# Mechanisms for Precise Positional Information in Bacteria: The Min system in E. coli and B. subtilis

**Martin Howard**

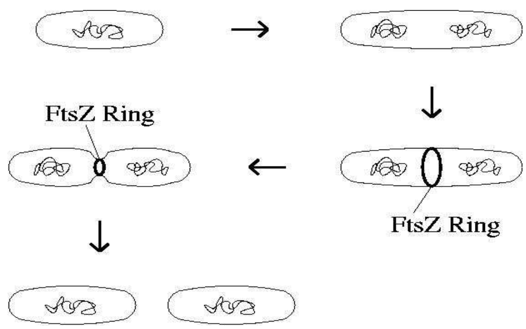
**Imperial College London**

## Bacterial Organization

- Many processes where bacterial cell needs accurate positional information in order to control and direct protein localization
- Examples: cell division, chromosome/plasmid segregation, sporulation, signal transduction, chemotaxis …
- How is this accurate positional information obtained?
- Focus on cell division … in E. coli and B. subtilis …

# Cell Division

- Targeting of cell division to cell midplane is very precise




- How is FtsZ ring directed to midcell?

# Division Models

- **Potential Division Sites:**
  Possible division sites distinguished by "topological markers"
  But how did they locate the cell centre?!

- **Nucleoid Occlusion:**
  Division prevented at sites adjacent to the nucleoid

- **The Min System:**
  Primary regulation of accurate cell division controlled by three proteins:
  → E. coli: MinC, MinD and MinE
  → B. subtilis: MinC, MinD and DivIVA

# E. coli: MinCDE Proteins

**MinC:**
- Prevents division by interfering with construction of FtsZ ring

**MinD:** (~1500 copies/cell)
- Self-associates to membrane
- Binds to MinC and recruits it to membrane where it can be effective
- MinC/MinD alone block division everywhere → filamentous cells

**MinE:** (~1500 copies/cell)
- Recruited to membrane by MinD, removing midcell division block

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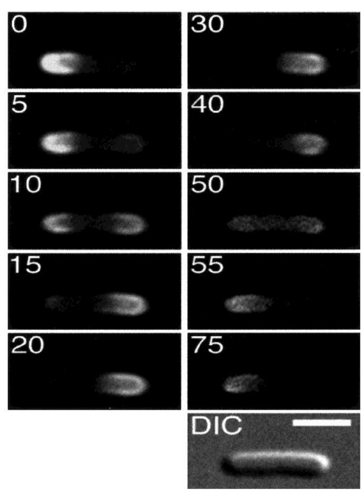
**MinC: Pichoff et al: J. Bacteriol. 183 6630 (2001)**
**Hu et al: Mol. Microbiol. 34 82 (1999)**
**de Boer et al: J. Bacteriol. 174 63 (1992)**
**MinE: Raskin, de Boer: Cell 91 685 (1997)**
**MinD: de Boer et al: EMBO J. 10 4371 (1991)**
**de Boer et al: Cell 56 641 (1989)**
**Huang et al: J. Bacteriol. 178 5080 (1996)**

# Min Oscillations

**MinD Oscillations**
**Hale, Meinhardt, de Boer:**
**EMBO J. 20 1563 (2001)**

- MinE stimulates coherent pole to pole oscillations of MinCDE
- Protein movement observed by attaching green fluorescent protein (GFP) to Min proteins
- MinD oscillations: period ~1 min
  **Raskin, de Boer:**
  **PNAS 96 4971 (1999)**

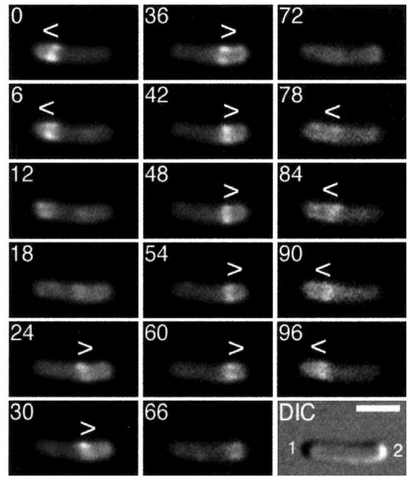


# Min Oscillations

**MinE Oscillations**

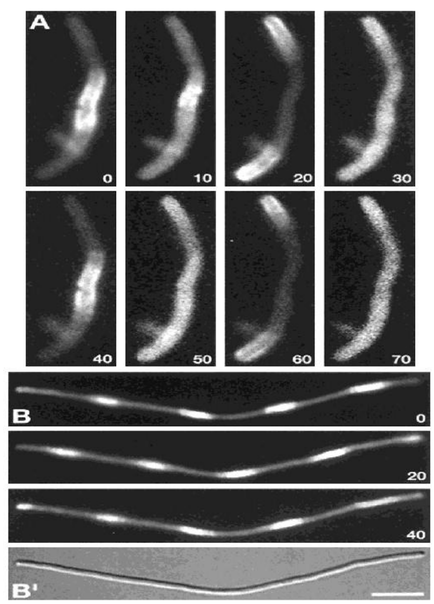
**Hale, Meinhardt, de Boer:**

**EMBO J. 20 1563 (2001)**




- Formation of oscillating MinE ring structure
  **Fu, Shih, Zhang, Rothfield: PNAS 98 980 (2001)**
- Centre of cell marked by minimum MinC/MinD concentration

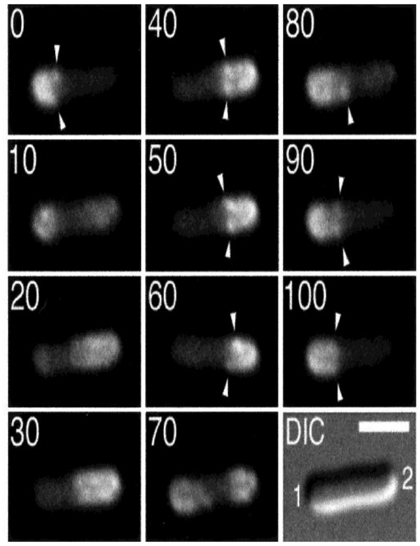
# MinD in Filamentous Cells




**Raskin, de Boer: PNAS 96 49 (1999)**

- Induce filamentous cells by deleting FtsZ protein
- Clear evidence for characteristic wavelength

# Min Oscillations




- Simultaneous imaging of MinD and MinE

**Hale, Meinhardt, de Boer: EMBO J. 20 1563 (2001)**

- MinE forms a dynamical ring that drives MinD off the membrane

# Model for MinCDE Oscillations

- Experiments indicate that MinC dynamics "slaved" to MinD
  → only model MinD/MinE
- Oscillations continue even if protein synthesis is blocked

  **Raskin, de Boer: PNAS 96 4971 (1999)**

- Model using reaction-diffusion equations:

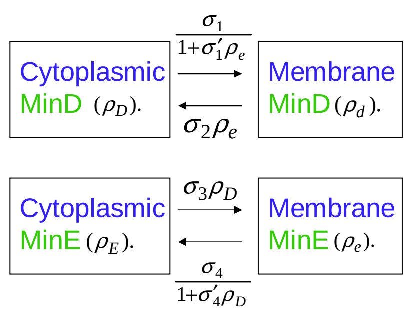
  **Howard, Rutenberg, de Vet: Phys. Rev. Lett. 87 278102 (2001)**

$$\frac{\partial \rho_D}{\partial t} = D_D \frac{\partial^2 \rho_D}{\partial x^2} - \frac{\sigma_1 \rho_D}{1+\sigma_1' \rho_e} + \sigma_2 \rho_e \rho_d$$

$$\frac{\partial \rho_d}{\partial t} = D_d \frac{\partial^2 \rho_d}{\partial x^2} + \frac{\sigma_1 \rho_D}{1+\sigma_1' \rho_e} - \sigma_2 \rho_e \rho_d$$

$$\frac{\partial \rho_E}{\partial t} = D_E \frac{\partial^2 \rho_E}{\partial x^2} - \sigma_3 \rho_D \rho_E + \frac{\sigma_4 \rho_e}{1+\sigma_4' \rho_D}$$

$$\frac{\partial \rho_e}{\partial t} = D_e \frac{\partial^2 \rho_e}{\partial x^2} + \sigma_3 \rho_D \rho_E - \frac{\sigma_4 \rho_e}{1+\sigma_4' \rho_D}$$



# Remarks on the Model

- Order of magnitude for diffusion constants obtained from
  **Elowitz et al: J. Bacteriol. <u>181</u> 197 (1999)**
- Values for reaction rates not constrained experimentally

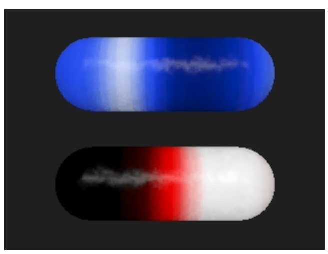
$$D_D = 0.28\mu m^2 s^{-1}, D_E = 0.6\mu m^2 s^{-1}, D_d = D_e = 0,$$
$$\sigma_1 = 20 s^{-1}, \sigma_1' = 0.028\mu m, \sigma_2 = 0.0063\mu m s^{-1},$$
$$\sigma_3 = 0.04\mu m s^{-1}, \sigma_4 = 0.8 s^{-1}, \sigma_4' = 0.027\mu m.$$

- For these parameters, linear instability to an oscillating state
  → any initial inhomogeneities/fluctuations amplified
  → subcellular "Turing" structure [crucial feature: disparity of membrane and cytoplasmic diffusion constants]

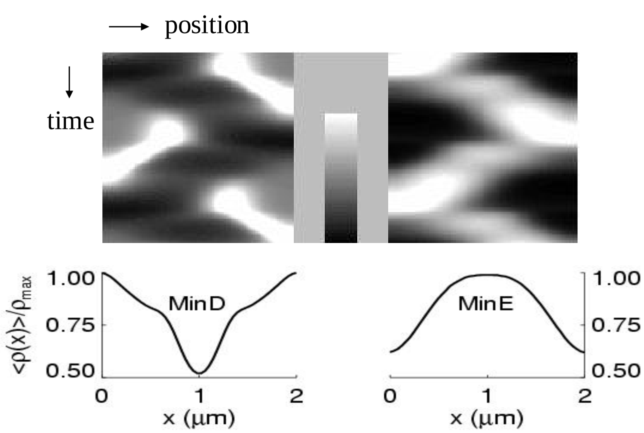
# Numerical Results for MinD & MinE

MinD:

MinE:



# Numerical Results for:
# MinD & MinE




# Other Models

Three other MinCDE oscillation models have been proposed
→ all share a fundamental reaction-diffusion mechanism

- **Meinhardt, de Boer: PNAS 98 14202 (2001)**
  → requires continuous protein production for oscillations
- **Kruse: Biophys. J. 82 618 (2002)**
  → quite similar to our model
- **Wingreen, Huang, Meir: unpublished**
  → no new principles; ATP dynamics also included
  → somewhat better agreement with experiment

# Fluctuations

**Howard & Rutenberg: Phys. Rev. Lett. 90 128102 (2003)**

- Relatively small number of MinD and MinE proteins
- Do small number fluctuations destroy the oscillations?
- Does E. coli use "optimal" concentrations of pattern forming proteins?

- Stochastic pde approach: $\partial_t \rho = D\nabla^2 \rho + f(\rho) + \xi$

  Noise is much bigger than deterministic term at low densities!
  $\longrightarrow$ negative densities + reactions driven "backwards"

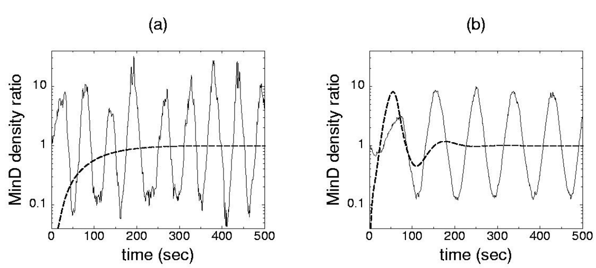
- Discrete particle approach:
  Monte-Carlo simulations of diffusing/reacting protein particles

# Fluctuation Driven Instability

- For some parameter values, noise is essential for the generation of patterns

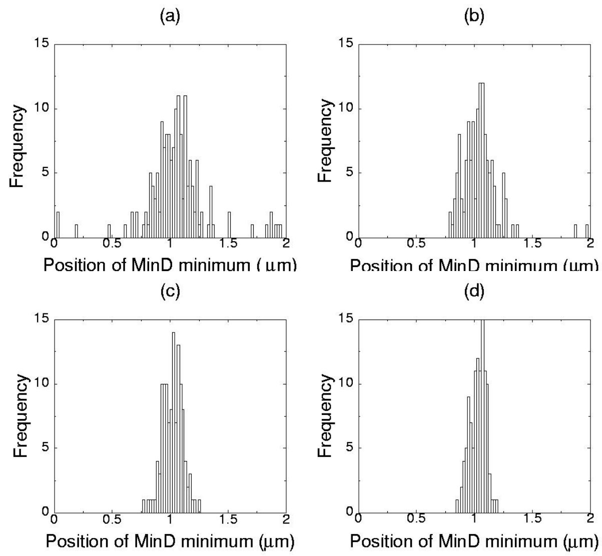
- Results for stochastic and deterministic models (with equivalent parameters) at equal copy numbers N of MinD and MinE: (a) N=200, (b) N=1500

- Cell can exploit fluctuation effects!



# Fluctuations and Optimisation

- Histograms showing the distribution of the position of MinD concentration minimum at number of protein copies=200,400, 800 and 1500
- Using substantially fewer proteins than in “wild type” cells degrades midcell accuracy; using more proteins does not usefully increase accuracy




# MinCDE Filaments

- Very recently Min proteins found to form helical filaments in E. coli

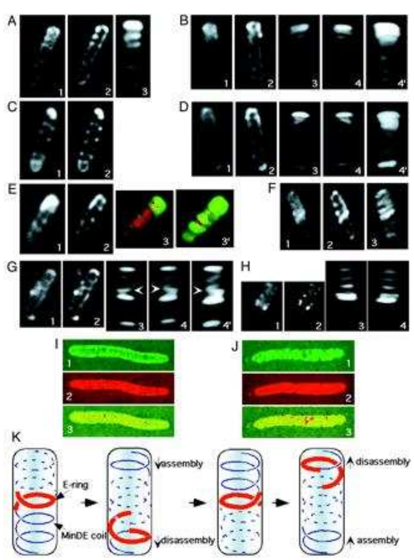
**Shih, Le, Rothfield: PNAS <u>100</u> 7865 (2003)**

- Not included in the above mathematical models, except that:

❑ Filaments ensure low membrane diffusion constants

- But what about cell division in other bacteria?

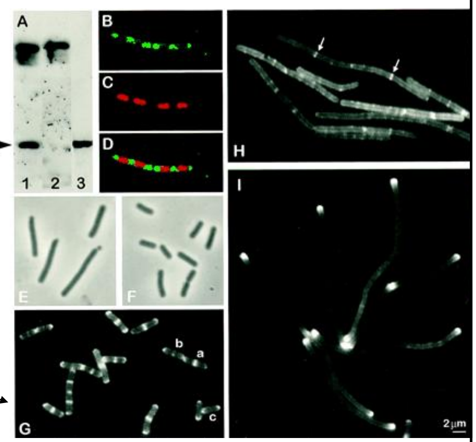
Let’s look at B. subtilis …



## Cell Division in B. subtilis

- MinC and MinD present, but no MinE
- Uses an unrelated protein: DivIVA

- No oscillations in B. subtilis…
- MinCD anchored to poles by DivIVA
- MinCD and DivIVA also have affinity for the division apparatus



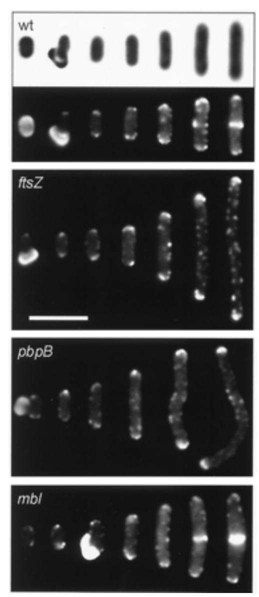

Marston et al: Genes & Dev. 12 3419 (1998)

- If MinCD/DivIVA are attracted to the division apparatus and then retained there, then any subsequent polar division in daughter cells will be blocked!

## Protein Localization in Outgrowing Spores

- But it can't be that simple: look at outgrowing spores
- Cells germinating from spores don't have pre-existing division apparatus from prior divisions
- But DivIVA can still locate poles rapidly…

- Absolutely no evidence for a characteristic wavelength: very different from E. coli
- So what is the regulatory mechanism?!




Hamoen & Errington: J. Bacteriol. 185 693 (2003)

# Model for MinCD/DivIVA function

- Assume that MinD membrane binding into filaments is inhibited at the cell poles
- Curvature effects?
- Incorporate into model, with similar structure to before:

**Howard, submitted (2003)**

MinD equations:

$$\frac{\partial \rho_D}{\partial t} = D_D \frac{\partial^2 \rho_D}{\partial x^2} - \frac{\lambda_D(x)\rho_D}{(1+\lambda_D'^2 \rho_d^2)} + \frac{\lambda_d \rho_d}{(1+\lambda_d' \rho_a)}$$

$$\frac{\partial \rho_d}{\partial t} = D_d \frac{\partial^2 \rho_d}{\partial x^2} + \frac{\lambda_D(x)\rho_D}{(1+\lambda_D'^2 \rho_d^2)} - \frac{\lambda_d \rho_d}{(1+\lambda_d' \rho_a)}$$

DivIVA equations:

$$\frac{\partial \rho_A}{\partial t} = D_A \frac{\partial^2 \rho_A}{\partial x^2} - \frac{\lambda_A \rho_A \left|\frac{\partial \rho_d}{\partial x}\right|}{(1+\lambda_A'^2 \rho_a^2)} + \frac{\lambda_a \rho_a}{(1+\lambda_a'^2 \rho_a^2)}$$

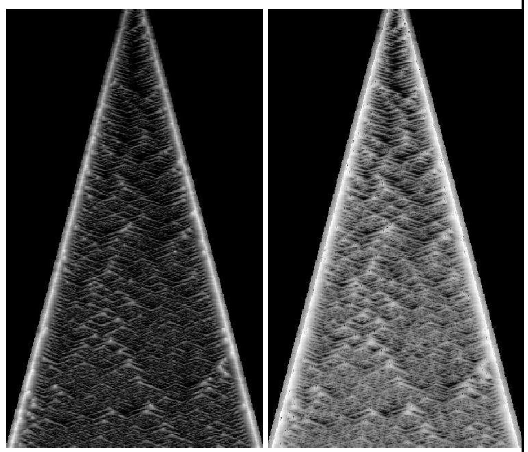
$$\frac{\partial \rho_a}{\partial t} = D_a \frac{\partial^2 \rho_a}{\partial x^2} + \frac{\lambda_A \rho_A \left|\frac{\partial \rho_d}{\partial x}\right|}{(1+\lambda_A'^2 \rho_a^2)} - \frac{\lambda_a \rho_a}{(1+\lambda_a'^2 \rho_a^2)}$$

- Note that DivIVA binds to the edges of MinD clusters: leading to a coupling to the MinD density gradient
- Use similar numbers as in E. coli model

# Numerical Results

- Grey scale spacetime plots of density in simulated outgrowing spore →
- Instantaneous densities at length 10 μm:
- DivIVA:

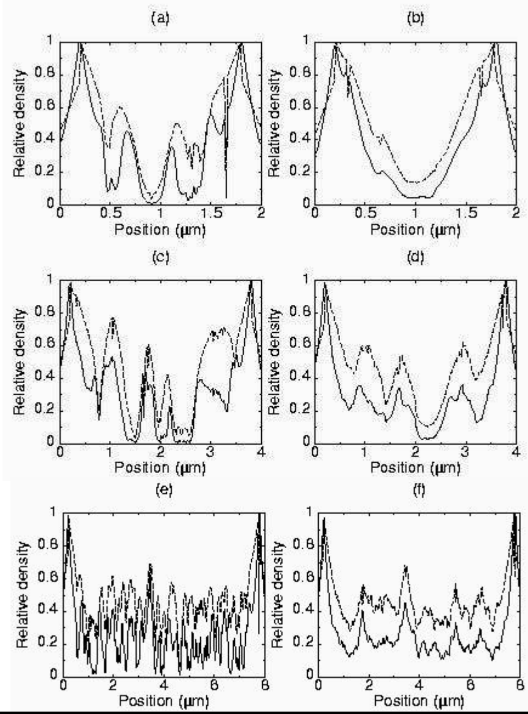
- MinD:



# Numerical Results

- Left column: instantaneous MinD (dotted), DivIVA (full line) concentrations
- Right column: 90sec average

- DivIVA more tightly localized to pole
- Cell loses ability to locate centre in long cells




# Conclusions

- Reaction-diffusion model for accurate cell division in E. coli
- Subcellular "Turing" pattern eliminates need for topological markers
- Analyzed role played by fluctuations

- Different system at work in B. subtilis...
- Relies on geometrical constraints and reaction-diffusion-polymeric dynamics

- Excellent examples of self-organised dynamics underpinning cellular architecture

- Different ways of solving the same problem!
- Could this be due to extra demands imposed by B. subtilis sporulation?

# Acknowledgements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→ E. coli partial differential equation & stochastic models

- Simon de Vet (Dalhousie)

→ E. coli partial differential equation model

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