Drug discovery

New antibiotics target bacterial envelope

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A double membrane protects certain bacteria from antibiotics, but compounds have now been generated that can overcome this obstacle, seemingly by targeting a crucial protein in the outer membrane. **See p.452 & p.459**

Antibiotic resistance is a growing global public-health problem[1]. One group of bacteria, called Gram-negative bacteria, is particularly difficult to treat, because the cells are shielded by a double-membrane envelope, which constitutes a formidable barrier to antibiotics[2]. When antibiotics do breach the membranes, these bacteria often use efflux pumps to remove the drugs[3,4]. Three papers (two in *Nature*[5,6] and one in the *Proceedings of the National Academy of Sciences*[7]) now describe antibiotics that overcome these obstacles by targeting, directly or indirectly, a protein integral to the outer membrane.

The outer membrane of Gram-negative bacteria contains lipopolysaccharide (LPS) molecules in its outer leaflet, with outer-membrane proteins (OMPs)[8] spanning the entire outer membrane. OMPs are folded into the membrane by a protein complex called the β-barrel assembly machine (BAM), the central component of which, BamA, is an OMP itself (Fig. 1). Because BamA is exposed to the extracellular space, it could be an Achilles heel in the bacterial shield — inhibitors that access BamA would not need to penetrate the cell. Indeed, a proof-of-concept study[9] has shown that this approach inhibits OMP folding and compromises membrane integrity, albeit by an unknown mechanism.

The three current studies took different approaches to develop antibiotics against Gram-negative bacteria. On page 459, Imai *et al.*[5] turned to Gram-negative bacteria that live symbiotically in the gut of nematode worms and can secrete antibiotics to fend off competing bacteria — including other Gram-negative species. A screen of the secretions from 22 of these symbionts revealed a Gram-negative-targeting antibiotic, which the authors named darobactin.

Darobactin displayed antibiotic activity against multiple Gram-negative bacteria, both *in vitro* and in infected mice, including against several drug-resistant human pathogens such as polymyxin-resistant *Pseudomonas aeruginosa* and β-lactam-resistant *Klebsiella pneumoniae* and *Escherichia coli*. Darobactin as not toxic to human cells at the concentrations at which it was an effective antibiotic.

Next, Imai *et al.* asked what bacterial molecule darobactin targets. The group identified three strains of *E. coli* that were resistant to darobactin and showed that each harboured mutations in the *bamA* gene. The mutations all changed amino-acid residues in the same region of BamA's protein structure, suggesting a putative binding site for darobactin that would be accessible from the extracellular space.

The authors provided evidence that darobactin and BamA bind to each other directly, using a technique called isothermal titration calorimetry, which measures the heat changes associated with physical interactions between molecules. The results of nuclear magnetic resonance (NMR) spectroscopy experiments were also consistent with direct binding, and suggested that the antibiotic stabilizes the protein in a potentially inactive conformation.

The researchers next showed that darobactin inhibits the ability of an isolated BAM complex to perform its OMP-folding function *in vitro*, consistent with direct BamA targeting. However, only one of the resistant BamA mutants showed reduced inhibition by darobactin in this assay. A test of whether darobactin–BamA binding is impaired in the *bamA* mutants could be used in the future to confirm BamA as the molecular target.

On page 452, Luther *et al.*[6] focused on analogues of an existing antibiotic, murepavadin[10], which targets a surface-exposed protein called LptD that is involved in assembling LPSs in the outer membrane[8]. Murepavadin displays potent but narrow antibiotic activity against *P. aeruginosa*[10]. The authors therefore screened for murepavadin analogues that had antibiotic activity against other Gram-negative species.

Luther and colleagues chemically linked the compounds identified through this screen to a portion of another antibiotic, polymyxin B, that binds to LPS directly[11]. Intact polymyxins efficiently disrupt bacterial membranes and kill cells, but are rather toxic to humans[12]. The researchers hoped that linking just the LPS-binding portion of polymyxin B could increase the membrane targeting of their murepavadin analogues. Indeed, their strategy produced several chimaeras that had potent activity, both *in vitro* and in mice infected with *K. pneumoniae*, *P. aeruginosa*, *E. coli* and other Gram-negative bacteria,

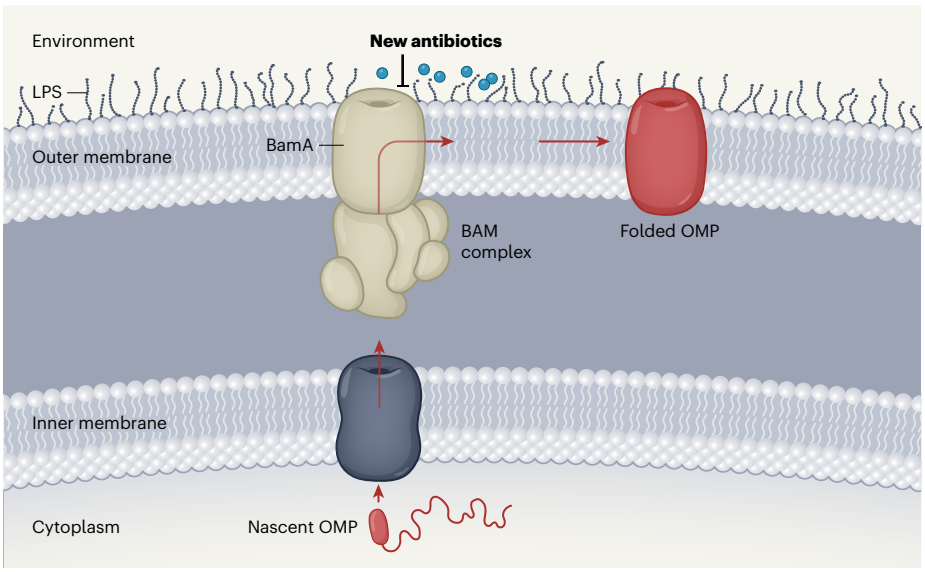

Figure 1 | Overcoming a double-membrane barrier. Gram-negative bacteria are protected by inner and outer membranes. The outer membrane contains lipopolysaccharide (LPS) molecules in the outer layer and integral outer-membrane proteins (OMPs). These proteins are synthesized in the cell's cytoplasm and transported to the space between the membranes by the translocation machinery (dark blue). From here, they are captured, inserted and folded into the outer membrane by the BAM protein complex (red arrows). BamA is the central component of BAM and is accessible from the bacterial surface. Three studies[5–7] describe new antibiotics that seem to target BamA, preventing the normal OMP folding that is required for bacterial survival.

including drug-resistant strains. Notably, the chimaeras showed low toxicity in mice.

It might be expected that the chimaeras would target LptD, but when Luther and colleagues tested for interacting partners, they found evidence of BamA targeting. The authors analysed strains of *K. pneumoniae* that showed resistance to the chimaeras. They found that resistant strains carried mutations in several genes, including *bamA* and genes responsible for LPS modification. Reintroduction of the wild-type *bamA* gene into the resistant strains led to increased sensitivity to the chimaera, indicating that BamA has a role in the antibiotic's mechanism of action.

Direct chimaera–BamA binding was confirmed with *in vitro* assays in which the authors fluorescently labelled the chimaeras and monitored changes in fluorescence that indicate binding to a large protein such as BamA. As with darobactin, NMR experiments suggested that chimaera binding stabilizes BamA in a potentially inactive conformation, consistent with direct BamA targeting. However, when the bacteria were treated directly with the chimaeras, both the outer and inner membranes were rapidly permeabilized; this suggests that the compounds might act directly on the membrane. The results raise the possibility that the chimaeras act in a similar way to polymyxins, with binding to BamA strengthening their membrane targeting.

In the third study, Hart *et al.*[7] identified a compound, MRL-494, that had similar antibiotic potency against both wild-type *E. coli* and a mutant defective in outer-membrane integrity and efflux mechanisms, suggesting that this antibiotic might not need to penetrate the cell to exert its activity. *In vitro*, MRL-494 exhibited moderate potency against Gram-negative pathogens, including *K. pneumoniae* and *P. aeruginosa*. The efficacy of MRL-494 in animal models remains to be tested.

The authors showed that treatment of *E. coli* with the compound resulted in decreased abundance of OMPs in the outer membrane, indicating BamA as a possible target. In support of this possibility, Hart *et al.* identified a *bamA* mutation that confers resistance to MRL-494 in *E. coli*. They showed that, whereas MRL-494 inhibited normal folding of a model OMP in *E. coli* cells expressing wild-type *bamA*, it had less effect on the resistant cells. The researchers found that MRL-494 stabilizes BamA against heat-induced protein aggregation in cells, suggesting an interaction between the two. However, MRL-494 stabilizes the resistant *bamA* mutant to a similar extent. Furthermore, MRL-494 displays similar potency against Gram-positive bacteria, which lack BamA. Therefore, in Gram-negative bacteria, MRL-494 might inhibit BamA directly or might target the outer membrane and affect BamA function indirectly.

Together, these studies describe new antibiotics that are active against difficult-to-treat Gram-negative bacteria. Given the compounds' size and chemistry, they are likely to act at the cell surface, bypassing the need to breach the permeability barrier. Imai *et al.* provided compelling evidence that BamA is the target of darobactin, including a putative binding site, to be confirmed by demonstrating reduced binding to resistant mutants. The chimaeric compounds both seem to bind BamA and LPS. But, as is also the case for MRL-494, further experiments will be required to determine whether their activity is caused by direct effects on BamA.

"These studies describe new antibiotics that are active against difficult-to-treat Gram-negative bacteria."

Future research to identify specific BamA binding sites for any of the compounds, and to examine the mechanism by which antibiotic binding impairs BamA activity, would provide a platform for further antibiotic development. Such research might also shed light on how BAM mediates the insertion and folding of OMPs, which is poorly understood.

Darobactin and MRL-494 are initial lead compounds, and medicinal-chemistry efforts could yield more-potent and effective analogues. Preclinical studies aimed at determining their toxicity in animal models will also be important. Luther and colleagues' chimaeras are at a more advanced stage of development, because, as the authors show, they have potent *in vivo* activity as well as favourable toxicity, pharmacokinetics and pharmacodynamics in animal models. The future looks promising for this newly discovered class of antibiotic.

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Condensed-matter physics

Magnetic and topological order united in a crystal

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A material that has electrically conducting surfaces has been found to show, when cooled, a type of magnetic ordering that reduces conduction at the surfaces. Such remarkable behaviour could have practical applications. **See p.416 & p.423**

An ordered arrangement of magnetic moments in a material normally prevents the formation of another kind of electronic order associated with an exotic state of matter known as a topological insulator. But Otrokov *et al.*[1] (page 416) and Rienks *et al.*[2] (page 423) report that manganese bismuth telluride integrates these two types of electronic behaviour. The complex layered structure of alternating magnetic and topologically non-trivial regions in this material leads to an intriguing and potentially technologically useful interplay between magnetic and topological order.

One of the earliest descriptions of electronic order in solids was of ferromagnetism, the existence of which was reported in natural minerals in Greece and China more than 2,000 years ago. In a simple ferromagnet, microscopic magnetic moments, arising predominantly from the spin (intrinsic angular momentum) of a material's electrons, align in the same direction, leading to an overall macroscopic magnetic moment. The concept of antiferromagnetism, in which spins align in alternating directions and the average magnetic moment is zero, was developed only in the 1930s. These two kinds of magnetic order are viewed theoretically as breaking time-reversal symmetry: when the direction of time is reversed, the pattern of spins