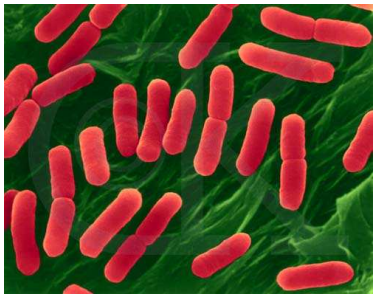
How does a cell finds its center ?



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אוניברסיטת בן-גוריון בנגב
Ben-Gurion University of the Negev

With Ned Wingreen (NEC)
KC Hwang (MIT)

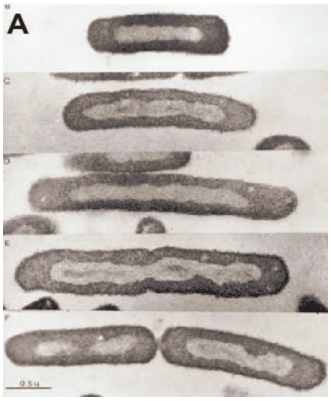
Thanks to: A. Yochelis (BGU)

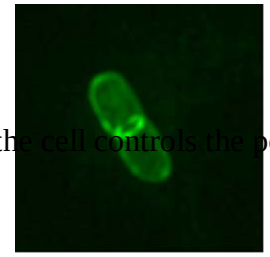
Outline:

- Introduction:
  - Cell division in E. Coli
  - Experiments
  - Existing Theories
- Minimal Theory:
  - Equations
  - Results
- Conclusions

E. Coli cell division

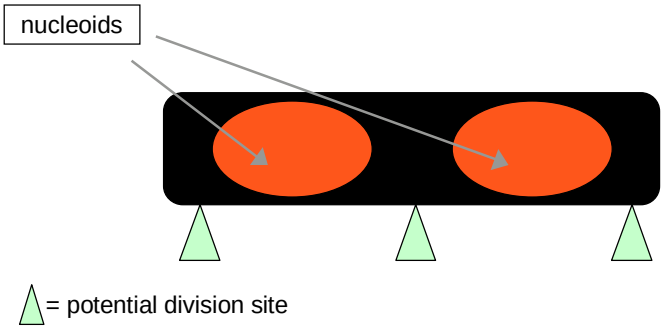
- Rod-shaped bacterium
- Grows longer and divides in two
- Division is very accurate (.50 +/- .02)
- First step in division-site selection is formation of FtsZ ring, also very accurate (.50 +/- .01)



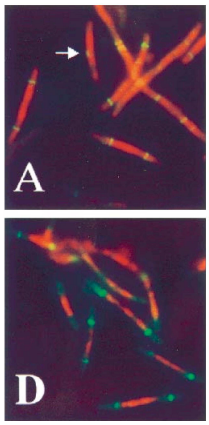


How does the cell controls the position of the FtsZ ring ?

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

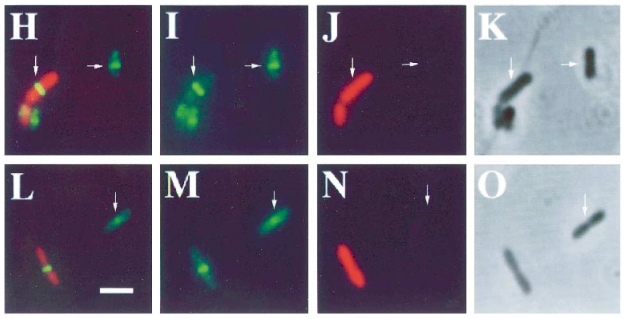


Sun et al., 1998: *parC* mutant – the chromosomes do not segregate



"Don't you think you've had enough coffee?"

- FtsZ ring forms on the two sides of the nucleoid
- no apriori defined partition sites
- exceptions



ΔmukB mutant

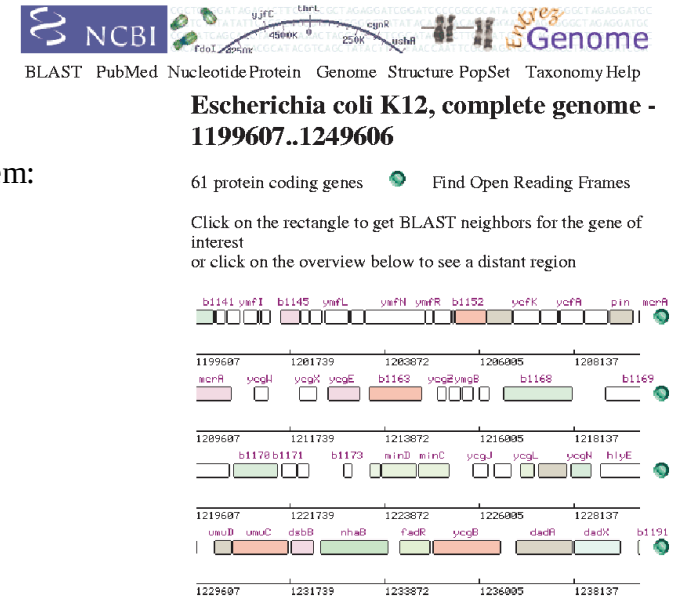
FtsZ is directed to the center even without the nucleoid !!

The min system:

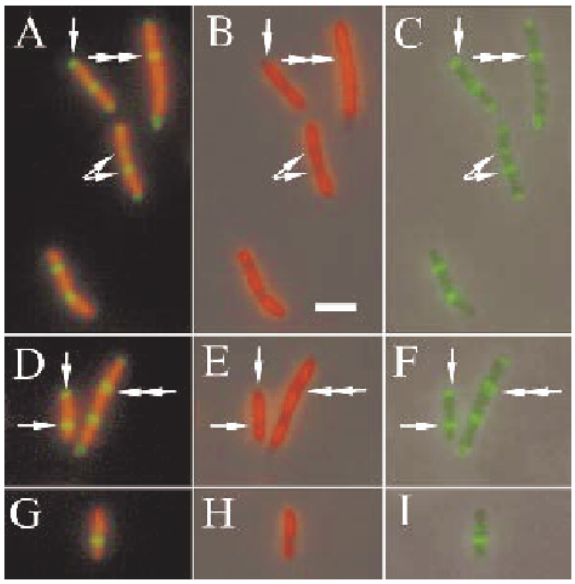
MinC

MinD

MinE



Yu and Margolin (1999)



ΔminCDE mutant

- FtsZ rings near poles
- multiple FtsZ rings

Phenotypes:

WT – wild type, natural
Minicells – division occurs also near the cell poles
Filaments – cell division is blocked

Observations:

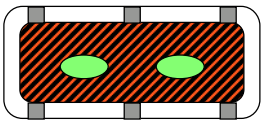
- Overexprssion of minC - Filaments
- minC interacts directly with ftsZ
- $minC^-$ - Minicells

- $minD^-$ - Minicells
- minD-ATP recruits minC to the membrane
- ~1000/micron

- $minE^-$ - Filamets
- ~700/micron

Essential interactions

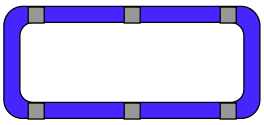
MinC or MinE without MinD exists in the cytoplasm



When MinC is overproduced, cell division is blocked

MinD associates on the membrane

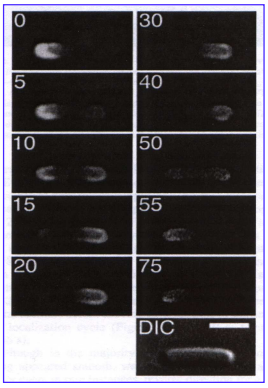
(needs ATP)



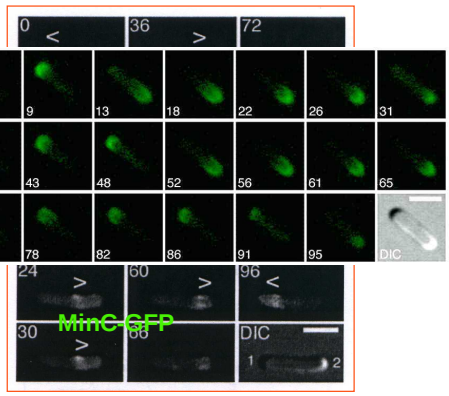
… and pulls MinC along with it.

What happens when all three proteins are present?

Oscillations !!



MinD-GFP

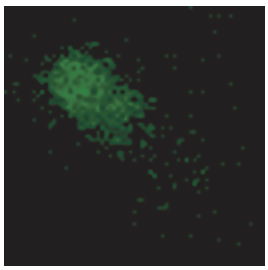


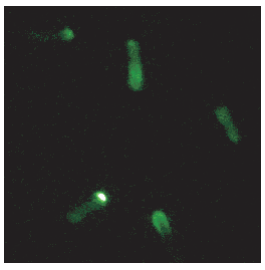
MinE-GFP

P. de Boer (Case Western)
J. Lutkenhaus (Kansas)
W. Margolin (Houston)
L. Rothfield (Connecticut)

MinD oscillations

P. de Boer (Case Western)
J. Lutkenhaus (Kansas)
W. Margolin (Houston)
L. Rothfield (Connecticut)



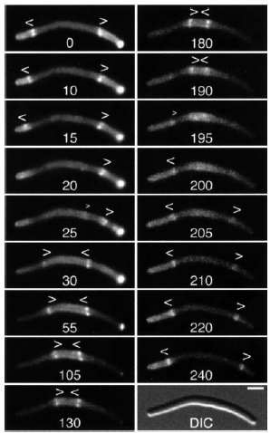


MinD + MinE oscillations

Phenomenology of Min oscillations

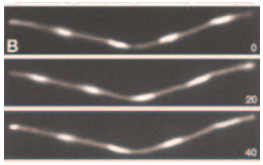
- MinD polar regions grow as end caps.
- MinE ring caps MinD polar region.
- Oscillation frequency:
  - [MinE] ↑ ⇨ frequency ↑,
  - [MinD] ↑ ⇨ frequency ↓.
- Filamentous cell has “zebra stripe” pattern of oscillations.
- MinE mutants may have no MinE ring.

Filamentous cell has “zebra stripe” pattern of oscillations



Hale *et al.* (2001)

MinE-GFP



Raskin and de Boer (1999)

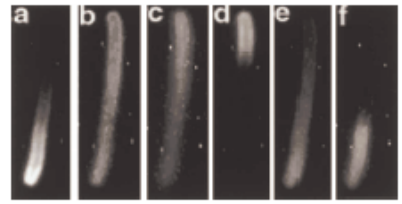
MinD-GFP

- Wavelength of oscillations is ~10 microns.

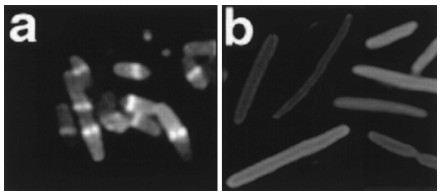
MinD oscillations in a long cell

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 – generate oscillations but fail to reproduce some of the observed behavior

Meinhardt and de Boer (2001)
- Requires new protein synthesis.
- MinE sticks to ad hoc preferential MinD concenetration

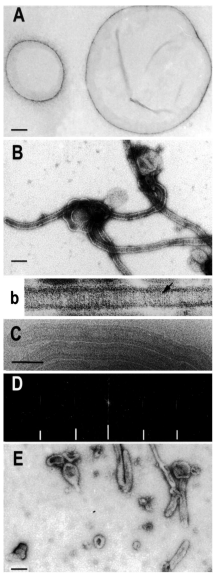
Howard *et al.* (2001)
- Ad hoc assumptions: “normalized” parameters, MinE is driven to the membrane by cytoplasmic MinD
- MinD polar region fails to reform from poles outward
- Incorrect dependence of frequency on concentration of MinE

Kruse (2002)
- No MinE ring, requires fast membrane diffusion.

How far can you go, based on available experimental information ?

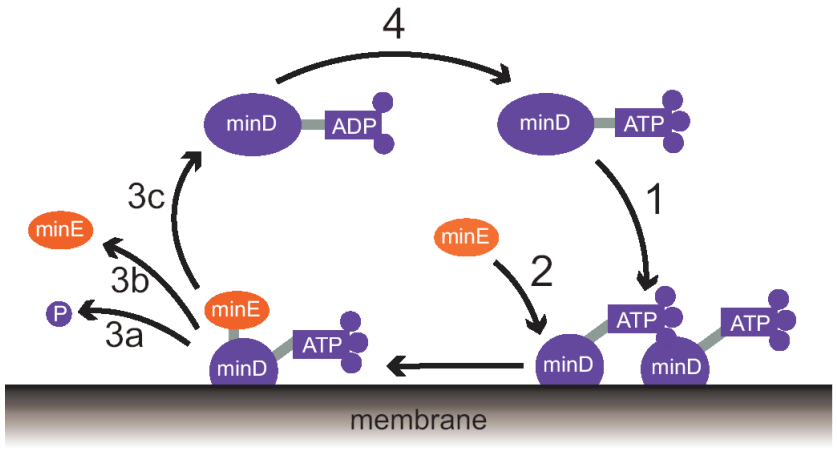
More data from *in vitro* studies

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)

A minimal model with only known interactions fully reproduces observed behavior



Equations:

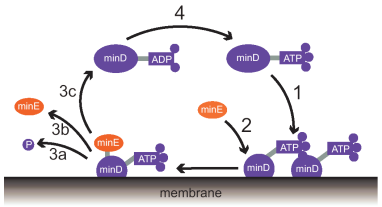
$$\frac{d\rho_{D:ADP}}{dt} = \mathcal{D}_D \nabla^2 \rho_{D:ADP} + \sigma_{de}\rho_{de} - \frac{1}{\tau_{ADP\to ATP}}\rho_{D:ADP}$$

$$\frac{d\rho_{D:ATP}}{dt} = \mathcal{D}_D \nabla^2 \rho_{D:ATP} + \frac{1}{\tau_{ADP\to ATP}}\rho_{D:ADP} - [\sigma_D + \sigma_{dD}(\rho_d + \rho_{de})]\rho_{D:ATP}$$

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

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

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



ρ_D = MinD in cytoplasm

ρ_E = MinE in cytoplasm

ρ_d = MinD:ATP in membrane

ρ_{de} = MinE:MinD:ATP in membrane

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

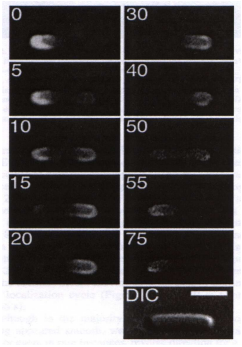
$$\sigma_E = 0.16\left(\frac{\mu m^3}{s}\right);\ \sigma_{de} = 0.8\left(\frac{1}{s}\right)$$

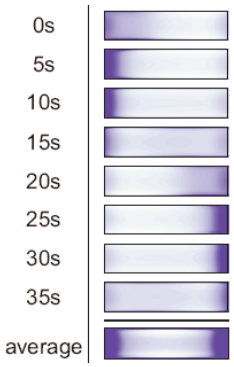
$$\mathcal{D}_D = \mathcal{D}_E = 2.5\left(\frac{\mu m^2}{s}\right)$$

Where does this get us?

Let's apply our model to a 4 micron cell with WT levels of MinD and MinE.

Experiment **Theory** →





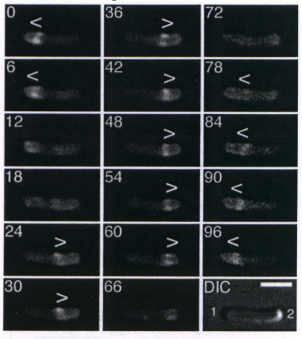
(These plots are showing the projection of the density onto a planar cross section to mimic experiment.

Thus a membrane-resident protein will have greater fluorescence at the cell boundary where the effective area is greater.)

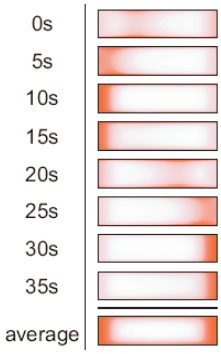
Note the lack of MinD in the middle of the cell, and the growth of the endcap from the **end** of the cell, rather than the middle.

How about the MinE ring?

Experiment



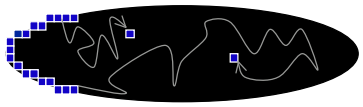
Theory

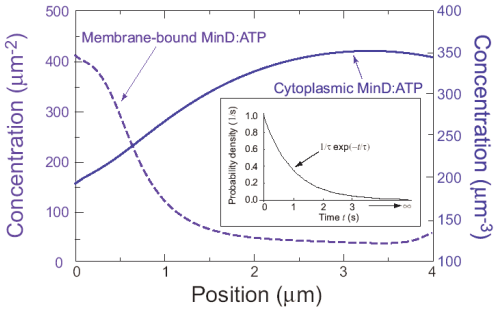


Note the clear presence of a MinE "ring", which is not really a ring at all, but merely a region of enhanced concentration. (More to come later…)

Mechanism for growth of MinD polar regions

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





MinD

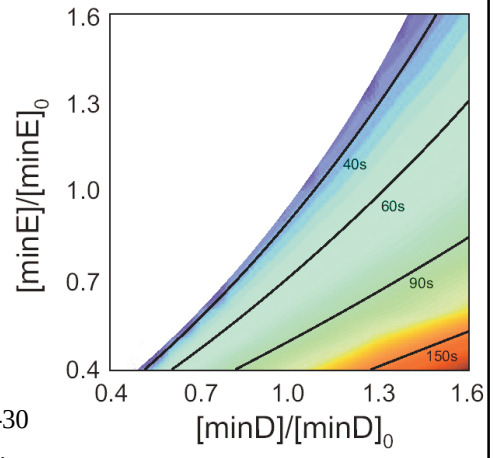
MinE

Concentration dependence

As in the experiment, the period T has an almost perfectly linear dependence on the ratio

$$x = \frac{[\mathrm{MinD}]}{[\mathrm{MinE}]}$$

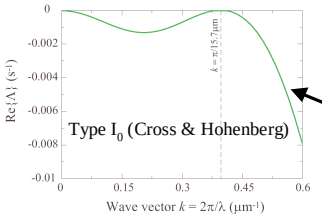
The period is determined by the rate at which MinE hydrolyzes the polar zone of MinD, hence increases with MinD concentration and decreases with MinE concentration

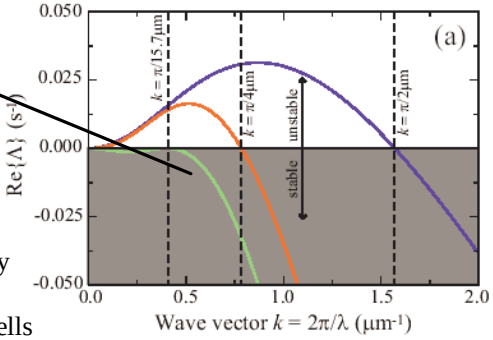


In agreement with experiments, there are no oscillations with period smaller than 20-30 seconds, while the period can be very large.

Where do the oscillations come from?

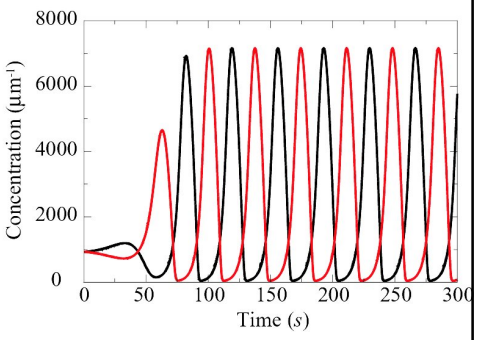
Linear stability analysis (infinite cell):





- (nonzero k and ω) oscillatory and periodic
- period doubling for longer cells
- quantitative estimates of period

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

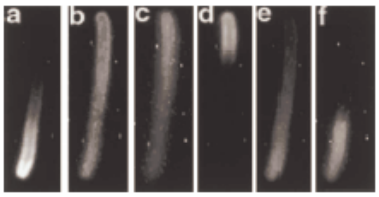


[MinD] in membrane at ends of cell

Robustness

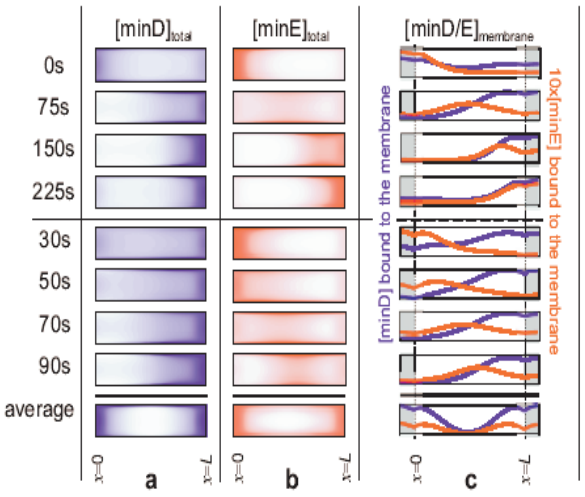
MinE fragments

Experiment



A cell with MinD and MinE$^{1-53}$ undergoes oscillations, albeit with a much slower period and the absence of an E-ring…

Fast diffusion of MinE attenuates MinE ring

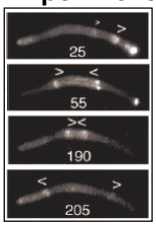


- Oscillations persist, but MinE is diffuse.
- Possible relevance to $MinE^{1-53}$ mutant?
- Similar results with changing the hydrolysis rate

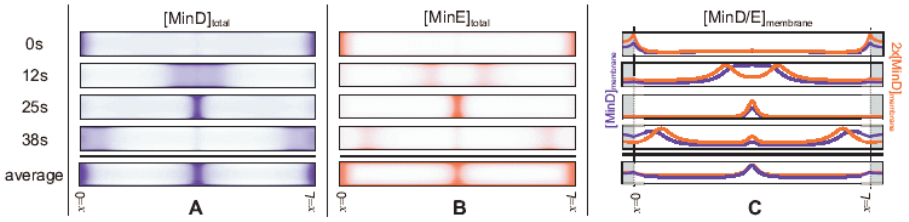
Filamentous cells

Knock out FtsZ so that the cell will grow long filaments and the following is observed.

Experiment

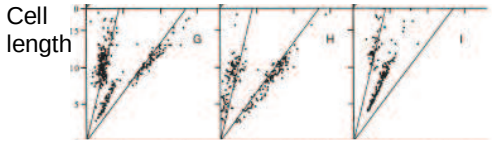


Theory



The results of our model show good agreement with experiment: The MinD alternates between a central concentration and two endcaps, while there are two MinE rings at the boundary between MinD test tubes and bare membrane.

Placement of FtsZ ring by Min system



Position along the cell of the FtsZ ring

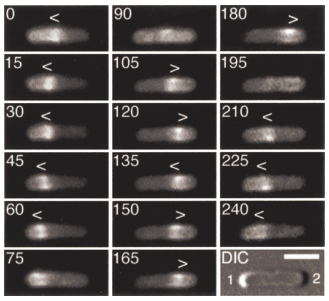
- In mutant with defect in nucleoid occlusion, FtsZ ring placement follows predicted nodes of MinD oscillations.

(see also Fishov et al.)

Results of model

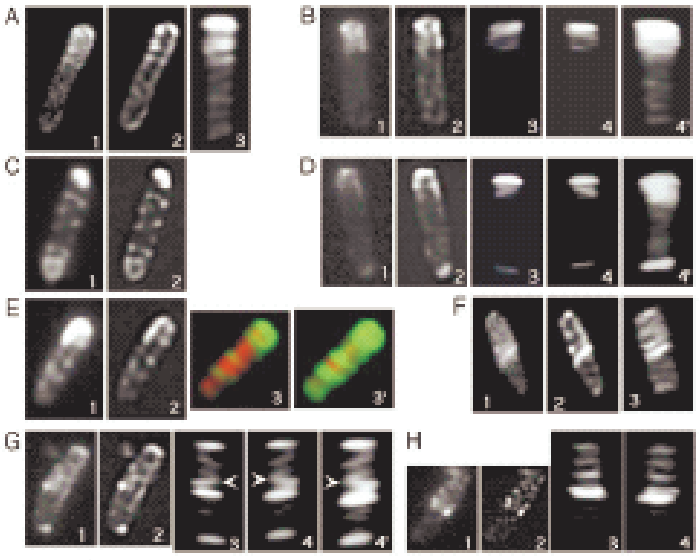
- Delay in MinD:ATP recovery is essential.
- Rate of hydrolysis of MinD:ATP by MinE sets oscillation frequency.
- Diffusion length of MinD before rebinding to membrane sets oscillation wavelength.

Open questions regarding Min oscillations



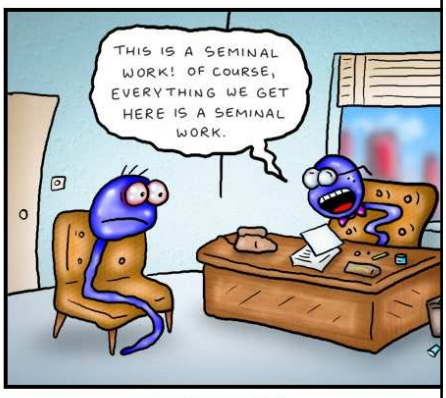
- MinE ring moving in reverse.
- Role of MinE dimerization
- Feedback between nuceloid and Min

Min proteins form helices: [Rothfield et al. (2003)]



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 (SOS ?).
- Experiments being planned to test role of interaction between Min proteins and nucleoid (BGU)



Sperm publishers

Table 4. *minCDE* homologues in completed prokaryotic genome projects

Species	Annotation* or reference	Identified in this study*
Aquifex aeolicus	*minCD1*, *minD2*	
Archaeoglobus fulgidus†	*minD1*, *minD2*	
Bacillus subtilis	*minCD* (*divIVA*)‡	
Borrelia burgdorferi	*minD* related genes: *ylxH-1*,-2, -3	
Chlamydia pneumoniae	*minD*	
Chlamydia trachomatis	*minD*	
Deinococcus radiodurans	*minD*, *divIVA*‡	*minC*
Escherichia coli	*minCDE*	
Haemophilus influenzae	Not present	
Helicobacter pylori	*minCDE* (Rothfield *et al.*, 1999)	
Methanobacterium thermoautotrophicum†	*minD* (Rothfield *et al.*, 1999)	
Methanococcus jannaschii†	*minD1*, *minD2*	
Mycobacterium tuberculosis	*minD* (Rothfield *et al.*, 1999)	
Mycoplasma genitalum	Not present	
Mycoplasma pneumoniae	Not present	
Neisseria gonorrhoeae	*minCDE*§	*minCDE*
Neisseria meningitidis	*minCDE*	
Pyrococcus horikoshii†	*minD*	
Rickettsia prowazekii	Not present	
Synechocystis sp.	*minCDE*	
Thermotoga maritima	*minCD*	
Treponema pallidum	*minD* (Rothfield *et al.*, 1999)	
Vibrio cholerae	*minCDE*	