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Positive Regulation of Localization of Cell Division Proteins in Escherichia coli

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Positive Regulation of Localization of Cell Division Proteins in *Escherichia coli*

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Abstract

Escherichia coli is one of the most studied model organisms in biology; however many aspects of its cellular organization are not clear. Regardless of the extent of research, it is still not understood how its cell division proteins assemble at the right time and location to carry out cytokinesis. Two molecular systems, the Min system and SlmA mediated nucleoid occlusion, play a role in the placement of division proteins, the divisome, to the center of the cell. However, work in Dr. Jaan Mannik's lab has indicated that when the Min system and SlmA-mediated nucleoid occlusion are not present in *E. coli*, then the cells can still successfully divide by their middle. The main purpose behind this thesis is to elucidate the mechanism that allows *E. coli* cells accomplish mid-cell division when both Min system and SlmA-mediated nucleoid occlusion are absent. We employed fluorescent protein markers to simultaneously track the division proteins and replication terminus region of the chromosome. GFP was fused to the ZipA protein, which is part of the divisome, and mCherry to MatP, which specifically binds to the replication terminus region of the chromosome. We were able to see co-localization between the two proteins in both the wild type (strain WD2) and the *ΔminΔslma* (strain WD1) cells. This movement of the proteins has led to a model of a positive regulator determining the position of cell division proteins, and has opened the doors for new research.

Introduction

At the microscopic level, bacteria grow in, on and all around us. These minute life forms perform important functions from nitrogen fixation to the digestion of nutrients in the mammalian intestine, which are necessary for the success of life on the planet. While there are species of bacteria that make life possible, there are some that cause harm to other life forms leading to the importance in understanding how they live and reproduce. The aim of this thesis is to elucidate an essential process in bacterial life and reproduction cycle – cell division.

Bacterial cells reproduce through binary fission. In most species, including *Echerichia coli*, which is the subject of this study, binary fission produces two almost equally sized daughter cells from a mother cell. The mechanism is shown through figure 1 courtesy of Margolin.[1] The division process starts with assembly of the cytokinetic ring in the middle of the cell. A highly conserved,[2] essential division protein, known as FtsZ, is first to localize at the site of division and forms the initial ring-like structure (called the Z-ring) onto which the remaining proteins that comprise the divisome bind.[2] The final location of this structure is where the cell divides, and in prokaryotic cells similar to *Escherichia coli*,

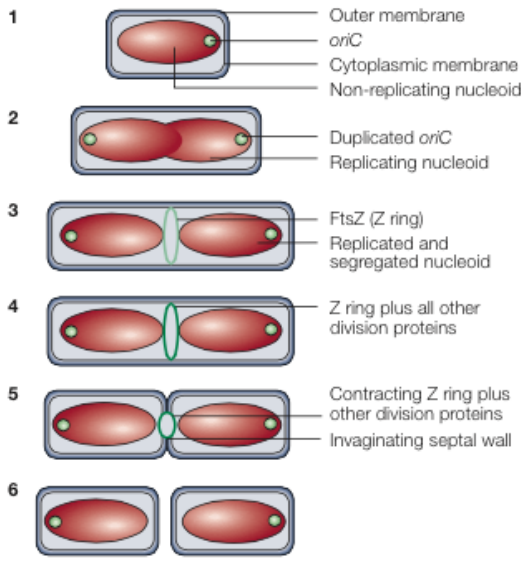


Figure 1: Shown above is the typical cell division cycle in *E. coli*. (1) Seen is a non-replicating nucleoid. (2) The chromosome begins to duplicate and the replication origin (*oriC*) moves to the poles. (3) The nucleoid segregates and the Z-ring forms led by FtsZ. (4) The Z-ring recruits the remaining division proteins to the divisome. (5) The Z-ring contracts and division into daughter cells begins as shown in (6).

the Z-ring forms in the geometric center of the cell.[3] It is of great importance that during the division of the cell that the genetic material of the cell is efficiently split between the two daughter cells, and the inappropriate partitioning of nucleic material inevitably leads to the death of the daughter cells.[2]

In *E. coli*, there are two known mechanisms explaining the division of cells during reproduction, the first being the Min System.[3] This negatively regulating system consists of three proteins – MinC, MinD, and MinE. Of these proteins, MinC has been observed to inhibit the polymerization of FtsZ and its subsequent proteins into the Z-ring.[1,3] MinD is a membrane binding ATPase, which is unbound by ATP hydrolysis promoted by MinE.[1] MinC associates heavily with the MinD protein, and, due to the MinD-MinE process occurring at the poles of the cell, the presence of MinC drives Z-ring formation into the central part of the cell.[1]

The second division system is SlmA-mediated nucleoid occlusion, which acts independently of the Min System.[3,1] This mechanism prevents the development of a Z-ring overtop of unduplicated chromosomes until it has been copied and pushed towards the poles of the cell to avoid destruction of genetic material during replication.[1] The mediating protein for occlusion is the DNA-binding SlmA, which either binds to or dissolves the protofilaments of the FtsZ protein.[3] This system allows for a Z-ring antagonist free middle of the cell.[2]

When a cell is produced having both of the division systems deleted yielding a *ΔslmaΔmin* double mutant cell, *E. coli* cells are still capable of performing cellular division along with chromosomal segregation efficiently.[4] Cells with these deletions are viable and successfully divide in slow growing, nutrient poor M9 media.[5] With

Δslma cells, this lone deletion does not show to have any effect on the accuracy of cell division and the relationship of chromosome segregation from the divisome.[6] Within double mutants, it is seen that Z-rings tend to localize toward nucleoid centers, which is occupied by the Ter region of the chromosome. [4] This Ter macrodomain serves as a form of scaffolding for the placement of division proteins making up the divisome.[4] While the Min system and SlmA-mediated nucleoid occlusion base their function from the inhibition of divisome formation, the Ter linkage shows a form of positive regulation.[4]

This paper describes the process through which I was able to engineer specific types of *ΔslmAΔmin E. coli* with fluorescent protein markers to allow for the greater ability to observe the localization of division proteins. The resulting cells allowed for the discovery and characterization of the Ter linkage in division protein positioning, opening up the idea of positive regulation in binary fission.

Overview of Methods

Bacterial Growth:

Cells were grown in a nutrient-rich lysogeny broth (LB) and two nutrient-poor M9 agars containing either glucose or glycerol. Some cells require different concentrations of nutrients to grow effectively. Strains that have both Min and nucleoid occlusion systems deleted are still capable of growing, but grow into a long cell incapable of dividing. These strains cannot be placed in a nutrient rich environment and require longer incubation periods; these strains require the M9 media. Other, less manipulated strains are capable of growing quickly with the LB agar.

In some cases, the use of antibiotic resistance is required for the construction of specific bacterial strains. Antibiotic resistance is used in research as a tool to select cells with the appropriate genetic modifications. An abundance of cells is required for genetic engineering, and we need to be able to select for cells that have successfully take in this modification out of billions of cells. Resistance can be obtained through chromosomal DNA or via plasmid DNA. We are able to group resistance with certain genetic modifications. If the cells are able to grow on the agar containing that antibiotic, then the trial was successful. The agar that the cells are grown on contains a concentration of antibiotic that the cell should be resistant to. Antibiotics commonly used in the trials are ampicillin (20μg/ml), kanamycin (20μg/ml), and chloramphenicol (35μg/ml).

P1 Transduction:

This is a form genetic manipulation that allows for the transferring of genetic material of interest from one bacterial cell to another with the use of a P1 bacteriophage – a bacteria specific virus. The P1 phage used in these trials has the tendency to incorporate bacterial DNA into its capsid during replication within a host cell.[7] Transductions are used to achieve a variety of different outcomes. In some cases, we want to delete either or both of the division mechanisms in an *E. coli* cell or place a fluorescent protein tag onto a protein of interest.

Transductions work off of homologous recombination; which is the recombining of two different DNA strands of similar nucleotide sequences. This is usually used in the repair of broken DNA strands allowing for the removal of

deleterious sequences. In research, it allows for the exchange of genetic material of interest with what is already in the cell.

To achieve a successful infection, a P1 lysate must be made using the host strain and the P1 bacteriophage. Within this lysate there are phages with either viral DNA or bacterial DNA, and once they are grown with a recipient bacterial strain, the genes of interest will be incorporated into the bacterial DNA of the new strain. An important component to include with the donor DNA is a selectable marker (i.e. antibiotic resistance) to show that the genetic material was taken up by the recipient strain successfully.[7]

Electroporation & Transformation:

This protocol allows for the entrance and incorporation of foreign genetic material other than a virus. It begins with electroporation; which uses millisecond long electric pulses to cause a temporary loss in semipermeability of cell membranes.[8] Once this occurs, it is possible for the cell to take in contents – plasmids, molecular probes, and antibiotics[8] – that were once not capable of diffusing across the membrane. Following electroporation, it is now possible for the cell to become transformed. In this experiment, transformations are used largely for the uptake of DNA in the form of plasmids with the main objective to remove a cell's resistance to the antibiotics kanamycin, ampicillin, or chloramphenicol. This affords us the ability to use the resistance as a marker for the change in the genetic makeup of our cells. This trial involves the use of the plasmid pCP20; which enables the removal of the kanamycin resistance gene from transduced cells. This plasmid codes for the flippase; which recognizes the frt sequence sites coding for antibiotic

resistance. The enzymes carries out recombination between two *frt* sites containing the antibiotic resistance gene thereby removing the gene.

Results

In the lab of Dr. Jaan Mannik at the University of Tennessee, Knoxville, past research has identified that the Z-ring co-localizes with the center of the nucleoid in *ΔslmaΔmin* strains. Prior studies by other groups have identified that the replication terminus occupies the nucleoid center of a cell at the end of the cell cycle. Developed from these findings comes the hypothesis that the replication terminus region may act as a site for the accumulation of cell division proteins. To test this, Ph.D candidate Matthew Bailey and I developed two separate strains of *E. coli* using transformation and P1 transduction methods described previously. The strains in question originated from the wild type strain JMBW5 and the double mutant TB86(λCH151).

We employed the use the GFP and mCherry fluorescent proteins to follow the movement of cellular division proteins and effectively analyze this phenomenon. The fluorescent markers were attached to two different division proteins. By tagging ZipA with GFP, we could watch the movement of the Z-ring during the replication process, and the mCherry allowed us to observe the replication terminus through MatP. The protein-marker constructs were in the form of lysates from previous strains provided by collaborating researchers Paola Bisicchia from the University of Oxford and P. A. J. de Boer of Case Western Reserve University.

Using transformations and P1 transductions, I was able to synthesize two strains of *E. coli* that allowed for the observation of the interaction between the Ter macrodomain and the divisome. Beginning with the wild type stain, JMBW5, which was previously transduced with a lysate from the TB86(λCH151) strain to yield the ZipA-GFP construct, I performed yet another P1 transduction. A lysate containing the MatP-mCherry construct was transduced into JMBW5. This yielded the strain WD2, which did not contain deletions of either division mechanism, but rather the addition of the constructs ZipA-GFP and MatP-mCherry. Figure 2 shows fluorescent images of both ZipA-GFP and MatP-mCherry along with an RGB image showing th e co-localization of the two proteins in WD2.

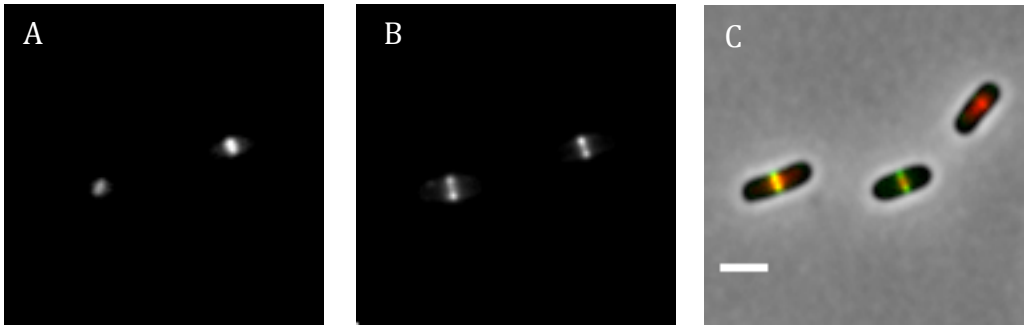
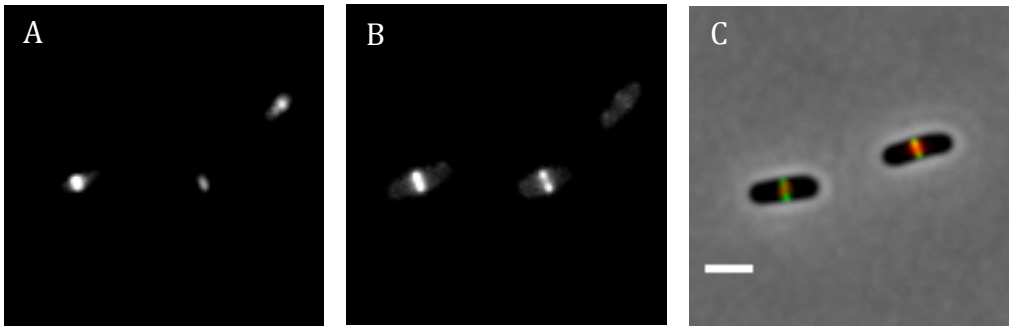


Figure 2: Co-localization of MatP-mCherry and ZipA-GFP in wild type cells (strain WD2). (A) The fluorescent image of matP-mCherry , which marks the replication terminus region of the chromosme. (B) The fluorescent image of ZipA-GFP, which marks the divisome. (C) Overlay image of the two fluorescent images and phase contrast image. Scale bar is 2 um.

With the TB86(λCH151) strain as a base, the kanamycin resistance cassette was removed via a transformation using the pCP20 plasmid. This allowed for the transduction of the strain using the same lysate used to create WD2. Following the successful P1 transduction, the WD1 strain was observed. This strain, unlike WD2,

was a double mutant lacking both the Min system and SlmA-mediated nucleoid occlusion. Shown in figure 2 are fluorescent images of both ZipA and MatP in WD1; the proteins observe to look similar to those of the wild type WD2. Also pictured is an RGB image of WD1, showing an equal co-localization of MatP and ZipA from WD2.



We **Figure 3:** Co-localization of MatP-mCherry and ZipA-GFP in Δ*slmA* Δ*min* cells (strain WD1). (A) The fluorescent image of matP-mCherry , which marks the replication terminus region of the chromosme. (B) The fluorescent image of ZipA-GFP, which marks the divisome. (C) Overlay image of the two fluorescent images and phase contrast image. Scale bar is 2 um.

To obtain these images, we excited the fluorescent markers using a 200W Hg lamp through an ND4 neutral density filter in Nikon Ti-E fluorescent microscope. To record the fluorescing proteins, Chroma 41004 and 41001 were used for mCherry and GFP images, respectively. To capture the images, we used an Andor iXon DU897 camera and recorded them on NIS-Elements software.

Prior assumptions suggested that a *ΔminΔslma* cell would be unable to localize the division proteins effectively enough to allow for the creation of the daughter cells. Figure 3, however, shows this to not be the case. The MatP-mCherry marked Ter region of the nucleoid centers with the ZipA-GFP marked Z-ring appear to co-localize together regardless of no division mechanism being in place. Figure 2

shows the wild type, WD2 strain, and by comparing to WD1 in figure 3, shows that the co-localization of the two structures is independent from both the Min system and slma-mediated nucleoid occlusion. From this arose the presence of a positive regulating Ter linkage guiding the nucleoid to the divisome.

Discussion

The Min system and nucleoid occlusion are both categorized as negative regulators of cell division through the inhibition of Z-ring formation in specific areas. Removal of these mechanisms, on the other hand, provides a look into the presence of a possible positive regulator.[4] Cells were seen to have Z-ring localization over the centers of nucleoids rather than the poles or gaps of the cells.[9] Regardless of whether the Min system or nucleoid occlusion factors were in place, the co-localization of the division proteins appears to be independent on the mechanisms.

Organized by MatP proteins, the replication terminus of *E. coli* cells forms into a structure termed the Ter macrodomain.[10] Anchoring of this Ter region to the Z-ring occurs through the interaction of MatP with the binding protein ZapB, and the binding of ZapB to another protein ZapA.[11] The structure serving as a form of anchor has been identified as the Ter linkage, connecting the terminus to the Z-ring as shown through figure 4 courtesy of Mannik and Bailey.[9] It attaches via a DNA-MatP-ZapB-ZapA-FtsZ chain with the components nearest the

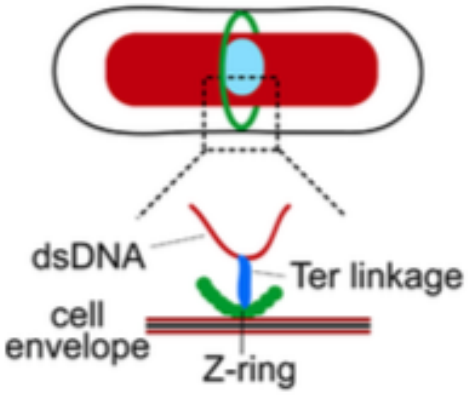


Figure 4: Shown above is a depiction of the Ter linkage actively guiding the nucleoid to the Z-ring and divisome prior to cell division.

linkage actually interacting with it.[9] The Ter linkage serves as an assurance that the positioning of chromosomes does not change following the formation of the Z-ring in relation to the divisome.

Thus we can identify that there are two steps involved in the localization of Z-ring and replication terminus. The first action is seen by the promotion of Z-ring formation by a signal from the replication terminus. This is followed by the connection of terminus to Z-ring by MatP, ZapB and ZapA, but how this occurs is not exactly known.[9] Figure 5, courtesy of Mannik and Bailey, depicts an illustration of how these three proteins interact with FtsZ and the terminus to make up the Ter linkage.[9] There could be some other form of molecular machinery driving this connection along with these three proteins; however, further research is needed to test this theory. At this time, research is underway to look more into the positive regulation in *E. coli* and the possibility of other components involved in the process of cellular division.

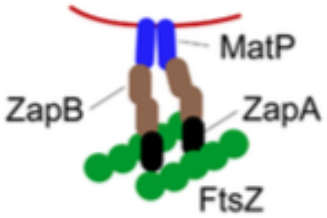


Figure 5: Shown above is the make up of the Ter linkage and its interaction with FtsZ and the cellular DNA. MatP appears to bind to the DNA and ZapA to FtsZ; while ZapB binds the other two together.

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