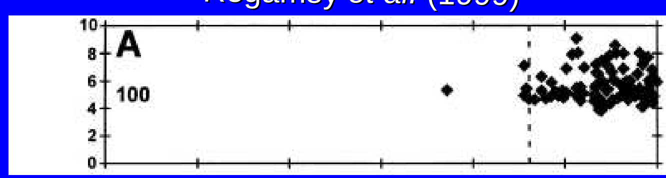
In *B. subtilis*, minicelling is prevented by MinCD homologs, but polar regions are static.



Marston *et al*. (1998)

FtsZ ring placement is 5 times less accurate In *B. subtilis* than in *E. coli*

Regamey *et al.* (1999)

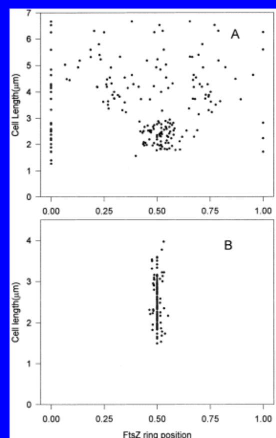


0.45 0.5

FtsZ ring

Accuracy of FtsZ ring placement in *E. coli* depends on Min proteins

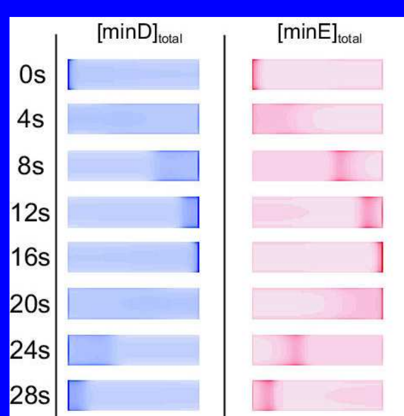
Yu and Margolin (1999)



FtsZ ring position

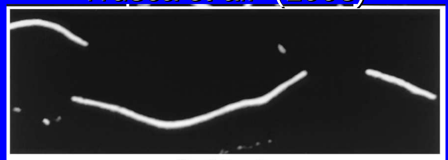
- In addition to producing minicells, Min$^-$ strain has inaccurate placement of medial FtsZ ring.
- Wild type cells (Min$^+$) have very accurate FtsZ ring placement.

Since same proteins oscillate from end-to-end, position of MinD minimum is insensitive to number fluctuations



Possible role of Min proteins in recovery from filamentous growth?

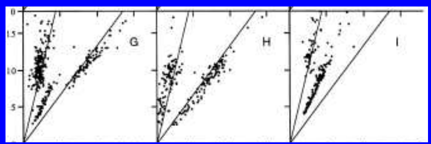
Trusca *et al.* (1998)



SulAwt

- In response to UV radiation, or other DNA damage, *E. coli* induces SOS response.
- As part of SOS response, filamentous growth is induced by SulA protein.
- "Zebra stripe" pattern of Min oscillations may help partition cell for recovery from SOS (T. Silhavy).

Placement of FtsZ ring by Min system



FtsZ ring

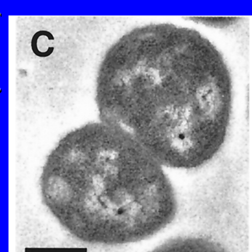
- In mutant with defect in nucleoid occlusion, FtsZ ring placement follows predicted nodes of MinD oscillations.

Conclusions

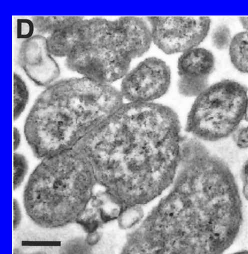
- Division-site placement in *E. coli* is regulated by Min proteins, which oscillate from pole to pole.
- A simple model reproduces the observed behavior:
  - MinD polar regions grow as end caps,
  - MinE ring sits at edge of MinD polar region,
  - Oscillation frequency ~ [MinE] / [MinD],
  - Filamentous cell has "zebra stripe" pattern.
- Experiments being planned to test role of Min proteins in division accuracy and SOS recovery.

Min proteins in spherical cells: Neisseria gonorrhoeae

Szeto *et al.* (2001)

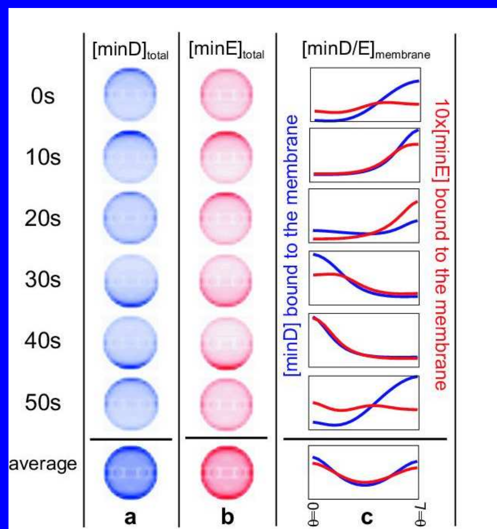


Wild type



$MinD_{Ng}^{-}$

Min-protein oscillations in a sphere



"Zebra-stripe" oscillations in a large sphere