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Bacterial cell division proteins as antibiotic targets

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ABSTRACT

Proteins involved in bacterial cell division often do not have a counterpart in eukaryotic cells and they are essential for the survival of the bacteria. The genetic accessibility of many bacterial species in combination with the Green Fluorescence Protein revolution to study localization of proteins and the availability of crystal structures has increased our knowledge on bacterial cell division considerably in this century. Consequently, bacterial cell division proteins are more and more recognized as potential new antibiotic targets. An international effort to find small molecules that inhibit the cell division initiating protein FtsZ has yielded many compounds of which some are promising as leads for preclinical use. The essential transglycosylase activity of peptidoglycan synthases has recently become accessible to inhibitor screening. Enzymatic assays for and structural information on essential integral membrane proteins such as MraY and FtsW involved in lipid II (the peptidoglycan building block precursor) biosynthesis have put these proteins on the list of potential new targets. This review summarises and discusses the results and approaches to the development of lead compounds that inhibit bacterial cell division.

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1. Introduction

Cell division in bacteria is accomplished by a large and very dynamic protein complex termed the divisome of which all proteins contribute to the simultaneously synthesis of all required compounds of the cell envelope [1–3]. The envelope of Gram-negative bacteria consist from inside to outside of a cytoplasmic membrane, the single layer of peptidoglycan embedded in the periplasmic space and the outer membrane. Gram-positive bacteria lack the outer membrane and make up for this lack of protection by having a multi-layered peptidoglycan wall. Peptidoglycan is a covalently closed network of glycan strands that are interconnected by peptide side bridges. Consequently to be able to insert new material, the peptidoglycan layer has to be opened by hydrolytic enzymes [4]. During cell pole synthesis several activities can be discriminated; peptidoglycan synthesis, peptidoglycan hydrolysis and modification, synchronization of the invagination of all envelope layers and spatial and temporal organization of the process. An imbalance in these activities leads to cell death as illustrated by the lysis of bacteria at their division site due to the peptidoglycan synthesis inhibiting activity of penicillins or β-lactams. Whether inhibition of the other important protein activities would also kill bacteria is the subject of this review.

In many bacterial species investigated, cell division is initiated by the assemblage of a scaffold made of FtsZ polymers bound to the cytoplasmic membrane by proteins such as FtsA and ZipA and organized by proteins such as ZapA, B, C and D [2]. This scaffold establishes the side of division by positioning new cell envelope material (peptidoglycan) without invagination [5,6]. This activity recruits a second set of cell division proteins that are the proteins that will do the real job of synthesis of two new cell poles (Fig. 1). Depending on the species the new cell pole synthesis is either accompanied by invagination of the cell envelop (i.e. *Escherichia coli*), or the two new cell envelopes are separated in a later hydrolytic process (i.e. *Bacillus subtilis*).

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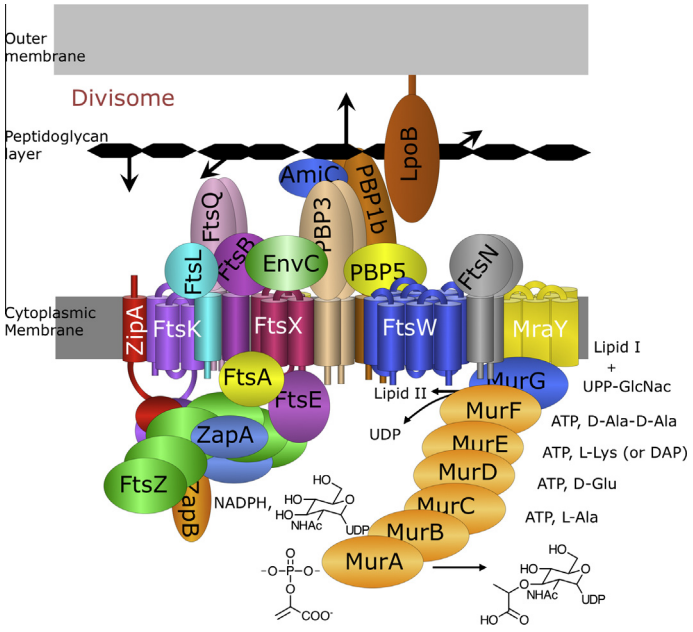

Fig. 1. Schematic overview of the three-layered cell envelop of *Escherichia coli* and the protein complex that drives new cell pole synthesis. For transparency not all known proteins that localize at mid cell during division are shown. Indicated are the Mur proteins and their substrates that might form a hyperstructure to synthesize lipid II.

2. Antibiotic inhibition of septal peptidoglycan synthesis

2.1. Transpeptidase activity

The Penicillin Binding Proteins or PBPs that synthesize peptidoglycan come in several variants. Some of the PBPs have D,D-carboxypeptidase activity, endopeptidase activity or D,D-transpeptidase (TP) activity that can all be inhibited by penicillins or β-lactams. These reactions all involve the binding of the last amino acids of the peptidoglycan peptide side chain, D-Ala-D-Ala, which has a structure similar to penicillin. To overcome the resistance to β-lactams alternative non-lactam drugs are investigated that inhibit the same reaction such as the γ-lactam Lactivicin and its derivatives ([7,8]) or compounds based on boronic acid that mimic the transition state of the enzymatic reaction, which show promising results (see for a review [9]).

2.2. Transglycosylase activity

The second reaction performed by PBPs is the polymerization of the dissacharide subunits of the peptidoglycan structure by transglycosylase activity (TG). The PBPs with TGase activity are bifunctional having TG and TP activity but enzymes with only TG activity are also present in many bacterial species. Clinically used inhibitors of the TG activity are not available. But the relatively recent publication of a number of crystal structures of bifunctional PBPs [10–12] (see for a review [13]) have given access to virtual drug screening and rational drug design. New high throughput TG assays have been described [14,15] that can replace the much more elaborate assays using fluorescently or radioactively labeled lipid II, followed by HPLC analysis of the synthesized compounds [16,17]. Lipid II (Fig. 2) is the precursor of the peptidoglycan-building unit and the substrate for the TG reaction. One of the screening assays is based on Förster Resonance Energy Transfer (FRET) to monitor the event of lipid II polymerization [14]. The lysine of the pentapeptide moiety of lipid II carries a donor fluorophore that is quenched by an acceptor fluorophore present in the lipid tail of the PG precursor. The transglycosylase reaction that attaches the dissacharide pentapeptide to the existing peptidoglycan will release the lipid moiety and increase the fluorescence of the peptide side-chain. The newly synthesized peptidoglycan is subsequently digested by N-acetylmuramidase to release the fluorescent peptidoglycan subunit in the soluble fraction of which an increase in fluorescence can be measured [14].

The only known inhibitor of the TGases is the natural product moenomycin (Fig. 2) [18] that mimics the structure of the donor substrate of the TG reaction (Fig. 2). It is a very effective antibacterial against Gram-positives that had a wide usage in the animal husbandry as growth stimulator [19]. Presently moenomycin is banned for this use, which allows its development for human treatment. A disadvantage of moenomycin is that the long lipid tail is essential for its activity but also gives the molecule poor phamacokinetics [19,20]. Therefore, attempts has been undertaken to make analogues with a shorter lipid tail [21]. Unfortunately the resulting molecules had low affinities for PBPs. However, a slightly shorter fluorescently conjugated moenomycin analogue with good PBP binding characteristics was successfully used in a replacement assay for the screening of a library of 110,000 small molecules [22]. Compound **10** (Fig. 2) of this collection replaced the fluorescent molecule in a dose depended manner and also inhibited the TG activity of several PBPs *in vitro* and bacterial growth *in vivo*, with IC_{50} and MICs in the tens of μM range, respectively. Although it has not yet been shown that these compounds also target the TG activity in the bacterial cell, this approach seems to be promising.

The acceptor substrate of the TG reaction is lipid-II (Fig. 2). Using the TG FRET assay [14] 120,000 natural and synthetic compounds were screened. About 25 hits were scored among which were well known inhibitors like vancomycin but also smaller molecules such as compound **24** (Fig. 2) that had a K_i of 2.3 ± 0.3 μM and a MIC of about 4 μM against several Gram-positive species. Others have synthesized Lipid II analogues to determine which parts of the precursor is essential for PBP binding, which resulted in some compounds with a K_i in the tens of μM and very weak antibacterial activity [23] and for a review see [24].

2.3. Lipid II synthesis

In *E. coli* and probably also in other bacterial species the proteins that synthesize Lipid II are shared by the elongation and the division machinery. The essential cytoplasmic proteins MurA,

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