Targeting the Bacterial Z-Ring

Waldemar Vollmer[1,*]
[1]Institute for Cell and Molecular Biosciences, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK
*Correspondence: W.Vollmer@ncl.ac.uk
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FtsZ is a prokaryotic homolog of eukaryotic tubulin and forms the essential bacterial cell division ring (Z-ring). A new study in this issue of *Chemistry & Biology*, Läppchen et al., provides further evidence that differences in nucleotide-binding properties of FtsZ and tubulin can be exploited to specifically target the bacterial Z-ring.

Bacterial cells are substantially smaller than eukaryotic cells and they lack much of the eukaryotic cellular complexity. Yet, it became very clear in recent decades that the degree of cellular organization in a bacterial cell is much higher than previously thought. For example, older microbiology textbooks contain the dogma that the absence of a cytoskeleton is one of the hallmarks of prokaryotic cells. Today, it is accepted knowledge that bacteria have cytoskeletal elements, which not only show structural similarity to their eukaryotic counterparts but also exhibit remarkably dynamic localization patterns inside the cell. Moreover, these cytoskeletal elements drive or organize essential physiological processes such as cell growth and division, plasmid segregation, or polar targeting of proteins (Vollmer, 2006).

One of these cytoskeletal elements is the Z-ring, which is formed by the FtsZ protein (Bi and Lutkenhaus, 1991). FtsZ is the prokaryotic homolog of the eukaryotic tubulin and is highly conserved in bacteria. Both FtsZ and tubulin share a distinct 2-domain fold, with a long, central α helix connecting an N-terminal nucleotide-binding domain and a C-terminal domain (Löwe and Amos, 1998) (Figure 1). Like tubulin, FtsZ binds to and hydrolyses GTP, and it polymerizes to form protofilaments in which the nucleotide is bound to the interface between two monomers. Unlike tubulin, FtsZ does not form microtubuli. FtsZ is essential for bacterial cell division but also contributes to cell elongation of rod-shaped species (Aaron et al., 2007). During division, it plays a central role in organizing more than a dozen other cell division proteins at the closing septum. Only a fraction (~30%) of the FtsZ molecules localize at mid-cell in a ring-like structure called the Z-ring. The Z-ring is surprisingly dynamic, with a constant exchange of free and ring-bound molecules occurring within seconds, presumably under consumption of GTP. Recent cryoelectron microscopy data indicated that FtsZ filaments might not form a complete ring at the division site, but mostly long, filamentous arches (Li et al., 2007). However, how exactly rapid FtsZ assembly and disassembly drives cell division in bacteria remains largely unknown.

Cell division has been recognized as a possible new target for antimicrobial therapy (Vicente et al., 2006). Blocking cell division is known to be lethal in many pathogenic cocci; for example, in *Staphylococcus aureus*. Even if inhibition of division does not result in cell death, it should still prevent proliferation of the bacteria within the host and the spread of bacteria from infection sites, thus enhancing the efficiency of host-defense factors. The assembly of the Z-ring by inhibition of the GTPase function of FtsZ became a prime target for antimicrobial research in recent years for several reasons. First, it is a process specific for bacterial cells. Second, hydrolysis of GTP by FtsZ is one of the few known enzymatic reactions occurring in septum formation; others are the (hypothetical) ATPase activity of FtsA and the peptidoglycan biosynthesis reactions, which are targeted by β-lactams and glycopeptides. Finally, the assays for FtsZ GTPase activity are relatively simple and can be adapted for medium- and high-throughput screenings. The aim is to identify FtsZ GTPase inhibitors that, at the same time, do not target eukaryotic tubulin, since inhibitors of tubulins are expected to be toxic. In fact, tubulin-inhibitors such as taxol have been shown to be cytotoxic and are used in cancer therapy.

To date, several small-molecule inhibitors of FtsZ have been identified (Vollmer, 2006). Among them is the GTP analog 8-bromo-GTP, which competitively inhibits GTPase activity of FtsZ (and its polymerization) and, most importantly, does not inhibit tubulin (Läppchen et al., 2005). A further extensive and multidisciplinary study on the effect of C-8-substituted GTP analogs on FtsZ and tubulin is published in this issue of *Chemistry & Biology* (Läppchen et al., 2008). Ten derivatives of GTP have been synthesized, which differ in the size and hydrophobicity of the group residing at C-8. All compounds inhibited GTPase activity and polymerization of FtsZ; the most potent inhibitor of this series was 8-methoxy-GTP, with an IC_{50} value for polymerization of 10 μM (at 60 μM GTP). The observed IC_{50} values of the different derivatives correlated with their affinities to nucleotide-free FtsZ and also with sterical parameters calculated for the substituent at C-8. Moreover, the authors present a crystal structure of FtsZ from *Aquifex aeolicus* with bound GTP-analog (8-morpholino-GTP) showing that the inhibitor indeed binds the active site in a way similar to GTP. Interestingly, when testing the effect of these C-8-substituted GTP analogs on tubulin, it turned out that none of them inhibited tubulin polymerization. Some of the more hydrophobic compounds were even more proficient than GTP in the induction of tubulin polymerization and, in fact, all compounds were hydrolyzed by tubulin. Thus, this type of compound specifically inhibits polymerization of FtsZ but not of tubulin.

The results of the study by Läppchen et al. confirm structural and biochemical data showing striking differences in the active-sites in tubulin or FtsZ polymers, primarily with respect to hydrophobicity and nucleotide exchangeability (Oliva et al., 2007). The study also proves that it is possible to find compounds specifically inhibiting FtsZ. The C-8-substituted GTP analogs will be important tools for basic research and could boost the future search for selective inhibitors of the bacterial Z-ring.

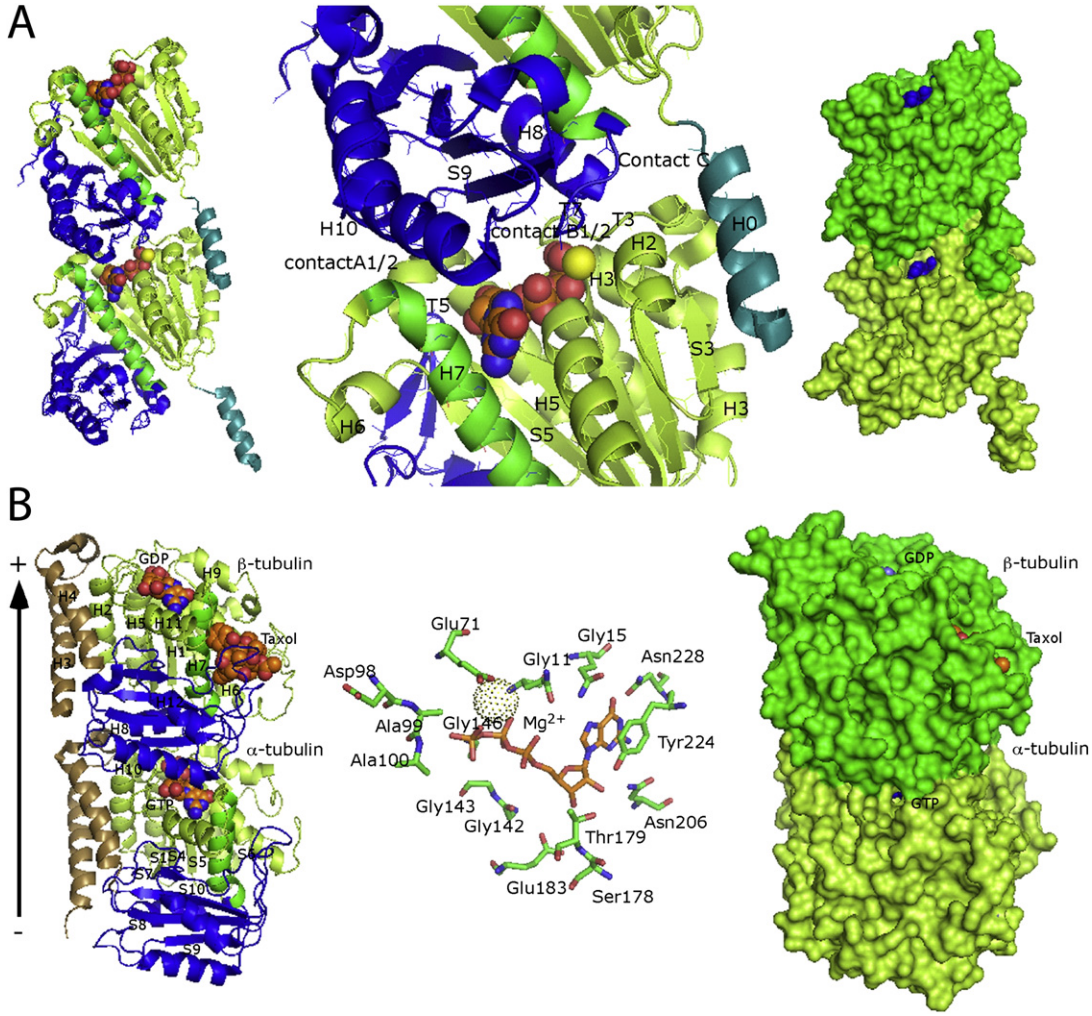

Figure 1. Structures of FtsZ and Tubulin
(A) Dimer of FtsZ (PDB accession code: 1W5A).
(B) α/β-tubulin (PDB accession code: 1JFF).
The left side shows a ribbon presentation with bound nucleotides and taxol as space-filling models. The middle part shows a detailed view on the nucleotide-binding site at the interface between the monomers. The space-filling models of the dimers (right side) illustrate differences in solvent-accessibility of the nucleotide-binding sites in FtsZ and tubulin. See Läppchen, 2007. Courtesy of Tanneke den Blaauwen and Tilman Läppchen.

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