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Ryann Murphy
ClpXP modulates cell growth and morphology in cell shape mutants of *E.coli*

Project Summary:

I completed my yearlong Honors research project in the laboratory of Dr. Jodi Camberg, of the Cell and Molecular biology department, URI. *ClpXP modulates cell growth and morphology in cell shape mutants of E.coli* culminated in the creation of a scientific research poster.

Background:

Escherichia coli, is a rod-shaped bacterium commonly found in the intestine of warm-blooded organisms. The bacterial cell shape is governed by the construction of the cell wall, comprised of peptidoglycan [3]. The rigid peptidoglycan is a large single cross-linked macromolecule that provides the structural integrity of the cell [3]. The antibiotic drug penicillin interferes with specific bacterial enzymes embedded in the cell wall known as penicillin-binding proteins (PBPs). These proteins are transpeptidases or enzymes involved in cell wall construction, which form cross-links in peptidogylcan chains. In the presence of penicillin, the antibiotic binds to PBPs in peptidoglycan of *E.coli* and destroys the bacterial cell wall. Researchers have linked PBPs to cell division, but specific biochemical roles of this family of proteins are unknown. During cell division, it is thought that PBPs function at the site of division to insert and remodel the outer cell layer.

In normal, wild type *E.coli* cells, the uniform rod shape is conserved across generations; meaning little variation of cell morphology is observed after cells divide. Cells have two main life phases: one in which cells grow to maximum potential and the other in which cells divide. *E.coli* undergoes the process of cell division known as binary fission. When cells begin the division process, two copies of genetic material, chromosomes, are made and lined up at the mid-point of the cell. One set of chromosomes is pulled to one pole of the cell, while the other set of chromosomes is pulled to the other pole of the cell. This equal segregation of material clears the midpoint area of the cell and allows for the formation of a Z-ring. The Z-ring is comprised of the protein FtsZ. It performs cell septation, in which the Z-ring constricts and pinches off. Two identical daughter cells result.

Normally, the protein FtsZ forms a Z-ring at the midpoint of the cell and guides perpendicular growth of the division septum [5]. Low molecular weight penicillin binding proteins (LMW PBPs) play a roll in synthesis and maintenance of cell wall peptidoglycan, which maintains cell structure [3]. Removing one or more LMW PBPs in *E.coli* results in irregularities in cell morphology, including: elongation, bending, and branching [5]. PBP5 has demonstrated a role in maintaining perpendicular geometry of Z ring in *E.coli* [1]. PBP5 removal reveals the strongest cell morphology phenotype, which is stronger when a second LMW PBPs is deleted [5]. Since double deletion mutants missing two PBPs show even more perturbed cell shape, research suggests that roles among the LMW PBPs overlap. When Z-ring formation is perturbed via lack of key PBPs, cell morphology varies from the normal rod shape.

LMW PBPs, in particular D,D-carboxypeptidase, PBP5 is a candidate to be a regulator of septal diameter [4]. Studies of mutants LMW PBPs by Dr.Young [4] provide strong evidence that PBP5 plays a key role in removal of ‘excess’ cell wall and the maintenance of normal rod shape [4]. Removal of cell wall requires degradation. PBPs are possible substrates, or reaction sites, for enzyme activity related to this removal. Specifically, PBP5 is a candidate for degradation by ClpXP. ClpXP is an ATP-dependent protease. A protease is an enzyme, which breaks down proteins and peptides [1]. Since protein degradation is a component of biological regulation and protein quality control [1] it’s role in cell division is relevant. *E.coli* ClpXP is an ATP-dependent intracellular protein [1]. The ClpX component is a hexameric AAA+ ATPase responsible for substrate recognition, unfolding, and translocation into ClpP [1].

Studies reveal clear morphological responsibilities of PBPs, yet questions regarding basic details of how bacterial cells produce a wall of a particular shape remains undetermined [4] Why PBP5 deficiencies cause the most morphological distortions is unclear [2]. Double and triple deletion mutant strains, deleted for PBP5, PBP 4, and PBP 7 have been constructed by Dr. Young, a collaborator of the Camberg lab. Since low molecular weight PBPs have overlapping rolls, double (PBP 4, 7 (-)) and triple deletion (PBP 4, 5, 7 (-)) mutants strains were used to discern their roles in cell division via interaction with ClpXP. The same strains will be used to discern the role of PBPs in cell division via interaction with FtsZ. Overall, the proposed research will further define the molecular mechanisms of PBPs that contribute to the cellular targeted protein degradation and control linked to cell division.

Research:

My original question of interest was: does ClpXP degrade PBP5 proteins? To study this, PBP deletion strains were created by Dr.Kevin Young and provided for use in Dr.Camberg's lab. *E.coli* strain CS109 is the parental strain used. CS109ΔPBP4,7 is the strain lacking PBP4 and PBP7. This strain is referred to as the PBP double deletion strain. CS109ΔPBP4,5,7 is the strain lacking PBP4, PBP5, and PBP7. This strain is referred to as the PBP triple deletion strain. The CS109 parental strain serves as a control, wild type strain to compare PBP deletion strains to. The difference between the PBP double deletion strain and the PBP triple deletion strain is the presence of functional PBP5 in the PBP double deletion strain and the absence of PBP5 in the PBP triple deletion strain. This allowed us to change expression levels of ClpXP in the three strains and study cell viability in the presence and absence of PBP 5. Using the parental, PBP double deletion strain, and the PBP deletion strain, the ClpXP was overexpressed. Growth over time was measured and cell morphology of the strains overexpressing ClpXP was observed using a microscope. My *in vivo* research cannot confirm ClpXP degradation of PBP5 definitively. ClpXP overexpression was associated with a change in viability of the double and triple PBP deletion strains.

To complement the ClpXP overexpression data set, the absence of ClpXP activity in the three strains was observed. In order to do this *clpx* deletion strains were created. The deletion of the *clpx* gene results in cells that cannot produce clpX protein, a component of the ClpXP molecular chaperone. In *clpx* deletion strains, cells do not have the ClpXP protease complex. The deletion of *clpx* resulted in a change in viability of the PBP triple deletion mutant.

Overall, looking at high levels of ClpXP and no levels of ClpXP, I was able to study whether PBP degradation is occurring and how this interaction affects growth and morphology of cells. Since the role of PBPs in cell division was of interest, characterizing PBP interaction with another major cell division protein, FtsZ took place. FtsZ protein forms the septal Z-ring during cell division. FtsZ is a known substrate for recognition of ClpXP. Research by the Camberg lab shows ClpXP modulated the septal ring dynamics. My question of interest was whether PBPs play a role in Z-ring placement and orientation. To do this, I introduced green fluorescent protein (GFP) tagged FtsZ in the parental, PBP double deletion strain, and PBP triple deletion strains and observed ring orientation using confocal fluorescent microscopy. This allowed me to determine if cells lacking PBPs have irregular placement and orientation of the Z-ring. If cells with mutated PBPs in fact have irregular Z-ring orientation, when the septal ring constricts during cell division two irregular daughter cells result. Z-ring placement could explain why cells with mutated PBPs have irregular sizes and shapes.

Rationale for Project:

Both ClpXP and FtsZ are major cell division proteins. Demonstrating interactions with PBPs has never been done. Information regarding PBPs has medical applications. Understanding *E.coli* cell division, an easily grown prokaryotic model organism, scientist can determine why PBP genes have been conserved across many bacterial species. Understanding components of cell division gives scientists a means to create new bacteriocidal medicines to target bacterial pathogens.

Project Value to Academic/Career Aims:

Research on PBPs is medically important since these enzymes are the primary targets for β-lactam antibiotics [2]. PBP genes are highly conversed in a wide range of bacteria, despite seemly redundant roles, suggesting importance in physiology yet to be clarified [2]. My interest lies in microbial research of pathogens as they relate to human health. This project provided me with invaluable research experience to perpetuate my career goals in microbiology.

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