Topics in Mathematical cell biology: final report Pancaldi Francesco

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Models for Min system in Escherichia Coli

Escherichia coli has been one of the most investigated bacteria in biology in the last 60 years. The scientific interest in this bacteria is both due to its role as a model organism and to the easy and inexpensive process to grow cultures in a laboratory setting. Moreover, the abundance of articles and papers on this bacteria also generated interest in the modeling community producing many different models to reproduce a variety of mechanism in E. coli life cycle.

One of the most studied aspects of this bacteria is the one allowing E. coli to select the location of its division cite. This process involves two main mechanism: formation of nucleoids, and spatio-temporal oscillation of a system of proteins in the cell, the so called Min system.

The interaction of these two mechanism allow the E. coli cell to individuate the center of its long axis, or *septum*, with surprising accuracy. The wild type bacteria as been observed to locate the division cite 0.5±0.013 of its length. Moreover, also in mutants in which nucleoids formation is turned off the location of the site is still observed at 0.5±0.062 of their length (average cell length between 2-6μm). On the other hand experiments on bacteria with defective or absent Min proteins have revealed that 40% of divisions produce defective minicells due to location of the division site too close to one of the poles.

Therefore the experimental evidence suggest that the Min system oscillation are of primary relevance to achieve correct cell division in E. coli.

These Min proteins concentrations has been observed to go through oscillations in time forming peaks at one pole of the cell that shrinks and reform at the other pole. This time patterns produce an average minimum concentration of MinCD complex at the center of the cell providing a mechanism to select the division site.

Similar oscillation and dynamical instabilities occur in many biological systems (e.g. Cdc42

oscillations in fission yeast) providing a broad range of mechanism that can be attempt to replicate using reactions similar to the one occurring between Min proteins.

This report first covers some general background on E. coli cell division focusing specifically on the Min system mechanism, then presents in details two models [1,2] that were proposed to reproduce the oscillations of MinCDE proteins observed in experiments. When presenting these models is also provided a Matlab code for the simpler 1D, simulations obtained with it, both to reproduce some of the results from the paper and to check the behavior of the model when some parameters are changed. Moreover, after introducing the 3D model and its results I will explain the attempts done to replicate this more complex model and the issues encountered. In the last section the paper discuss pros and cons of the two models and provides some consideration on the possible use of stochastic or semi-stochastic models to simulate the Min system.

1 - Cell division, Ftsz, Z-ring and MinCDE

Ftsz (Filamenting temperature-sensitive mutant Z) is a protein encoded by the corresponding gene ftsZ. This is the first protein to move to the cell division site, where it assembles into a ring on the inner membrane of the cell called the Z-ring. Then, FtsZ recruits other proteins responsible for the formation of the new walls of the daughter cells. Moreover, the filaments forming the Z-ring tighten and pull on each other to divide the cell.

The formation of the Z-ring and consequent localization of the cell division site (or septum) is regulated by the formation of nucleoids, and a group of proteins called Min system.

The nucleoids originated during DNA replication contain the genetic material of the two future daughter cells. These physically occlude the formation of the Z-ring in the parts of the cell between the poles and the center of the cell. Therefore the only location where FtsZ can polymerize are the zones near the two end of the cell and the one around the center.

The actual location of the Z-ring is then selected by the three Min proteins: MinC, MinD, MinE.

MinD is present in the cell in an ADP form and an ATP form. This second form can bound to the membrane where tends to form clusters. These clusters than activate the protein MinC binding with it. The activated MinC is an inhibitor for FtsZ polymerization, so an high concentration of MinC stops the formation of a Z-ring. Finally MinE can bind to the MinCD complex on the membrane activating the phosphatase domain of MinD converting it to the ADP form and hence inducing the release of both MinD-ADP and MinC in the cytoplasm. Before being able to bound again to the membrane the MinD-ADP molecule will need to phosphorylate changing back to MinD-ATP .

The time between conversion from ADP to ATP forms generate a periodicity in peaks of MinCD complex oscillating between the two poles of the cell.

This mechanism induce the formation of peaks in the average concentration of the MinCD complex in the cell at the poles as well as a minimum close to the center. This average time profile avoids the formation of a Z-ring close to the ends of the cell, where the inhibition is stronger, while allowing its formation at the septum, where the concentration of active MinC is minimal.

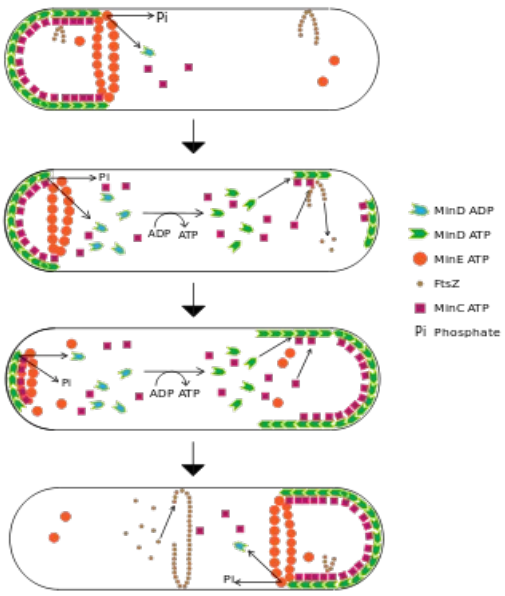

Fig.1: Min system and Z-ring formation(http://en.wikipedia.org/wiki/FtsZ)

2 – 1D-model

In this section we introduce a simple 1D PDE model developed in Howard et.al. 2001[1].

In this model there is no production or decay of proteins and the coupled PDEs system conserves the total amount of each protein. These features are present also in the 3D model presented in the next section.

This model gives rise to what the authors call a linear "Turing like (Hopf) instability", and the resulting spatio-temporal oscillation will produce a minimum for the time average concentration of the MinCD complex at the septum.

MinC is not modeled directly since as been observed that its concentration profile closely follows the one for MinD.

The system is composed of two pairs of coupled PDE with no flux boundary condition, one for each protein MinD. In each pair the first equations describes the evolution of the cytoplasmic species of the protein while the seconds represents the membrane bound protein.

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

$$\frac{\partial \rho_d}{\partial t} = \frac{\sigma_1 \rho_D}{1 + \sigma_1' \rho_e} - \sigma_2 \rho_e \rho_d , \qquad (2)$$

$$\frac{\partial \rho_E}{\partial t} = D_E \frac{\partial^2 \rho_E}{\partial x^2} - \sigma_3 \rho_D \rho_E + \frac{\sigma_4 \rho_e}{1 + \sigma_4' \rho_D} , \qquad (3)$$

$$\frac{\partial \rho_e}{\partial t} = \sigma_3 \rho_D \rho_E - \frac{\sigma_4 \rho_e}{1 + \sigma_4' \rho_D} . \qquad (4)$$

Here ρ_D and ρ_E are the cytoplasmic MinD and MinE respectively, while ρ_d and ρ_e are the membrane bound forms. Note that the authors make no difference between ATP and ADP bound MinD.

The first term of eq. 1 and 3 represent diffusion of the cytoplasmic proteins, the corresponding terms for the membrane bound MinD and MinE are considered to be negligible since diffusion in the interior of the cell is much faster than on the membrane.

The second term in eq. 1 and 2 (notice Michaelis-Mentin form) represent the transfer of MinD from the

cytoplasm to the membrane, this is proportional to the cytoplasmic concentration and has a negative feedback from the concentration of MinE in the membrane. The last term of these equations represent the hydrolysis reaction initiated by ρ_e that release ρ_d in the cytoplasm.

The second term in eq. 3 and 4 illustrates the recruitment of MinE to the membrane by cytoplasmic MinD, while the last term model its release from the membrane, proportional to the membrane bound concentration and with a negative feedback by ρ_D

The authors used cell length of 2 μm and average concentration of 1500 μm^{-1} for MinD and 85 μm^{-1} for MinE. All the other parameters are defined as follows:

the following values for the parameters in Eqs. (1)–(4):

D_D=0.28 $\mu m^2/s$, D_E=0.6 $\mu m^2/s$, σ_1= 20 s^{-1}, σ_1'= 0.028 μm, σ_2= 0.0063 μm/s, σ3= 0.04 μm/s, σ_4= 0.8 s^{-1}, and σ_4'= 0.027 μm.

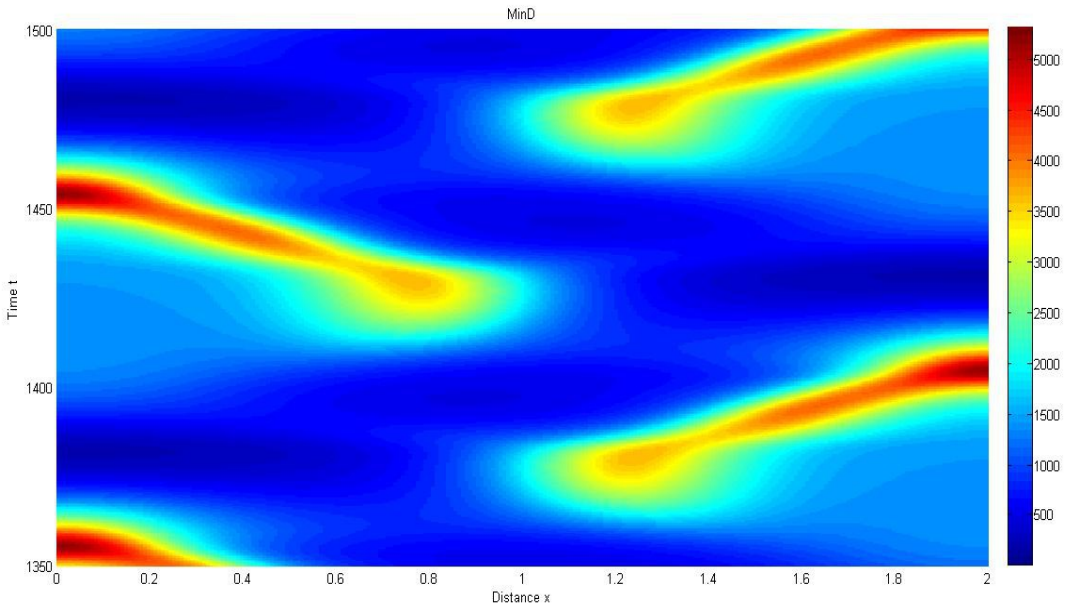

Fig. 2: last 150 seconds of a 1500 seconds simulation for the concentration of MinD.

They describe the initial condition used as 'almost constant random', so in my code I took 0 for the membrane bound species and normal distribution with adequate standard deviations (100 for MinD and

25 for MinE) for the cytoplasmic species. I also tested other random initial conditions with no significant change in the behavior of the system.

Using my Matlab implementation I was able to replicate the oscillation pattern and the time average profiles of Howard et.al.

In Fig. 2 we can clearly observe the formation of a polar zone of high concentration of MinD protein oscillating in time from one end of the cell to the opposite one within a time period of around 50 seconds.

Similarly in Fig.3 we observe an analogous pattern formation for MinE.

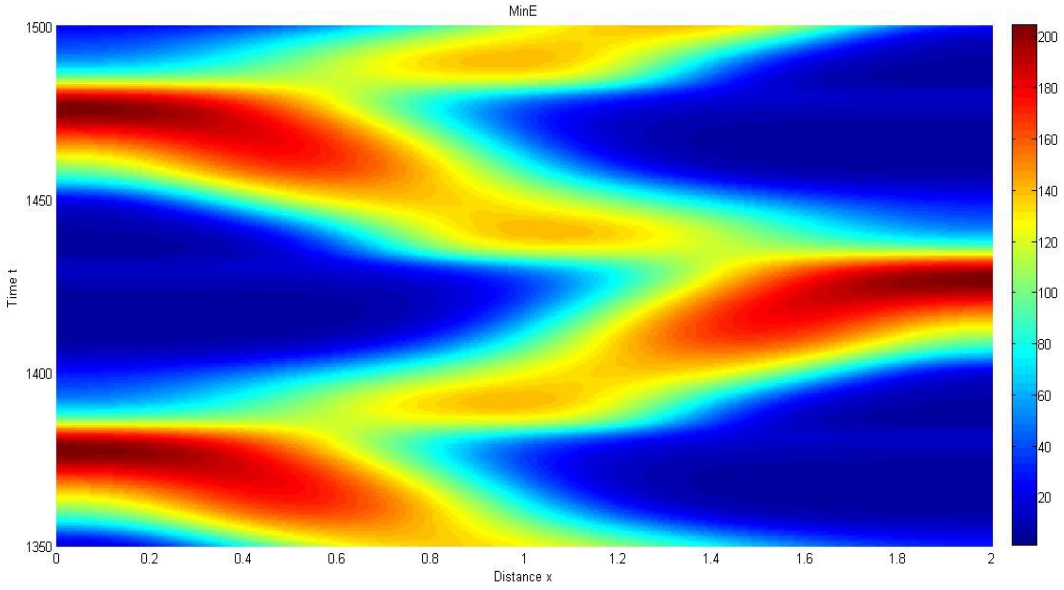

Fig. 3: last 150 seconds of a 1500 seconds simulation for the concentration of MinE.

Finally in Fig. 4 and 5 we can observe how the average-time concentrations form a minimum of MinD protein around the mid point of the cell with peaks at the poles, while MinE have a maximum around the center and is minimal at the poles.

Howard et. al. also found a linear dependence of the oscillation time to the concentration of MinD as well as an increasing dependence on cell length (fig.6).

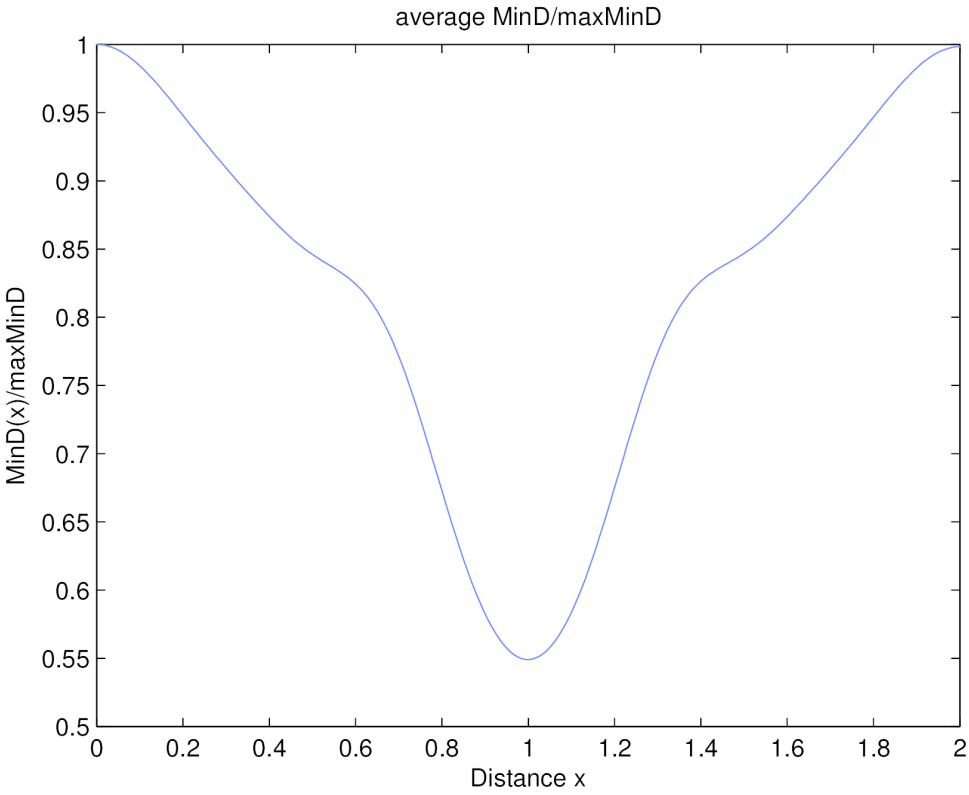

Fig. 4: time-average MinD concentration normalized to maximum value (10,000 seconds simulation, typical cell division time)

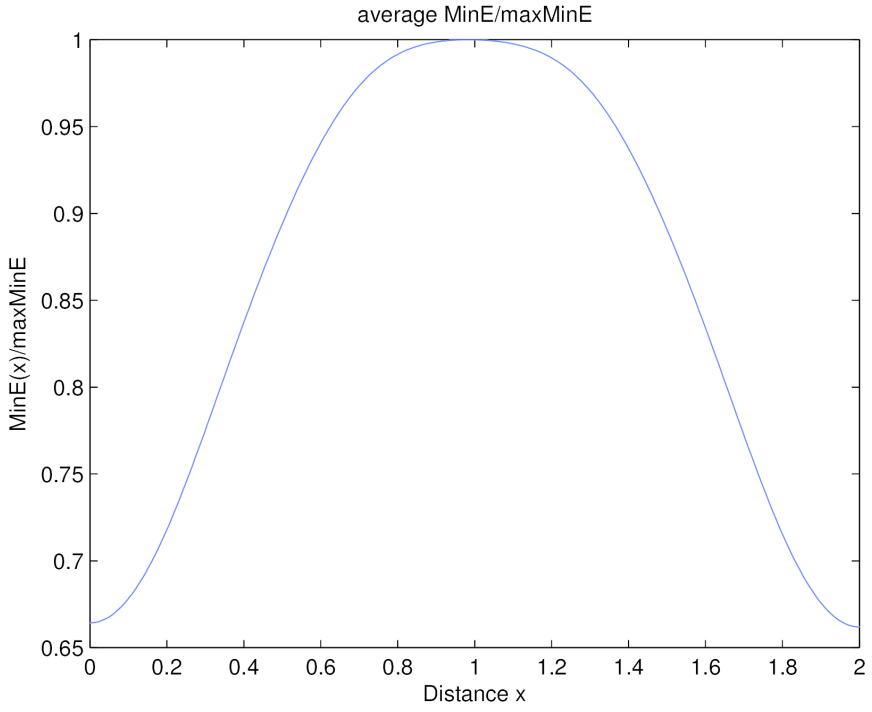

Fig. 5: time-average MinE concentration normalized to maximum value (10,000 seconds simulation, typical cell division time)

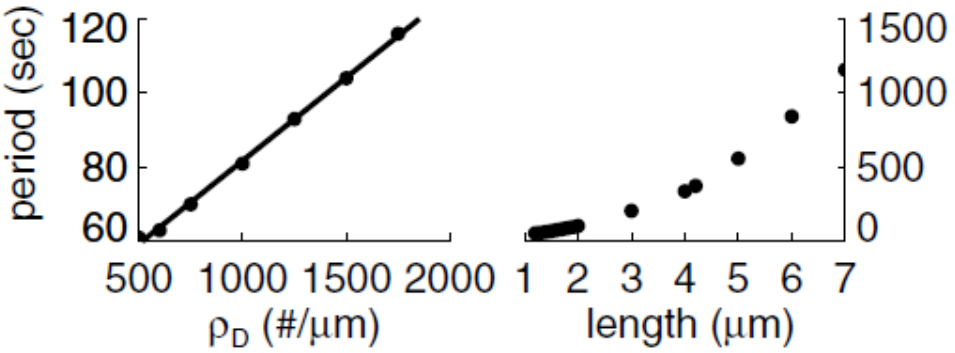

Fig.6: MinD and length dependence of the oscillation time

Since the authors mentioned a Turing like instability generating the oscillations I have performed additional simulation to understand at least qualitatively the dependence of the results on the diffusion coefficients. For this purpose I kept D_E=0.6 μm^2/s and varied D_D.

What I found is that taking D_D as low as 0.175 or has high as 0.387 the behavior of the system is unchanged. Instead lower values, while still producing oscillations, generate local maxima of MinD at the center and two minima located between center and each pole, in opposition with the minimum observed normally (fig. 7). Moreover, taking even lower value will make the local maxima become a global one (fig. 8).

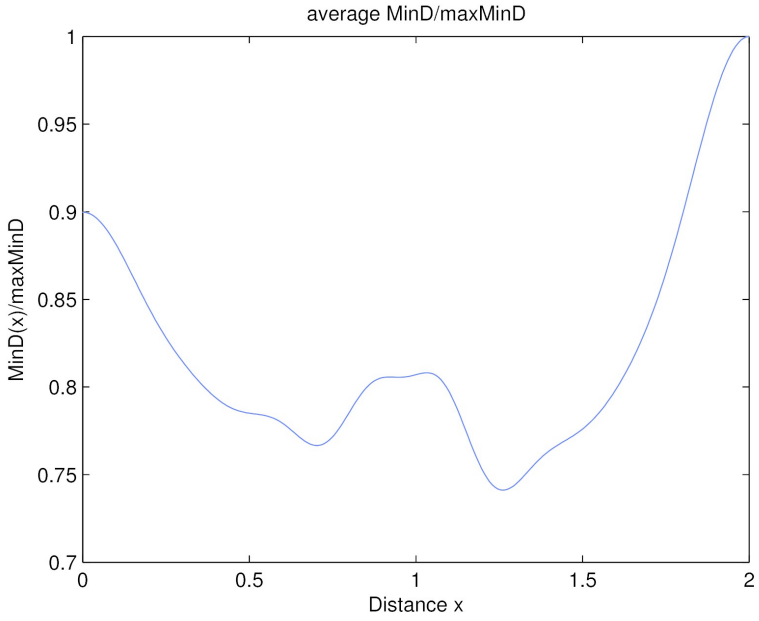

Fig.7: D_D=0.16 D_E=0.6

On the other hand higher values first produce a zone highly sensitive to the initial condition, where oscillation form later in time or may not form at all. Then for even higher values we have no oscillation and constant stable steady states. Similar results in opposite directions where obtained when I fixed D_D and changed D_E.

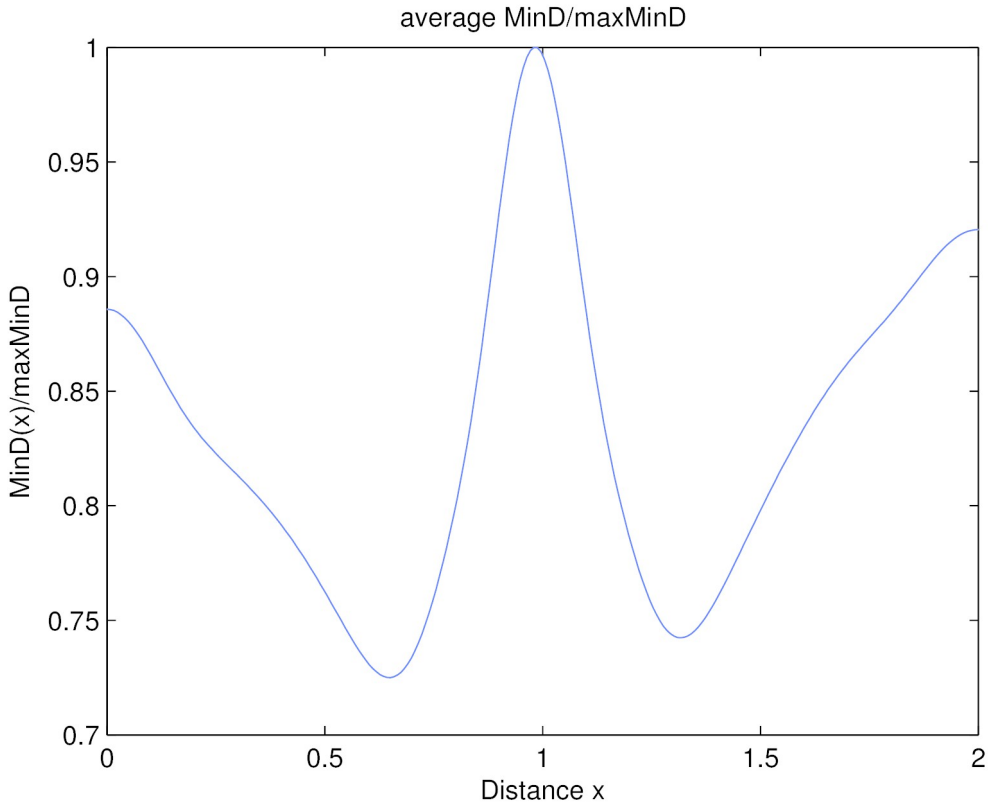

Fig. 8:D_D=0.1 D_E=0.6

Therefore my conclusion is that to obtain the wanted behavior from this system once we fix the diffusion coefficient for MinE (or MinD) the other diffusion coefficient need to be into a certain range interval of values, for example as already said if D_E=0.6 $\mu m^2/s$ we need D_D in [0.172,0.387].

This is likely due to the overlapping of an interval (bounded from above) to obtain oscillations and one (bounded from below) to obtain a minimum for MinD at the center.

More analysis may be done to evaluate other parameters range. Moreover, the authors are not providing any reference to explain why they chose any of the parameters. Therefore would be appropriate to try

estimate the parameters from experiments and see if they correspond to an adequate range in which the model can reproduce the wanted results. This observation is true also for the next model.

3 – 3D model

In this 3D PDE model Huang et. al. are considering the cell as a cylinder of length 4 μm and radius 0.5 μm. Moreover they keep track of both MinD-ADP and MinD-ATP and they consider MinE membrane bound as a complex with MinD.

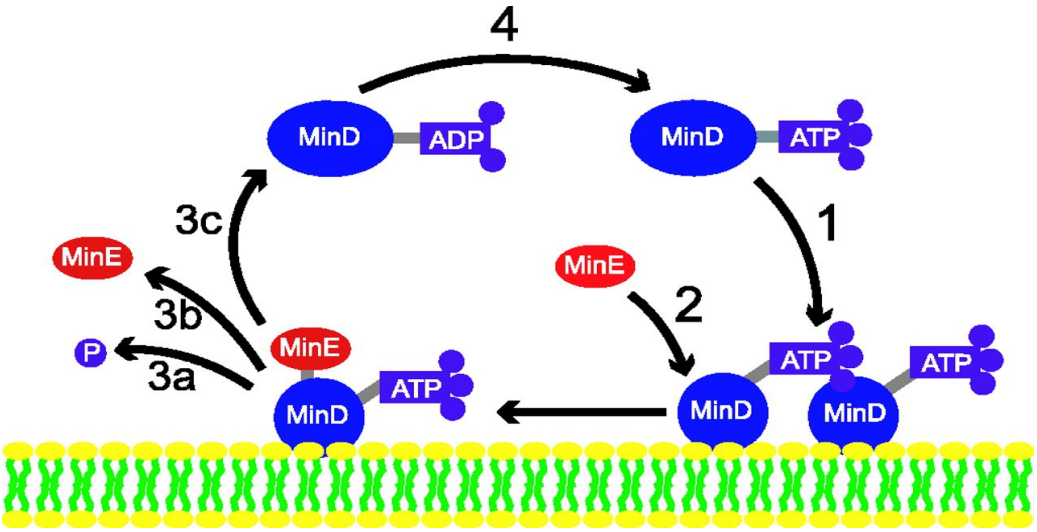

Fig. 9: Min system in the 3D-model

The following system of PDEs was used to model the concentrations of cytoplasmic MinD-ATP, MinD-ADP, MinE and the membrane bound MinD and MinDE complex.

$$\frac{\partial \rho_{D:\mathrm{ADP}}}{\partial t} = \mathcal{D}_D \nabla^2 \rho_{D:\mathrm{ADP}} - \sigma_D^{\mathrm{ADP}\to\mathrm{ATP}} \rho_{D:\mathrm{ADP}} + \delta(r-R)\sigma_{de}\rho_{de} \qquad [1]$$

$$\frac{\partial \rho_{D:\mathrm{ATP}}}{\partial t} = \mathcal{D}_D \nabla^2 \rho_{D:\mathrm{ATP}} + \sigma_D^{\mathrm{ADP}\to\mathrm{ATP}} \rho_{D:\mathrm{ADP}} - \delta(r-R)[\sigma_D + \sigma_{dD}(\rho_d + \rho_{de})]\rho_{D:\mathrm{ATP}} \qquad [2]$$

$$\frac{\partial \rho_E}{\partial t} = \mathcal{D}_E \nabla^2 \rho_E + \delta(r-R)\sigma_{de}\rho_{de} - \delta(r-R)\sigma_E \rho_d \rho_E \qquad [3]$$

$$\frac{\partial \rho_d}{\partial t} = -\sigma_E \rho_d \rho_E(R) + [\sigma_D + \sigma_{dD}(\rho_d + \rho_{de})]\rho_{D:\mathrm{ATP}}(R) \qquad [4]$$

$$\frac{\partial \rho_{de}}{\partial t} = -\sigma_{de}\rho_{de} + \sigma_E \rho_d \rho_E(R), \qquad [5]$$

Diffusion is modeled like in the 1D case, with diffusion coefficients: $\mathcal{D}_D = \mathcal{D}_E = 2.5\mu\mathrm{m}^2/\mathrm{sec}$,

In equation 1 the second term in the second member represent conversion of MinD-ADP to MinD-ATP, while the third term is the uptake of MinD-ATP into the membrane driven by the membrane bound species. Note in this model MinD bound to the membrane preferentially where other MinD is already present.

In the third equation the second member is the same as the hydrolysis term in the previous model, while the third one represent transfer of MinE to the membrane induced by the membrane bound MinD.

Here the total concentrations of MinD and MinE are 1000/μm and 350/μm.

For the other parameters the following values where used:

$$\sigma_D^{\mathrm{ADP}\to\mathrm{ATP}} = 1/\mathrm{sec}, \quad \sigma_D = 0.025\ \mu\mathrm{m/sec},$$
$$\sigma_{dD} = 0.0015\ \mu\mathrm{m}^3/\mathrm{sec},$$
$$\sigma_{de} = 0.7/\mathrm{sec}, \quad \sigma_E = 0.093\ \mu\mathrm{m}^3/\mathrm{sec},$$

No flux boundary conditions are imposed on the surface of the cylinder.

In fig. 10 Huang et al. illustrate their results visualizing the concentration of the proteins similarly to the way it is done in experiments.

They still observe formation of polar zones for MinD switching from one pole to the other in time and a corresponding MinE cap at the edge of the polar zone that makes it shrink. Moreover in the right part of Fig. 10 we can see again the formation of a MinD minimum at the center of the cell marking it as the location for the formation of the Z-ring.

In this model the oscillations are induced by the difference of the concentration profiles for membrane bound MinD-ATP and cytoplasmic MinD-ATP (fig.11). This is due to the fact that MinD-ADP need to reconvert into ATP form after being released from the membrane before being able to bound with it. again.

As for Howard et. al they also derived a dependence relation for the oscillation time but in terms of both MinD and MinE concentration (fig.12).

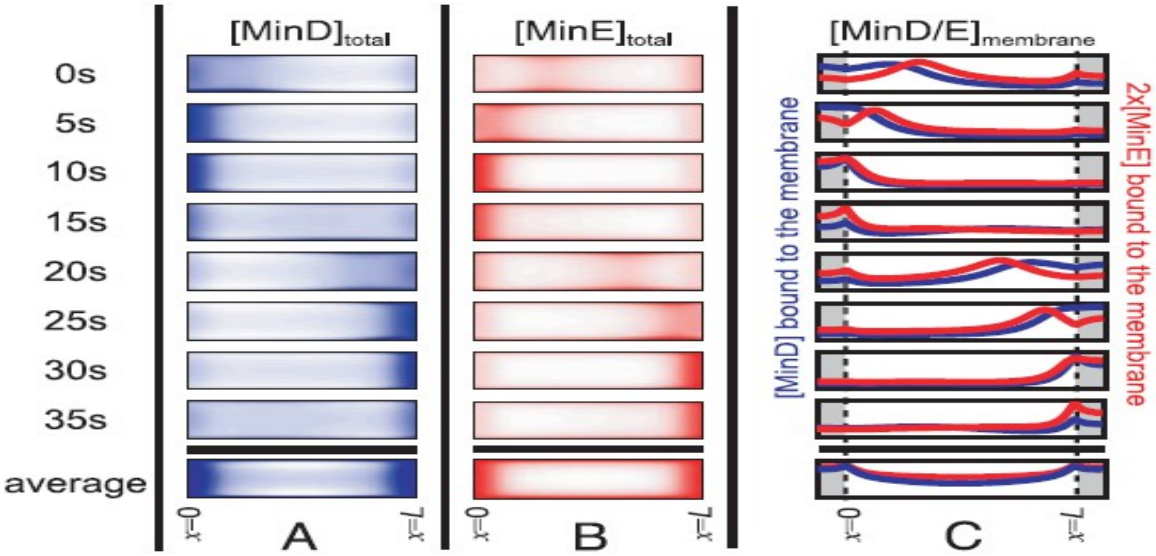

Fig. 10: MinD and MinE concentrations (5-sec time slices)

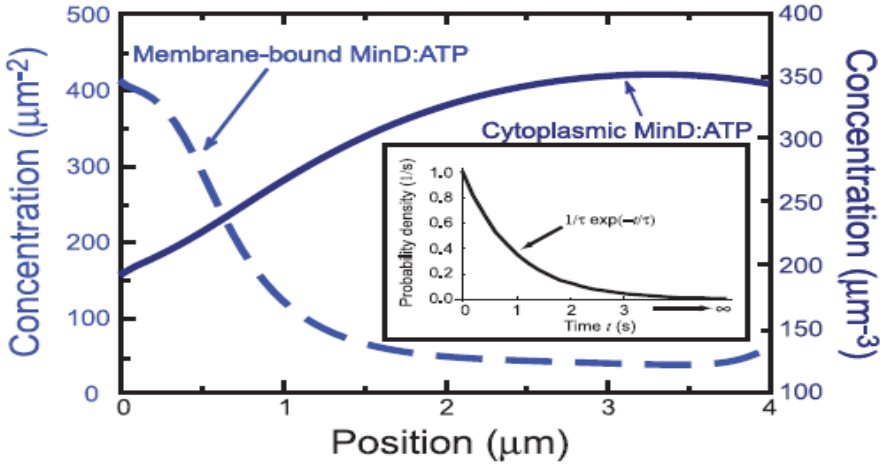

Fig.11: t=5sec concentrations of MinD-ATP membrane bound and cytoplasmic

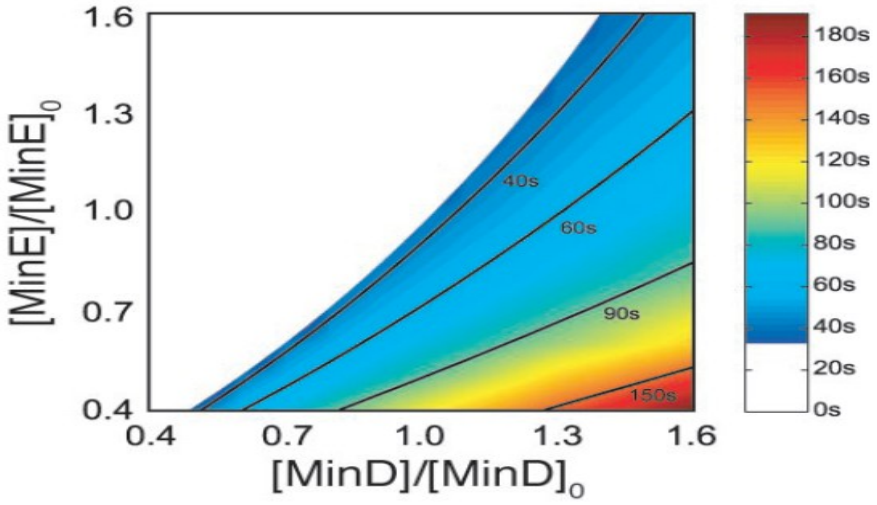

Fig.12: Dependence of oscillation period on the average concentration of MinD and MinE

They also provided simulation for a filamentous cell (length 10 μm), in which they obtained formation of two minima between the center and each poles, and for a MinE defective bacteria with reduced hydrolysis power, in which no clear minimum was formed (fig. 13-14).

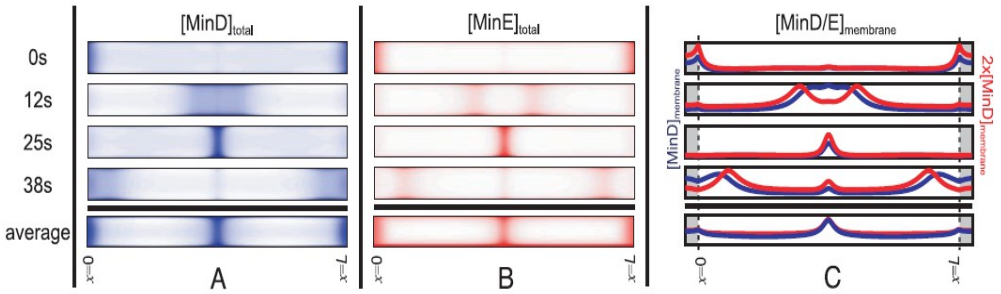

Fig. 13: simulation for a filamentous cell (length 10 μm)

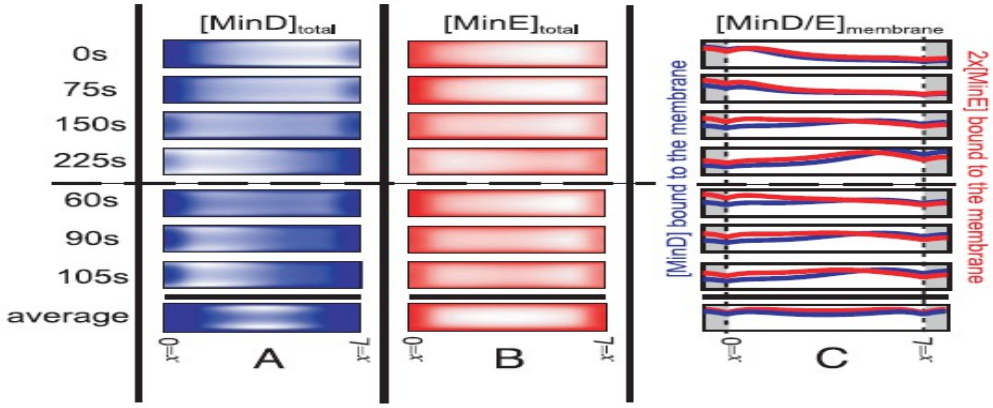

Fig. 14: MinE defective bacteria with reduced hydrolysis power

In my attempt of replicate this model I first tried to simplify it to 1D substituting the delta functions with a new parameter representing the fraction of cytoplasmic protein involved in the reaction, and then tried to implement the system in an existing 3D solver (flexpde6).

The first approach results only in constant steady states solutions with no oscillations independently on the value chosen for the new parameter. The second approach was not possible, at least in the solver I tried to use, due to the nature of the system. In fact we have both 3D equations with 2D components introduced by the delta functions, and 2D equations (on the surface of the cylinder) where 3D solutions are evaluated only on the membrane. These specificity of the system proved to be too hard to implement into flexpde6.

4 – Conclusions and reflections

The two models where both able to replicate features observed in experiments: peaks of MinD concentration at the pole, oscillating from one pole to an other with times around 50 seconds; formation of a time average minimum for MinD at the center of the cell, indicating selection of a preferential site for Z-ring formation; dependence of the oscillation time on the MinD average concentration.

The first model is easy to implement and may be analyzed to determine the stability or a range for parameters that will still provide the right behavior when changed like what I did for the diffusion coefficient D_D.

On the other hand, the 1D model is less biologically relevant, since we are reducing a 3D system as an Escherichia coli cell into a 1D segment, reducing drastically the possible complex effects generating the oscillations observed in the experiments. Moreover, Howard et. al in their model suppose that MinE is recruited to the membrane by cytoplasmic MinD against experimental evidence that showed how membrane bound MinD is the one to induce this effect, and also they are not distinguishing between MinD-ADP and ATP species, against the fact that MinD needs to be in its activated form to bound to the membrane.

The second model is more biologically accurate both because of the fact that the cell is actually a 3D object and because the authors are including in the model only experimentally verified reactions to model the Min system. However the 3D model as previously explained is more complicated both to implement and to analyze, and also was not able to replicate the maximum for MinE at the center.

Furthermore, both models are not providing any experimental reference for the value of the parameters used in their simulation. Henceforth a more accurate estimate of these parameters will be necessary.

Finally these PDEs model are useful to get a general idea of the behavior of the system and explain the way spatio-temporal oscillation and minimum for MinD are originated, but we need to remember that the number of proteins for cell is only few thousands for MinD and a few hundreds for the MinE protein. Therefore stochastic effects may be heavily relevant in these system. Moreover a PDE model

can only provide the average behavior of the system, while stochastic models may be used to explain more uncommon events (e.g. minicelling) and to evaluate the accuracy of the cell in forming the MinD minimum at the center of the cell.

All these consideration are valid as well for any other system where a system of molecules interact with each other forming spatio-temporal oscillation. Therefore, when modeling this kind of systems, we should first approach the problem with a system of PDE, simplifying the problem as much as possible like in the 1D model, then proceed with a more complex PDE model, like in Huang et. al., and if the number of molecules involved is low or we want to be able to simulate uncommon realization of the system we should develop a stochastic model.

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