Instituto Juan March
de Estudios e Investigaciones

146 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Manufacturing Bacteria: Design, Production and Assembly of Cell Division Components

Organized by

P. de Boer, J. Errington and M. Vicente

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Introduction
M. Vicente

The process of division (cytokinesis) is vital to all living cells. In prokaryotes, the process initiates with the assembly of a key division protein, FtsZ, into a polymeric ring (Z-ring) just underneath the cytoplasmic membrane at a precisely selected site to ensure that partition satisfies the physiological requirements of either cell division or differentiation. Other essential division proteins are recruited to this ring in a specific order, resulting in a mature division apparatus (septal ring, septator or divisome) which mediates cell constriction (Margolin. 2000. *FEMS Microbiol. Rev.* 24: 531-548).

Significant progress in the molecular description of prokaryotic cytokinesis has taken place recently and includes:

i) Division proteins FtsZ and FtsA share biochemical properties and tertiary structures with the major eukaryotic cytoskeletal proteins (tubulin and actin, respectively), indicating common ancestries (Löwe and Amos. 1998. *Nature* 391: 203-206; van den Ent and Löwe. 2000. *EMBO J.*19:5300-5307).

ii) Advances in genomics have revealed an almost universal presence of FtsZ in a wide variety of prokaryotic organisms (eubacteria and archaea), as well as in eukaryotic organelles (plastids and some mitochondria). In addition, many of the other division genes, as well as their chromosomal arrangement, have been conserved among dissimilar prokaryotic phyla (Tamames *et al*, 2001. *TIG* 17: 124-126).

iii) A combination of genetic, biochemical, and microscopic techniques has provided a stream of new information on the spatial and temporal regulation of the key initiating event (Z-ring assembly), the composition and order of assembly of the mature apparatus, and on the coupling of cell constriction with synthesis of the peptidoglycan portion of the cell wall (Hale and de Boer. 1999. *J. Bacteriol.* 181: 167-176; Meinhardt and de Boer. 2001. *Proc. Natl. Acad. Sci. USA* 98: 14202-14207; Erickson, 2001. *Curr. Opinion Cell Biol.* 13:55–60).

iv) Activities of the division apparatus have been shown to be intimately coupled with sister chromatid separation, as well as with cell cycle progression and cell differentiation in developmental model organisms (Edwards and Errington. 1997. *Mol. Microbiol.* 24:5 905-5915).

These developments are not only crucial to our understanding of bacterial physiology, but will have far-reaching consequences on the future management of important social issues as diverse as the conservation of the environment, and public health.

Miguel Vicente

Session 1: Design: structure and function of septum components
Chair: E. Lucile White

The (4→3) and (3→3) peptidoglycans, the acyltransferases of the SxxK superfamily and the bacterial life cycle

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The bacterial acyltransferases of the SxxK superfamily vary enormously in sequence and function, with conservation of particular amino-acid-groups and all-α and α/β folds. They occur as independent entities (free-standing polypeptides) and as modules linked to other polypeptides (protein fusions). SxxK D,D-acyltransferases penicillin-binding proteins (PBPs) are implicated in the synthesis of wall peptidoglycans of the (4→3) type. They invariably bear the motifs SxxK, SxN(D), KT(S)G. They are components of morphogenetic apparatuses which, as a whole, control multiple parameters such as shape and size and allow the bacterial cells to enlarge and duplicate their particular pattern. Class A PBP fusions comprise of glycosyltransferase module fused to an SxxK acyltransferase of class A. Class B PBP fusions comprise a linker, *i.e.* protein recognition, module fused to a SxxK acyltransferase of class B. Free standing PBPs are auxiliary cell-cycle proteins. SxxK acyltransferases also exit which are indistinguishable from the PBP fusions in motifs and membrane topology, but they resist penicillin. They are referred to as Pen^r protein fusions. Plausible hypotheses are put forward on the roles that the Pen^r protein fusions acting as L,D-acyltransferases, may play in the (3→3) peptidoglycan-synthesizing molecular machines of *Enterococcus faecium*, *Escherichia coli* and *Mycobacteria spp*. Shifting the wall peptidoglycan from the (4→3) type to the (3→3) type could help *Mycobacterium tuberculosis* and *Mycobacterium leprae* survive in a penicillin-resistant manner.

References:

Goffin C. and J.-M.Ghuysen. Microbiol. Mol. Biol. Rev., 2002, 66, (December issue 4)

Structures of FtsZ:SulA and FtsN

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We have solved the X-ray crystal structure of the SOS cell division inhibitor SulA with and without FtsZ bound to it. We have also isolated the folded domain of the periplasmic domain of FtsN and solved its structure by NMR.

Upon DNA damage, RecA protein induces the SOS response that leads to activation of a large number of genes, mainly involved in DNA repair, via activation of auto-proteolysis of the repressor lexA. In *E. coli*, *sulA* is derepressed as part of the SOS response. SulA has been found to induce filamentation and binds directly to FtsZ, the key component of cell division. The crystal structure of SulA from *P. aeruginosa* shows a tight dimer and demonstrates surprising homology to the ATPase domain of RecA. The 2.1Å crystal structure of the complex of FtsZ and SulA from *P. aeruginosa* shows again a dimer with two SulA molecules binding two FtsZ molecules in a Ftsz:SulA:SulA:FtsZ arrangement. SulA binds to the T7-loop side of FtsZ, perfectly blocking one of the two protofilament contacts. No major structural changes occur upon complex formation on either FtsZ or SulA. Contacts between SulA and FtsZ are restricted to the C-terminal domain of FtsZ, no contacts exists between SulA and the GTPase domain of FtsZ. FtsZ contains GDP but in a different crystal form of the complex has also been observed containing GTP highlighting the fact that the nucleotide state in the complex is not important. FtsZ and SulA are arranged in a way that would make it possible to grow FtsZ protofilaments in exactly opposing directions as SulA only blocks one of the two FtsZ protofilament contacts.

FtsN is a late recruit to the divisome, the protein complex that forms after FtsZ polymerisation during cell division. FtsN from *E. coli* contains about 30 cytosolic residues, a single trans-membrane helix and a periplasmic domain of about 250 residues. While trying to obtain structural information of the C-terminal, periplasmic domain we noticed by using N^{15} labelled protein and NMR that a large proportion of the periplasmic domain, more that 200 residues, are disordered in solution. Only residues 243-319 are in fact ordered. We found that the structered, C-terminal part of FtsN shows some sequence homology to N-acetylmuramoyl-L-alanine amidases (LytC related family) that are involved in modifying the murein layer. The structure of the folded part of FtsN resembles U1A and related proteins. Based on these findings we propose that FtsN, anchored in the inner membrane, bridges periplasmic space with its very long linker segments and has a role in organising or making contact to the murein layer.

FtsZ folding, activation, self-association and assembly

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We have investigated the folding and assembly processes (1) of purified archaeal and bacterial FtsZ proteins, in comparison with the eukaryotic structural homologue tubulin. The guanidinium chloride-induced unfolding of FtsZ from *M. jannaschii* and *E. coli* is essentially reversible, unlike tubulin. Isolated FtsZ polypeptide chains are thus capable of spontaneous folding, which has been confirmed by their GTP-dependent self-assembly. On the other hand, tubulin does not fold spontaneously, but with the assistance of eukaryotic chaperonin CCT. It is not known whether FtsZ folding may be further facilitated by molecular chaperones. Analysis of the extensive tubulin insertion loops in the tubulin/FtsZ common core, and of the contacts in model microtubules and in model CCT-tubulin complexes, suggest that the loop insertions of tubulin and its need of a CCT-assisted folding coevolved with the lateral association interfaces responsible for extended two-dimensional polymerization into microtubules (2). This proposal is supported by comparison of microtubules with the distinct FtsZ polymers (see below).

The activation for assembly of FtsZ from *M. jannaschii* by the bound nucleotide was studied with molecular dynamics and site-directed mutagenesis. The molecular dynamics indicated that the γ-phosphate of GTP induces a conformational perturbation in loop-T3 (Gly88-Gly99 segment), in a position structurally equivalent to switch II of Ha-*ras*-p21. In the simulated GTP-bound state, loop-T3 is pulled by the γ-phosphate into a more compact conformation than with GDP, related to that observed in α and β tubulin. A nucleotide-induced structural change in loop-T3 was confirmed by mutating Thr92 into Trp (T92W-W319Y FtsZ). This tryptophan (12 Å away from the γ-phosphate) shows large differences in fluorescence emission, depending on whether GDP or GTP is bound to FtsZ monomers. Loop-T3 is located at a side of the contact interface between two FtsZ monomers in the current model of FtsZ filament. Such a structural change may bend the GDP-filament upon hydrolysis by pushing against helix-H8 of next monomer, thus generating force on the membrane during cell division. A related curvature mechanism may operate in tubulin activation (3).

The magnesium induced self-association of FtsZ from *E. coli* was studied with sedimentation equilibrium and velocity. The oligomer formation mechanism is best described as an indefinite self-association, and compatible with a linear single-stranded arrangement of FtsZ monomers with a tubulin-like 4 nm spacing. It was thus proposed that *E coli* FtsZ linear oligomerization involves the same interaction as protofilament formation in cooperative bidimensional assembly. Whereas FtsZ oligomerization was weakly modified by the GDP or GTP nucleotide, FtsZ assembly was induced by GTP and inhibited by GDP (4).

The self-association of FtsZ from *M. jannaschii* proceeds in a nucleotide unregulated fashion. The reversible assembly of FtsZ from *M. jannaschii* has been quantified by sedimentation. It is cooperative, and it is regulated by nucleotide, magnesium and temperature (S. Huecas and J.M. Andreu, unpublished results; 5). The assembly of FtsZ is polymorphic. FtsZ from *M. jannaschii* assembles into filamentous cable like polymers, which hydrolyze the GTP in the solution and then disassemble. A GlySerHis6 purification tag at the C-end of FtsZ enhances laminar polymerization. The mutant W319Y-FtsZ-His6 is an inactivated GTPase, which assembles into large ordered sheet polymers, in a GTP and magnesium regulated equilibrium (M.A. Oliva et al. and J.M. Andreu, unpublished results; 6). These sheets are made of double stranded filaments of two symmetrical tubulin-like protofilaments. This thick filament is a repeated pattern in the assembly of FtsZ from *M. jannaschii* (7). The lateral contacts between these symmetric protofilaments cannot form the larger two-dimensional polymers, since the thick filaments have different contact interfaces. This is in contrast with microtubule assembly, in which the protofilaments are parallel and practically the same lateral contacts are propagated in each tubulin molecule across the polymer. Purified bacterial FtsZ typically forms polymers made of few protofilaments, except with polymer-binding additives (8). We favor the notion that the dynamic FtsZ polymers in the Z ring form by cooperative assembly of FtsZ molecules into polymers of limited two-dimensionality, such as double stranded filaments, which are stabilized by the other septal components which bind them.

References and notes:

1. Association refers to the formation of quaternary structure and oligomers, whereas macromolecular assembly refers to the formation of large polymers and biological superstructures. Jaenicke R, Lilie H (2000) Adv Protein Chem 53, 329-401.
2. Andreu JM, Oliva MA, Monasterio O (2002) J Biol Chem papers in press M20623200
3. Diaz JF, Kralicek A, Mingorance J, Palacios JM, Vicente M and Andreu JM (2001) J Biol Chem 276, 17307-17315.
4. Rivas G, Lopez A, Mingorance J, Ferrandiz MJ, Zorrilla S, Minton Ap, Vicente M, Andreu JM (2000) J Biol Chem 275, 11740-11749.
5. See poster by S Huecas at this workshop
6. See poster by MA Oliva at this workshop
7. Löwe J and Amos LA (1999) EMBO J 18, 2364-2371.
8. Lu C, Stricker J, Erickson HP (1998) Cell Motil Cytoskel 40, 71-86.

Dynamics of FtsZ assembly

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1. The assembly dynamics of *E. coli* FtsZ have been demonstrated by FRAP of bacterial cells expressing FtsZ-gfp. Our previous study reported that FtsZ was exchanging in and out of the Z-ring with a half time of 30 sec (1). We have now found that the exchange is even faster, with a half time of 8 sec. The exchange rate seems to be strongly affected by the method of immobilizing the bacteria. The 30 sec exchange was found for bacteria stuck to polylysine coated cover slips, while the 8 sec exchange was found for bacteria immobilized on an agar pad. We believe the agar pad is gentler and best preserves the bacteria, and the 8 sec exchange represents the physiological exchange.

2. We repeated the FRAP measurements on *B. subtilis* and obtained the same 8 sec exchange half time for the Z-ring. We also determined that only 30% of the FtsZ is in the Z-ring (the remainder is cytoplasmic), the same as found for *E. coli*. This has a very important implication. For *E. coli* we estimated that the Z-ring was only 6-7 protofilaments thick on average. However, *B. subtilis* has only 1/3 as much FtsZ as *E. coli* (5,000 vs 15,000 molecules per cell (2,3)), so its Z-ring should be only 2 protofilaments thick on average. This implies that a Z-ring of only 2 protofilaments can function for cell division.

3. We have done FRAP on cytoplasmic FtsZ and found that the recovery was very fast. The first frame after the FRAP (~0.5 sec) showed bleaching of most of the FtsZ in one half of the cell, but 1 sec later the two halves seemed fully equilibrated. The rapid recovery is consistent with cytoplasmic FtsZ diffusing as single subunits or short protofilaments.

4. Assembly dynamics *in vitro* have not been measured directly, but the rate of GTP hydrolysis, ~ 5 GTP per min per FtsZ, provides an indirect measure. This steady state hydrolysis requires that the GDP exchange for GTP following hydrolysis. Although there is one report that GTP can exchange directly into intact protofilaments(4), we favor the tubulin paradigm, where exchange can only occur when the GTP-binding interface is exposed. In this paradigm GTP exchange requires subunit release or protofilament fragmentation, and the steady state GTP hydrolysis will equal the rate at which interfaces are exposed. The *in vitro* GTPase of 5 per min corresponds closely to 8 sec half time for *in vivo* polymer turnover.

5. In order to explain the assembly of protofilaments one subunit thick, we proposed a model of isodesmic assembly (5). One prediction of this theory is that the K_D should be ~ 3 nM, which would mean that there should be substantial assembly at FtsZ concentrations above 10-

30 nM. We have use Isothermal Titration Calorimetry to measure assembly as a function of protein concentration. In Mg and GTP there was no assembly until a concentration of 0.4 μM FtsZ, after which assembly increased abruptly and plateaued above 0.6 μM. This behavior appears characteristic of a cooperative assembly, not isodesmic. So far, we have no model that can explain the apparent cooperative assembly of protofilaments one subunit thick.

References:

1. Stricker, J., Maddox, P., Salmon, E. D., and Erickson, H. P. (2002) PNAS 99, 3171-3175
2. Feucht, A., Lucet, I., Yudkin, M. D., and Errington, J. (2001) Mol Microbiol 40, 115-125.
3. Lu, C., Stricker, J., and Erickson, H. P. (1998) Cell Motility & the Cytoskeleton 40, 71-86
4. Mingorance, J., Rueda, S., Gomez-Puertas, P., Valencia, A., and Vicente, M. (2001) Mol Microbiol 41, 83-91.
5. Romberg, L., Simon, M., and Erickson, H. P. (2001) J Biol Chem 276, 11743-11753.

The concentrations of the division ring proteins FtsZ, FtsA and ZipA are constant during the *Escherichia coli* cell cycle

Sonsoles Rueda, Miguel Vicente and Jesús Mingorance

The cellular levels of essential cell division proteins FtsZ, FtsA and ZipA during the cell cycle have been measured using synchronous cultures obtained by the membrane elution technique (Helmstetter, 1969) and found to be constant.

During the cell cycle of exponentially growing *E. coli* cells there are several events that occur in an ordered and periodic fashion; among them assembly of the Z-ring and cell division. This suggests that the levels of some proteins might oscillate during the cycle in order to regulate these processes, and in fact it has been shown that the levels of the ftsZ transcripts oscillate in a cell-cycle dependent manner (Garrido et al., 1993; Zhou and Helmstetter, 1994). On the contrary, no evidence of a developmental program has been found by examination of protein levels in the *E. coli* cell cycle (Lutkenhaus et al., 1979; Wientjes et al., 1983; Hupp et al., 1994; Bechtloff et al., 1999). We have analyzed by Western blotting the relative concentrations of the division proteins during one complete cell cycle and found them constant.

The assembly of these proteins in the septal ring was analyzed in the same cultures by immunofluorescence microscopy. In a culture with a doubling time of 49 minutes assembly of the Z-ring started around minute 25, and was seen first as a diffuse ring that later became more compact and sharply defined. The localization of FtsA and ZipA is known to depend on Z-ring assembly, and therefore it is thought to occur after the localization and assembly of FtsZ. Immunofluorescence microscopy showed that these two proteins localized into the division ring nearly at the same time that FtsZ (whithin the resolution of the technique), and followed the same time course.

We conclude that the periodicity in the assembly of these proteins at the division ring is not due to periodic oscillations in the cell division protein levels but to other undescribed mechanisms.

References:

-Bechtloff, D., Grünenfelder, B., Åkerlund, T and Nordström, K. (1999) Analysis of protein synthesis rates after initiation of chromosome replication in *Escherichia coli*. J. Bacteriol. 181: 6292-6299.
-Garrido, T., Sánchez, M., Palacios, P., Aldea, M. and Vicente, M. (1993) Transcription of ftsZ oscillates during the cell cycle of *Escherichia coli*. EMBO J. 12: 3957-3965.
-Helmstetter, C. E. (1969) Methods for studying the microbial division cycle. Methods Microbiol. 1: 327-363.
-Hupp, T. R., Keasling, J. D., Cooper, S. and Kaguni, J. M. (1994) Synthesis of DnaK protein during the division cycle of *Escherichia coli*. Res. Microbiol. 145: 99-109.

-Lutkenhaus, J., Moore, B. A., Masters, M. and Donachie, W. D. (1979) Individual proteins are synthesized continuously throughout the *Escherichia coli* cell cycle. J. Bacteriol. 138: 352-360.
-Wientjes, F. B., Olijhoek, T. J. M., Schwarz, U. and Nanninga, N. (1983) Labeling pattern of major penicillin-binding proteins of *Escherichia coli* during the division cycle. J. Bacteriol. 153: 1287-1293.
-Zhou, P. and Helmstetter, C. (1994) Relationship between ftsZ gene expression and chromosome replication in *Escherichia coli*. J. Bacteriol. 176: 6100-6106.

Session 2: The use in symbiosis of the bacterial division apparatus
Chair: Jo-Anne R. Dillon

Biochemistry of *Mycobacterium tuberculosis* FtsZ

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FtsZ is one of the key elements in the process of bacterial cell division (de Boer, Cook et al. 1990; Lutkenhaus and Addinall 1997). Bacteria divide by first selecting potential division sites with the MinCDE system. FtsZ is the first non-regulatory element to appear at the septum site, and the function of the septum has been shown to depend on correct FtsZ function. FtsZ is a cytosolic protein that polymerizes in a GTP dependent manner and has been shown to be the bacterial tubulin homolog (Erickson 1995; Lowe 1998; Nogales, Downing et al. 1998; Nogales, Wolf et al. 1998). The sequence similarity between FtsZ and tubulin, however, is generally low (<20% identity). However, both proteins share a short stretch of sequence, called the tubulin-signature motif, which is involved in GTP binding and hydrolysis. They also have a very similar three-dimensional fold, as shown by electron crystallography of tubulin sheets and the X-ray crystal structure of FtsZ from *Methanococcus jannaschii* (Lowe 1998; Nogales, Wolf et al. 1998). *In vitro*, both proteins first assemble into linear protofilaments and these protofilaments then associate into larger structures such as sheets and tubes. FtsZ is a very promising target for new antimicrobial drugs because of its central role in bacterial cell division.

Southern Research Institute has an extensive background in designing inhibitors of eukaryotic tubulins (Temple, Rose et al. 1968; Temple, Rose et al. 1970; Temple 1990). Over the course of a thirty-year program to design synthetic inhibitors of tubulin polymerization as anticancer compounds, thousands of these analogs have been prepared at Southern Research Institute and screened as polymerization inhibitors (Temple 1990). These compounds were shown to compete with colchicine for its binding site (Bowdon, Waud et al. 1987). Some of our lead agents did not derive out of the anticancer drug design program, but were developed in a separate program with the United States Army relating to the preparation of new antimalarial agents (Temple, Rose et al. 1968; Temple, Rose et al. 1970). One compound in this class has been reported as curative for *P. berghei* in a murine model, and generally, these compounds have been reported to show low toxicity in mice. Our initial hypotheses were that this class of compounds, the 2-alkoxycarbonylaminopyridines, should inhibit *M. tuberculosis* FtsZ and that the low degree in sequence homology between FtsZ and tubulin was consistent with finding compounds specific for TB. Over 200 compounds from the Southern Research Institute repository were submitted to the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (National Institute of Health, USA) for screening and several were found to have antimicrobial activity (White, Suling et al. 2002). Two compounds, SRI-7614 and SRI-3072, were selected for further study. Both compounds inhibited *M. tuberculosis* FtsZ polymerization and GTP hydrolysis. SRI-7614 was equipotent against tubulin, whereas

SRI-3072 failed to inhibit tubulin polymerization. When *M. bovis* (BCG) was treated with SRI-3072 for 1 hour, the elongated cells contained multiple DNA staining sites. Clearly, SRI-3072 disrupted normal cell division, consistent with our hypothesis that these compounds were acting on FtsZ.

We recognized that a key component in improving the selective activity of our compounds for *M. tuberculosis* FtsZ was gaining a better understanding of the physical and biochemical properties of FtsZ. Electron micrographs showed that *M. tuberculosis* FtsZ polymerized to form long strands (3.7-nm diameter) that combined as protofilaments two subunits wide (White, Ross et al. 2000). Although these protofilaments often combined to form bundles or sheets, the preferred conformation seemed to be two subunits wide. Polymerization required GTP and magnesium and was markedly reduced at neutral or alkaline pH.

Numerous studies with *E. coli* FtsZ and tubulin have shown that polymerization and GTP hydrolysis are connected, but at least for tubulin they may not be kinetically linked (Carlier and Pantaloni 1981; Carlier, Didry et al. 1987; de Boer, Cook et al. 1990; Bramhill and Thompson 1994; Erickson and Stoffler 1996; Mukherjee and Lutkenhaus 1998; Rivas, Lopez et al. 2000). Understanding the link between *M. tuberculosis* FtsZ GTP hydrolysis and assembly should provide important insights into the molecular processes involved in the *in vivo* function of FtsZ. Mtb FtsZ appears to be uniquely suited to exploring this function. Unlike *E. coli* FtsZ, the only other extensively studied FtsZ, all stages of assembly, steady state, and depolymerization, occur on a convenient time scale. Unlike tubulin, all three phases occur after the addition of GTP, with only modest changes in the initial conditions altering the length and rate of each phase. The addition of KCl to the reaction increases the initial rate of polymer formation and the rate of GTP hydrolysis. When KCl is absent, there is a pronounced uncoupling between filament elongation and GTP hydrolysis as a function of FtsZ concentration. This uncoupling does not occur when KCl is added to the reaction. Other aspects of the GTP hydrolysis and protofilament formation will be presented.

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References:

Bowdon, B. J., W. R. Waud, et al. (1987). "Comparison of 1,2-dihydropyrido[3,4-b]pyrazines (1-deaza-7,8-dihydropteridines) with several other inhibitors of mitosis." Cancer Research **47**(6): 1621-6.

Bramhill, D. and C. M. Thompson (1994). "GTP-dependent polymerization of Escherichia coli FtsZ protein to form tubules." Proc Natl Acad Sci U S A **91**(13): 5813-7.

Carlier, M. F., D. Didry, et al. (1987). "Microtubule elongation and guanosine 5'-triphosphate hydrolysis. Role of guanine nucleotides in microtubule dynamics." Biochemistry **26**(14): 4428-37.

Carlier, M. F. and D. Pantaloni (1981). "Kinetic analysis of guanosine 5'-triphosphate hydrolysis associated with tubulin polymerization." Biochemistry **20**(7): 1918-24.
de Boer, P. A., W. R. Cook, et al. (1990). "Bacterial cell division." Annual Reviews of Genetics **24**: 249-74.
Erickson, H. P. (1995). "FtsZ, a prokaryotic homolog of tubulin?" Cell **80**(3): 367-70.
Erickson, H. P. and D. Stoffler (1996). "Protofilaments and rings, two conformations of the tubulin family conserved from bacterial FtsZ to alpha/beta and gamma tubulin." J Cell Biol **135**(1): 5-8.
Lowe, J. (1998). "Crystal structure determination of FtsZ from Methanococcus jannaschii." Journal of Structural Biology **124**(2-3): 235-43.
Lutkenhaus, J. and S. G. Addinall (1997). "Bacterial cell division and the Z ring." Annual Reviews of Biochemistry **66**: 93-116.
Mukherjee, A. and J. Lutkenhaus (1998). "Dynamic assembly of FtsZ regulated by GTP hydrolysis." Embo J **17**(2): 462-9.
Nogales, E., K. H. Downing, et al. (1998). "Tubulin and FtsZ form a distinct family of GTPases." Nature Structural Biology **5**(6): 451-8.
Nogales, E., S. G. Wolf, et al. (1998). "Structure of the alpha beta tubulin dimer by electron crystallography [published erratum appears in Nature (1998) **393**, 191]." Nature **391**(6663): 199-203.
Rivas, G., A. Lopez, et al. (2000). "Magnesium-induced linear self-association of the FtsZ bacterial cell division protein monomer. The primary steps for FtsZ assembly." J Biol Chem **275**(16): 11740-9.
Temple, C. G. (1990). Synthesis and Evaluation of 1-Deaza-7,8-dihydropteridines and Ring Analogues. Chemistry and Biology of Pteridines. H.-C. Curtis, S. Ghisla and N. Blau. Berlin, Walter de Gruyter: 1009-1014.
Temple, J., C., J. D. Rose, et al. (1968). "Synthesis of potential antimalarial agents. II. 6,8-disubstituted pyrido[2,3-b]pyrazines." Journal of Medicinal Chemistry **11**: 1216-8.
Temple, J., C., J. D. Rose, et al. (1970). "Synthesis of potential antimalarial agents. V. pyrido[2,3-b]pyrazines." Journal of Medicinal Chemistry **13**: 853-7.
White, E. L., L. J. Ross, et al. (2000). "Slow polymerization of Mycobacterium tuberculosis FtsZ." Journal of Bacteriology **182**(14): 4028-34.
White, E. L., W. J. Suling, et al. (2002). "2-Alkoxycarbonylaminopyridines: inhibitors of Mycobacterium tuberculosis FtsZ." J Antimicrob Chemother **50**(1): 111-4.

Genomics and the cell division process in the chlamydiae

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The chlamydiae are obligate intracellular bacteria that cause disease in animals and humans. All chlamydial species undergo a unique developmental cycle involving two major alternating forms: the infectious, metabolically inactive, elementary body (EB) and the noninfectious, metabolically active, reticulate body (RB). Molecular genetic studies of these unique organisms are hampered by the lack of a genetic system for introducing and analyzing mutants. In light of this limitation, the completion and analysis of chlamydial genome sequences become very important. The sequences of five chlamydial genomes have been published, and additional projects are near completion (6). These data have provided key information about chlamydial biology both within and among the different chlamydial species. One area in which this is clearly evident is the study of cell division and cell wall assembly processes. Although chlamydial EBs are highly resistant to physical disruption, peptidoglycan (PG) has not been identified in the chlamydial cell wall. Instead, the structural integrity of the EB cell wall is supplied by a unique set of cysteine-rich outer membrane proteins (4). The lack of detectable PG is paradoxical, as each completed genome sequence encodes a nearly complete PG-synthesis pathway (3,6). The fact that productive chlamydial development is acutely sensitive to inhibitors of PG synthesis adds another twist to the paradox. Treatment of chlamydia-infected cells with beta-lactam antibiotics results in the production of aberrant enlarged RBs that persist in a nonculturable state (3,5). Removal of the antibiotic from the culture medium leads to the resumption of the productive developmental cycle. Therefore while both genomics data and experimental studies indicate that some form of PG should be present within chlamydiae, its location and function remain uncharacterized.

Genomics analyses also indicate that the chlamydial cell division process is unique among bacteria. While chlamydial binary fission is evident microscopically, the process is poorly understood. All sequenced chlamydial genomes lack the gene encoding FtsZ, a ring-forming protein that is critical for cytokinesis in most bacterial systems (1). Our laboratory identified and partially characterized a unique chlamydial structure, labeled SEP, which is present at the plane of RB division within dividing cells (2). SEP is redistributed during EB maturation and during aberrant growth. The localization of SEP within dividing chlamydiae appears similar to the FtsZ rings observed in other bacteria. We continue to investigate the molecular nature of SEP and the role of SEP in the chlamydial division process.

References:

1. Addinall, S.G. and B. Holland. 2002 The tubulin ancestor, FtsZ, draughtsman, designer and driving force for bacterial cytokinesis. J Mol. Biol. 318: 219-236.
2. Brown, W. J. and D. D. Rockey. 2000. Identification of an antigen localized to an apparent septum within dividing chlamydiae. Infect. Immun. 68: 708-715.
3. Ghuysen, J.M. and C. Goffin. 1999. Lack of cell wall peptidoglycan versus penicillin sensitivity: new insights into the chlamydial anomaly. Antimicrob Agents Chemother 43:2339-2344
4. Hatch, T.P. 1996. Disulfide cross-linked envelope proteins: the functional equivalent of peptidoglycan in chlamydiae? J. Bacteriol. 178:1-5.
5. Matsumoto, A. and G.P. Manire. 1970. Electron microscopic observations on the effects of penicillin on the morphology of *Chlamydia psittaci*. J. Bacteriol. 101:278-285.
6. Rockey, D. D., J. Lenart, and R. S. Stephens. 2000. Genome sequencing and our understanding of chlamydiae. Infect. Immun. 68: 5473-5479.

Endoskeletal function and asymmetrical rotation of *Borrelia burgdorferi* periplasmic flagella

Nyles W. Charon

Spirochetes have a unique structure, and as a result their motility is different from that of other bacteria. These organisms can swim in a highly viscous, gel-like medium, such as that found in connective tissue, that inhibits the motility of most other bacteria. In spirochetes, the organelles for motility, the periplasmic flagella, reside inside the cell within the periplasmic space. A given periplasmic flagellum is attached only at one end of the cell, and depending on the species, may or may not overlap in the center of the cell. The number of periplasmic flagella varies from species to species. These structures have been shown to be directly involved in motility and function by rotating within the periplasmic space (1).

The present talk focuses on the spirochete that causes Lyme disease, *Borrelia burgdorferi.* In many bacterial species, cell shape is usually dictated by the peptidoyglycan layer of the cell wall. In the first part of the talk, results will be presented that the morphology of *B. burgdorferi* is the result of a complex interaction between the cell cylinder and the internal periplasmic flagella resulting in a cell with a flat-wave morphology. Backward moving, propagating waves enable these bacteria to swim and translate in a given direction. Using targeted mutagenesis, we inactivated the gene encoding the major periplasmic flagellar filament protein FlaB. The resulting *flaB* mutants not only were non-motile, but were rod-shaped. Western blot analysis indicated that *flaB* was no longer synthesized, and electron microscopy revealed that the mutants were completely deficient in periplasmic flagella. Our results indicate that the periplasmic flagella of *B. burgdorferi* have a skeletal function. These organelles dynamically interact with the rod-shaped cell cylinder to enable the cell to swim, and to confer in part its flat-wave morphology (see http:/www.uic.edu/orgs/blast/videos for animated model, 2).

The latter part of the talk concerns the basis for asymmetrical rotation of the periplasmic flagella of *B. burgdorferi* during chemotaxis. In translational motility, the bundles of periplasmic flagella rotate in opposite directions. When not translating, they rotate in the same direction, and the cells flex. We present evidence that asymmetrical rotation of the bundles during translation does not depend upon the chemotaxis signal transduction system. The histidine kinase CheA is known to be an essential component in the signaling pathway for bacterial chemotaxis. Mutants of *cheA* in flagellated bacteria continually rotate their flagella in one direction. *B. burgdorferi* has two copies of *cheA*. We reasoned that if chemotaxis were essential for asymmetrical rotation of the flagellar bundles, and if the flagellar motors at both cell ends were identical, inactivation of the two *cheA* genes should result in cells that constant flex. To test this hypothesis, the signaling pathway was completely blocked by construction of a double *cheA* mutant. This mutant was completely deficient in

chemotaxis. Rather than flexing, it failed to reverse, and it continually translated only in one direction. The results indicate that asymmetrical rotation does not depend upon the chemotaxis system but rather upon differences between the two flagellar bundles. We propose that certain factors within the spirochete localize at flagellar motors at one end of the cell to effect this asymmetry (3).

References:

1. Li C., Motaleb A., M. Sal, S.F. Goldstein, N.W.Charon. 2000. Spirochete periplasmic flagella and motility. Mol. Microbiol Biotechnol **2**:345-54.

2. Motaleb M.A., L. Corum, J.L Bono, A.F. Elias, P. Rosa, D.S. Samuels, N.W. Charon. 2000. Borrelia burgdorferi periplasmic flagella have both skeletal and motility functions. Proc Natl Acad Sci. 2000 **97**:10899-10904.

3. Li, C. R. Bakker, M. M2otaleb, F. Cabello, M.L. Sartakova, and N.W. Charon. 2002. Asymmetrical flagellar rotation in Borrelia burgdorferi non-chemotaxis mutants. Proc. Natl. Acad. Sci. **99**:6169-6174.

FtsZ in plants

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Despite the weak sequence similarity of FtsZ and tubulins, both protein families share convincing structural and biochemical similarity, suggesting FtsZ as the evolutionary ancestor of tubulin (e.g., 1).

In plants, several FtsZ proteins are encoded by two small nuclear gene families designated FtsZ1 and FtsZ2. For some of these homologues it has been proven experimentally that they are essential for chloroplast division (2, 3). Furthermore, certain FtsZ proteins polymerise to a ring-like structure at the division site of plastids (e.g., 4). Therefore, bacterial cell division and eukaryotic plastid division share at least the essential role of FtsZ in the division process. However, while most eubacteria possess only one *ftsZ* gene, plants harbour several different *ftsZ* homologues encoded by two different gene families, indicating a more complex role of FtsZ in plants when compared to bacteria (5, 6).

In transiently transformed moss (Physcomitrella) protoplasts, FtsZ2::GFP fusion proteins polymerised to highly organised networks within the plastids (7). Strikingly, models drawn from TEM results demonstrating microtubule-like structures within plastids match with the FtsZ networks found in chloroplasts (8). Thus, we suggested the term 'plastoskeleton' for these filamentous networks. This plastoskeleton could help the chloroplasts to keep or change their shape in different tissues.

We will present a phylogenetic analysis of the FtsZ-families in the ancient land plant Physcomitrella as well as a detailed analysis of the sub-cellular localisation of each single member utilising *ftsZ::gfp* fusion constructs and transient transformation techniques.

Acknowledgements
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References:

(1) Erickson, H.P. (2001): Cytoskeleton. Evolution in bacteria. Nature 413, 30.
(2) Strepp, R., S. Scholz, S. Kruse, V. Speth, R. Reski (1998): Plant nuclear gene knockout reveals a role in plastid division for the homolog of the bacterial cell division protein FtsZ, an ancestral tubulin. Proc. Natl. Acad. Sci. U.S.A. 95, 4368-4373.

(3) Osteryoung, K.W., K.D. Stokes, S.M. Rutherford, A.L. Percival, W.Y. Lee (1998): Chloroplast division in higher plants requires members of two functionally divergent gene families with homology to bacterial *ftsZ*. Plant Cell 10, 1991-2004.
(4) Vitha, S., R.S. McAndrew, K.W. Osteryoung (2001): FtsZ ring formation at the chloroplast division site in plants. J. Cell Biol. 153, 111-119.
(5) Reski, R. (2002): Rings and networks: the amazing complexity of FtsZ in chloroplasts. Trends in Plant Science 7, 103-105.
(6) Rensing, S.A., J. Kiessling, R. Reski, E.L. Decker (2002): Diversification of *ftsZ* during early land plant evolution. Submitted.
(7) Kiessling, J., S. Kruse, S.A. Rensing, K. Harter, E.L. Decker, R. Reski (2000): Visualization of a cytoskeleton-like FtsZ network in chloroplasts. J. Cell Biol. 151, 945-950.
(8) McFadden, G.I. (2000): Skeletons in the closet. How do chloroplasts stay in shape? J. Cell Biol. 151, F19-22.

Composition and evolution of the chloroplast division apparatus

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The chloroplasts of plant cells are descendents of cyanobacterial endosymbionts. Consistent with this evolutionary origin, we have identified several chloroplast division proteins with counterparts that function in cyanobacterial cell division. FtsZ1 and FtsZ2 are nuclear-encoded, chloroplast-targeted forms of the tubulin-like bacterial protein FtsZ. Immunofluorescence microscopy has shown that FtsZ1 and FtsZ2 assemble into rings and are tightly colocalized. Experiments to establish the topology of these rings with respect to the chloroplast envelope membranes indicate they are positioned in the stromal compartment. Quantitative measurements show that FtsZ1 and FtsZ2 are present in a molar ratio of 1:2. Recent data suggest the two proteins interact directly, and may be components of the same ring structure. Studies to define the functional relationship between FtsZ1 and FtsZ2 are underway. ARC6 is a new chloroplast division protein with homology to the recently identified cyanobacterial cell division protein Ftn2. ARC6 is encoded in the nuclear genome, imported to the chloroplast by a cleavable transit peptide, and is probably localized in the inner envelope membrane. ARC6 bears a motif found in DnaJ proteins, which act as co-chaperones in HSP70-mediated reactions. The *Arabidopsis* arc6 mutant exhibits severe chloroplast division defects and fragmented FtsZ1 and FtsZ2 filaments, suggesting the ARC6 protein may play a role in FtsZ1 and FtsZ2 ring assembly. ARC5 is another new chloroplast division protein we have recently identified by positional cloning in *Arabidopsis*. Chloroplasts in *arc5* mutants constrict slowly and often remain dumbbell-shaped. ARC5 is related to the dynamin family of proteins, which mediate vesicle budding and mitochondrial fission in eukaryotes. Unlike FtsZ1, FtsZ2 and ARC6, ARC5 has no clear homologues in cyanobacteria or other prokaryotes, but instead appears to be derived from a dynamin-like protein present in the eukaryotic host cell. Although ARC5 is not imported to the chloroplast, a GFP-ARC5 fusion protein localizes to the chloroplast division site. ARC5 is thus the first cytosolic component of the chloroplast division machinery to be identified.

References:

Osteryoung, K.W. 2001. Fission of eukaryotic organelles. *Curr. Opin. Microbiol.* 4: 639-646.

Osteryoung, K.W. and R.S. McAndrew. 2001. The plastid division machine. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52: 315-333.

Vitha, S., R.S. McAndrew and K.W. Osteryoung. 2001. FtsZ ring formation at the chloroplast division site in plants. *J. Cell Biol.* 153: 111-119.

McAndrew, R.S., J.E. Froehlich, S. Vitha, K.D. Stokes and K.W. Osteryoung. 2001. Colocalization of plastid division proteins to the chloroplast stromal compartment establishes a new functional relationship between FtsZ1 and FtsZ2 in higher plants. *Plant Physiol.* 127: 1656-1666.

Session 3: Septum production and assembly for binary fission I
Chair: Keith Chater

Models of biological pattern formation as applied to bacterial morphogenesis

Hans Meinhardt

Local self-amplifying processes that are coupled with long-ranging antagonistic reactions are the driving force in biological pattern formation. For bacterial morphogenesis, this is proposed to be realized by a self - re-enforced binding of molecules to the membrane while the depletion of the precursor molecules in the cytoplasm works antagonistically and provides the spatial confinement. This leads to localized concentration maxima at the cell membrane. The condition for polar, symmetric and periodic patterns will be worked out. Both poles of a bacterium can obtain different signals. Polar oscillating patterns and traveling waves can be generated by a local destabilization of once established concentration maxima.

The MinD/MinE oscillation can beexplained in this way in great detail. This mode of pattern formation will be compared with other intercellular patterning processes, for instance, the orientation of chemotactic cells.

ATP-dependent interactions between the *E.coli* Min proteins and phospholipid membrane *in vitro*

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Equipartitioning of cell components during division requires that Z ring formation be accurately confined to a narrow zone around midcell. Positioning of the Z ring in *E.coli* is in part determined by the cellular distribution of MinC. MinC inhibits Z ring assembly and undergoes an oscillatory localization cycle during which the protein alternately accumulates on the membrane at either cell end every other 25 seconds, effectively forcing proper positioning of the Z ring to midcell (1-4).

The cellular location of MinC is directly dictated by that of MinD, which undergoes a similar oscillatory localization cycle (5, 6). MinD is a self-interacting ATPase which associates with the cytoplasmic membrane in a peripheral manner (4-11). MinD also interacts directly with both MinC and MinE, and recruits both to the membrane (2, 12-14). MinE is required for MinD oscillation, and itself undergoes a dynamic localization cycle which is coupled to that of MinD (15-17).

Several models for how the interactions between MinD, ATP, membrane, and MinE might culminate in the observed oscillating behavior have been formulated (11, 15-20).

We studied the interactions between the Min proteins and phospholipid vesicles *in vitro*. In support of recent work by Hu et al (21), we found that the ATP form of MinD (MinD.ATP) readily binds phospholipid vesicles, that MinE stimulates the dissociation of MinD from the vesicles, and that MinD becomes resistant to MinE-stimulated dissociation when ATP is substituted with ATPγS. In addition, equilibrium binding analyses show cooperativity in MinD.ATP binding to vesicles, indicating that MinD.ATP self-interactions enhance its assembly on membrane.

We further found that MinC is directly recruited to MinD-decorated vesicles in the presence of ATP or ATPγS. As expected, addition of MinE to MinC-MinD.ATP-membrane complexes stimulated dissociation of both MinD and MinC from the vesicles. In contrast, addition of MinE to MinC-MinD.ATPγS-membrane complexes led to the specific release of MinC, and the formation of relatively stable MinE-MinD.ATPγS-membrane complexes. This, and the work by Hu et al (21), support models wherein MinD.ATP directly associates with the phospholipid bilayer in a self-enhancing manner, and wherein MinE induces Min protein dynamics by stimulating the conversion of the membrane-bound form of MinD to the

cytoplasmic form (MinD.ADP). The results further suggests that, *in vivo*, MinE-stimulated dissociation of MinC from the MinC-MinD.ATP-membrane complex may occur prior to hydrolysis of the nucleotide.

References:

1. Hu, Z. & Lutkenhaus, J. (1999) *Mol. Microbiol.* **34,** 82-90.
2. Raskin, D. M. & de Boer, P. A. J. (1999) *J. Bacteriol.* **181,** 6419-6424.
3. Pichoff, S. & Lutkenhaus, J. (2001) *J. Bacteriol.* **183,** 6630-6635.
4. Johnson, J. E., Lackner, L. L. & de Boer, P. A. J. (2002) *J. Bacteriol.* **184,** 2951-2962.
5. Raskin, D. M. & de Boer, P. A. J. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 4971-4976.
6. Rowland, S. L., Fu, X., Sayed, M. A., Zhang, Y., Cook, W. R. & Rothfield, L. I. (2000) *J. Bacteriol.* **182,** 613-619.
7. de Boer, P. A. J., Crossley, R. E., Hand, A. R. & Rothfield, L. I. (1991) *EMBO J.* **10,** 4371-4380.
8. Szeto, J., Ramirez-Arcos, S., Raymond, C., Hicks, L. D., Kay, C. M. & Dillon, J. A. (2001) *J. Bacteriol.* **183,** 6253-6264.
9. Hayashi, I., Oyama, T. & Morikawa, K. (2001) *EMBO J.* **20,** 1819-1828.
10. Marston, A. L. & Errington, J. (1999) *Mol. Microbiol.* **33,** 84-96.
11. Hu, Z. & Lutkenhaus, J. (2001) *Mol. Cell* **7,** 1337-1343.
12. Huang, J., Cao, C. & Lutkenhaus, J. (1996) *J. Bacteriol.* **178,** 5080-5085.
13. Hu, Z. & Lutkenhaus, J. (2000) *J. Bacteriol.* **182,** 3965-3971.
14. Raskin, D. M. & de Boer, P. A. J. (1997) *Cell* **91,** 685-694.
15. Fu, X., Shih, Y. L., Zhang, Y. & Rothfield, L. I. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 980-985.
16. Hale, C. A., Meinhardt, H. & de Boer, P. A. J. (2001) *EMBO J.* **20,** 1563-1572.
17. Shih, Y. L., Fu, X., King, G. F., Le, T. & Rothfield, L. (2002) *EMBO J.* **21,** 3347-3357.
18. Howard, M., Rutenberg, A. D. & de Vet, S. (2001) *Phys. Rev. Lett.* **87,** 278102.
19. Kruse, K. (2002) *Biophys. J.* **82,** 618-627.
20. Meinhardt, H. & de Boer, P. A. J. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 14202-14207.
21. Hu, Z., Gogol, E. P. & Lutkenhaus, J. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 6761-6766.

Reversible interaction of the Min proteins with the membrane in *E. coli*

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In *E. coli* the *min* system prevents division from occurring away from midcell through topological regulation of MinC, an inhibitor of Z-ring formation. The topological regulation involves oscillation of MinC between the two halves of the cell under the direction of the MinDE oscillator. During an oscillatory cycle a GFP-fusion to either MinC or MinD displays a horseshoe pattern of fluorescence in one cell half (reviewed in 1). The arms of the horseshoe recede towards the pole and a new pattern is established in the other cell half emanating from the cell pole. GFP-MinE is present as a ring that is at the receding arms of the MinCD horseshoe. We have been attempting to understand the biochemical basis for this oscillation.

MinD is an ATPase whose activity is essential for the spatial regulation of division (2). Previously, we have shown that the ATPase activity of MinD is stimulated by MinE in the presence of phospholipid vesicles and provided evidence that this stimulation is required for the oscillation to occur (3). We also demonstrated that MinD binds to phospholipid vesicles in an ATP-dependent manner and assembles into polymers that deform the vesicles into tubes. Addition of MinE stimulates ATP hydrolysis leading to a breakdown of the tubes and the release of MinD from the membrane (4). This work provided the biochemical basis for the reversible binding of MinD to the membrane.

Our present work reveals that MinD dimerizes in the presence of ATP and interacts with MinC. In the presence of a phospholipid bilayer and ATP, MinD recruits MinC to the membrane. Addition of MinE to the MinCD-bilayer complex resulted in release of both MinC and MinD. The release of MinC did not require ATP hydrolysis indicating MinE could displace MinC from the MinD-bilayer complex. In contrast, MinC was unable to displace MinE bound to the MinD-bilayer suggesting that MinE and MinC do not compete for binding to MinD. Instead, it suggests that MinE induces a conformational change in MinD bound to the bilayer that results in the release of MinC. Also, we argue that MinD binding to the membrane not only concentrates MinC at the membrane but must also activate MinC.

The basis of MinD binding to the membrane was also investigated. A conserved, short C-terminal region of MinD was found to be essential for binding. Truncations of 3 or 10 amino acids from the C-terminus dramatically decreased the ability of MinD to localize to the membrane and spatially regulate division. These truncations bound MinC but were deficient in targeting MinC to the septum. *In vitro* the mutants dimerized, but were deficient in binding to phospholipid vesicles and undergoing MinE stimulation. Additional experiments

demonstrate that the C-terminal region interacted directly with the membrane. We suggest a model in which the ATP-dependent dimerization of MinD affects the conformation of the C-terminal region, a potential amphipathic helix, triggering membrane binding.

References:

(1) L.I. Rothfield, Y.L. Shih, and G. King (2001) Cell 106:13-16.
(2) P.A. de Boer, R.E. Crossley, L. Rothfield. (1991) EMBO J. 10:4371-80.
(3) Z. Hu and J. Lutkenhaus. (2001) Mol. Cell 7:1337-43.
(4) Z. Hu, E.P. Gogol, and J. Lutkenhaus. (2002) PNAS 99:6761-6.

The *Escherichia coli* amidase AmiC is a periplasmic component of the septal ring exported by the twin arginine transport pathway

Thomas G. Bernhardt and Piet A.J. de Boer

The septal ring is a dynamic membrane-associated organelle that drives cell wall invagination during cytokinesis in bacteria. Evidence indicates that the constriction process is tightly coupled to highly localized synthesis of septal murein So far, the DD-transpeptidase FtsI (PBP3) is the only *Escherichia coli* septal-ring component known to be directly involved in murein metabolism. It is clear, however, that a number of other murein synthetic, as well as degrading, activities must be present at the ring in order to generate septal murein and shape new polar caps.

The N-acetylmuramoyl-L-alanine amidases are periplasmic enzymes that remove murein crosslinks by cleaving the peptide moiety from N-acetylmuramic acid. It was recently shown that Ami$^-$ cells form chains, indicating amidase activity contributes to daughter cell separation by helping to split septal murein.

To investigate whether amidases are specific components of the septal ring complex, we studied the sub-cellular localization of AmiA and AmiC in live cells using functional fusionsto GFP. GFP exported to the periplasm via the general secretion pathway does not fold properly and is not fluorescent. In contrast, it was recently found that pre-folded (fluorescent) GFP can be exported to this compartment through the twin-arginine transport (Tat) pathway. Interestingly, Tat$^-$ cells show a cell chaining phenotype very similar to that of *ΔamiA ΔamiC* cells. Moreover, we noted that the signal sequences of AmiA and AmiC contain motifs resembling a Tat-targeting consensus. These observations suggested that these amidases might use Tat to reach the periplasm, and we showed this is indeed the case. Thus, examination of live cells by fluorescence microscopy showed that both AmiA-GFP and AmiC-GFP dispersed throughout the cytoplasm of *ΔtatC* cells, but accumulated in the periplasm of Tat$^+$ cells. Moreover, both fusions corrected the chaining phenotype of a double *ΔamiA ΔamiC* mutant, showing that both retained amidase activity.

Interestingly, the periplasmic localization patterns of the two fusions differed markedly. AmiA-GFP appeared distributed throughout the periplasm in all cells. In contrast, AmiC-GFP was similarly present throughout the periplasm in small cells, but localized almost exclusively to a ring at the site of constriction in dividing cells. This result indicated that AmiC is a periplasmic component of the septal ring organelle. Accordingly, we found that the accumulation of AmiC-GFP at the site of constriction requires the prior assembly of other septal ring proteins, including FtsN, placing AmiC as the latest known recruit to the organelle. This and other evidence suggests that AmiC moves to the septal ring very close to the time of

constriction initiation and remains associated throughout the process. Comparison of the AmiA and AmiC peptides suggested that the latter contains an N-terminal non-amidase domain that is missing in the former. Using deletion derivatives of AmiC, we showed that this domain is both necessary and sufficient to target periplasmic proteins to the septal ring, and that targeting is important for full AmiC activity *in vivo*.

In short, we have localized the first entirely periplasmic septal ring component in live bacteria. We expect that Tat-targeted GFP-fusions will be valuable tools for the study of other periplasmic proteins involved in cell division and other important cell envelope-associated processes.

Role of Min proteins in Gram negative cocci

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Cell division or cytokinesis is a process central to all living cells. In bacteria the process has been mostly studied in *Escherichia coli or Bacillus subtilis*, two rod-shaped organisms that divide along parallel planes. Our laboratory has pioneered the study of cell division using *Neisseria gonorrhoeae*, comprising round cells that divide in alternating perpendicular planes, as a model coccal organism. We have focused on the role of the Min proteins (MinC, MinD, and MinE) in the gonococcus. These proteins have been implicated in specifying the midcell division site through a type of negative regulation of septum formation in rods.[1] How the Min proteins might function in cells with no obvious middle (i.e. in round cells) is an intriguing dilemma.

We determined that *minC*, *minD*, and *minE* homologues exist in *N. gonorrhoeae* (Ng) as part of a 27 gene cluster.[2] Disruption of either $MinC_{Ng}$ or $MinD_{Ng}$ alters the cell division phenotype of gonococcal cells and reduces their viability.[2,3] Overexpression of both of these proteins in *N. gonorrhoeae* causes cell enlargement, supporting a role for each protein in gonococcal cell division inhibition.[3] The Min_{Ng} proteins exhibit cross-genus functionality, inhibiting cell division when expressed in either rod-shaped or mutant round (*rodA*) *E. coli* cells.[2,3] *E. coli minC* and *minD* mutants are complemented by their respective gonococcal orthologues.[2,3] Green fluorescent protein (GFP) fusions to MinD and MinE from *N. gonorrhoeae* localized dynamically in different *E. coli* backgrounds.[5] GFP-$MinD_{Ng}$ moved from pole-to-pole in rod-shaped *E. coli* cells with a 30-45 sec localization cycle when $MinE_{Ng}$ was expressed *in cis*. The oscillation time of GFP-$MinD_{Ng}$ was reduced when wild-type $MinE_{Ng}$ was replaced with $MinE_{Ng}$ carrying a R30D mutation, but lengthened by almost 2-fold when activated by $MinE_{Ec}$. Several mutations in the N-terminal domain of $MinD_{Ng}$, including K16Q and 4- and 19-amino acid truncations, prevented oscillation; these $MinD_{Ng}$ mutants showed decreased or lost interaction with themselves and $MinE_{Ng}$. Like $MinE_{Ec}$-GFP, $MinE_{Ng}$-GFP formed MinE rings and oscillated in *E. coli* cells when $MinD_{Ec}$ was expressed *in cis*. Finally, in small round *E. coli* cells, GFP-$MinD_{Ng}$ appeared to move parallel to completed septa. This pattern of movement is predicted to be similar in gonococcal cells that also divide in alternating perpendicular planes.[6]

Using bioinformatic approaches, we have recently initiated structure/function analyses of the C-terminus of MinC. By comparing MinC sequences from 22 different prokaryotes, we noted a highly conserved C-terminus that contained four identical glycines (G138/G135, G157/G154, G164/G161 and G174/G171 of *N. gonorrhoeae* and *E. coli* MinC, respectively).

Each of these residues was found to be essential for MinC function since point mutations at these sites resulted in protein incapable of inducing cell division arrest upon overexpression in *E. coli* (Ec). Results from yeast two-hybrid assays suggested that the loss of $MinC_{Ec}$ functionality in these mutants was likely due to loss of self-interaction (G161S and G171E mutants) or loss of interaction with $MinD_{Ec}$ (G135D, G154D and G171E mutants). We are currently investigating the effects of these mutations on the secondary structure of the protein, and are focusing on the analysis of the domain that we believe may be involved in MinC-MinD interaction. We have also shown that MinD from both *N. gonorrhoeae* and *E. coli* MinD self-associate.[3] Using genomic and structural modeling approaches we are investigating the domains involved in $MinD_{Ng}$ self-interaction and function. We have mutated a predicted exposed loop region (R92L, D93L, K94I) and a highly conserved ATP-binding site (K16Q) in $MinD_{Ng}$ which rendered the protein unable to induce cell filamentation in *E. coli*. Circular dichroism indicated that the mutant proteins do not have significant changes to their secondary structure; hence, the observed phenotype was likely not due to misfolded protein. The K16Q mutation led to decreased ATPase activity, while mutation of the predicted loop region resulted in increased enzyme activity. In addition, GFP-fusions to the mutant $MinD_{Ng}$ derivatives no longer displayed intracellular movement in *E. coli*. Yeast two-hybrid assays suggested that the mutant $MinD_{Ng}$ proteins were compromised for self-association and/or lost interaction with MinE and MinC. These results are being confirmed by other analytical methods.

Our investigation of Min_{Ng} protein function in round *E. coli* cells, as well as *N. gonorrhoeae*, led to the development of a model of Min protein function in *N. gonorrhoeae*.[5] This model presently assumes that gonococci contain only one nucleoid per cell, that the Min_{Ng} proteins will oscillate within gonococcal cells. Oscillation of the $MinCD_{Ng}$ complex from one hemisphere of the coccus to the other results in division inhibition at those areas. The axis of oscillation would depend on the position of the nucleoid, which would itself inhibit oscillation across its surface. Stimulation of the ATPase activity of $MinD_{Ng}$ by $MinE_{Ng}$ at either cell half would lead to the continued oscillation of $MinCD_{Ng}$. Our model also incorporates the nucleoid as a regulator of cell division by preventing $FtsZ_{Ng}$-ring formation where the bulk of the chromosome is located. We have shown that $FtsZ_{Ng}$ gathers at the asymmetric invagination site, which may be is opposite to the location of the nucleoid. Completion of septation following chromosome segregation would result in a $MinCD_{Ng}$ oscillation pattern perpendicular to the first, yet parallel to the newly-formed cell wall that separates daughter cells. Ultimately, a tetrad of daughter cells is formed.

We have demonstrated that the Min proteins are essential for proper cell division in gonococci. However, several important questions remain to be answered with this model, and are the subjects of our current investigations.

References:

1. de Boer, P., and Crossley, R. (1989) A division inhibitor and a topological specificity factor T.coded for by the minicell locus determine proper placement of the division septum in *Escherichia coli*. *Cell* 56: 641-649.
2. Ramirez-Arcos, S., Szeto, J., Beveridge, J., Victor, C., Francis, F., and Dillon, J.R. (2001) Deletion of the cell division inhibitor MinC results in lysis of *Neisseria gonorrhoeae*. *Microbiol.* 147: 225-237.
3. Szeto, J., Ramirez-Arcos, S., Raymond, C., Hicks, L.D., Kay, C.M., and Dillon, J.A. (2001) Gonococcal MinD affects cell division in *Neisseria gonorrhoeae* and *Escherichia coli* and exhibits a novel self-interaction. *J.Bacteriol.* 183: 6253-6264.
4. Begg, K., and Donachie, W. (1998) Division planes alternate in spherical cells of *Escherichia coli*. *J.Bacteriol.* 180: 2564-2567.
5. Ramirez-Arcos, S., Szeto, J., Dillon, J.R. and Margolin, W. (2002) Conservation of dynamic localization among MinD and MinE orthologs: oscillation of *Neisseria gonorrhoeae* proteins in *Escherichia coli*. *Mol. Microbiol.* 46: 493-504.
6. Westling-Häggström, B., Elmros, T., Normark, S., and Winblad, B. (1977) Growth pattern and cell division in *Neisseria gonorrhoeae*. *J.Bacteriol.* 29: 333-342.

Session 4: Septum production and assembly for binary fission II
Chair: Yves V. Brun

Physical and physiological aspects of nucleoid compaction and segregation in *Escherichia coli*

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Many recent reviews in the field of bacterial chromosome segregation propose that newly replicated DNA is actively separated by the functioning of specific proteins. This view is primarily based on an interpretation of the position of fluorescently-labelled DNA regions and proteins in analogy to the active segregation mechanism in eukaryotic cells, i.e. to mitosis. So far, physical aspects of DNA organization like the diffusional movement of DNA supercoil-segments and their interaction with soluble proteins leading to a phase separation between cytoplasm and nucleoid have received relatively little attention (1, 2).

Here we will describe a quite different view taking into account DNA-protein interactions, the large variation in the cellular position of fluorescent foci and the compaction and fusion of segregated nucleoids upon inhibition of RNA or protein synthesis. It is proposed that the random diffusion of DNA supercoil segments (Brownian movement) is transiently constrained by the process of cotranscriptional translation and translocation (transertion) of membrane proteins (3). After initiation of DNA replication a bias in the positioning of transertion areas creates a bidirectionality in chromosome segregation that becomes self-enhanced when neighbouring genes on the same daughter chromosome are expressed. This transertion-mediated segregation model (3) is applicable to multifork replication during rapid growth and to multiple chromosomes and plasmids that occur in many bacteria. In this model the driving force for segregation is Brownian movement in contrast to most other models (4) in which forces and direction of movement are generated by specific protein assemblies.

References:

1. Woldringh, C.L., and T. Odijk. 1999. Structure of DNA within the bacterial cell: physics and physiology. p. 171-187. In R.L. Charlebois (ed.), Organization of the prokaryotic genome. Chapter 10. American Society for Microbiology, Washington, D.C.
2. Cunha, S., C.L. Woldringh, Th. Odijk (2001) Polymer-mediated compaction and internal dynamics of isolated Escherichia coli nucleoids. J. Struct. Biol. 136:53-66.
3. Woldringh, C.L. (2002) The role of co-transcriptional translation and protein translocation (transertion) in bacterial chromosome segregation. Mol. Microbiol. 45: 17-29.
4. Lemon, K.P. and Grossman, A.D. (2001) The extrusion-capture model for chromosome partitioning in bacteria. Genes Develop. 15: 2031-2041.

MreB and PBP2 localize at the site of division of *Escherichia coli*

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In analogy to the protein complex responsible for constriction of the cell envelope of *E. coli* during division, we predict the presence of a similar complex for the synthesis of the lateral cell envelope. Such a complex should consist of peptidoglycan synthetases and hydrolases, such as PBP1B, PBP2, and one of the amidases, integral membrane protein such as RodA and cytosolic components. For instance MreB, which helical structure (1) could provide a topographical marker for the complex. To validate this hypothesis we have studied the localization of several proteins that are putatively part of the complex.

Green-Fluorescent-protein fusions to PBP2, MreB, and RodA were expressed from a medium-copy low-expression plasmid to ensure near to wild type levels of fusion protein production. All fusion proteins were able to complement either deletion strains or temperature sensitive strains at the non-permissive temperature and therefore are proven to be functional.

GFP-PBP2 localized in a distinct pattern similar to the MreB helix, but did not seem to be a helix itself. It localizes at the site of division somewhat before a constriction can be visualized by light miscroscopy.

Apart from a small dot that possibly can be regarded as a remnant from the last division similar to the bud scar in yeast cells, it was absent from the cell poles. Time resolved analysis of the morphology of a culture growing in the presence of Mecillinam that inhibits PBP2 specifically, suggest an active role for PBP2 in the synthesis of the new cell pole during division.

GFP-MreB localized as expected as a helix underneath the membrane and the pitch of the helix appeared to be correlated with the diameter of the cells. This indicated that MreB is involved in the maintenance of the diameter apart from other possible functions.

GFP-RodA was uniformly distributed in the cytoplasmic membrane and perhaps somewhat less at the site of constriction. Whether is also functions at all sites were it localizes has yet to be determined.

References:

1. Jones, L. J. F., Carbadillo-López, R., and Errington, J. (2001) Cell 104, 913-922.

Cell shape and gene clustering in cell division

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Clustering of the main cell division and wall synthesis genes in bacterial genomes (the *dcw* cluster[1]) is correlated to cell shape, particularly in rod-shaped bacteria[2]. Contrary to spherical cells, rod shaped cells posses a selective advantage in certain environments as their volume to surface ratio stays constant as they grow. The pattern of conservation of the cluster suggests that it is an ancient feature of bacterial genomes. Although the conservation extends both to the presence of genes and to their relative order in the cluster, several lines of evidence indicate that integrity of the *dcw* cluster is not required to maintain the rod shape.

To explain the remarkable conservation of the cluster we propose a model in which the gene order would confer a selective advantage in rod-shaped cells, while it would be of no significant benefit in cells with other shapes. Rods would profit from a mechanism involving: i) co-translational assembly of the protein complexes involved in the synthesis of peptidoglycan precursors and cell division and ii) the precise localization of the assembled complexes in the cell to participate in the synthesis of the septal peptidoglycan and cell division, or alternatively in the synthesis of the lateral cell wall.

The selective pressure to maintain the cluster would then arise from the need to efficiently co-ordinate the processes of elongation and septation in rod-shaped bacteria. These two processes can be viewed as competing reactions leading to cell growth and cell division sharing several common peptidoglycan precursors that are not abundant. The pools of lipids I and II in particular are very low (700 and 1000-2000 molecules/cell respectively), and seem to be a limiting factor for the synthesis of peptidoglycan. Competition does not occur in cocci as they do not depend on lateral wall elongation for their growth.

A hypothetical ancestral bacteria would be a rod-shaped cell in which the need to funnel sufficient precursors towards the synthesis of the septum at a discrete time during the cell cycle would favour the assembly of the *dcw* genes into a precisely ordered cluster. This hypothetical ancestor would maintain its rod shape due to the presence of genes, as *rodA*, specifically dedicated to the elongation pathway. As soon as a cell adapted to circumstances, including accidental loss of the elongation genes, in which the advantages of the peptidoglycan rod shape were not important the maintenance of a well structured *dcw* cluster no longer provided an advantage and therefore the cluster lost compactness or disappeared altogether. An example of this drift towards segregation of a well structured *dcw* cluster is

offered by *Neisseria*, in which together with the loss of the rod shape the transcriptional continuity observed in the *dcw* cluster of rod-shaped cells is lost.

References:

1. J. A. Ayala, T. Garrido, M. A. de Pedro, and M. Vicente. 1994. Molecular biology of bacterial septation. p 73-101.*In* R. Hakenbeck, and J.-M. Ghuysen (eds) *New comprehensive Biochemistry* vol. **27**: *Bacterial cell wall*. Elsevier Science Publishers, Amsterdam, The Netherlands.

2. J. Tamames, M. González, J. Mingorance, A. Valencia, and M. Vicente. 2001. Bringing gene order into bacterial shape. *Trends in Genetics* **17**: 124-126.

Cell division proteins FtsL and FtsB of *Escherichia coli* exist in a complex and localize to the division site in a co-dependent fashion

Nienke Buddelmeijer, Dana Boyd and Jon Beckwith

FtsB, also known as YgbQ, was identified in a computer screen designed to find potential interaction partners of cell division protein FtsL of *E. coli*. FtsL and FtsB are bitopic integral membrane proteins that share size, topology and a unique leucine zipper-like motif that is predicted to adopt a coiled coil structure. The role of FtsB in cell division is supported by the following findings. Cells depleted of FtsB form long filaments while DNA segregation is not affected. FtsB localizes to the constriction site in wild type *E. coli* cells. Localization of FtsB to the constriction site depends on cell division proteins FtsQ and FtsL but not FtsW and FtsI, placing FtsB in the sequential dependency order of proteins localizing to the division site. Localization of GFP-FtsL is also dependent on FtsB indicating that FtsL and FtsB co-localize to the division site. Our results present the first instance of co-localization of proteins to the bacterial mid-cell in *E. coli*. FtsB of *E. coli* and *Vibrio cholerae* are highly conserved (50% identical) and are functional homologues. Co-immunoprecipitation experiments suggest that FtsL and FtsB exist as a complex *in vivo*. Our hypothesis is that this complex is involved in membrane fusion during cell division.

References:

1. Buddelmeijer, N., Judson, N., Boyd, D., Mekalanos, J. J. & Beckwith, J. (2002) Proc. Natl. Acad. Sci. U S A. 99, 6316-6321.
2. Ghigo, J.-M. & Beckwith, J. (2000) J. Bacteriol. 182, 116-129.
3. Ghigo, J. M., Weiss, D. S., Chen, J. C., Yarrow, J. C. & Beckwith, J. (1999) Mol. Microbiology 31, 725-737.
4. Guzman, L.-M., Weiss, D. S. & Beckwith, J. (1997) J. Bacteriol. 179, 5094-5103.
5. Guzman, L.-M., Barondess, J. & Beckwith, J. (1992) J. Bacteriol. 174, 7716-7728.

Molecular interactions between R1-par components

Jakob Møller-Jensen, Jonas Borch, Mette Dam & Kenn Gerdes

The partitioning system (par) of *Escherichia coli* plasmid R1 mediates active segregation of its replicon. The par locus consists of two genes, parM and parR, and a cis-acting centromere-like site, called parC. The parC site contains two sets of five direct repeats (iterons) separated by a region containing the R1 par promoter. The R1 par promoter is autoregulated by ParR and the ten iterons are potential operator sites.

ParM protein forms dynamic F-actin-like filaments that are required for active partitioning of replicated plasmid progeny. Nucleation of ParM filaments in vivo requires ParR and parC, whereas the recycling of ParM through multiple rounds of filament formation is governed by its ATPase activity. Here we study the molecular interactions between purified components of the R1 par system. The pattern of ParR binding to parC DNA indicated at least ten binding sites for ParR and that binding is co-operative. ParM did not bind to parC nor did the protein affect the binding of ParR, indicating that ParM filaments interact with the plasmid DNA via ParR. Using BIA-CORE we show that co-operative binding of ParM to the ParR/parC complex is strictly dependent on the presence of ATP, indicating that the ParM nucleotide switch is important for filament interaction with the plasmid DNA as well as for the recycling of ParM.

Together these data support a model for active plasmid transport, in which the growing ends of the ParM filaments moves the ParR/parC DNA complex towards opposite cell poles.

Cell surface topological domains and bacterial morphology

Miguel A. de Pedro

Recently we showed that the poles of *E. coli* sacculi are made of metabolically inert murein (peptidoglycan). Polar murein is neither subjected to turn-over nor to insertion of new precursors. Therefore the poles constitute stable domains in the sacculus, which are inherited upon successive rounds of cell division in a conservative fashion. As the murein sacculus and the outer membrane (OM) interact strongly by at least three different bridging complexes (Braun´s lipoprotein, PAL/TOL and OmpA) it was likely that mobility of OM elements able to interact with murein-bound proteins, or with murein itself, would be restricted at the cell poles. To investigate this hypothesis the cell surface was chemically labeled *"in vivo"* with the fluorescent reagent Texas Red X-succinimidyl ester (TR)(Molecular Probes) and the fate of label upon subsequent cell growth was studied by microscopy and biochemical techniques. TR efficiently labeled OM-proteins, but not major components of the periplasmic compartment (murein) or cytoplasmic membrane (phosphatidylethanolamine). The results clearly showed that TR-labeling was much more persistent in the cell poles than in the rest of the cell body. Fluorescence intensity at the poles of cells chased for three generations still higher than 60% of the initial value, in opposition to a maximum of 12% expected if labeled molecules could diffuse freely throughout the chase period. Furthermore, the boundary between the high (pole) and low (cylindrical cell body) fluorescence intensity areas was extremely sharp. Analysis of mutants with altered morphologies supported a close relation between areas of inert murein in the sacculus and areas of restricted protein mobility in the OM. Such topological domains are likely to play crucial roles in *E. coli* morphogenesis.

Session 5: Using septa for differentiating asymmetric compartments
Chair: Katherine W. Osteryoung

Chromosome segregation during asymmetric cell division in *B. subtilis*

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Bacillus subtilis is a rod-shaped bacterium that proliferates by a classical medial cell division. When starved it can switch to a modified, asymmetric form of division that is a prelude to the formation of an endospore (10). Asymmetric division requires all of the proteins needed for normal cell septation, as well as at least one sporulation-specific protein, SpoIIE (2, 3). Although formation of the division septum resembles vegetative division quite closely, chromosome segregation is considerably altered by the asymmetry of the system. Thus, the chromosome destined for the small prespore cell needs to travel right to the pole of the sporulating organism. Previous work in this and other labs has resulted in the identification of several proteins required for chromosome segregation in sporulating cells. Spo0J, DivIVA and the recently discovered YwkC protein are all involved in a mechanism that anchors the chromosome to the pole of the cell soon after the onset of sporulation (4, 5, 7, 8, 11, 13, 14, 16, 17). Two of the proteins, Spo0J and probably YwkC, bind to DNA over a large (several hundred kbp) region of DNA centred roughly on *oriC*. DivIVA is an anchor protein targeted to the cell pole. This system helps to ensure that a large (> 1 Mbp) *oriC*-proximal segment of DNA is already positioned at the pole of the cell when the polar septum is formed.

Having positioned part of the chromosome near the pole of the cell, the sporulation septum closes around the chromosome near the cell pole, partitioning it into two portions, the major one of which lies in the large "mother cell" compartment. The SpoIIIE protein then drives or facilitates transfer of the remaining 3 Mbp of *oriC*-distal DNA into the prespore compartment (1, 15, 18). SpoIIIE (called FtsK in *E. coli*), Spo0J (ParB) and DivIVA all have important functions in vegetative cells of *B. subtilis* and probably a wide range of bacteria (6, 9, 12). Recent progress in understanding the molecular basis for chromosome segregation in *B. subtilis* and related organisms will be described.

References:

1. Bath J, Wu LJ, Errington J, Wang JC. 2000. Role of *Bacillus subtilis* SpoIIIE in DNA transport across the mother cell-prespore division septum. *Science* 290: 995-997.
2. Ben-Yehuda S, Losick R. 2002. Asymmetric cell division in *B. subtilis* involves a spiral-like intermediate of the cytokinetic protein FtsZ. *Cell* 109: 257-266.
3. Errington J. 2001. Septation and chromosome segregation during sporulation in *Bacillus subtilis*. *Curr Opin Microbiol* 4: 660-666.
4. Glaser P, Sharpe ME, Raether B, Perego M, Ohlsen K, Errington J. 1997. Dynamic, mitotic-like behaviour of a bacterial protein required for accurate chromosome partitioning. *Genes Devel.* 11: 1160-1168.

5. Graumann PL, Losick R. 2001. Coupling of asymmetric division to polar placement of replication origin regions in *Bacillus subtilis*. *J. Bacteriol.* 183: 4052-4060.
6. Ireton K, Gunther NWI, Grossman AD. 1994. spo0J is required for normal chromosome segregation as well as the initiation of sporulation in *Bacillus subtilis*. *J. Bacteriol.* 176: 5320-5329.
7. Lin DC-H, Grossman AD. 1998. Identification and characterization of a bacterial chromosome partitioning site. *Cell* 92: 675-685.
8. Lin DC-H, Levin PA, Grossman AD. 1997. Bipolar localization of a chromosome partition protein in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. USA* 94: 4721-4726.
9. Marston AL, Thomaides HB, Edwards DH, Sharpe ME, Errington J. 1998. Polar localization of the MinD protein of *Bacillus subtilis* and its role in selection of the mid-cell division site. *Genes Devel.* 12: 3419-3430.
10. Piggot PJ, Losick R. 2001. Sporulation genes and intercompartmental regulation. In *Bacillus subtilis* and its relatives: from genes to cells, ed. L Sonenshein, R Losick, JA Hoch, pp. 483-517. Washington, D.C.: American Society for Microbiology
11. Pogliano J, Sharp MD, Pogliano K. 2002. Partitioning of chromosomal DNA during establishment of cellular asymmetry in *Bacillus subtilis*. *J Bacteriol* 184: 1743-1749.
12. Sharpe ME, Errington J. 1995. Postseptational chromosome partitioning in bacteria. *Proc. Natl. Acad. Sci.* USA 92: 8630-8634.
13. Sharpe ME, Errington J. 1996. The *Bacillus subtilis soj-spo0J* locus is required for a centromere-like function involved in prespore chromosome partitioning. *Mol. Microbiol.* 21: 501-509.
14. Thomaides HB, Freeman M, El Karoui M, Errington J. 2001. Division-site-selection protein DivIVA of *Bacillus subtilis* has a second distinct function in chromosome segregation during sporulation. *Genes Devel.* 15: 1662-1673.
15. Wu LJ, Errington J. 1994. *Bacillus subtilis* SpoIIIE protein required for DNA segregation during asymmetric cell division. *Science* 264: 572-575.
16. Wu LJ, Errington J. 1998. Use of asymmetric cell division and *spoIIIE* mutants to probe chromosome orientation and organization in *Bacillus subtilis*. *Mol. Microbiol.* 27: 777-786.
17. Wu LJ, Errington J. 2002. A large dispersed chromosomal region required for chromosome segregation in sporulating cells of *Bacillus subtilis*. *EMBO J* 21: 4001-4011.
18. Wu LJ, Lewis PJ, Allmansberger R, Hauser PM, Errington J. 1995. A conjugation-like mechanism for prespore chromosome partitioning during sporulation in *Bacillus subtilis*. *Genes Devel.* 9: 1316-1326.

Response to replication fork arrest in *Bacillus subtilis*

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Growing cells have a variety of regulatory processes that respond to the arrest of DNA replication. In bacteria, arrest of a replication fork induces the well characterized SOS response, the RecA-mediated inactivation of the repressor LexA and subsequent induction of transcription of many genes. We have found that replication fork arrest affects expression of many genes in addition to those controlled by the well understood *rec*A-dependent SOS response. In *Bacillus subtilis*, replication fork arrest caused induction of the *rec*A-dependent SOS response, but also caused inhibition of sporulation, and a global regulatory response independent of *rec*A, that is likely to be conserved in a wide range of bacteria.

In *B. subtilis*, the initiation of sporulation is controlled by DNA replication: arrest of replication forks, DNA damage, and defects in replication initiation all inhibit the initiation of sporulation by inhibiting activation (phosphorylation) of the central transcriptional regulator of sporulation, Spo0A. There are both *rec*A-dependent and *rec*A-independent mechanisms for inhibiting sporulation in response to defects in replication status (Ireton and Grossman, 1992; Ireton and Grossman, 1994).

Inhibition of sporulation in response to defects in replication initiation genes (*dna*A, *dna*B, and *dna*D) is due to increased expression of a small gene called *sda* (Burkholder et al., 2001). *sda* encodes an inhibitor of at least 2 of the histidine protein kinases, KinA and KinB, needed for activation of Spo0A and initiation of sporulation. Deletion of *sda* bypasses the effects of defects in the replication initiation genes. The *sda* gene product interacts directly with KinA (and probably KinB) and inhibits autophosphorylation. In this way, *sda* serves to inhibit the initiation of sporulation in response to certain defects in replication initiation.

Transcription of *sda* is directly activated by the replication initiation protein (and transcription factor) DnaA. In the replication initiation mutants, there is probably an accumulation of the active form of DnaA, DnaA-ATP, causing increased expression of *sda*.

sda is also regulated by RecA and LexA. There is a LexA binding site in the *sda* promoter region and transcription of *sda* is induced in response to DNA damage. Promoter fusions and deletion analysis indicate that some of the induction of *sda* is dependent on the LexA binding site and is RecA-mediated.

Upon arrest of replication forks, there is strong induction of transcription of *sda*. As expected, the *rec*A-dependent SOS response contributes to that induction. In addition, there

is significant induction in the absence of *rec*A. This induction depends on the DnaA-binding sites in the *sda* promoter region and by inference on DnaA. We suspect that DnaA-ATP accumulates upon replication fork arrest, as is the case in *E. coli* (Katayama et al., 1998). In this way, DnaA activity is coupled to the status of the replication fork and changes in the active form of DnaA then influences sporulation by controlling transcription of *sda*.

To evaluate the effects of replication fork arrest on gene expression in *B. subtilis* we used DNA microarrays to monitor RNA levels from virtually all of the known *B. subtilis* genes. We found that there are many changes in RNA levels in response to replication fork arrest. As expected, many of these are *recA*-dependent. However, expression of over 100 genes in more than 40 operons is affected by replication fork arrest, independently of *recA* and *sda*. At least 20 of the operons affected have recognizable DnaA binding sites, indicating that DnaA is a mediator of a global transcriptional response to arrest of DNA replication. Many of the genes affected in *B. subtilis* have homologues in other organisms, and some of these also have putative DnaA binding sites, indicating that aspects of this response might be highly conserved.

References:

Burkholder, W.F., Kurtser, I., and Grossman, A.D. (2001). Replication initiation proteins regulate a developmental checkpoint in *Bacillus subtilis*. Cell 104, 269-279.

Ireton, K. and Grossman, A.D. (1992). Coupling between gene expression and DNA synthesis early during development in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA 89, 8808-8812.

Ireton, K. and Grossman, A.D. (1994). A developmental checkpoint couples the initiation of sporulation to DNA replication in *Bacillus subtilis*. EMBO J. 13, 1566-1573.

Katayama, T., Kubota, T., Kurokawa, K., Crooke, E., and Sekimizu, K. (1998). The initiator function of DnaA protein is negatively regulated by the sliding clamp of the *E. coli* chromosomal replicase. Cell 94, 61-71.

Coordination of cell growth and division in *Bacillus subtilis*

Richard A. Daniel

Cell growth and division are the most fundamental processes undertaken by all living cells. Generally it involves a series of distinct but interconnected events, including; achievement of a doubling in cell mass, complete replication of the genomic DNA, segregation of the sister chromosomes, and finally cytokinesis, which can occur either by constriction or by formation of a division septum. Some aspects of cell division are better understood in eukaryotic cells because the process of mitosis involves a series of prominent cytological changes easily visible by microscopy. However, cell division is no less important in bacterial cells and is a crucial step in the phenomenal rates of proliferation that these simple cells achieve.

Great advances have recently been made in the determination of assemble of proteins at the division site in bacteria. However, little is known about the function of the majority of these proteins or how they interact at the division site. To try and dissect the assembly of the division complex in *B. subtilis* a null mutant of the divIB gene was used to select for mutations that suppressed the division defect associated with the loss of this protein. Using this selection process a set of mutant strains were isolated that provide genetic evidence for a complex set of interactions occurring between 4 essential division proteins (FtsL, DivIB, DivIC and PBP 2B). Work to obtain biochemical evidence for this "interaction network" is now in progress.

To further extent our understanding of the cell division process attempts have been made to characterize the function of the essential membrane proteins FtsW and RodA in cell division and cell growth respectively. This analysis lead to the development of a cell staining method that provides evidence for the localized export of peptidoglycan precursors to the surface of the growing cell. Work is now concentrated on the confirmation of the precise nature of the staining pattern and how it is linked to other cellular processes involved in cell shape and division.

Sporulation-associated cell-division in *Streptomyces coelicolor*

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In *Streptomyces coelicolor*, there is an extraordinary morphological and cellular change associated with the production of dispersive spores: filamentous hyphae with many tens of genomes per cell compartment grow into the air, and then undergo synchronous and regular cell division to form unigenomic compartments, which in turn go on to become rounded, thick-walled spores. The spores are pigmented (grey), and mutants defective in sporulation have been readily collected because their aerial mycelium remains white on prolonged incubation. Such sporulation-defective *whi* mutants generally fall into a small number of groups, identifying seven regulatory *whi* genes (*whiA, B, D, G, H, I, J*). Several other mutations giving a white colony phenotype have been identified more recently, including some affecting sporulation-specific aspects of FtsZ function ((Flardh *et al.*, 2000 ; K. Flardh, pers. comm.), indicating that the regulatory cascade emerging from studies of *whi* mutants (Chater, 2001) also involves aspects of morphological coupling. The underpinning regulatory cascade must ultimately bring about an orderly sequence of cell-division and morphological events, coupled with metabolic changes. We have been studying two aspects of this: interactions among the regulatory *whi* genes, and chromosome partitioning during sporulation.

Commitment of aerial hyphae to sporulation requires the action of the sigma factor encoded by *whiG*. This protein directs RNA polymerase to the promoters of *whiH*, which encodes a GntR-like regulatory protein (Ryding *et al.*, 1998), and *whiI*, which encodes a response regulator-like protein (Ainsa *et al.*, 1999). There is evidence of auto- and cross-regulation of *whiH* and *whiI*. Some evidence points to their close involvement in regulating genes for sporulation septation and associated processes involving the cellular DNA. The activities of *whiH* and *whiI* appear to be modulated by two *whiG*-independent genes, *whiA* (Ainsa *et al.*, 2000) and *whiB* (Davis and Chater, 1992). Progress in understanding the interplay of these *whi* genes will be described

The accurate partitioning of chromosomes from the multigenomic aerial hyphal compartments into unigenomic pre-spore compartments involves the *parAB* gene pair located near the replication origin of the linear chromosome – *parAB* mutations show no obvious effects on hyphal growth and branching, but they cause about 13% mis-segregation of genomes into spores (Kim *et al.*, 2000). The ParB protein has been found to interact in vitro and in vivo with a number of conserved ParS sequences located within a few hundred

kilobases of OriC, and it seems that the protein can assemble along the DNA in between these sites (Jakimowicz, 2002). The implication that there should be a large transient requirement for *parAB* expression at the time of sporulation septation is supported by the finding of a sporulation-associated strong upshift in the use of one of two well-defined *parAB* promoters (Kim *et al.*, 2000). The involvement of a postulated autoregulatory circuit and of the *whi* gene regulatory cascade in this upshift is being investigated.

References:

Ainsa, J.A., Parry, H.D., and Chater, K.F. (1999) A response regulator-like protein that functions at an intermediate stage of sporulation in Streptomyces coelicolor A3(2). *Mol Microbiol* **34**: 607-619.

Ainsa, J.A., Ryding, N.J., Hartley, N., Findlay, K.C., Bruton, C.J., and Chater, K.F. (2000) WhiA, a protein of unknown function conserved among gram-positive bacteria, is essential for sporulation in Streptomyces coelicolor A3(2). *J Bacteriol* **182**: 5470-5478.

Chater, K.F. (2001) Regulation of sporulation in Streptomyces coelicolor A3(2): a checkpoint multiplex? *Curr Opin Microbiol* **4**: 667-673.

Davis, N.K., and Chater, K.F. (1992) The Streptomyces coelicolor whiB gene encodes a small transcription factor-like protein dispensable for growth but essential for sporulation. *Mol Gen Genet* **232**: 351-358.

Flardh, K., Leibovitz, E., Buttner, M.J., and Chater, K.F. (2000) Generation of a non-sporulating strain of Streptomyces coelicolor A3(2) by the manipulation of a developmentally controlled ftsZ promoter. *Mol Microbiol* **38**: 737-749.

Jakimowicz D, Chater K., Zakrzewska-Czerwinska J. (2002) The ParB protein of Streptomyces coelicolor A3(2) recognizes a cluster of parS sequences within the origin-proximal region of the linear chromosome. *Mol Microbiol* **45**: 1365-1377.

Kim, H.J., Calcutt, M.J., Schmidt, F.J., and Chater, K.F. (2000) Partitioning of the linear chromosome during sporulation of Streptomyces coelicolor A3(2) involves an oriC-linked parAB locus. *J Bacteriol* **182**: 1313-1320.

Ryding, N.J., Kelemen, G.H., Whatling, C.A., Flardh, K., Buttner, M.J., and Chater, K.F. (1998) A developmentally regulated gene encoding a repressor-like protein is essential for sporulation in Streptomyces coelicolor A3(2). *Mol Microbiol* **29**: 343-357.

Essential role of a DivIVA homologue in polar growth and morphogenesis in *Streptomyces coelicolor*

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Streptomycetes grow as highly polarized hyphae. In analogy to filamentous fungi, extension of the cell wall occurs at the tip of the apical cell, which is long and contains multiple copies of the genome. After cell division, the subapical cell is unable to grow until a lateral branch with a new hyphal tip has been created. The basis for such asymmetric growth and marked cell polarization in a prokaryotic cell is unknown. We have found that the *Streptomyces coelicolor* homologue of *Bacillus subtilis* DivIVA has an important role in hyphal tip growth. A DivIVA-EGFP hybrid protein was shown to localise distinctively at vegetative hyphal tips, and nascent lateral branch points. No or only very weak signals were seen at other cell poles or septation sites. The gene could not be disrupted unless the gene product was provided *in trans*. A strain was constructed in which expression of the *divIVA* homologue could be strongly reduced. This underexpression produced a phenotype reminiscent of some nuclear migration or tip growth mutants in fungi (irregularly shaped, crooked hyphae, with branching in the apical cell). On the other hand, overexpression of the gene dramatically altered the cell shape. Hyphal tips became rounded and swollen, but continued growing to produce pear-shaped cells, much shorter and wider than normal hyphae. These characteristic cells were able both to form septa and to branch, albeit often aberrantly. They also had a cell wall which appeared to be of normal thickness and which retained the unusual cell shape after lysis. In summary, the results show that the *divIVA* homologue is essential and has important roles in hyphal tip growth and cell shape determination in *Streptomyces*. In contrast to the situation in *B. subtilis*, the *S. coelicolor* protein does not primarily affect cell division.

Checkpoints and the regulation of cell division in *Caulobacter*

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In *Caulobacter*, each cell division gives rise to two morphologically and functionally different progeny cells: a motile swarmer cell and a sessile stalked cell (Martin and Brun, 2000). The progeny stalked cell is immediately capable of initiating a new round of DNA replication and cell division, whereas the swarmer cell is unable to do so. After approximately one-third of the cell cycle, the swarmer cell initiates DNA replication as it differentiates into a stalked cell. The different cell division capacity of the two progeny cells is the result of combined transcriptional and proteolytic regulation of cell division proteins. The cell division initiation protein FtsZ is degraded at the end of each cell division cycle (Quardokus et al, 1998 and 2001). After cell division, *ftsZ* is only transcribed in stalked cells (Kelly et al, 1998). The differential transcription of *ftsZ* is negatively controlled by the global response regulator CtrA. CtrA is degraded in the stalked cell compartment of late predivisional cells, whereas it remains stable in the swarmer cell where it represses *ftsZ* transcription (Kelly et al, 1998). CtrA is also a repressor of DNA replication initiation. The degradation of CtrA during swarmer to stalked cell differentiation allows *ftsZ* transcription to begin at the start of the DNA replication cycle. However, coordination of FtsZ synthesis with DNA replication, while required for the proper positioning of Z-rings, is not required to couple cell division initiation with replication; Z-rings are still formed when DNA replication is blocked, even at the initiation stage (Quardokus and Brun, 2002). Thus, there is a need to couple later stages of cell division to DNA replication. One mechanism for this coupling is through the transcription of late cell division genes. *ftsQ* and *ftsA* are are specifically transcribed towards the end of the DNA replication period (Sackett et al, 1998). *ftsQ* and *ftsA* are co-transcribed by the promoter Pqa whose transcription is positively regulated by CtrA (Wortinger et al, 2000). The burst of Pqa transcription thus occurs following the completion of DNA replication when CtrA synthesis begins in predivisional cells. Inhibition of DNA replication prevents resynthesis of CtrA and activation of *ftsQA* transcription in predivisional cells (Wortinger et al, 2000). Consistent with this checkpoint model is the fact that FtsQ and FtsA are degraded after the completion of each cell division cycle. Thus, the differential regulation of cell division proteins by cell cycle dependent transcription and proteolysis allows the establishment of checkpoints that coordinate cell division and DNA replication.

References:

Din, N., E. M. Quardokus, M. Sackett, and Y.V. Brun. 1998. Dominant-lethal C-terminal deletions of FtsZ that affect its ability to localize in *Caulobacter* and its interaction with FtsA. Molecular Microbiology, 27: 1051-1063.

Kelly, A.J., M. Sackett, N. Din, E. M. Quardokus, and Y.V. Brun. 1998. Cell cycle dependent transcriptional and proteolytic regulation of FtsZ in *Caulobacter*. Genes and Development, 12: 880-893

Martin, M. and Y. V. Brun. 2000. Coordinating development with the cell cycle in *Caulobacter*. Current Opinion in Microbiology, 3: 589-595.

Quardokus, E. M., N. Din, and Y.V. Brun. 1996. Regulation and cell type specific localization of the FtsZ division initiation protein in *Caulobacter*. PNAS USA, 93, p. 6314-6319.

Quardokus, E. M., N. Din, and Y. V. Brun. 2001. Cell cycle and positional constraints on FtsZ localization and the initiation of cell division in *Caulobacter crescentus*. Molecular Microbiology. 39: 949-959.

Quardokus, E. M. and Y. V. Brun. 2002. DNA replication initiation is required for mid-cell positioning of FtsZ rings in *Caulobacter crescentus*. Molecular Microbiology, 45: 605-616.

Sackett, M., A. J. Kelly, and Y.V. Brun. 1998. Ordered expression of ftsQA and ftsZ during the *Caulobacter* cell cycle. Molecular Microbiology, 28: 421-434.

Wang, Y., B. Jones, and Y. V. Brun. 2001. A set of FtsZ mutants blocked at different stages of cell division in *Caulobacter*. Molecular Microbiology, 40: 347-360.

Wortinger, M., M. Sackett, and Y. V. Brun. 2000. CtrA mediates a DNA replication checkpoint that prevents cell division in *Caulobacter*. EMBO J., 19: 4503-4512.

POSTERS

A missense mutation in *ftsZ* differentially affects vegetative and developmentally controlled cell division in *Streptomyces coelicolor* A3(2)

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Streptomyces coelicolor A3(2) undergoes at least two different kinds of cell division: vegetative division by crosswall formation in the substrate mycelium, and the developmentally regulated sporulation septation only in sporogenic aerial hyphae. Both types of cell division require the FtsZ protein, but are differentially regulated in time, space and cell-type specificity. By isolation and characterisation of a missense mutation in *ftsZ*, we could genetically distinguish between the two types of septation at the level of FtsZ-ring formation. The *ftsZ*(spo)*1* allele specifically prevented sporulation and gave rise to a classical *white* phenotype. The mutant grew as well as the parent on plates, and was able to lay down apparently normal vegetative hyphal crosswalls. In contrast, it was deficient in making sporulation septa and had a phenotype reminiscent of *whiH* and *ftsZΔ2p* mutants. The *ftsZ*(spo)*1* allele was partially dominant, and had no detectable effect on the cellular amount of FtsZ. As judged from both an *ftsZ-egfp* translational fusion and immunofluorescence microscopy, the mutation prevented assembly of FtsZ rings at the correct time and place in sporulating aerial hyphae, despite normal developmental up-regulation of *ftsZ* expression. The mutation was an A249T change in the C-terminal domain of FtsZ, probably affecting the conformation on the lateral side of FtsZ protofilaments, rather than being directly involved in their polymerization. The results indicate that, in addition to transcriptional regulation, cytokinesis may be developmentally controlled also at the level of FtsZ-ring assembly during sporulation of *S. coelicolor* A3(2). Suppressor mutations that restore sporulation to the *ftsZ*(spo)*1* mutant have been isolated and are being characterised.

FtsA & FtsZ families of proteins. Bioinformatic analysis of sequences and structures

Paulino Gómez-Puertas

Structure-based sequence alignments of the FtsA and FtsZ families of proteins, including their eucharyotic counterparts actin and tubulin, were analysed using bioinformatic approaches to extract functional knowledge from evolutive and structural information.

Molecular models for FtsA and FtsZ homodimeric interaction were performed using, well structural data from their eucharyotic homolog (FtsZ and tubulin; Mingorance et al. 2001), well according to structure docking approaches in addition to correlated mutations data and prediction of residues responsible for protein-protein interaction using neural network-based methodology (FtsA; Carettoni et al, 2002).

In addition to structural data, functional characteristics of both proteins were extracted from sequence comparison to other members of their respective families, including differences in evolutive adaptation to the interaction to molecular chaperones, as the eucharyotic chaperonin CCT (Llorca et al. 2001).

Experimental results supporting the bioinformatic models-based predictions and speculation on their functional significance will be discused.

References:

1.- Mingorance, J., Rueda, S., Gomez-Puertas, P., Valencia, A. & Vicente, M. (2001). Escherichia coli FtsZ polymers contain mostly GTP and have a high nucleotide turnover. Molecular Microbiology 41, 83-91. 2.- Carettoni, D., Gomez-Puertas, P., Yim, L., Mingorance, J., Massida, O., Vicente, M., Valencia, A., Domenici, E., & Anderluzzi, D. Phage-display and correlated mutations identify an essential region of subdomain 1C involved in homodimerization of Escherichia coli FtsA. Proteins: Structure, Function and Genetics, 2002. (in press) 3.- Llorca, O., Martin-Benito, J., Gomez-Puertas, P., Ritco_Vonsovici, M., Willison, K. R., Carrascosa, J. L. & Valpuesta, J. M. (2001). Analysis of the interaction between the eukaryotic chaperonin CCT and its substrates actin and tubulin. Journal of Structural Biology 135, 205-218.

Temporal and spatial regulation of Z ring assembly in *Bacillus subtilis*

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Cell division in vegetatively growing rod-shaped bacteria occurs at midcell between two replicated chromosomes. The first protein to assemble at the future division site is FtsZ, which polymerizes into an annular structure called the Z ring. To understand how cell division is regulated, one of the key questions is what determines the timing of assembly and positioning of the Z ring?

Although it has been known for many years that the Min system and nucleoid occlusion play a role in division site (and Z ring) placement, it is still unclear how these mechanisms cooperate to achieve precise placement of the Z ring at midcell and whether any other factors are involved. We have found that in *B. subtilis*, midcell Z ring assembly is intimately linked to the early stages of chromosome replication1,2 and this could be at least one way in which coordination between replication and division is achieved. Recently we published a comprehensive model proposing how the Min system, nucleoid occlusion and chromosome replication all work together to ensure correct temporal and spatial regulation of Z ring assembly1. We have tested a highly speculative aspect of this model: the blocking and unblocking of Z ring assembly at midcell by the replisome2.

We have compared the precision of Z ring positioning with that of the midcell replisome during the first cell cycle in a synchronous population of *B. subtilis* outgrown spores using FtsZ and PolC fused to fluorescent proteins (YFP and GFP). Interestingly, it appears that Z rings are more precisely positioned than the midcell replisome (and other elements including the origin and terminus regions of the chromosome). So it may not be the replisome as such that defines the midcell Z ring assembly site but rather, a site or structure to which it is anchored. We have also tested various other aspects of our model As predicted, we have shown that the Min system is not required for precise positioning of the Z ring at midcell in *B. subtilis*. Rather its primary role appears to be the prevention of Z rings at the nucleoid-free poles. By examining the position of non-central Z rings, under conditions which block midcell Z ring assembly, we have more also more clearly defined the roles of nucleoid occlusion and the Min proteins in division site placement.

References:

1 Harry, E. J., Rodwell, J., and Wake, R. G. (1999). Mol. Microbiol. 33, 33-40
2 Regamey, A., Harry, E. J., and Wake, R. G. (2000). Mol. Microbiol. 38, 423-434

Energetics of archaeal FtsZ assembly

Sonia Huecas and Jose Manuel Andreu

FtsZ, the homologue of eukaryotic tubulin, is the first protein recruited to the division site where it assembles into the Z ring which is required for the cytokinesis in prokaryotic cells. FtsZ is ubiquitous in eubacteria and archaea, has GTPase activity and forms a number of different polymers *in vitro* depending on the assay conditions [1]. Although the oligomerization of FtsZ from *E. coli* has been well characterized [2], apparently contradictory reports have either confirmed [3, 4, 5] or questioned [6] whether the polymerization of FtsZ and the induction of the GTPase are cooperative. We have quantified the assembly of FtsZ from *Methanococcus jannaschii* with the guanosine nucleotides GMPCPP, GTP, GMPCP or GDP, and Mg^{2+}, at temperatures ranging from 30 to 55 C. The polymerization was monitored with light scattering and electron microscopy, and the concentration of polymers formed was measured by sedimentation. In this work, we show that FtsZ cable polymers form cooperatively in a nucleated condensation polymerization reaction [7], with critical concentration values in the micromolar range. The effects of temperature and solution variables including pH, Mg^{2+}, and ionic strength on the affinity of FtsZ polymer elongation have also been measured.

Assembly requires magnesium ions and is characterized by an apparent change in heat capacity of -800 ± 100 cal mol-1 K-1 and positive enthalpy and entropy changes, compatible with the loss of ordered water upon the formation of each FtsZ-FtsZ contact.

References:

1. Rothfield L, Justice S, and García-Lara J. (1999) Annu. Rev. Genet. 33, 423.
2. Rivas G, Lopez A, Mingorance J, Ferrandiz MJ, Zorrilla S, Minton AP, Vicente M, and Andreu JM. (2000) J. Biol. Chem. 275, 11740.
3. Lu C, Stricker J and Erickson HP. (1998) Cell Motility and Cytoskeleton 40, 71.
4. Mukherjee A, and Lutkenhaus J. (1998) EMBO J. 17, 462.
5. White L, Ross LJ, Reynolds RC, Seitz LE, Moore GD, and Borhani DW. (2000) J. Bacteriol. 182, 4028.
6. Romberg L, Simon M and Erickson HP. (2001) J. Biol. Chem. 276, 11743.
7. Oosawa and Asakura S. (1975) Thermodynamics of the polymerization of protein, Academic Press, London.

Molecular mechanism of unipolar targeting of the *Shigella* actin assembly protein IcsA

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Three-dimensional localization of proteins to specific sites within bacteria is essential to many bacterial functions, including flagellar assembly, cell division, and pathogenesis. An example of such protein localization is found in *Shigella*, the causal agent of bacillary dysentery. The *Shigella* protein that polymerizes host cell actin, IcsA (VirG), is targeted to the old pole (Goldberg et al., 1993). Actin tail assembly occurs behind this pole and generates a force sufficient to propel the bacterium through the cytoplasm of infected cells and into adjacent cells. Two distinct regions of IcsA, located at amino acids 1-104 (targeting region 1) and 507-620 (targeting region 2), are each necessary and sufficient to mediate polar targeting (Charles et al., 2001). Notably, IcsA targeting regions localize to the pole in a variety of *Enterobacteriacae* and *Vibrio*, which indicates that IcsA uses a mechanism of targeting that is common among these organisms (Charles et al., 2001). As of yet, the basic mechanisms that mediate IcsA targeting, including the identification of the target structure it recognizes at the pole, are unknown.

Currently, we are attempting to identify those amino acid residues within each of the two IcsA targeting regions that are important in the interaction with the putative polar target structure. We are altering the amino acid composition within the targeting regions, initially by clustered charged-to-alanine scanning mutagenesis, as charged residues are typically exposed on the surface of folded proteins. Each mutant construct's ability to localize to the pole is determined by the creation of translational fusions to GFP, and visualization using fluorescent microscopy. None of the charged-to-alanine changes in the IcsA targeting regions display loss of polar localization. These results suggest that hydrophobic residues in the IcsA targeting regions could be mediating the interaction with the polar target structure. Presently, we are evaluating amino acid changes in the hydrophobic stretches of the two targeting regions of IcsA using site-directed-mutagenesis. Any non-targeting mutants identified will be used to screen for extragenic suppressors of IcsA localization. Such analysis may provide clues to what IcsA could be sensing at the pole and/or other proteins that interact with IcsA and are involved in its targeting.

References:

Charles, M., M. Perez, J. H. Kobil, and M. B. Goldberg 2001. Polar targeting of Shigella virulence factor IcsA in Enterobacteriacae and Vibrio. Proc. Natl. Acad. Sci. USA. 98:9871-9876.

Goldberg, M. B., O. Barzu, C. Parsot, and P. J. Sansonetti 1993. Unipolar localization and ATPase activity of IcsA, a Shigella flexneri protein involved in intracellular movement. J. Bacteriol. 175:2189-2196.

FtsZ in the moss *Physcomitrella patens*

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In procaryotes, cell division is mediated by the protein FtsZ, which assembles into a ring structure at the future site of cytokinesis (1). As tubulin and FtsZ share similar three-dimensional structures and polymerize to related structures *in vitro*, FtsZ is supposed to be the ancestor of tubulin (2). In general, only one *ftsZ* gene is found in nearly all eubacteria (3). As plastids are endosymbiotic organelles of cyanobacterial origin (4), the mechanism of organelle division by FtsZ proteins was conserved while the corresponding gene was transferred to the nucleus. However, in contrast to procaryotes, plant species comprise several nuclear-encoded FtsZ homologs suggesting functional diversity for these homologs. Plant FtsZ proteins cluster in two families designated FtsZ1 and FtsZ2. So far, it has been shown in several plant species that members of both families are localized within plastids. In Arabidopsis, FtsZ homologs of both families polymerize to ring structures near the inner envelope membrane within plastids (5).

The moss *Physcomitrella patens* harbors four FtsZ homologs. Using GFP fusions driven by the 35 S-promoter, members of the PpFtsZ2 family were shown to be imported into plastids forming network-like structures within these organelles (6).
A detailed analysis of the subcellular localization of each single member utilizing *ftsZ::gfp* fusion constructs will be presented.

References :
1. E. F. Bi and J. Lutkenhaus, Nature **354**, 161 (1991)
2. J. Löwe and L.A. Amos, Nature **391**, 203 (1998)
3. J. Lutkenhaus and S. G. Addinall, Annu. Rev. Biochem. **66**, 93 (1997)
4. W. Martin and R.G. Herrmann, Plant Physiol. **118**, 9 (1998)
5. Vitha et al., J. Cell Biol. **153**, 111 (2001)
7. Kiessling et al., J Cell Biol. **151**, 945 (2000)

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Genetic and biochemical evidence for FtsZ-associated proteins in the filamentous bacterium *Streptomyces coelicolor* A3(2)

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Homologues of many division genes, first identified as essential genes in the unicellular bacteria *Escherichia coli* and *Bacillus subtilis*, have been identified, studied and shown to be dispensable for viability of the sporulating filamentous organism *Streptomyces coelicolor*. The complete sequence of the *S. coelicolor* genome has been determined and obvious homologues coding for two essential *E. coli* proteins, FtsA and ZipA, are absent in *S. coelicolor*. Both of these proteins are known to interact directly with a short conserved block of amino acids in the C-terminal domain of tubulin-like FtsZ. A paradox exists because although the C-terminal block of FtsZ is conserved in *S. coelicolor*, genes encoding important interacting proteins are absent. In order to probe its functional importance, we constructed an allele of ftsZ encoding a protein lacking the final 12 amino acids (FtsZD12), which includes the conserved C-terminal block of amino acids. This deletion allele does not support division on its own and behaves as a dominant-negative mutation in the presence of the wild-type allele. Alanine-scanning mutations have been constructed for 6 residues in the conserved C-terminal block to further dissect the requirement. All six alanine mutants have at least a partial dominant negative phenotype and cannot support division when present as the only allele. Our data are consistent with the interpretation that either functional homologues of FtsA and/or ZipA are present in *S. coelicolor* or the C-terminal domain of FtsZ is also important for an unrecognized function. As a complementary strategy, we have used a biochemical approach to search for proteins that directly interact with FtsZ in streptomycetes. Putative FtsZ-associated proteins have been identified. Experiments are underway to purify the proteins. Finally, wehave used a unique screen to identify a mutant containing a dominant-negative allele of ftsZ that appears to be blocked at the level of FtsZ-ring assembly. We are attempting to isolate reversion mutants containing intragenic suppressors by site-directed and random mutagenesis, which may contribute to the further understanding of this key cytoskeletal protein.

Functional analysis of *Escherichia coli* FtsW

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The polytopic membrane protein FtsW is essential for cell division in *Escherichia coli*. The protein could interact with the other cell division proteins during septation. To investigate its role in septum formation, we analysed the effect of point mutations on its in vivo activity by complementation and on its localization after fusion to the Green Fluorescent Protein (GFP).

Mutations have been introduced in regions susceptible to interact with other proteins and in conserved regions of the protein.

The results allowed to define regions with distinct functions.

Artificial microbiology and the bacterial cell cycle

Maurice Demarty, Derek Raine, Camille Ripoll and Vic Norris

Bacterial cells can be considered autocatalytic networks that increase in mass and then divide. We have written a program of artificial microbiology that simulates a cell fed by amino acids. These amino acids are symbols that can be assembled into linear polymers to give different lengths. A reaction is catalysed by a particular polymer or 'enzyme' that may itself be a reactant of that reaction (autocatalysis). These reactions are only studied within the confines of the "cell" or "reaction chamber".

There is a flux of material through the cell since monomers and polymers may be both acquired by and lost from the cell. Eventually, the mass of polymers in the cell reaches a threshold at which cell division occurs.

The two daughter cells can then be competed against one another and the faster growing cell retained. In real bacterial cells, we have proposed that hyperstructures, alias large collections of diverse molecules, assemble to perform particular functions; we have also proposed that the dynamics of these hyperstructures drives the cell cycle. In our program, we can attribute increased probabilities of reactions to polymers that are colocalised and thereby, we hope, evaluate the consequences of hyperstructure dynamics on the relationship between cell cycle events and evolution.

Reversible assembly and GTP hydrolysis by FtsZ from *M. jannaschii*

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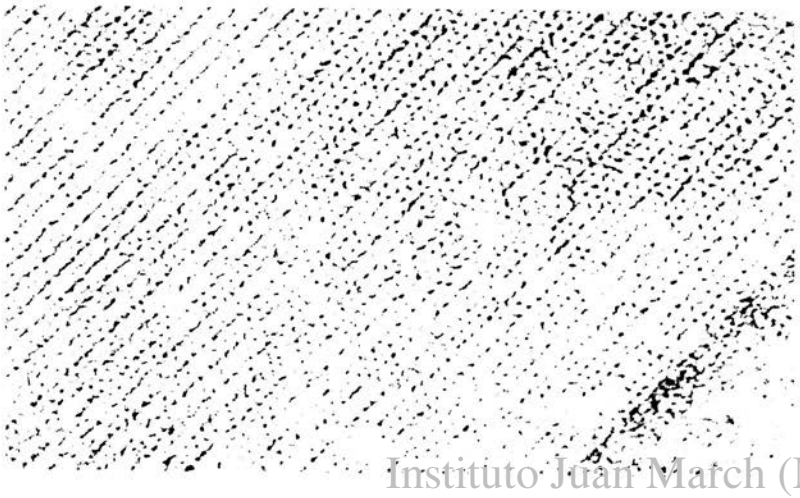
We have studied the assembly and GTPase activity of FtsZ from the hyperthermophilic archaeon *Methanococcus jannaschii*, a structural homologue of eukaryotic tubulin. Four different protein constructs have been employed: FtsZ (FtsZ1, wild type protein), FtsZ-His_6 (FtsZ1 with a C-terminal GlySerHis_6 tag, originally employed for purification and crystallisation by Löwe and Amos, 1998), and the point mutants FtsZ-W319Y and FtsZ-W319Y-His_6. W319 is the single tryptophan of FtsZ, located in the beta-sheet strand S9, less than 1nm away from residue Asp238 and the co-catalytic loop T7. The four protein forms have indistinguishable secondary structures (circular dichroism) and stabilities (unfolding with guanidinium hydrochloride). Their polymerisation was characterised with light scattering and electron microscopy.

Archaeal FtsZ assembly is induced by GTP (or GMPCPP) and Mg^{2+} above 40 C, and it is inhibited by GDP. FtsZ polymers rapidly disassemble after hydrolysing the GTP in the buffer (or slowly consuming GMPCPP), suggesting that the nucleotide is rapidly exchanged in the polymer. The polymerisation-induced GTPase of FtsZ- His_6 is inhibited by substituting Na^+ for K^+ in the buffer. The assembly of purified FtsZ is polymorphic. FtsZ assembles predominantly into filamentous cable-like polymers, and the His_6 extension enhaces laminar polymerisation. The FtsZ-W319Y-His_6 mutant is an inactive GTPase whose assembly is also regulated by GTP and Mg^{2+}. It easily forms large ordered two-dimensional polymers made of symmetrical pairs of tubulin-like protofilaments asociated in an antiparallel array, similar to the minoritary Ca^{2+}-induced sheets of FtsZ- His_6 (Löwe and Amos, 1999).

References:
Löwe J and Amos LA (1998) Nature 391, 203-206
Löwe J and Amos LA (1999) EMBO J 18, 2364-2371



Four conserved glycines in the C-terminus of MinC are essential for protein functionality

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MinC functions as a cell division inhibitor in *Neisseria gonorrhoeae* (Ng) and *Escherichia coli* (Ec). It has been reported that the C-terminus of $MinC_{Ec}$ is essential for interaction with itself and with $MinD_{Ec}$. By sequence alignment, we show that the MinC C-terminus is highly similar and contains four completely conserved glycines. Site-directed mutagenesis of these residues was performed on both *E. coli* and *N. gonorrhoeae minC* homologs. Since we have shown that wild-type $MinC_{Ng}$ is active in *E. coli*, we evaluated the functionality of both gonococcal and E. coli MinC mutants by their abilities to induce filamentation in rod-shaped *E. coli* upon protein overexpression. Using microscopy and flow cytometry, we demonstrate that the overexpression of the MinC C-terminal mutants do not affect cell morphology. The G161S and G171E $MinC_{Ec}$ mutants lost their ability to self-interact as determined by yeast two-hybrid assays, and the G135D, G154D and G171E $MinC_{Ec}$ mutants showed lost or decreased interaction with $MinD_{Ec}$. The crystal structure of *Thermotoga maritima* MinC ($MinC_{Tm}$) has been solved, showing that the C-terminus of MinC is a β-helix comprising three different surfaces (A, B and C), with the "A" surface being involved in MinC self-interaction. Upon protein modeling analysis of our mutants using $MinC_{Tm}$ as a template, the conserved glycines were found to reside within the "C" surface and at the "B" and "C" surface junctions. It is therefore possible that these regions, containing the conserved glycines, are involved in MinC-MinD interaction. Structural conservation of the MinC mutants is currently being tested by circular dichroism. We also constructed chimeric proteins containing combined N- and C-termini from $MinC_{Ng}$ and $MinC_{Ec}$. By comparison of the predicted structures of *E. coli*, gonococcal and chimeric MinC with $MinC_{Tm}$ we found differences in the β-strands of the "C" surface in the C-terminus. These results along with differences between MinC-MinD interactions of the chimeras supports our hypothesis that this surface is implicated in the MinC-MinD protein association, and that the conserved glycines of the C-terminus of MinC are essential for its proper function as a cell division inhibitor.

Cloning and characterization of the *Brevibacterium lactofermentum* homologue of the mycobacterial antigen 84 gene involved in cell morphology

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The *Brevibacterium lactofermentum* orf8 gene, an homologue of the *Mycobacterium tuberculosis* antigen 84 gene (wag31), was cloned and sequenced, and in both organisms this gene is located downstream from the dcw cluster. The protein ORF8 showed week similarities with DivIVA coiled-coil proteins, which are analogous to eukaryotic tropomyosins. The orf8 gene is expressed mainly during the exponential growth phase of *B. lactofermentum* and it is expressed in *E. coli* from its own promoter. The protein ORF8 from *B. lactofermentum* was detected in *E. coli* using monoclonal antibodies against M. tuberculosis antigen 84, which also specifically detected a single protein in *B. lactofermentum*, *M. tuberculosis* and *M. smegmatis*, two proteins in *S. coelicolor*, and none in *B. subtilis* or *E. coli*.

Disruption experiments using an internal fragment of the orf8 gene or a disrupted orf8 cloned in a suicide conjugative plasmid were unsuccessful, suggesting that orf8 gene is needed for cell viability in *B. lactofermentum*. Transformation of *B. lactofermentum* with a multicopy plasmid containing the orf8 gene altered drastically the morphology of the corynebacterial cells, becoming bigger and bulky cells.

ORF8-GFP fusions accumulated largely at one end of the corynebacterial cell, suggesting that coryneform bacteria might have an asymmetrical growth, which seems to be a reminiscence of the mycelial growth.

Dynamic assembly of MinD into filament bundles modulated by ATP, phospholipids, and MinE

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Accurate positioning of the division septum at the equator of *Escherichia coli* cells requires a rapid pole-to-pole oscillation of the division inhibitor MinC together with MinD ATPase (1-4). MinD recruits MinC to the membrane and colocalizes with MinC into a polar horseshoe in either half of the cell. Both MinCD assembly and oscillation depend on MinE, which localizes into a ring structure near the medial edge of the MinCD horseshoe and also accumulates as a polar zone along the horseshoe arms (5-6). The mechanisms underlying membrane assembly and acrobatics of the Min proteins remain poorly understood. Here we demonstrate that purified MinD assembles into protein filaments in the presence of ATP. Incubation with phospholipid vesicles further stimulates MinD polymerization. Addition of purified MinE in the presence of lipids promotes bundling of MinD filaments as well as their disassembly via activation of MinD ATPase. MinE thus provokes a net decay in the steady-state MinD polymer mass. Taken together, these results provide a biochemical mechanism for the dynamic processing of positional information in a bacterium to precisely identify the nascent site for cell division.

References:

1. Raskin, D. M. & de Boer, P. A. (1999) *J. Bacteriol.* **181**, 6419-24.
2. Hu, Z. & Lutkenhaus, J. (1999) *Mol. Microbiol.* **34**, 82-90.
3. Raskin, D. M. & de Boer, P. A. (1999) *Proc. Natl. Acad. Sci. USA* **96**, 4971-4976.
4. Rowland, S. L. *et al.* (2000) *J. Bacteriol.* **182**, 613-619.
5. Fu, X., Shih, Y. L., Zhang, Y. & Rothfield, L. I. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 1332-1334.
6. Hale, C. A., Meinhardt, H. & de Boer, P. A. J. (2001) *EMBO J.* **20**, 1563-1572.

Ribosomal RNA synthesis in *Mycobacterium fortuitum* growing *in vitro* and into the macrophages

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The synthesis of ribosomal RNA has been identified as a rate-limiting step in ribosome synthesis, and it has consequently a main influence on the growth-rate regulation of bacteria. *Mycobacterium* is a bacterial genus characterized by its rather slow growth. The genus is formally subdivided into two groups: rapid growers mycobacteria (growth-rate about 2-3 hours) and slow growers *mycobacteria* (growth-rate about 15-20 hours). Differences when comparing *rrn* operons between mycobacterial species account not only in the number of *rrn* operons, but mainly in the number of promoters controlling their synthesis. This number is ranging from two promoters in *M. tuberculosis* to five promoters in *M. chelonae*.

M. fortuitum is an opportunistic rapid grower mycobacteria, having two *rrn* operons per genome and up to five *rrn* promoters leading their expression. We have investigated the differential *rrn* promoter usage in this species under different growing conditions.

Primer-extension analysis has been applied on total RNA isolated from mycobacterial cultures. Mycobacteria were recovered from three *in vitro* media with different nutritional content: from a minimal to a rich medium. Promoter usage was quantified by Instant Imager and results compared to each other. Quantitative PCR (Real Time PCR) has been also tested for comparison. On the other hand, RNA isolated from bacteria recovered from infected macrophages, was analysed by using quantitative PCR. Different post-infection times were tested.

Differential usage of *rrn* promoters has been analysed and discuss. Growth-rate of *M. fortuitum* infecting macrophages could be calculated.

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The roles of specific domains of the cell divison protein FtsA of *Escherichia coli*

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Alterations in a region of FtsA that belongs to a domain structurally conserved in the sugar kinase/hsp70/actin superfamily (b-chains S12 and S13) may have an effect on the placement of the septum, and consequently on the localization of the FtsZ ring. Additionally, the domain 1C, that differs most in its position within the structure of FtsA relative to the rest of the family, although required for the activity and for the self-interaction of the protein, may not be necessary for the interaction of FtsA with FtsZ.

FtsA is an essential *E. coli* cell division protein that integrates into the septator, and is widely conserved in bacteria. Based on sequence homology and structure it has been proposed that FtsA belongs to the sugar kinase/hsp70/actin superfamily (1 and 2). FtsA may interact directly or indirectly, with other proteins, such as FtsZ, PBP3, FtsQ, FtsN and itself (3, 4, 5, 6 and 7).

FtsA protein lacking b-chains (S12 and S13) in domain 2B (FtsADS12-13) cause septa to be placed at abnormal sites along the cell length.

Misplacement of the Z-ring could be either a result or a cause of faulty nucleoid segregation, which is also affected. As a consequence *E. coli* cells expressing FtsADS12-13 from a plasmid contain a small but noticeable proportion of round cells devoid of nucleoid some may be classified as minicells. Cell viability is lost when the FtsADS12-13 proteins are overproduced in the presence of FtsA+. The viability loss is accompanied by a more severe phenotype that includes curved swollen cells. Finding an effect of FtsA mutations on segregation and/or septum placement is intriguing. A plausible explanation could be that FtsADS12-13 could stabilize the FtsZ polymers causing them to assemble into a ring at aberrant positions independently of the Min and nucleoid occlusion systems.

In FtsA domain 1C is positioned differently, relative to other members of the family, and is near the carboxy-end, implicated in the biological function and in the self-interaction of the protein (7). We find that this domain, although required for activity and for the self-interaction of the protein, may not be necessary for interaction of FtsA with FtsZ. An FtsA lacking domain 1C (FtsAD1C) is unable to interact with wild-type FtsA in a yeast two hybrid assay and is not functional in cell division. This truncated protein is able to localize at the potential division site (presumably by interacting with the Z ring), but it fails to allow progression of ring constriction.

The isolated domain 1C is inert (it fails to complement, is not toxic, does not induce any change in cell morphology when overexpressed, it fails to localize as a ring, and does not interfere with the FtsZ polymerization).

References:

1. Bork, P., Sander, C. and Valencia, A. 1992. PNAS, 89: 7290-7294.
2. van den Ent, F. and Löwe, J. 2000. EMBO J. 19: 5300-5307.
3. Dai, K., Xu, Y. and Lutkenhaus, J. 1993. J. Bacteriol. 175: 3790-3797.
4. Descoteaux, A. and Drapeau. 1987. J. Bacteriol. 169: 1938-1942.
5. Ma, X., Sun, Q., Wang, G., Singh, G., Jonietz, E. L. and Margolin, W. 1997. J. Bacteriol. 179: 6788-6797.
6. Tormo, A., Ayala, J.A., de Pedro, M.A. Aldea, M. and Vicente, M. 1986. J. Bacteriol. 166: 985-992.
7. Yim, L., G. Vandenbussche, J. Mingorance, S. Rueda, M. Casanova, J-M. Ruysschaert, and M.Vicente. 2000. J. Bacteriol. 182: 6366-6373.

Macromolecular crowding favours the GTP-dependent dynamic assembly of the *E. coli* cell division protein FtsZ into polymer networks

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In contrast to the experimental solution conditions commonly used in the laboratory, the intracellular environment in which the bacterial cell division protein FtsZ [1,3] is located and functions *in vivo* is highly volume-occupied ('crowded'), with a total concentration of proteins and nucleic acids on the order of 400 g/L [5]. Although excluded volume is likely to have a substantial effect on the energetics and dynamics of functional assembly processes in such a medium [2,4], the influence of crowding on the structural organization and functional interactions of FtsZ with other components of the septator complex has been largely unexplored.

In the presence of GTP, FtsZ forms a variety of polymers in vitro by means of poorly understood mechanisms [1,8]. The magnesium-linked formation of linear oligomers of FtsZ in the presence of GDP rather than GTP is in contrast fairly well understood [6]. It was shown previously that high concentrations of unrelated proteins facilitated the formation of these GDP-FtsZ oligomers in accordance with predictions of excluded volume theory [7]. In the present investigation we begin to explore crowding effects upon the more complex assembly processes taking place in the presence of GTP.

By means of analytical centrifugation, light scattering, optical and electron microscopy, and assays of biochemical activity, we have found that high concentrations of unrelated macromolecules favour the formation of polymer networks of *E. coli* FtsZ in solutions containing the appropriate content of the main physiological osmolytes [5]. This self-organization process, which is GTP dependent, and does not occur in the presence of GDP, reduces significantly the kinetics of the FtsZ GTPase activity and the polymer dynamics of assembly/disassembly, but not the GTP turnover within the polymer when compared with the FtsZ protofilaments normally observed *in vitro*. Our results will be compared with those of parallel studies carried out on tubulin, the eukaryotic relative of FtsZ.

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References:

1. Addinall, S.G., & Holland, B. (2002) J. Mol. Biol. 318:219-236
2. Ellis, R.J. (2001) TIBS 26:597-604
3. Margolin, W. (2000) FEMS Microbiol. Rev. 24:531-548
4. Minton, A.P. (2001) J. Biol. Chem. 276:10577-10580
5. Record Jr., T.M., Courtenay, E.S., Cayley, S., & Guttman, H.J. (1998) TIBS 23:190-194

6. Rivas, G., López, A., Mingorance, J., Ferrándiz, M.J., Zorrilla, S., Minton, A.P., Vicente, M., & Andreu, J.M. (2000) J. Biol. Chem. 275:11740-11749.
7. Rivas, G., Fernández, J.A., & Minton, A.P. (2001) PNAS USA 98:3150-3155
8. Scheffers, D.J., & Driessen, A.J.M. (2001) FEBS Lett. 25254:1-5

Cell cycle parameters of haloarchaea are affected by drugs that act on eukaryotic cytoskeleton

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Colchicin, podophyllotoxin and vincristine are drugs that exert its action over eukaryotic tubulin. We analysed the effect of these drugs in the cell cycle of the extreme halophilic euryarchaeon *Halobacterium salinarum*. Microscopy observation of a exponential culture of this microorganism shows a rod-shaped haloarchaea with an average lenght of 5 m. Using exponential cultures, different parameters were assessed: Cell growth, cell viability, cell size and morphology, frequency of septa, and distribution of septa along the cell.

We found that the three drugs produce growth inhibition and loss of cell viability. By microscopy analysis we found that these drugs also produced a decrease in the percentage of septated cells. In addition, exponential cultures treated with colchicin or podophyllotoxin show a decrease in cell size that is not observed when vincristine is used. Nevertheless, vincristine produces an anomalous distribution of septa along the cells.

An increase in the percentage of cells with aberrant morphology was also observed in all three cases.

Other drugs (paclitaxel, cytochalasin, nodocodazol) known by its effect in eukaryotic cytoskeleton were used, but not effect was observed.

Preliminary results from our group shows that vincristine has effect on a haloarchaeal cellular structure known as the Fibrocrystalline Body (Alba et al. 2001, Extremophiles, 5: 169-175). Further experiments are now conducted in our laboratory in order to identify the molecular target of these drugs.

Cell shape determination in bacteria: Localisation of the cell-wall synthesizing machinery

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Two related proteins from *Bacillus subtilis*, MreB and Mbl, form filamentous helical structures that lie close to the cell surface and are essential for correct cell shape. MreB and Mbl are homologues of eukaryotic actin, and have a cytoskeletal, actin-like role in bacterial cell morphogenesis. MreB-like proteins are present in both bacteria and archaea that have a complex, i.e. non-spherical, shape, implying a conserved role for these proteins. An important question is how an intracellular cytoskeletal structure determines the shape of bacteria. The MreB/Mbl filaments are not strong enough to support the cell shape itself, but are likely to confer structural information to the machinery that assembles the cell wall, which supports the cell structure by counteracting the force of the turgor pressure. Penicillin-binding proteins (PBPs) are a major component of the cell wall synthesizing machinery, as well as the targets of a main class of antibiotics, penicillin and penicillin derivatives. We have determined the position of all (putative) PBPs involved in vegetative growth of *B. subtilis* using fluorescence microscopy to determine whether the position of these proteins is determined by the MreB/Mbl cytoskeleton. We show that during vegetative growth there are two localisation classes of PBPs: one class that is associated with synthesis of cell wall material at the place of the septum, and one class that localises as distinct foci at the circumference of the cell. The dependence of both classes on the MreB/Mbl cytoskeleton will be discussed. Our results point to an organised machinery for cell wall synthesis during bacterial growth.

Cell cycle-dependent expression of an essential SMC-like protein and dynamic chromosome localization in the archaeon *Halobacterium salinarum*

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The genome of *Halobacterium salinarum* encodes four proteins of the SMC protein superfamily. Two proteins form a novel subfamily and are named "SMC-like proteins of *H. salinarum*" (Sph1 and Sph2). Northern blot analyses revealed that *sph1* and *hp24*, the adjacent gene, are solely transcribed in exponentially growing, but not in stationary phase cells.

A synchronization procedure was developed, which makes use of the DNA polymerase inhibitor aphidicolin and leads to highly synchronous cultures. It allowed for the first time to study cell cycle-dependent transcription in an archaeon. The *sph1* transcript was found to be highly cell cycle-regulated, with its maximal accumulation around the time of septum formation. The Sph1 protein level was also elevated at that time, but a basal protein level was found throughout the cell cycle. The *hp24* transcript was sharply up regulated about one hour prior to *sph1*, and already declined at the time of *sph1* induction. These and additional transcript patterns revealed that precisely controlled transcriptional regulation is involved in haloarchaeal cell cycle progression.

A DNA staining protocol was developed, which opened the possibility to follow the dynamic intracellular localization of haloarchaeal nucleoids using synchronized cultures. After an initial dispersed localisation the nucleoid is condensed at mid-cell. Subsequently DNA is rapidly transported to the 1/4 and 3/4 positions. All staining patterns were also observed in untreated exponentially growing cells, excluding synchronization artifacts.

The Sph1 concentration is elevated when segregation of the new chromosomes is nearly complete, therefore it is proposed to play a role in a late step of replication, e.g. DNA repair, similar to eukaryotic Rad18 proteins.

Murein synthesis during cell division and elongation in *Escherichia coli*

Ute Meisel, Tobias Kessler, Joachim-Volker-Hoeltje and Waldemar Vollmer

Various murein synthases participate in the enlargement of the thin, stress-bearing murein sacculus during growth and division of *E. coli*. In addition to synthases, murein hydrolases (autolysins) are active resulting in the loss of 50% of the existing murein material per generation (murein turnover). Murein hydrolases are also essential for cutting the septum during cell division. Both, the coordination of murein synthesis reactions and the tight regulation of the potentially dangerous autolysins might be achieved by combining the synthetic and hydrolytic activities in murein synthesis holoenzymes.

We have isolated the four four major high-molecular weight murein synthases PBP1A, 1B, 2 and 3 and characterized the protein-protein interactions between them. The results support the hypothesis that the activities of murein synthases and hydrolases are coordinated by the formation of multienzyme complexes.

In addition, we overproduced different variants of PBP1B in wild-type cells. By site-directed mutagenesis, we produced expression plasmids which allowed the production of PBP1B proteins containing single amino acid exchanges inactivating either the transpeptidase or transglycosylase domain or both domains. Overproduction of the inactive PBP1B variants - but not of the active enzyme - leads to the cell lysis, proving the tight functional connection between PBP1B and autolytic enzymes. Differences in the lytic response following the overproduction of inactive PBP1B or the N-terminally truncated form (PBP1Bg) with simultaneous inhibition of PBP2 or PBP3 indicate a different cellular function of the two forms in cell elongation and cell division.

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145 **Workshop on The Ubiquitin-Proteasome System.**
Organizers: A. Ciechanover, D. Finley, T. Sommer and C. Mezquita.

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