Cell division in walled Bacillus subtilis and cell wall-lacking Acholeplasma laidlawii

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CHAPTER

7

Summary and Discussion

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Summary

This thesis deals with three questions: (i) How are early and late cell division connected, (ii) can FtsZ constrict the cell membrane, and (iii) do cell division proteins function in other cellular processes.

Link between early and late division proteins

Bacterial cell division is initiated by polymerization of the tubulin homologue FtsZ into a ring-like structure at midcell [1], and after some time, which can last ~20% of the cell cycle [2,3], the late division proteins involved in synthesis of the septal wall are recruited [4]. However, in Gram-positive bacteria it is unclear which proteins in the Z-ring recruit the late cell division proteins. For the Gram-negative bacterium *E. coli*, it was shown that the two membrane anchors of the Z-ring, FtsA and ZipA, function as the main linkers connecting Z-ring with the rest of divisome complex. However, FtsA is not essential in *B. subtilis*, and Gram-positive bacteria do not contain ZipA homologues [5-7]. **In chapter 2**, I found that overexpression of protein SepF, which is the alternative membrane anchor of the Z-ring in Gram-positive bacteria, blocks assembly of late cell division proteins, without interfering with Z-ring assembly, suggesting that SepF might support the recruitment of late cell division proteins. In fact, extra SepF aggregates into large clusters at the cell membrane, triggering large membrane invaginations, and late proteins tend to accumulate at these sites, whereas FtsZ is not recruited to these membrane invaginations. I also showed that SepF, FtsA and EzrA compete for FtsZ binding, and that the balance in this competition is important for the recruitment of late cell division proteins. **In Chapter 4**, I used an artificial FtsZ-anchoring system to test whether FtsZ itself can recruit late cell division proteins. For this I used FtsZ and SepF from *Acholeplasma laidlawii*, a cell wall lacking mycoplasma-like bacterium that I examine in **Chapter 3**. These proteins are able to assemble into Z-rings when expressed in *B. subtilis*, without support of *B. subtilis* own early cell division proteins FtsZ, FtsA, SepF, EzrA and ZapA. Intriguingly, this artificial Z-ring can form a mixed Z-ring together with native *B. subtilis* FtsZ, even in absence of its own membrane anchors *Bs*FtsA and *Bs*SepF. Based

on this artificially mixed Z-ring system, I found that *B. subtilis* FtsZ alone is unable to recruit the late cell division protein Pbp2B, which is a representative for the late cell division proteins. The recruitment of Pbp2B requires the presence of FtsA and EzrA. In addition, the absence of either ZapA or SftA reduced the recruitment of Pbp2B slightly. This data suggests that there are multiple interactions between early and late cell division proteins in *B. subtilis*, but the details of these interactions are still obscure.

Constriction of the cell membrane by FtsZ

The second problem dealt with was the question whether FtsZ can constrict the cell membrane. Currently, there are two models proposed to describe the constrictive force for cytokinesis. One is the so called 'FtsZ-centric' model, in which hydrolysis of GTP bends FtsZ polymers, thereby constricting the Z-ring and pulling the cell membrane inwards [8-13]. However, constriction of only the cell membrane has never been observed in bacteria, and the invagination of the cell membrane is always closely linked to septal peptidoglycan synthesis [14,15], and the consensus is now shifting towards a 'peptidoglycan-centric' model, whereby the membrane is pushed inwards by synthesis of the septal cell wall [16]. To address this, I tried to examine cell division in natural existing, cell wall-lacking bacteria, such as mycoplasmas, which lack peptidoglycan so cell division cannot be driven by peptidoglycan synthesis. Yet these species contain an FtsZ homologue and appear to divide by binary fission [17,18], suggesting that they divide according to the FtsZ-centric model. **In Chapter 3**, I used *Acholeplasma laidlawii* as the mycoplasma model organism because it uses the canonical genetic code for protein translation and grows reasonably fast [19,20]. I identified the *A. laidlawii* gene *acl_0703* as SepF homologue, and found that *A. laidlawii* SepF and FtsZ can form Z-rings in *B. subtilis* devoid of its native division proteins FtsZ, FtsA, SepF, EzrA and ZapA. However, no Z-rings were detected in *A. laidlawii* itself, instead, *Al*FtsZ forms a cluster attached to the *A. laidlawii* cell membrane. In fact, time-lapse movies indicated that *A. laidlawii* propagates by budding instead of binary fission, and the *Al*FtsZ-*Al*SepF clusters initiate bud formation. Interestingly, when *B. subtilis* was transformed into round-shaped cells

by forming either spheroplasts, L-forms or by deleting *mreD*, the Z-rings formed by *Al*FtsZ-*Al*SepF switched to clusters, similar to its localization in *A. laidlawii*. So in this chapter we propose that round-shaped cells cannot divide according to the FtsZ-centric model for the reason that a contractile Z-ring cannot be stably maintained at midcell in round-shaped cells without cell wall anchoring. That also gives an explanation for the failed attempts to stimulate Z-ring triggered cell division in artificial liposomes [21].

Early cell division proteins and other cellular processes

Cell division is considered to be an autonomous process, and the inactivation of early cell division proteins and its regulators does not affect growth, with the exception of *ftsA* mutants that grow slower. **In chapter** 5, I tried to confirm this by measuring the global gene transcription, using RNA-seq, of a clean Δ*ftsA,* Δ*sepF,* Δ*ezrA,* Δ*zapA,* Δ*minC,* Δ*noc* and Δ*sftA* mutant, and also that of FtsZ-depleted cells. This gave a surprising result and showed that all mutants affected the expression of genes unrelated to cell division. Some of the same regulons were affected, including prophages, biofilm formation (*epsA-O*) and the SigB-triggered stress response. In addition, many genes involved in carbon metabolisms were influenced and belonged to the CcpA regulon. However, I could not find a regulator that was associated with the Z-ring, although I have not explored all possibilities. However, when verifying the transcriptome data by qRT-PCR and *lacZ* promoter fusions, I found that the qRT-PCR data was in line with RNA-seq data, whereas in several cases the *lacZ* expression was unaffected. We speculate that the mRNA stability is affected in many of these mutants.

Of the tested mutants, only an *ftsA* deletion showed a clear effect on the overall growth rate. **In Chapter 6**, I tried to determine the genetic factors involved in this, by finding suppressor mutations using transposon mutagenesis. I found that deletion of *yczN* and *yczM*, partially restored growth. YczN and YczM are classified as type I toxins [22,23], but we did not find any indication that the expression of these toxins was affected, and it remains unclear how a Δ*ftsA* mutation affects growth.

Future studies

In this thesis I have answered the question whether FtsZ is able to function as membrane constrictor, and I showed that cell division proteins are involved in processes that are unrelated to cell division, and that FtsZ alone is not able to recruit the late cell division proteins in *B. subtilis*. But my work also generated many new questions. In **Chapter 4**, it was shown that the recruitment of late division proteins requires the presence of FtsA and EzrA. It is now time to elucidate how these proteins bind to the late proteins in detail. The mixed Z-ring system can be a first step as it provides the possibility to make domain deletions and swaps to determine which regions in these proteins are important, and the same can be done for the key late proteins. Another possibility is to use chemical crosslinking to try to find the interaction domains in the early and late cell division proteins. In **Chapter 5**, the inconsistency between qPCR results and some *lacZ* promotor fusions suggested that mRNA stability might be affected in a number of cell division mutants. This should be confirmed by determining the half-life of related mRNAs, using the RNA polymerase inhibitor rifampicin [24]. If this is the case, it might be possible to check strains that have been mutated for different RNases and check which strains shows the same effect as the division mutants. Two-hybrid or pull down experiments can then be used to examine whether there is an interaction between the relevant RNase and cell division protein. However, the RNAseq data showed a plethora of different gene regulation effect that differed between the mutants and it is likely that mRNA instability is not the only explanation. Thus, there is still plenty of research to do in order to explain the transcriptome data. In conclusion, my PhD research have provide a few answers, but it will take many more years of research before we can say that we fully understand bacterial cell division.

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