Investigation of protein interactions in the cell division complex of Bacillus subtilis reveals a possible mechanism for cell size homeostasis

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Appendix

Summary

Investigation of protein interactions in the cell division complex of *Bacillus subtilis* reveals a possible mechanism for cell size homeostasis

Cell division is essential for bacterial survival. The increase in bacterial resistance against conventional antibiotics necessitates the development of new antibiotics. For the development of antibiotics that specifically interfere with cell division, a deeper understanding of the molecular mechanism of cell division is essential. In this work, the cell division machinery, or divisome, of the gram positive, rod shaped bacterium *Bacillus subtilis* is investigated.

The divisome is a protein complex that consists of over a dozen different subunits that localize dynamically to the future division site. The divisome is build up sequentially, starting with four proteins assisting the highly conserved tubulin homologue FtsZ to form a ring-like structure (Z-ring). After a delay, a second wave of proteins (late-proteins) are recruited to the division site, responsible for the synthesis of the crosswall between the daughter cells. In this work, the dynamic protein interactions within the divisome have been investigated with the help of crosslinking mass-spectrometry (chapters 2 and 3) and fluorescence time-lapse microscopy (chapter 4).

To investigate the dynamic protein interactions in the divisome, we made use of protein crosslinking. Succinimidyl esters, such as BAMG, react with amine groups present in lysine residues and thus crosslink residues that are within 25 Å distance from each other. After trypsin digestion, crosslinked peptides can be identified by mass-spectrometry. Crosslinking a protein complex such as the divisome will identify residues from proteins that are in close proximity, which subsequently can be used to reconstruct their position within the complex. To

crosslink the dynamic protein interactions in the divisome we developed a method, described in chapter 2, which allows for the direct crosslinking of cells in their growth medium. A proteome-wide analysis of crosslinked residues identified 82 unique crosslinks between proteins. However, these crosslinks are restricted to mainly abundant complexes, such as ribosomes and RNA polymerases. We report a new interaction orientation between the β' and δ subunits of RNA polymerase.

A more directed approach to investigate protein interactions in the divisome was taken by studying the interaction between FtsZ and the membrane anchor SepF, described in chapter 3. SepF assists FtsZ in Z-ring formation and together these proteins form a microtubule structure *in vitro*. The interaction between purified proteins was investigated using crosslinking mass-spectrometry. Based on the crosslinks identified, we propose that SepF dimers possibly form a tetramer. *In silico* docking simulations suggest that the FtsZ C-terminal peptide binds to the globular surface of a SepF dimer. The absence of crosslinks in the FtsZ C-terminal peptide could indicate that it is not surface exposed, possibly by being trapped between two SepF dimers forming a tetramer.

Finally, we also address the delay between Z-ring formation and the recruitment of late-proteins, described in chapter 4. Fluorescent fusions of ZapA (Z-ring) and Pbp2B (late-protein) were imaged in growing cells with time-lapse microscopy. We observed a large variation in the delay between Z-ring formation and late-protein recruitment and this variation was further investigated in the context of size-homeostasis. Bacteria such as *B. subtilis* and *Escherichia coli* grow a fixed amount per cell cycle, as an 'adder', which results in a decrease of variation in cell size in the population. Since larger cells grow faster than smaller cells, larger cells will also have to divide quicker. We observe that the variation in delay between Z-ring formation and late-protein recruitment negatively correlates with

cell size, as larger cells have a shorter delay than smaller cells. We also observe that a higher concentration of Z-ring proteins at the division site results in a faster cell division, and that larger cells recruit more Z-ring proteins and recruit Z-ring proteins faster. This size-dependent Z-ring accumulation therefore could be the molecular mechanism of cell size homeostasis in *B. subtilis*.

In this thesis we investigated the dynamic protein interactions in the divisome of *B. subtilis*. Both the proposed interaction site between FtsZ and SepF and the size-dependent accumulation of Z-ring proteins increase our insight in the mechanism of cell division, thereby indirectly contributing to the development of novel antibiotics that could disrupt cell division.

Samenvatting

Onderzoek naar eiwit interacties in het celdelingscomplex van *Bacillus subtilis* onthult een mogelijk mechanisme voor celgrootte homeostase.

Celdeling is essentieel voor het voortbestaan van bacteriën. Toenemende bacteriële resistentie tegen conventionele antibiotica maakt het ontwikkelen van nieuwe antibiotica noodzakelijk. Voor de ontwikkeling van antibiotica die specifiek celdeling verstoren is meer inzicht naar het moleculaire mechanisme van celdeling essentieel. Het celdeling machinerie, of divisoom, van de gram positieve, staafvormige bacterie *Bacillus subtilis* staat centraal in dit proefschrift.

Het divisoom is een eiwit complex dat uit meer dan een dozijn verschillende componenten bestaat die dynamisch lokaliseren naar het toekomstige delingsvlak. Het divisoom wordt sequentieel opgebouwd, en deze opbouw start met de tubiline homoloog FtsZ die geassisteerd door vier andere eiwitten een ringvormige structuur vormt, de Z-ring. Na een vertraging zal een tweede set eiwitten, late-eiwitten, worden gerekruteerd naar het delingsvlak. Deze late-eiwitten zijn verantwoordelijk voor de synthese van het delingsseptum tussen de dochtercellen. In dit proefschrift onderzoeken wij deze dynamische eiwit interacties in het divisoom met behulp van crosslinking-massaspectrometrie (hoofdstuk 2 en 3) en fluorescentie time-lapse microscopie (hoofdstuk 4).

Om de dynamische eiwit interacties in het divisoom te bestuderen maken wij gebruik van eiwit crosslinking. Succinimidyl esters, zoals BAMG, reageren met amine groepen zoals die aanwezig in lysine residuen en crosslinken zo residuen die minder dan 25Å van elkaar verwijderd zijn. Na trypsinedigestie worden de gecrosslinkte peptiden geïdentificeerd met massaspectrometrie. Het crosslinken van een eiwitcomplex zoals het divisoom laat zien welke residuen van eiwitten zich

dichtbij elkaar bevonden, en deze crosslinks kunnen vervolgens worden gebruikt om hun positie in het complex te reconstrueren. Om de dynamische interacties in het divisoom te kunnen crosslinken hebben wij een methode ontwikkeld, beschreven in hoofdstuk 2, waarbij cellen direct in het groeimedium kunnen worden gecrosslinkt. Een proteoom-brede analyse van gecrosslinkte residuen resulteerde in 82 unieke crosslinks tussen eiwitten. Echter, deze crosslinks zijn voornamelijk aanwezig in abundante eiwitcomplexen zoals ribosomen en RNA polymerasen. We rapporteren een nieuwe interactie oriëntatie tussen de β' en δ subunits van RNA polymerase.

Om toch meer inzicht te krijgen in eiwitinteracties in het divisoom onderzochten we de interactie tussen FtsZ en het membraan anker SepF, beschreven in hoofdstuk 3. SepF assisteert FtsZ in het vormen van de Z-ring en samen vormen deze eiwitten een microtubulus structuur *in vitro*. De interactie tussen opgezuiverde eiwitten werd onderzocht met crosslinking-massaspectrometrie. Aan de hand van de gevonden crosslinks postuleren wij dat SepF dimeren mogelijk een tetrameer vormen. *In silico* docking simulaties suggereren dat de FtsZ C-terminale peptide het globulaire oppervlak van de SepF dimeer bindt. De afwezigheid van crosslinks in de FtsZ C-terminale peptide zou kunnen indiceren dat deze ontoegankelijk is voor de crosslinker, en mogelijk opgesloten is tussen twee SepF dimeren in.

Tot slot adresseren we in hoofdstuk 4 ook de vertraging tussen de Z-ring formatie en het rekruteren van de late-eiwitten. Fluorescente fusies van ZapA (Z-ring) en Pbp2B (late-eiwitten) werden gevolgd in groeiende cellen met behulp van time-lapse microscopie. We observeerden een zeer variabele vertraging en deze variatie onderzochten we vervolgens binnen de context van celgrootte homeostase. Bacteriën zoals *B. subtilis* en

Escherichia coli groeien een vaste hoeveelheid per celcyclus, als een 'adder', wat resulteert in een afname van variatie in celgrootte binnen de populatie. Aangezien grote cellen sneller groeien dan kleine moeten deze daardoor ook eerder delen. Wij vonden dat de variatie in vertraging tussen Z-ring formatie en rekruteren van late-eiwitten negatief correleert met celgrootte, waarbij grote cellen een kortere vertraging hebben dan kleine cellen. Ook zien we dat een hogere concentratie Z-ring eiwitten zorgt voor een snellere celdeling, en dat grote cellen meer en sneller Z-ring eiwitten rekruteren. Deze grootte afhankelijke Z-ring accumulatie verzorgt zo mogelijk het mechanisme voor celgrootte homeostase in *B. subtilis*.

In dit proefschrift onderzochten we de dynamische interacties in het divisoom van *B. subtilis*. Zowel de mogelijke interactie site tussen FtsZ en SepF als de grootte-afhankelijke accumulatie van celdelingseiwitten geven meer inzicht in de mechanistische werking van het celdelingsmachinerie. Deze bevindingen dragen zo mogelijk indirect bij aan de ontwikkeling van nieuwe antibiotica die celdeling kunnen verstoren.

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