Dynamic Compartmentalization of Bacteria: Accurate Division in E. Coli

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Abstract: brief summary of your write-up (150-250 words)

## Introduction

Escherichia coli is a gram- negative bacterium that is normally found in the lower intestine, but in some instances is found within the gut of animals. For this research the main focus was based on the division site within Escherichia coli . The two systems that regulate the division site are the nucleoid occlusion and the Min proteins. Nucleoid occlusion is a defense mechanism that prevents the chromosome from being bisected and broken by the division septum. 1 It does this by preventing the formation of the Z ring near the nucleoid. Min proteins are divided into three categories, MinC, MinD, and MinE. All three of these proteins are required in order to prevent minicelling. Minicells are achromosomal cells that are products of cell division, that produce one small daughter cell that contains RNA and protein, but very little chromosomal DNA. 2

In vivo observations reveal spatial oscillations of the three main proteins that were mentioned. During the oscillation period, minD accumulates at one end of the cell and then shrinks, forming at the other end of the cell. This process occurs for minC as well, creating complexes between the proteins. The interaction between the minD proteins and minE protein ring is inversely proportional, with minE occurring at opposite ends of the cell. Although the minC protein follows the spatial pattern of minD and is important to prevent minicelling, it is not required for the oscillations to occur within a cellular system. The “polar” properties of minC and minD appear to prevent ftsZ ring formation at either end of the cell, preventing minicelling to occur during the cellular division process. The problem at hand is that there are models that depict the Min protein oscillations, but none fully explain the in vivo observations. Multiple models have been provided but each requires different scenarios with different circumstances such as synthesized proteins and unrealistically rapid diffusion across membranes to be able to account for the oscillations.2 Huang et al. was successful in explaining the aforementioned interactions including only reported in vitro interactions, making the model a much more likely candidate in explaining Min protein oscillations.

## Table Description automatically generatedMathematical Model

The mathematical model designed by Howard et al is a set of four coupled reaction diffusion equations. Each equation models the densities of different proteins, such as mind on the cytoplasmic membrane (pd), minD in the cytoplasm (pD), MinE on the cytoplasmic membrane (pe) and minE in the cytoplasm (pE). (figure 1)

Figure 1. Howard et al’s coupled set of equations describing the density of minD and minE

It is worth noting that although the density of minC is not modeling with this set of equations, minC and minD travel with each other. With this assumption in mind, although the density of minC is not calculable the spatial coordinates are.

The reactions in figure 1 can be dissected into spatial fluxes (1/area/time) and reaction fluxes (1/volume/time). In the above figure only equation 1 and 3 contain spatial fluxes, which are the first term in both equations. The reaction fluxes all other terms within the equations, e.g. the last two terms in equation 1 and both terms in equation 2. Within the constructed MATLAB model (available in supplementary material), extra terms were added to equation 2 and 4 for the spatial fluxes, with a diffusion constant multiplied by the second derivative of the equation. This allowed for alterations to diffusion and made the model have a higher degree of customizability.

Boundary conditions were set at “zero flux” conditions, with no proteins entering or exiting. This lead to conserved dynamics of the total amount of minD and minE, calculated by the addition of pd+pD and pe+pE respectively. Since the model is a set of deterministic 1-dimensional rate equations, fluctuation effects are neglected. In order to correct for some amount of this, noise is added to the initial conditions, with varying starting amounts for minD and minE within the cytoplasm and the cytoplasmic membrane. As stated before, although the amount varies for each protein within the cytoplasm and cytoplasmic membrane, the total amounts of minD and minE are conserved. Parameters for the models are as follows:

Text, letter

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Figure 2. Howard et al’s designated parameters for the above model.

## Results

*Recreating Figures*

Figures by Howard et al. were replicated using the developed MATLAB code from the Howard et al. model (supplemental information). The first replicated figure was the space-time plot of MinD and minE densities, demonstrating oscillation patterns across the cell for both proteins.

Graphical user interface, application

Description automatically generatedGraphical user interface, application, PowerPoint

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Figure 3. Space-time plots by howard et al. (left) and their recreation (right). Time increases from bottom to top in the figures on the right.

The general pattern is clearly captured within the recreation when comparing to the original with oscillations of the proteins acting inversely with one another. That being said, there is a clear loss of definition within the recreated figure. That being said, although there is information being lost through definition, the recreated figures allow for the quantification of each protein with a higher degree of accuracy, as a density value bar is shown with values. This allows for the insight that values are much higher for minD compared to minE, suggesting that minE requires less protein density to carry out its functions.

An additional space-time plot was created on a longer time scale to show additional features of the oscillating proteins:

Graphical user interface, application

Description automatically generated

Figure 4. Space time plot of mind and minE on a longer time scale (0-500 secs)

Figure 4 is useful for showing the consistency of oscillations within the system. Both minD and minE have a clear period that they stick to for the entire range of time modeled. That being said, a deterministic 1-dimensional system is unlikely to capture all the properties of the minCDE proteins and the consistency of oscillations should be checked against observation and experimentation. Additionally, it shows the gap of density for minD in the center of the cell, pointing to properties that will be visualized below, as minD must not be present in the center in order for the ftsZ ring to form properly in the center:

Chart, diagram

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Chart, line chart

Description automatically generatedDiagram

Description automatically generated

Figure 5. The time-average MinD (left) and MinE (right) densities, p(x)/pmax, relative to their respective time-average maxima, as a function of position x (in µm) along the bacterium.

The recreation of this figure is another case that the MATLAB code recreates the model of Howard et al. That being said, there are inconsistencies between the graphs. For example, minD contains a flattening slop above 0.75 on the y-axis and then steeply drops below that point. This property was not recreated, as the minD plot has more of a parabolic curve to it. Based on the figure by Howard et al. it seems that minD concentrations would stay higher for greater distances and then steeply drop off closer to the center of the cell. This would lead to a more constrained area for the ftsZ ring to function.

Another recreation of the figures within Howard et al is the analysis of the period:

Chart, scatter chart

Description automatically generated Chart, scatter chart

Description automatically generated

Figure 6. Left: Plot of the period of oscillation (in seconds)

against MinD density (in mm21), at fixed average MinE concentration

of 85 mm21. The solid line is a linear best fit. Right: Plot of oscillation period against cell length, for fixed MinD and MinE concentrations. Below bacterial lengths of 1.2 mm, oscillation is not observed.

The plot of oscillation period against cell length was recreated and the same general dynamics were confirmed. There is a clear increase in oscillation as cell length increases, likely due to increased travel time of the proteins.

*Additional Figures*

The below findings are novel and not within the Howard et al. paper. As discussed above in the mathematical model section, fluctuation effects in the initial conditions are needed for the model to work properly. This led to the questions: “What’s the optimal amount of noise in the initial conditions?” and “Is there such a thing as too much noise?” These questions led to the following figure:

Chart, line chart

Description automatically generated

Figure 7. Percent success of 30 kymograph runs against incidence of noise added from 0 to 1.

The plot shows the percentage of successful results displayed by the kymograph for each value of noise added to the IC function. The % success is based on 30 runs at each interval of 0.05. The finding of this graph show that between 0.65 and 0.7, the kymograph runs reach 100% success rate and stay in the general range of 100% as noise continues to increase. This is important because, at least in the context of noise added to the initial conditions of the model, there is no such thing as too much. This demonstrates how important cellular noise is to the minCDE system in order to function properly.

Another avenue of novel figures utilized the spatial modeling set-up that was done previously. The cell was lengthened from 2 micrometers to 6 and then 8.3 micrometers. The findings of these plots were discussed in the Howard et al. paper, however the figures were not visualized. Spatial modeling with a cell length of 6 micrometers first yielded the below figure:

A screenshot of a computer

Description automatically generated with low confidence

Figure 8. Space-time plots of minD and minE. Plotting density from 0-500 s and from 0-6 micrometers.

The figure demonstrates similar findings to that of figure 6, showing that the period of oscillation increases as the length of the cell increases. This makes sense as the travel time of the proteins will increase as the length of the cell increases. However, every so often that the exact same parameters and conditions (including cell length) are run, the following is generated:

Graphical user interface

Description automatically generated

Figure 9. Space-time plots of minD and minE. Plotting density from 0-500 s and from 0-6 micrometers.

Although nothing has changed in terms of the model from figure 8 to figure 9, an entirely new output has been generated. Howard et al. defines this as “long-lived metastable states” and begins to see these two-band figures at a cell length of 6 micrometers. The state becomes more common and even stable at roughly 8.3 micrometers:

Graphical user interface, application

Description automatically generated

Figure 10. Space-time plots of minD and minE. Plotting density from 0-500 s and from 0-8.3 micrometers.

Although the period has lengthened for the two-band state in figure 10, the properties are nearly identical. This points to the model outputting results similar to these as the length of the cell increases. These results surprised the model creators themselves and are explained as a “turing-like hopf instability.” (howard et al.)

Spontaneous formation of patterns in reaction diffusion systems have been defined before by Alan Turing. Figure 8 and Figure 9 point to the Turing pattern being present for the minCDE system as long as the cell has reached a certain length. Bonny et al. Demonstrates these properties for the minCDE proteins in an observational photograph: A screenshot of a computer

Description automatically generated with medium confidence

Figure 11. Observing minD; A) standing wave with one node; B) standing wave with two nodes. Top: dic image followed by snapshots from a time lapse recodrding of mind-GFP; bottom: corresponding kymograph. (Bonny et al.)

This figure demonstrates that the double node patterns have been experimentally observed and point to a Turing-like pattern. This is extremely interesting due to the rare and chaotical nature of Turing-patterns. The implications for this finding could be differences in how certain diseases are treated, as drugs to lengthen the cell could be used and the Turing patten harnessed as a tool.