Bacteria utilize multiple strategies to circumvent antibiotics, producing broad specificity exporters or enzymes that catalyze the modification of either antibiotics or their targets. A report in this issue of *Structure* provides the structural and catalytic mechanisms of LinB, an adenylyltransferase of *E. faecium* that confers resistance to the lincosamide antibiotic clindamycin.

The emergence of pathogenic bacteria that are resistant to many commonly used antibiotics has led to a renewed interest in understanding the biochemical basis of antibiotic action and resistance. Historically, resistant strains have been identified very soon after the introduction of a novel compound into the clinical setting. Resistance develops primarily from the ability of bacterial export proteins to rid the cell of the antibiotic by modification of the target protein to reduce antibiotic activity, or by the production of specific enzymes that are responsible for the chemical inactivation of the antibiotic molecule (Fischbach and Walsh, 2009). The facility of transferring resistance factors between organisms and species, and the evolutionary pressure to do so, has led to the current state, where multidrug-resistant bacteria are posing serious clinical problems (Nordmann et al., 2007).

With a few notable exceptions, the development of new antibiotics over the last few decades has focused primarily on the chemical modifications of known antibiotic scaffolds (Fischbach and Walsh, 2009). In this process, new compounds are identified from chemical libraries that are then screened for efficacy. A more directed approach involves the rational design of new compounds while simultaneously considering effectiveness and resistance. This prospect of true structure-guided antibiotic design, however, relies on a clear understanding of structures of the antibiotic bound to both its target, as well as to the enzyme that confers resistance by chemical modification or breakdown of the active drug. The availability of these structures enables the design of improved compounds that maintain necessary interactions for the binding of drug to the target, yet prevent interactions with the modifying enzymes. In this issue of *Structure*, a report by Morar et al. (2009) takes us one step closer in this regard with the lincosamide antibiotic clindamycin (Figure 1).

Clindamycin is a member of the lincosamide family of antibiotics. Lincosamides are classified with other macrolide and streptogramin (MLS) antibiotics, which all share a common binding site on the 23S rRNA of the 50S subunit of the bacterial ribosome (Roberts, 2008). Resistance is often conferred by ribosomal methylation catalyzed by members of the erythromycin ribosome methylase (erm) enzymes. In modifying the ribosome target, these enzymes confer resistance to chemically diverse drugs (Leclercq, 2002). Resistance to the lincosamides is also provided by chemical modification of the drugs with either phosphate or adenylate groups; resistance provided by these antibiotic-modifying enzymes is thus limited to chemically similar compounds that are recognized by the enzyme active site (Leclercq, 2002).

Structures of clindamycin bound to the ribosome are known (Schlunzen et al., 2001; Tu et al., 2005), providing an understanding of the functional groups on the drug that is responsible for target binding and the inhibition of ribosomal translation. In this issue of *Structure*, Morar et al. (2009) provide the structural and mechanistic insights into the enzymatic modification of clindamycin. The authors present two X-ray structures of LinB, the lincosamide antibiotic adenyllytransferase from *Enterococcus faecium*. The two models are structurally very similar with the exception of different ligands bound in the active site. In one model, using the methylene-bridged nonhydrolyzable ATP mimic, AMPCPP, two Mg$^{2+}$ ions, and the cognate antibiotic clindamycin, the authors were able to trap LinB primed for adenylation of the antibiotic. The active site of the second model includes pyrophosphate, which adopts the same orientation as the β and γ phosphates of ATP.

The structures show that LinB is composed of two domains: an N-terminal six-strand β sheet surrounded by two α helices, and a C-terminal α-helical bundle. The helical domain of one protomer rests in the groove between the two domains of the partner monomer, forming a swapped dimer. Catalysis of clindamycin adenylation occurs in a cleft at the dimer interface. Clindamycin binds wholly to one monomer, stacked between the AMPCPP molecule and the N-terminal β sheet. Alternatively, residues from both monomers contribute to AMPCPP binding. One Mg$^{2+}$ ion is coordinated by the three phosphates of the nucleotide. Interestingly, a second Mg$^{2+}$ ion is observed bridging the α-phosphate and the nucleophilic 3′-hydroxyl of clindamycin. This second cation appears to orient the 3′-hydroxyl for attack on the α-phosphate and was not observed in the related structure of kanamycin nucleotidyl transferase (Pedersen et al., 1995). This homolog contains conserved residues at the positions of the three ligands for the second Mg$^{2+}$, raising the possibility that other members of this family use two ions in the catalytic mechanism as well.

Structural and kinetic analyses point to key residues responsible for substrate binding, specificity, and nucleotidylation transfer.
from ATP. Geometry and distance values for these residues and the active site ligands, along with product inhibition studies and solvent isotope effects, point to a direct in-line adenylation reaction. These data are also consistent with prior enzymatic characterization of diverse members of this family (Gerratana et al., 2001; Magnet and Blanchard, 2005).

The kinetic characterization, in addition to structural comparisons using the distance alignment matrix method, allowed Morar et al. (2009) to classify LinB as a member of the nucleotidyl transferase superfamily, joining aminoglycoside nucleotidyl transferases and nucleotide polymerases. This classification further supports the proposed LinB mechanism, as several crystal structures of these related enzymes with characterized mechanisms have been solved with trapped active site intermediates bound to conserved residues (Pedersen et al., 1995).

The structural and mechanistic data presented by the authors solidify the evolutionary relationship between antibiotic nucleotidyl transferases and the nucleotide polymerases found throughout all forms of life. As only a few of the former are known to exist, the relationship suggests that they evolved in response to the small molecule antibiotics produced by competing bacteria or even as a self-defense mechanism within the bacteria that produce the antibiotics. Resistance in virulent strains results from the transfer of these genes from environmental bacteria to their pathogenic counterparts.

Finally, the authors propose an interesting analogy from the field of cancer biology. Oncogenes are mutated or truncated eukaryotic genes, often incorporated into cancer-causing viruses, that have the capability of transforming a normal cell into a cancerous one. The wild-type genes from which these oncogenes evolved are termed proto-oncogenes. Adopting this nomenclature, Morar et al. (2009) term the normal bacterial precursors of antibiotic resistance genes as proto-resistance elements. This is an intriguing classification that may lead to new ways of thinking about the enzymatic strategies that confer resistance. A more complete understanding of the evolution of enzymes that confer antibiotic resistance may also contribute to the design of novel compounds that maintain therapeutic activity and are less susceptible to enzymatic degradation.

Hopefully, this study, and others like it, will result in new strategies to overcome antibiotic resistance.

REFERENCES


Figure 1. Binding of Clindamycin to Its Target and Resistance Element
Clindamycin, a lincosamide antibiotic, has now been structurally characterized bound to both the 24S rRNA of the ribosome (left), the therapeutic target, as well as to the LinB (right), the enzyme that catalyzes the inactivation of the antibiotic through an adenylyltransferase reaction.