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Force generation in dividing *E. coli* cells: A handles-on approach using optical tweezers

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Citation

Verhoeven, G. S. (2008, December 2). *Force generation in dividing E. coli cells: A handles-on approach using optical tweezers*. Retrieved from <https://hdl.handle.net/1887/13301>

Version: Corrected Publisher's Version

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Note: To cite this publication please use the final published version (if applicable).

Summary

This thesis is about cell division in bacteria. What (to me) is fascinating about a bacterium such as *Escherichia coli* is that it can multiply at an incredibly fast pace by growing in length and then simply splitting itself in two. It has been known for decades that our own cells divide by making use of force-generating proteins similar to those present in our muscle cells. However, how a bacterium manages to divide is still a mystery. One of the problems when studying bacteria is their small size (one hundredth of the thickness of a human hair). This was one of the reasons that led scientists to think that bacteria were small “bags of protein”, without an internal cytoskeleton (built from proteins) such as possessed by plant and animal cells. This view has changed in recent years, as it turns out that bacteria have more in common with our own cells than previously thought. For instance, it was found that a protein called FtsZ forms a ring in the middle of a dividing bacterium. There are strong indications that this “Z-ring” generates force to constrict the bacterium. However, this has not yet been proven experimentally. This thesis describes the development of a method to perform force measurements on a single dividing bacterium.

To be able to measure the very small forces that can be produced by single cells, we make use of a technique called “optical tweezers” or “optical trapping”. An optical trap is created by a focused laser beam, and can hold (‘trap’) small beads in liquid. The trapped bead, when calibrated properly, can become an extremely sensitive force sensor. Because it works in a fluid environment, optical tweezers are ideally suited to study cells and proteins. Our optical tweezers set-up consists of a strong laser beam coupled into a commercial microscope. Plastic or glass beads, as well as bacteria (length scale: a thousandth of a millimeter) can be optically trapped. When a force in the order of a millionth of a millionth Newton (one Newton is the force that the earth exerts on a not too heavy book) is exerted on the bead it will slightly displace out of the trap (on a scale of nanometers, a millionth of a millimeter). The strength of the trap can be calibrated by measuring the spatial fluctuations of the trapped bead.

The challenge now is to attach such a bead to a bacterium roughly at the site where the bacterium will divide, because we suspect that at that location, forces are generated. The idea that has been worked out in this thesis is to genetically alter the bacteria in such a way that they produce molecular handles which end up on the bacterial surface, but only

at the division site. We believe this is possible because proteins often consist of multiple domains, which often have their own separate functions. Since protein domains exist that are present on the bacterial surface, as well as protein domains that localize to the division site, it should in principle be possible to create a new fusion protein which is present on the bacterial surface, but only at the division site.

A complicating factor in bacteria is the presence of a rigid cell wall, which is similar in many ways to the cell wall that gives shape to plant cells. When a bacterium divides in two daughter cells, also the new cell poles need to be formed. Or to put it differently: the creation of the new cell poles of the two daughter cells is the process of division. **Chapter 2** summarizes what is known about the processes that shape bacterial cell walls. For these processes, the length scale is that of the single protein molecule (1-10 nm). The focus is on the relatively new hypothesis that forces exerted by cytoskeletal proteins such as FtsZ and MreB play an important role in shaping the cell wall.

The three chapters that follow deal with the creation of the molecular handle. In **Chapter 3** we present experiments in which a surface protein (OmpA, for 'outer membrane protein A') of the bacterium *Escherichia coli* is genetically altered such that a small part of another protein (an epitope) is displayed on the bacterial surface. This is required for bead attachment. Three different epitopes are compared. We show that all three epitopes are displayed on the cell surface, but that the efficiency of protein insertion into the outer membrane differs. The so-called FLAG and myc epitopes are partially degraded by the cell. In contrast, virtually no degradation was observed for the SA-1 epitope. In addition, experiments are described which demonstrate that OmpA with the SA-1 epitope present can insert in the outer membrane with unaltered efficiency when its cell wall binding domain is absent. With this result, we have demonstrated that the OmpA-177 protein domain with SA-1 epitope is a suitable starting point for gene fusions to a second domain with mid-cell affinity.

In **Chapter 4** we study whether the cell division protein FtsQ is able to localize (bring) another unrelated protein domain to mid-cell (the division site). If we would like to localize OmpA to mid-cell using FtsQ, a bridging domain is required to connect the two membranes, since FtsQ is present in the inner membrane, and the OmpA-177 domain in the outer membrane. Initially, the AcrA protein was used as bridging domain. Two issues arose: during the course of our research, the molecular structure of AcrA was published,

and it became clear that it could not function as a bridging protein. Furthermore, fusion proteins between FtsQ and AcrA lost their ability to localize to mid-cell after further extension with extra protein domains (such as OmpA-177). Subsequently, the ALBP protein was chosen as bridging protein. The results are encouraging, as FtsQ-ALBP appears to be present at the division site.

However, after a publication of a study on (yet) another protein, the Pal protein, we decided to change our strategy. Fusions based on FtsQ and ALBP consist of at least five protein domains (GFP, FtsQ, two ALBP bridging domains and the OmpA-177 domain). It is known that as more and more domains are fused in series, the risk that protein domain misfolding and degradation occurs increases rapidly. What makes the Pal protein special is its structural similarity to the cell wall binding domain of OmpA, with the crucial difference that it localizes specifically to the division site. Simply fusing the OmpA-177 domain to the Pal domain could create the desired molecular handle at the division site, with just two protein domains. The experiments in **Chapter 5** however suggest additional complications: *E. coli* can grow without the Pal protein, albeit with a defect in outer membrane attachment to the cell wall. These defects are most severe at the division site. Although we demonstrate that internally, a (OmpA-177)-Pal fusion protein was present at mid-cell in cells lacking native Pal, on the cell surface the fusion protein was not concentrated at the division site. It is tempting to attribute this to the outer membrane attachment problems in these cells. When both the fusion protein and the native Pal protein are present, the native Pal occupies all available spots at the division site, and the opposite of what was aimed at occurs: the molecular handle is now present everywhere on the cell surface, except at the division site. In addition, **Chapter 5** contains results on fusions between various other protein domains and OmpA-177. It seems that when protein domains are fused behind (C-terminal to) OmpA-177 the probability that fusions end up in the outer membrane is greater than when domains are fused in front of (N-terminal to) OmpA-177.

Then the second part of thesis follows, which covers the force measurements. In **Chapter 6** the optical tweezers set-up is described. What makes this optical tweezers set-up special is that the laser light can enter the sample from opposite sides. This allows trapping of particles with a higher refractive index, generating stronger trapping forces on the particle compared to e.g. conventional polystyrene (plastic) particles. In turn, this

allows the measurement of higher forces. Improvements to the set-up, such as the ability to measure at elevated temperatures (required to study bacterial cell division), and the ability to trap and manipulate multiple particles with a single laser beam (so-called ‘time sharing’) are described. The latter can be likened to the circus act ‘plate spinning’, in which multiple plates on sticks are kept rotating by a single person by giving them extra spin from time to time. In the same manner, multiple particles can be kept in place by re-directing a single laser beam rapidly between multiple positions. This technique allows the attachment of two beads to a single bacterium.

Chapter 7 describes experiments in which beads are attached via a DNA molecule to bacteria displaying the SA-1 epitope on their surface. We measure force-extension curves of DNA molecules held between two beads and compare these curves to curves of DNA molecules attached to bacteria. In this way, we obtain information about the properties of the molecular handle on the cell surface. In our experiment, the bead is pulled out of the trap under an angle. Unexpectedly, this has a large effect on the measured forces. However, the results suggest that the molecular handle contained in the outer membrane is pulled off the cell via a membrane tube. Experiments in which the height of the bead with respect to the bacterium is carefully controlled are needed to provide a definitive answer whether tubes are pulled, and if so, whether the presence of the cell-wall binding domain of OmpA can reduce the probability of such an event. These experiments are important, because if the molecular handle can indeed be pulled away from the cell it will have consequences for the experiments aimed at in this thesis.

Finally, in **Chapter 8**, the feasibility of creating a molecular handle at the division site is discussed. An important prerequisite is the ability of the protein domain in the outer membrane to move around to bring the internal domain with mid-cell affinity to the division site. Literature on this topic (although sparse) suggests that this should be possible. In addition, we present alternative approaches explored in this research. These approaches include using a special *E. coli* mutant that has lost its outer membrane and PG cell wall, as well as indirectly trapping a single growing bacterium by attaching beads directly to its poles, and trapping these beads. We recommend a continued search for a fusion protein that localizes to the division site on the cell surface, as the best option to realize the planned experiments on force generation in dividing bacteria.