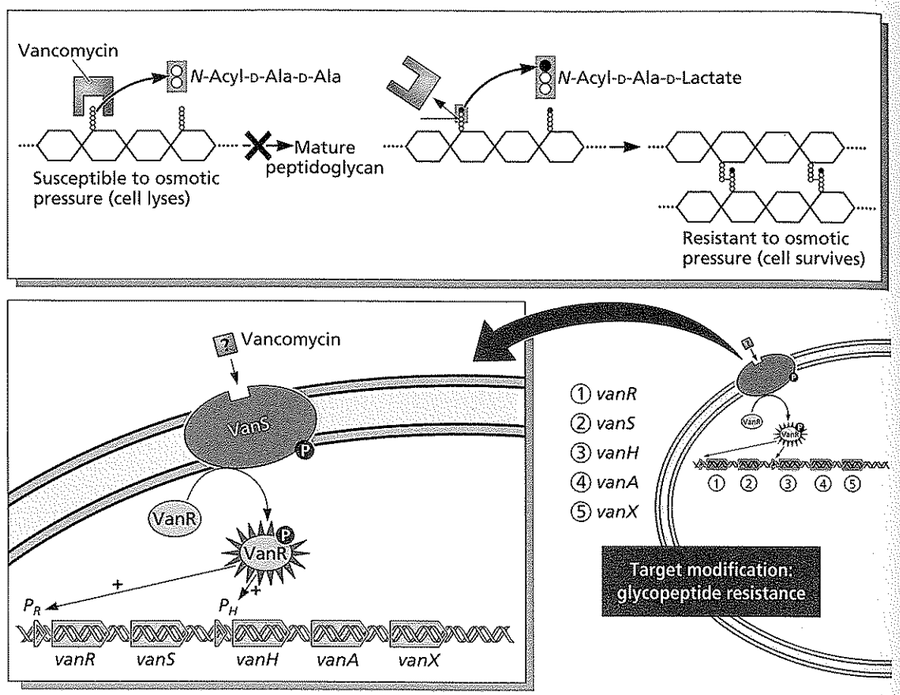


Antibiotic resistance by modification of the target.

chapter 10

Antibiotic Resistance by Replacement or Modification of the Antibiotic Target

The last of the three major routes to clinically important resistance in pathogenic bacteria is the ability of drug-resistant pathogens to modify the drug target to insensitivity while still retaining its essential cellular function. The chapter opening figure is a blowup of that section of Fig. 2.2 that exemplifies the principles of antibiotic resistance arising from replacement or modification of the target to an insensitive form.

This can be achieved by mutation at one or more sites in the target gene or by importation of a gene that specifies a new replacement enzyme that has markedly decreased sensitivity to the drug. β-lactam resistance in the gram-positive *Streptococcus pneumoniae* and *Staphylococcus aureus* strains represent these two variations on a theme. The erythromycin family of macrolides and the streptogramin B family both have decreased affinity in response to methylation of a single adenine in the 23S rRNA in the 50S ribosomal subunit. Finally, we shall note the cell wall reprogramming in vancomycin-resistant enterococci (VRE) phenotypes A, B, and C. Micrographs of *S. aureus, S. pneumoniae,* and *Enterococcus faecalis* are shown in **Fig. 10.1.**

Methicillin resistance in *S. aureus*

Methicillin (**Fig. 10.2**), with a bulky 2,5-dimethoxybenzoyl substituent on the 6-aminopenicillin scaffold, was introduced in 1950 to treat gram-positive bacterial infections that had become resistant to penicillin via inducible β-lactamase hydrolysis of the antibiotic. The bulky side chain substituent in the penicilloyl-O-lactamase acyl enzyme intermediate selectively slows the deacylation hydrolytic step (see chapter 8) and lengthens the lifetime of the covalent acyl enzyme, effectively deactivating β-lactamase during that interval. This strategy was effective for a decade before outbreaks of methicillin-resistant *S. aureus* (MRSA) developed in Europe in 1961, and by the 1980s MRSA was widespread globally.

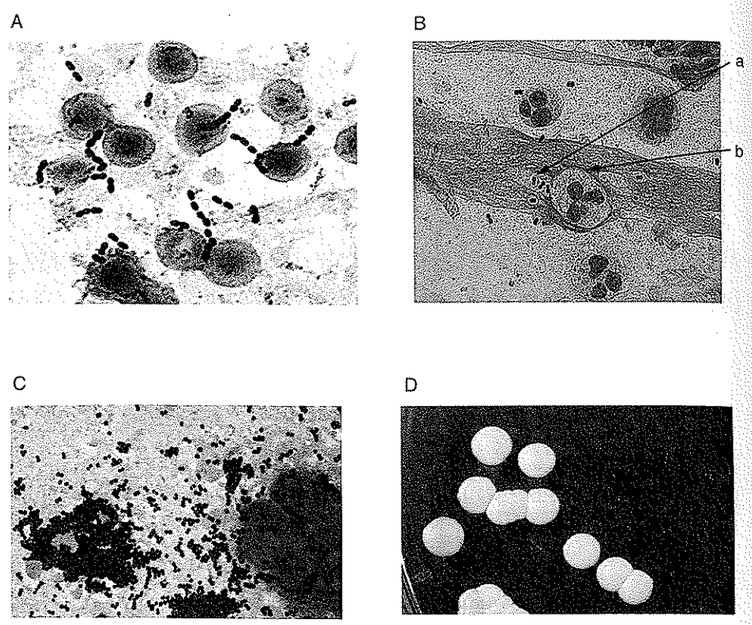
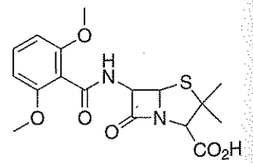


Figure 10.1 Gram-positive pathogens that become drug resistant by target alterations: (A) *Enterococcus faecalis* in a blood culture; (B) encapsulated *Streptococcus pneumoniae*, including (a) gram-positive diplococci surrounded by a capsule and (b) a polymorphonuclear leukocyte with multilobed nucleus; (C) Gram stain of sputum of patient with *Staphylococcus aureus* pneumonia; (D) *Staphylococcus aureus* "golden" colonies on blood agar plates. (From Elliot et al. [1997], with permission.)

MRSA is not elaborating an improved version of β-lactamase that is more efficient at chewing up methicillin, but rather has acquired the *mecA* gene, which encodes a new penicillin-binding protein (PBP), termed PBP2A (also PBP2′), in greater than 90% of the drug-resistant clinical isolates (see Hiramatsu et al., 2001). In hospital environments in the United States, MRSA can reach an incidence of 20 to 40%; in Japan an incidence of up to 60% incidence has been reported (Chu et al., 1996). MRSA can be particularly prevalent in burn centers, but is also present in other long-term care facilities. MRSA poses problems for

Figure 10.2 Structure of methicillin.



Methicillin

treatment because it is resistant to essentially all β-lactam molecules, including penicillins, cephalosporins, carbapenems, and penems. This pan-β-lactam insensitivity stems from the low binding affinity of the *mecA*-encoded PBP2A, a 76-kDa bifunctional transglycosylase/transpeptidase. In contrast, the normal high-molecular-weight PBPs, PBP1 through 4, may remain sensitive to acylation by β-lactams in methicillin-sensitive *S. aureus* (MSSA).

The molecular basis of the insensitivity of PBP2A to lactams while still carrying out peptidoglycan (PG) cross-linking functions is not yet clear and will probably require an X-ray structure comparison with the sensitive PBPs of the same organism. The origin of the *mecA* gene is also unknown, although horizontal transmission from some other *Staphylococcus* species has been postulated (chapter 7). It is known that the MRSA phenotype arises from transfer of a 30- to 40-kb mobile DNA element with the *mecR1-mecI-mecA* triad of genes at the core of the methicillin-inducible phenotype. We noted in chapter 8 (Fig. 8.12) the close parallel of the logic of the *blaR1-blaI-blaZ* circuitry, in that case for β-lactamase induction, to the *mecR1-mecI-mecA* circuitry for the MRSA phenotype (by a proteolytic two-component gene-activating cascade). The MecR1 protein is a 68-kDa transmembrane sensor/transducer, with a classical PBP exo domain that can be acylated by methicillin, and this covalent occupancy transduced to the endo domain, a zinc protease zymogen, which undergoes autoproteolysis. The released cytoplasmic MecR1 fragment, now an active protease, then cleaves the MecI repressor such that it cannot dimerize and bind DNA. Repression of transcription of the *mecA* gene is relieved and the PBP2A protein is made, is transported to the cell surface, and functions unimpaired for PG cross-linking in the presence of the extracellular methicillin and other lactam antibiotics. The morphology of the PG being synthesized by PBP2A in the absence of the other functional PBPs is somewhat altered but clearly sufficient to allow MRSA growth. There are auxiliary genes in MRSA, the *fem* genes, that add the pentaglycyl cross bridges to PG strands before cross-linking, which also contribute to the phenotype (Berger-Bachi and Tschierske, 1998; Filipe et al., 2000; Scholar and Pratt, 2000). An estimate of the number of PBP molecules has been made for an MSSA cell and the same cell transformed to the MRSA phenotype with the *mec* DNA (Pucci and Dougherty, 2002). The MSSA cell had about 1,100 copies of PBPs (PBP1–4), with PBP2 making up about 45% of the total. The MRSA cell had about 1,900 to 2,000 PBPs per cell, with PBP2A comprising 40% and PBP2 25% of the total.

Intensive medicinal chemistry activity to produce lactams that will target PBP2A and reverse the MRSA phenotype to MSSA have led to some new lactam structures with promising potency. Examples in the carbapenem series are shown in **Fig. 10.3A**, with 2-aryl or 2-carbolinyl substituents, as well as the β-methyl-substituted aryl carbapenem MK-826 (Chu et al., 1996; Lee and Hecker, 1999). MK-826 has pharmacokinetic properties that suggest once-a-day dosing. One carbapenem had been designed at Merck to be active against MRSA by virtue of a large lipophilic side chain. It was observed that such large hydrophobic substituents were antigenic and caused hypersensitivity, so Rosen et al. (1999) devised the carbapenem L-786,392 that, on attack by the active-site serine of PBP2A, underwent chemical fragmentation and expulsion of the antigenic side chain to reduce immunogenicity (**Fig. 10.3B**) (Rosen et al., 1999).

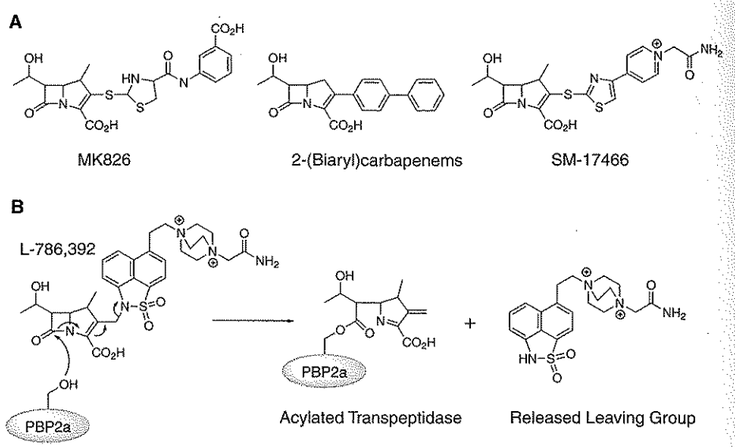


Figure 10.3 Carbapenems with activity against MRSA: (A) molecules with aryl side chain substituents; (B) release of the immunogenic side chain of carbapenem L-786,392 on attack of the β-lactam by the active-site serine of PBP2A.

Such molecules could satisfy a pressing clinical need since MRSA clinical isolates have also gained other drug resistance determinants from the efforts to treat them with varied antibiotics. For example, in MRSA clinical isolates in Japan in 1992, two-thirds had additional drug resistances (Chu et al., 1996). Selection to additional resistance can occur quite rapidly. After the widespread use of the fluoroquinolone ciprofloxacin for treatment of MRSA infections, the incidence of combined MRSA and quinolone resistance went from 5% to >85% in one year.

β-Lactam-resistant *S. pneumoniae*

S. pneumoniae has been an important causative agent in community-acquired pneumonia, meningitis, otitis media, and sinusitis. Unlike the *S. aureus* strains and many other pathogens, *S. pneumoniae* does not use β-lactamases as the major route to penicillin resistance. On the other hand, resistance to penicillin rose 240-fold over the five decades from 1941 to 1991 (see Chu et al., 1996) due to resistance development in the PBP targets themselves. An initial outbreak of lactam-resistant *S. pneumoniae* in South Africa in 1977 has now spread worldwide (Chu et al., 1996). Analysis of transpeptidases/transglycosylases in *S. pneumoniae* reveal five high-molecular-weight PBPs which contribute to killing by β-lactams: PBP1A, 1B, 2A, 2B, and 2X. A low-molecular-weight PBP3 is not a killing target, whereas PBP2B and 2X are essential. Low-level resistance to penicillins involves PBP2X, while cephalosporins elicit a PBP2B of reduced affinity, and these are preludes to further changes in high-resistance phenotypes. In clinical isolates with high lactam resistance there are mutations in all five high-

molecular-weight PBPs that cause reduced affinities for β-lactams (Nagai et al., 2002). This acquisition of five kinds of mutant proteins would seem to have very low probability if each required independent mutation. There is evidence that at least PBP2B and 2X have undergone homologous recombination in various parts of the encoding genes to create mosaic genes in which resistance is developed by a cassette mechanism (Hakenbeck, 1998; Spratt, 1994). This would represent a natural gene shuffling and could speed evolution of mosaic-resistant PBP proteins.

The X-ray structure of a soluble form of PBP2X has been reported (Pares et al., 1996) after truncation of the N-terminal membrane anchor, revealing a three-domain structure, with the transpeptidase domain in between an amino- and a carboxyl-terminal domain. The gene sequence for *pbp2x* from 35 clinical isolates of penicillin-resistant *S. pneumoniae* (Asahi et al., 1999) has been determined and mapped onto the X-ray structure for the transpeptidase domain, reflecting a clustering of side chain alterations in the penicillin binding site. The modular nature of these proteins may facilitate a gene shuffling strategy to generate diversity and provide an evolutionary route to resistance. In sum, the most remarkable feature in *S. pneumoniae* penicillin resistance is the large number of PBP targets that have been altered to insensitivity to express the phenotype (Hakenbeck et al., 1999). This reflects the remarkably rapid genetic plasticity of bacteria when facing extinction by an antibiotic.

Resistance to macrolides by 23S rRNA methylation

The macrolide class of antibiotics, including erythromycin and the expanded-spectrum agents azithromycin and clarithromycin, have been widely used for respiratory tract infections, but erythromycin resistance has become problematic. Pneumococcal clinical isolates are substantially resistant to erythromycin, and in one study in South Africa in 1992, 70% of MRSA isolates were also resistant to erythromycin (Chu et al., 1996). The major route of resistance is modification of the 23S rRNA in the 50S ribosomal subunit to insensitivity, while efflux, as noted in chapter 9, can also be significant.

The recent X-ray determination of the 50S subunit of the ribosome soaked with erythromycin (Fig. 4.5) visualized the antibiotic bound in the peptidyltransferase cavity, in the vicinity of both A loops and P loops and near A_{2058} (see chapters 4 and 7). It is monomethylation or dimethylation of this N_6 exocyclic amino group of A_{2058} (Fig. 7.6) by an erythromycin ribosome methylation (Erm) modification enzyme that produces the Erm phenotype and reduced affinity of the RNA for the antibiotic, without affecting the role of A_{2058} in peptidyltransferase architecture or function. The cosubstrate is the common biological methyl donor *S*-adenosylmethionine, for both the first and second N-methylations. There are many cellular *N*-methyltransferases known, including RNA *N*-methyltransferases, and evolution of the Erm methyltransferases from such a precursor is likely. More particularly, one such Erm, ErmE, is a constitutively active enzyme in the erythromycin producer *Saccharopolyspora erythraea*, where it provides autoimmunity to the antibiotic producer (chapter 7) and may be a recent progenitor of the Erms in the resistant pathogens. More than two dozen

Erm enzymes have been described in resistant bacteria and an X-ray structure of ErmX (Bussiere et al., 1998) has been reported.

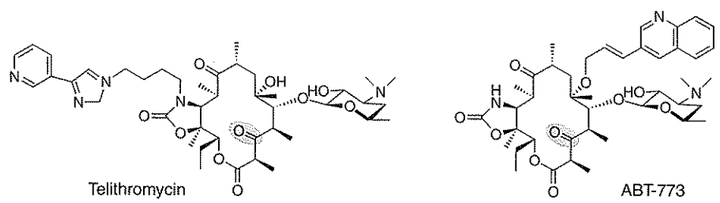
The methyl-A_{2058}-specific enzymatic modification in 23S rRNA not only decreases affinity for macrolide antibiotics of the erythromycin class, but also for those of the lincomycin/clindamycin class, as well as for a third group, the streptogramin B (pristinamycin) family, and this has been described as the macrolide-limcosamide-streptogramin B (MLS_B) phenotype of ribosomal drug resistance (see chapter 4, Fig. 4.5). While the ErmE enzyme in *S. erythraea* is produced constitutively, the MLS_B phenotype is usually inducible by erythromycin in resistant pathogens. The *ermC* gene transcription in *S. aureus* has been well studied (Chu et al., 1996) and indicates that a 141-bp leader sequence just upstream of the *ermC* start codon adopts a secondary structure that sequesters the ribosome binding site so *ermC* transcription is blocked. In the presence of low levels of erythromycin, by mechanisms not yet clear, the secondary structure of the leader is postulated to refold, exposing the ribosome binding site, permitting *ermC* transcription, and producing the ErmC methyltransferase, which methylates A_{2058} and protects the ribosome before lethal concentrations of erythromycin build up in the cell.

One of the goals of medicinal chemistry in developing broad-spectrum erythromycins is to overcome the Erm phenotypes by creating semisynthetic or altered versions of the macrolides that can still bind to methylated A_{2058} versions of the 23S rRNA. Modifications to the expanded-spectrum drug clarithromycin, including oxidation of the 3-hydroxy to a 3-oxo group, produce the ketolide series (**Fig. 10.4**), which are active against erythromycin-resistant *S. pneumoniae* as they do not induce the MLS_B phenotype (Chu et al., 1996). Additional modification of the right-hand side of the macrolactone scaffold and tethered aryl substituents provide sufficient affinity for methylated ribosome 50S subunits to make ketolides promising new drugs. Telithromycin has recently been approved for human use and ABT-773 is in advanced clinical evaluation.

VRE reprogramming the peptidoglycan termini

The increasing use of vancomycin to treat infections caused by the gram-positive MRSA in the 1980s and 1990s selected for drug-resistant enterococci, less potent pathogens than staphylococci but opportunistic in the space vacated by other

Figure 10.4 Structure of 3-ketolides telithromycin and ABT-773, broad-spectrum erythromycin derivatives.



bacteria and in patients with compromised immune systems. *E. faecalis* species account for about 90 to 95% of vancomycin-resistant clinical isolates and *E. faecium* another 5%, with minor species accounting for the rest. Enterococci are the leading causes of endocarditis and are common pathogens in patients with indwelling catheters, including dialysis patients and those undergoing cancer chemotherapy who have chemotherapy-induced white cell depletion in the middle of treatment cycles (Murray, 2000). **Figure 10.5** shows a rise in VRE incidence from below 0.5% in 1989 to 26% in 1994 in hospital wards and in intensive care units, where enterococci can contaminate and multiply in surgical wounds (see Poole, 2001, and references therein). There had been few therapeutic choices for VRE treatment, but the recent approvals of both the Synercid combination and the oxazolidinone linezolid (chapter 4) have come with indications of efficacy against VRE.

The first major clinical phenotype of VRE was termed VanA, followed by the closely related VanB, which has essentially the same molecular mechanism but differs in the continuing sensitivity to teicoplanin (**Table 10.1**) (see Fig. 7.8 for teicoplanin structure). The VanC phenotype has been found in *Enterococcus gallinarum*, and subsequent variations of these three phenotypes have been reported (Cetinkaya et al., 2000).

The VanA and VanB phenotypes are plasmid borne and the relevant genes are often found on transposable elements that account for their rapid spread through enterococcal populations. Five tandemly arranged genes have been found to be necessary and sufficient for both VanA and VanB phenotypes (**Fig. 10.6**), with three enzymes, VanH, VanA, and VanX, involved in reprogramming of the PG termini from *N*-acyl-D-Ala-D-Ala to *N*-acyl-D-Ala-D-lactate (**Fig. 10.7**) and two proteins, VanS and VanR, comprising a two-component regulatory pair that are sensor and response regulator for inducible reprogramming to vancomycin resistance. The switch from D,D-dipeptide to D,D-depsipeptide at the uncross-linked PG terminus effects a thousand-fold decrease in the binding constant for vancomycin (Bugg et al., 1991) and mirrors the thousand-fold increase in vancomycin MICs seen in VRE (**Fig. 10.8**). The affinity loss is in large part due to the loss of the middle hydrogen bond from the peptide carbonyl on

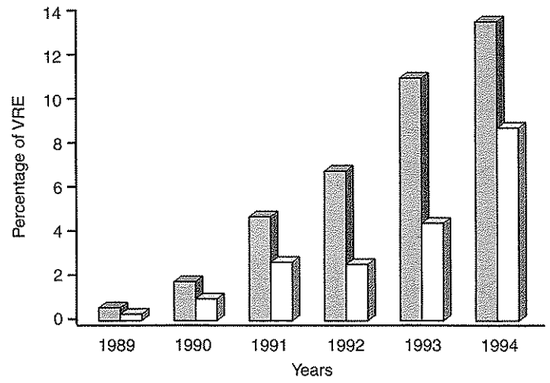


Figure 10.5 Incidence of VRE in intensive care units (shaded bars) and nonintensive care units (unshaded bars) in the early 1990s. (From Hughes and Tenover [1997], with permission.)

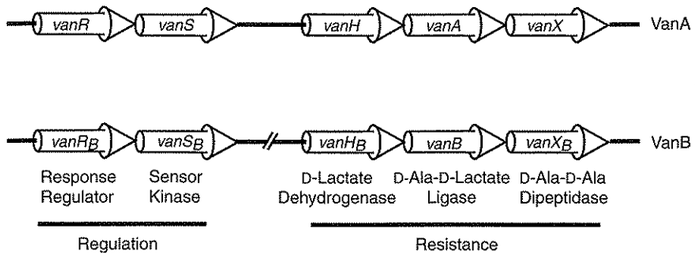
Table 10.1 Phenotypes of glycopeptide-resistant enterococci

Phenotype	Sample species	MIC (mg/liter)		Transferable resistance	Induction
		Vancomycin	Teicoplanin		
VanA	*E. faecium*	64–>1,000	16–512	Yes	Yes
	E. faecalis				
	E. avium				
	E. gallinarum				
VanB	*E. faecium*	4–1,024	0.25–2	Yes	Yes
	E. faecalis				
VanC	*E. gallinarum*	2–32	0.12–2	No	Some
	E. casseliflavus				

the underside of the cup-shaped vancomycin molecule to the amide N-H of the D-Ala-D-ala terminus, along with probable ground state repulsions between the depsipeptide lone pair electrons on the ester oxygen of D-Ala-D-Lac.

VanH is a pyruvate reductase (D-lactate dehydrogenase in the reverse direction) using NADH to reduce the C_2 ketone to the C_2-OH in lactate, with chirality control to produce D-lactate. The availability of this D-hydroxy acid in substantial amounts now allows the VanA enzyme to function as a D-Ala-D-lactate depsipeptide ligase, with a 150-to-1 preference at pH 6 for making D-Ala-D-Lac over D-Ala-D-Ala (Lessard et al., 1999). Meanwhile, the native D-Ala-D-Ala ligase has been working to produce its product, D-Ala-D-Ala, so there will be both D,D-dipeptide and D,D-depsipeptide (Fig. 10.6) in the VRE cell. These would normally compete with each other for elongation by MurF, the D-Ala-D-Ala-adding enzyme which converts UDP-muramyl tripeptide to UDP-muramyl pentapeptide to conclude the cytoplasmic phase of peptidoglycan biosynthesis (chapter 3). The presence of the third enzyme, VanX, is required for high-level VRE phenotypes, and VanX acts specifically as a D,D-dipeptidase while sparing the D-Ala-D-lactate from hydrolysis (Lessard and Walsh, 1999). The selectivity on a k_{cat}/K_m basis is almost 10^{10}, a staggering difference that ensures only D-Ala-D-lactate persists in a cell expressing VanH, VanA, and VanX. Then MurF has no competition from D-Ala-D-Ala when it uses D-Ala-D-lactate to make the UDP-muramyl-L-Ala-D-Glu-L-Lys-D-Ala-D-lactate. The subsequent enzymes in the PG

Figure 10.6 A five-gene cluster is necessary and sufficient to confer the VanA and VanB phenotypes of VRE.



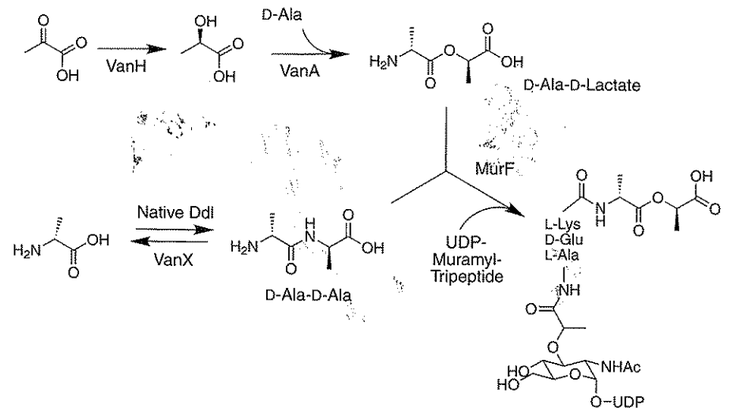
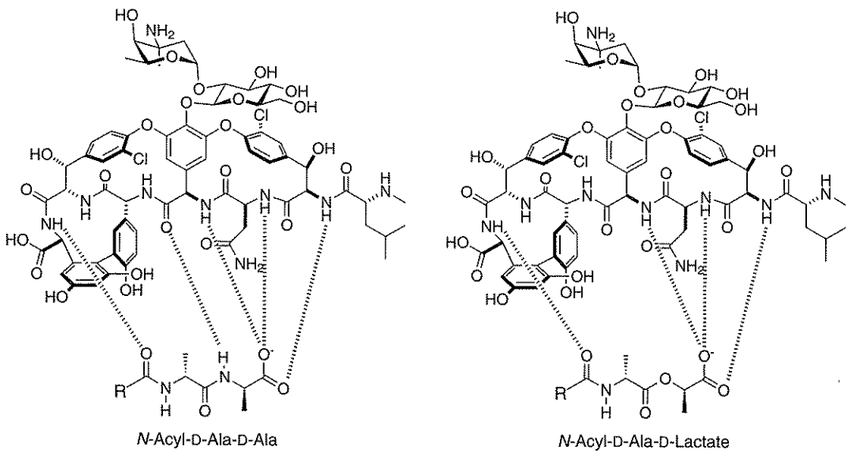


Figure 10.7 Reprogramming of the PG termini from D-Ala-D-Ala to D-Ala-D-lactate by the three-enzyme cassette VanH-VanA-VanX; role of VanX and MurF in partitioning of D-Ala-D-Ala versus D-Ala-D-Lac for destruction or elongation.

pathway take the depsipeptide through to the lipid II stage. This analog is a good substrate for transpeptidase cross-linking (**Fig. 10.9**), enabling the covalently cross-linked, mechanically sound PG layer to be produced such that VRE are not labile to osmotic lysis.

We noted in chapter 7 that the glycopeptide antibiotic producers use a similar strategy to switch on VanH, VanA, and VanX homologs to reprogram their PG layers and create protective self-immunity to the actions of the glycopeptide

Figure 10.8 Loss of one hydrogen bond between vancomycin and D-Ala-D-lactate provides a 1,000-fold drop in binding affinity.



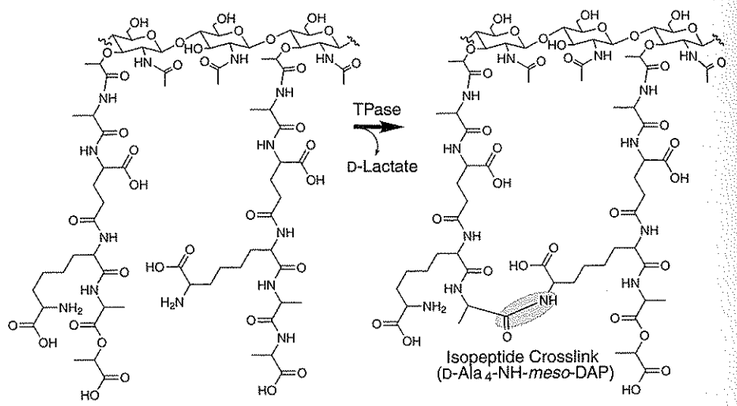


Figure 10.9 PG-D-Ala-D-Lac termini are substrates for transpeptidase-mediated cross-linking.

antibiotics they are making and exporting (Fig. 7.9). Such producer genes may be the source of the three-enzyme cassette in the opportunistically pathogenic VREs. The X-ray structures of a D-Ala-D-Ala ligase from *Escherichia coli* (Fan et al., 1994), a D-Ala-D-lactate ligase from the soil bacterium *Leuconostoc mesenteroides* (Kuzin et al., 2000) that has innate immunity to vancomycin, and the VanA ligase from VRE (Roper et al., 2000) have been determined, validating their close architectural homologies and indicating changes in the active site from a Tyr_{216} to a Phe_{216} to a His_{216} as one progresses through the three enzymes (**Fig. 10.10A**) and gains increasing selectivity for D-Ala-D-lactate over D-Ala-D-Ala formation. All three of the X-ray structures have a phosphinophosphate inhibitor bound, produced from the phosphinate reacting with ATP in the active site (**Fig. 10.10B and C**). The phosphinates are potent against the D-Ala-D-X ligases in vitro, but these charged molecules do not penetrate into bacterial cytoplasms and so are not active against whole bacteria.

Extending the hypothesis that VRE phenotypes reflect molecular reprogramming of D-Ala-D-Ala ligases is the VanC ligase from *E. gallinarum* (Navarro and Courvalin, 1994). This enzyme is a D-Ala-D-Ser ligase, showing about 350-fold selectivity for D-Ser in place of $D\text{-}Ala_2$ in the ligase active site (Park et al., 1997). The carry forward of D-Ala-D-Ser by MurF and subsequent PG biosynthetic enzymes yields a PG terminus with D-Ala-D-Ser where the CH_2OH side chain of the D-Ser interferes with the recognition by vancomycin compared to its complementary surface fit of the smaller CH_3 group of the terminal D-Ala. The affinity drop is more modest, about 10-fold.

The VanB operon has a comparable five-gene cluster—$VanS_B$, $VanR_B$, VanH, VanB, and $VanX_B$—and the molecular logic of the PG reprogramming enzymes is identical to that of the VanA operon. What differs is the observation that while both vancomycin and teicoplanin induces the transcription of the five genes of the VanA operon, only vancomycin but not teicoplanin induces the VanB operon (Table 10.1), explaining why VanB strains remain sensitive to teicoplanin (Arthur

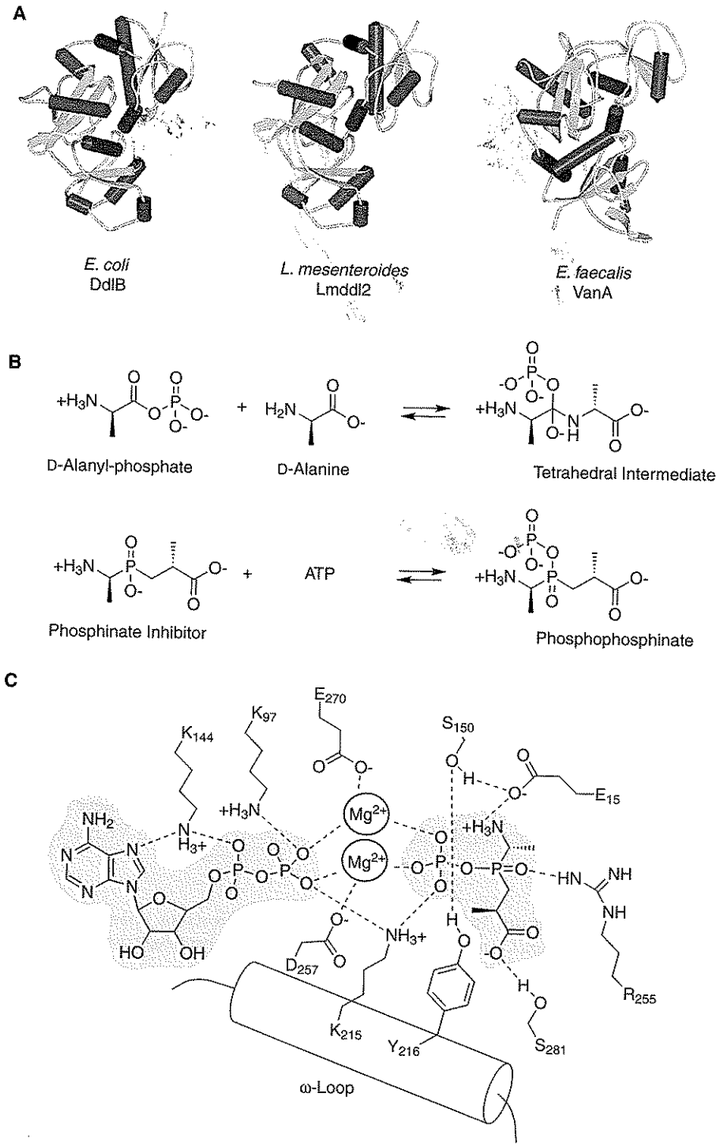
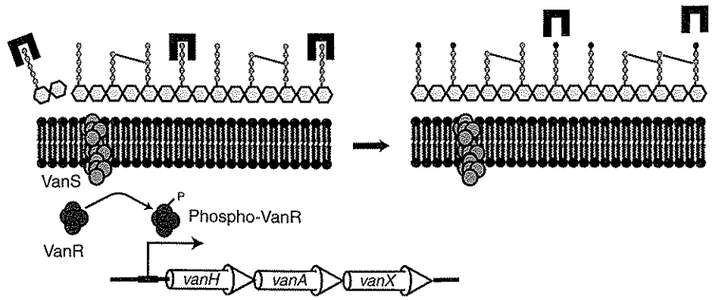


Figure 10.10 (A) X-ray structures of the D-Ala-D-Ala ligase from *E. coli*, the D-Ala-D-Lac ligase from *L. mesenteroides*, and the VanA D-Ala-D-Lac ligase from *E. faecalis*. (B) The phosphorylation of the dialkylphosphinate analog in the active site of the ligase produces a transition-state analog that behaves like a slow, tight-binding inhibitor. (C) Active-site architecture of *E. coli* DdlB with ADP and phosphophosphinate bound. (From Shi and Walsh [1995], with permission.)

and Courvalin, 1993). These results suggest that the glycopeptide antibiotic(s) are the inducers for VanA or VanB (vancomycin only) and indeed, mutants in the $VanS_B$ sensor protein can convert it to respond to extracellular teicoplanin. The two-component VanS-VanR logic seems typical of these bacterial sensor/transducer systems (Walsh et al., 1996b). Vancomycin in the extracellular microenvironment is sensed by the exo domain of VanS, whether directly or indirectly by some PG fragment as in the AmpC-sensing system (see chapter 8). This signal is transduced across the membrane to the cytoplasmic histidine kinase domain of VanS, which is now autoactivated to phosphorylate its dimeric subunit partner in *trans* (**Fig. 10.11**). The phospho-His form of VanS can transfer the $-PO_3$ group to the specific response regulator protein VanR, a two-domain transcription factor, to an Asp side chain in the N-terminal domain. The phospho-Asp form of the N-terminal domain of VanR communicates this change in phosphorylation state to the C-terminal DNA binding domain, and transcriptional activation of *vanH, vanA*, and *vanX* occurs to start the PG reprogramming. There is some evidence that in the default state in VanA phenotypes, the VanS acts in net fashion as a VanR kinase, while the $VanS_B$ may act at rest mostly as a phosphatase for phospho-Asp $VanR_B$.

Given that VanA and VanB phenotypes of VRE collect five genes to make a PG reprogramming that alters one hydrogen bond to vancomycin, there are several approaches for reversing the phenotype. One approach is to use VanS, VanR, VanH, VanA, and VanX as targets for molecules that inactivate them, and some inhibitors of VanX have been reported (Araoz et al., 2000). Such an inhibitor in combination with vancomycin would mimic the Augmentin strategy for β-lactam antibiotics (see chapter 8). A second strategy has been to screen against VRE for glycopeptides that retain antibiotic activity. This has led to semisynthetic versions of lipoglycopeptides such as LY333328 (see chapter 15, Fig. 15.6) that are 80- to 100-fold more active than the parent glycopeptides that lack the lipid chain, restoring two of the three logs of activity lost against the VRE phenotype. This compound is in clinical development under the name ortavancin. The hydrophobic chlorobiphenyl moiety can be moved around the disaccharide chain (Ge et al., 1999) and retain VRE activity, suggesting the lipid

Figure 10.11 Proposal for the VanS-VanR sensor kinase/response regulator to turn on the *vanH, vanA*, and *vanX* genes to reprogram peptidoglycan biosynthesis.



is a membrane anchor to preconcentrate the lipoglycopeptide and provide a higher effective concentration at the external surface of the membrane where the PG cross-links occur. It has been proposed that such hydrophobic derivatives of glycopeptides are selectively inhibiting transglycosylases in VRE, redirecting these drug candidates from transpeptidase inhibition to new targets (Ge et al., 1999; Sun et al., 2001).