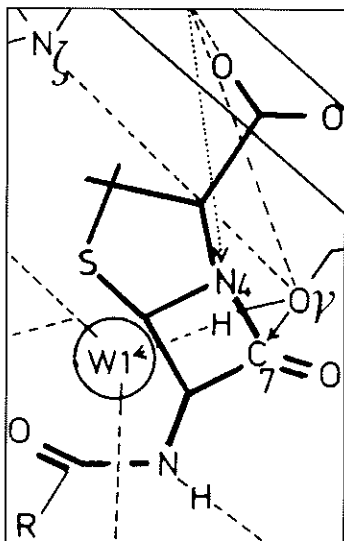

This section examines certain mechanisms by which drug resistance is mediated. The ß-lactamase genes are an example of a mechanism acting directly on the drug. Their relationship to penicillin-binding proteins has led to insights into their mode of action and to approaches to designing new antibiotics. This is particularly exciting when the structure of the proteins involved in resistance is understood at the three-dimensional level. With the benefit gained by drug resistance, the organism may pay a price for its survival. The appearance of stably resistant, virulent isolates of *Staphylococcus aureus* and *Streptococcus pneumoniae* argues against decreased virulence as a consequence of the acquisition of drug resistance. However, the possibility exists that other changes occur that could make these organisms subject to other antibacterial approaches. A novel and newly recognized mechanism for resistance is that carried by the enterococci against vancomycin and other glycopeptides. Several different determinants specify this drug resistance and illustrate how a combination of genes that alter cell-wall structure can render the host insensitive to the antibiotic. In the case of resistance to methicillin, full expression of the *mec* gene requires other genes on the chromosome, but other resistance genes can act alone. A multiplicity of chromosomal mutations form the molecular basis of drug resistance in mycobacteria. They provide a picture not only of the variety of genes involved in drug resistance, but also of the targets of antituberculosis drugs.

Resistance also confounds therapy of parasitic, fungal and viral diseases. Drug efflux via ATP-binding transporters, like those in mammalian tumor cells, has been found among parasites. The findings confirm the commonality of biological themes and the similarity of genes associated with resistance among different kinds of cells. Among viruses, point mutations in essential virus genes, the targets of antimicrobial agents, lead to drug resistance. Examples exist in DNA and RNA viruses with very different means of replication and very different mammalian host cells. Among fungi, several mechanisms are emerging, involving cytoplasmic and membrane proteins, but clearly efflux systems and reduced drug accumulation via decreased membrane permeability are most evident.

Molecular structures of penicillin-binding proteins and β-lactamases

Jean-Marie Ghuysen

In the past, new antibacterial agents have been selected either from natural sources or by 'trial and error' modification of existing antibacterials. Future therapeutic strategies are likely to depend on increased knowledge of existing drug targets and the search for new targets. The machinery for the assembly of bacterial-cell-wall peptidoglycan is an ideal place to look.

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The most familiar antibacterial agents, the penicillins, exert their effects by interacting with a class of enzymes, the penicillin-binding proteins (PBPs), that is responsible for the polymerization of precursor disaccharide-pentapeptide molecules to form the cell-wall peptidoglycan (Fig. 1). Resistance to penicillins, which are β-lactams, is most commonly due to the presence of β-lactamases (see p. 421 for a glossary of antibiotics). Both PBPs and the majority of β-lactamases are members of a family of 'penicilloyl serine transferases' (except for the zinc β-lactamases, which are not discussed here). Penicilloyl serine transferases catalyse the cleavage of the cyclic amide bond of penicillin by binding penicillin to their 'active-site' serine. With PBPs, the cleaved substrate remains bound to the enzyme; with the β-lactamases, it is released as penicilloate. Careful study of the steps in the reaction using the methods of theoretical chemistry may aid the design of non-lactam inhibitors of the penicilloyl serine transferases.

Other enzymes involved in the earlier stages of cell-wall peptidoglycan synthesis might also be useful in the search for new antibacterial agents. Possible candidates are the MraY and MurG transferases[1], which catalyse the attachment of the *N*-acetylmuramylpeptide to the intracellular end of its transmembrane lipid carrier and the subsequent addition of *N*-acetylglucosamine, respectively (see Fig. 1).

The close interactions between PBPs and several other proteins involved in the assembly of cell-wall peptidoglycans (FtsW, its homologue RodA, and the triad FtsZ, FtsQ and FtsA in *Escherichia coli*) are regulated in a cell-cycle-dependent fashion. The eluci-

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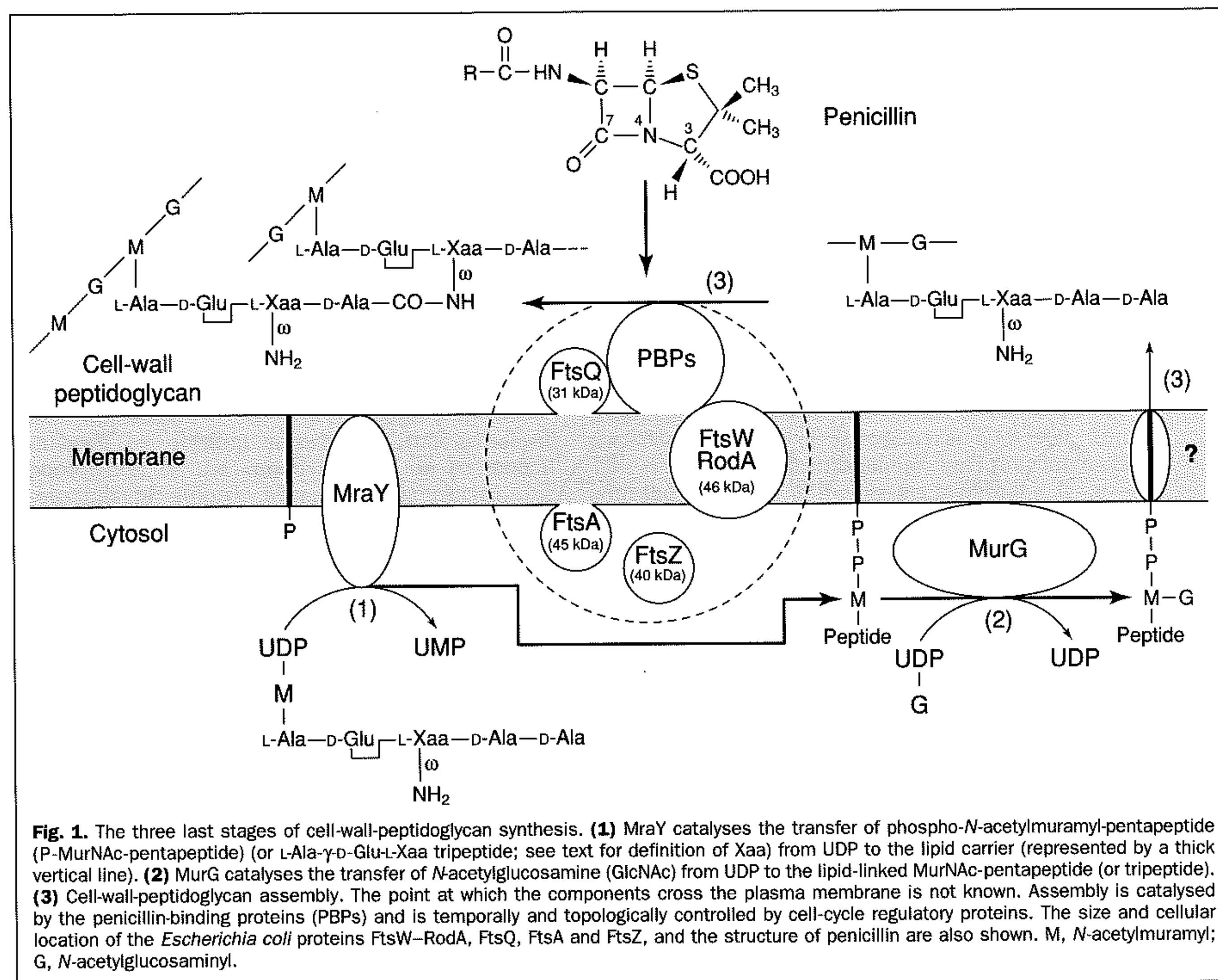


Fig. 1. The three last stages of cell-wall-peptidoglycan synthesis. **(1)** MraY catalyses the transfer of phospho-*N*-acetylmuramyl-pentapeptide (P-MurNAc-pentapeptide) (or L-Ala-γ-D-Glu-L-Xaa tripeptide; see text for definition of Xaa) from UDP to the lipid carrier (represented by a thick vertical line). **(2)** MurG catalyses the transfer of *N*-acetylglucosamine (GlcNAc) from UDP to the lipid-linked MurNAc-pentapeptide (or tripeptide). **(3)** Cell-wall-peptidoglycan assembly. The point at which the components cross the plasma membrane is not known. Assembly is catalysed by the penicillin-binding proteins (PBPs) and is temporally and topologically controlled by cell-cycle regulatory proteins. The size and cellular location of the *Escherichia coli* proteins FtsW–RodA, FtsQ, FtsA and FtsZ, and the structure of penicillin are also shown. M, *N*-acetylmuramyl; G, *N*-acetylglucosaminyl.

dation of such morphogenetic networks using molecular and structural biology methods may lead to the identification of novel targets for antibacterial agents.

PBP and β-lactamase active sites

As shown in Fig. 2a, the penicilloyl serine transferases (E) catalyse the transfer of the penicilloyl moiety of penicillin (D, for carbonyl donor) to the γOH of their active-site serine, forming a serine-ester-linked acyl (penicilloyl) enzyme (step 1), and from this, the transfer to water (step 2). K is the dissociation constant of the Michaelis complex E•D, k_{+2} and k_{+3} are first-order rate constants and k_{+2}/K is the second-order rate constant of enzyme acylation. For the β-lactamases, the values of k_{+2}/K and k_{+3} are large; penicillin is hydrolysed into penicilloate. For the PBPs, the value of k_{+2}/K is large, but k_{+3} is very small; the reaction stalls at the level of the penicilloyl enzyme.

Mechanistically, the penicilloyl serine transferases are similar to other serine transferases, such as chymotrypsin. Steps 1 and 2 each require a precise proton abstraction–donation that is carried by the charge-relay system of the enzyme (Fig. 2b). In step 1, the penicillin molecule binds to the active site of the enzyme in a position that allows the proton of the γOH of the active-site serine (S*) to be abstracted, the activated OγS* to attack the β-lactam carbonyl C-7 and the abstracted proton to be back-donated to the adjacent N-4. In step 2 (hydrolysis by the β-lactamases), the serine-ester-linked penicilloyl enzyme adopts a conformation that allows the proton of a water molecule to be abstracted, the activated OH to attack the carbonyl C-7 of the ester bond and the abstracted proton to be back-donated to the OγS* atom (with the concomitant re-entry of a water molecule). Backbone amino groups of the enzyme cavity (E–NH) polarize the carbonyl group of the β-lactam amide bond in step 1 and that of the penicilloyl-enzyme ester bond in step 2.

The three-dimensional structures of several β-lactamases of classes A (Refs 2–6) (Fig. 3a) and C (Refs 7,8) (Fig. 3b) and the low-molecular-mass PBP of *Streptomyces* R61 (Ref. 9) (Fig. 3c) are known. They each consist of one domain of α helices and another domain, the core of which is a five-stranded β sheet that is protected by additional α helices. The active-site-defining motifs (with x denoting any amino acid and using the numbering of the class A β-lactamases) are:

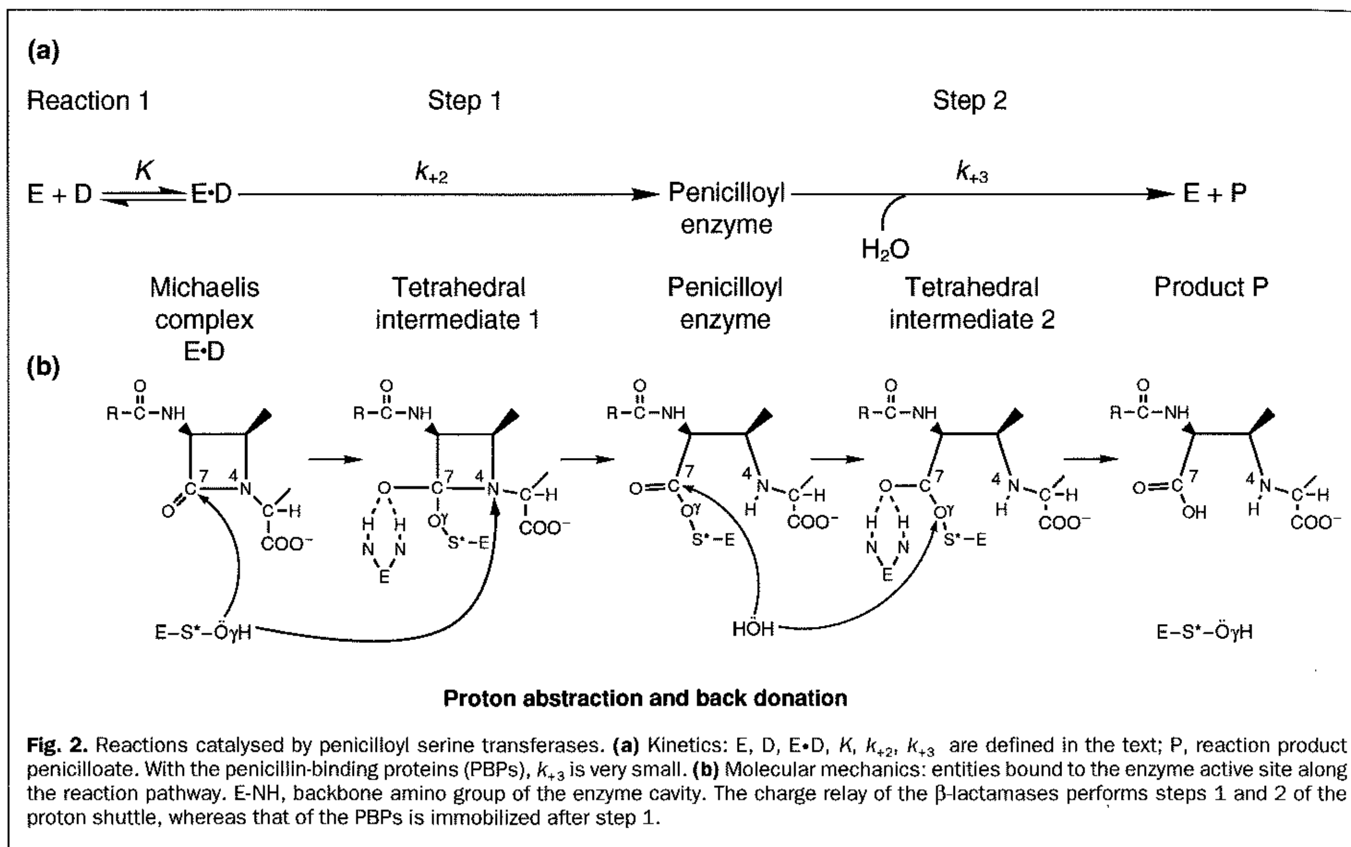


Fig. 2. Reactions catalysed by penicilloyl serine transferases. **(a)** Kinetics: E, D, E•D, K, k_{+2}, k_{+3} are defined in the text; P, reaction product penicilloate. With the penicillin-binding proteins (PBPs), k_{+3} is very small. **(b)** Molecular mechanics: entities bound to the enzyme active site along the reaction pathway. E-NH, backbone amino group of the enzyme cavity. The charge relay of the β-lactamases performs steps 1 and 2 of the proton shuttle, whereas that of the PBPs is immobilized after step 1.

the tetrad S*70xxK73 at the amino end of an α helix; the triad S130xN132, or its equivalent YxN of the class C β-lactamases and the R61 PBP, on a loop that connects two helices on one side of the cavity; and the tetrad K234[T/S]Gx237, or its equivalent HTGT of the R61 PBP, on the innermost strand (β3) of the β sheet on the other side of the cavity. The class A β-lactamases have an additional active-site-defining motif, the pentapeptide E166xELN170, located on a loop near the bottom of the β3 strand (Fig. 3a). This 'ω loop' might be related to the high penicillinase activity of the class A β-lactamases. Compared with the class A β-lactamases, the class C β-lactamases and the R61 PBP have additional loops and secondary structures at the surface of the all-α domain, away from the active site. For all the enzymes, the active sites are dense hydrogen-bonding networks interconnecting water molecules and the side chains of amino acid residues at the immediate boundary of the cavity (see Fig. 3d).

Penicillin binds to the active sites of the classes A and C β-lactamases and the R61 PBP in the same general orientation through hydrogen-bonding interactions that involve the three functional groups of the penicillin molecule. In all cases, the carboxylate points towards the top of the cavity, and the exocyclic amide bond at the other end of the molecule points towards the bottom of the cavity (Fig. 3e). The central carbonyl group of the β-lactam amide bond is hydrogen bonded in an oxyanion hole created by the backbone NH groups of the active-site S* and the amino acid residue x of the [K/H][T/S]Gx motif of the β3 strand, in a way that is reminiscent of the oxyanion-hole hydrogen bonds of chymotrypsin. As a result of these interactions, the OγS*70 atom, while losing its proton, can attack the β-lactam carbonyl C-7 on the well-exposed α face of the optimally positioned penicillin molecule.

Drug design

Knowing the route of the proton during catalysis should help to design molecules that could perturb the mechanism of the penicilloyl serine transferases. At this level, in the range of 10^{-10} m, this can only be achieved using the methods of theoretical chemistry[10].

Molecular mechanics allows the energy of molecular systems to be calculated using empirical force fields and simple functions of the internal coordinates of the system. Molecular mechanics cannot describe the entire energy profile of the catalysed reaction, but it can provide views of several energy-optimized entities (penicillin, penicilloyl, tetrahedral intermediates and product; see Figs 2b and 3e) bound to the active site, and it can be applied to large systems of up to 5000 atoms. Quantum chemistry allows all the geometric parameters and electronic redistributions involved in the molecular rearrangements undergone by the interacting partners to be described in the form of potential-energy hypersurfaces. The reaction can be seen as a continuum of transition states along the reaction coordinate. However, as the dimensionality of the hypersurface is $3N-6$ (where N is the number of atoms), *ab initio* calculations can only be applied to small systems of up to a few tens of atoms. Semiempirical calculations (in which the more intractable integrals are

replaced by simple functions) are used for systems of up to 300 atoms.

The design of enzyme inhibitors is an iterative two-step selection procedure that combines the methods of quantum chemistry and molecular mechanics. The intrinsic reactivity of a 'lead' compound towards a putative reactive centre of the enzyme can be estimated using as a starting structure 50-atom models that mimic the spatial disposition of the molecule within the active site. Algorithms have been developed that allow the hypersurfaces (of about 150 dimensions) to be described, the transition states to be identified and the associated activation barriers to be calculated[11]. The accuracy of fit within the active site of the lead compound, and of more-structured molecules derived from the lead compound, can be evaluated by docking experiments and geometric optimization of the complexes (of up to several thousands of atoms). Moreover, the electrostatic energy of the interaction (the main component of the total energy of interaction when no covalent bonds are made or broken) can be estimated by semiempirical quantum-chemical calculations. Recently, for the first time, a 244-atom model of the Michaelis complex *N*-acetyl-L-tryptophanamide•chymotrypsin (which is mechanistically analogous to the penicilloyl serine transferases) has been optimized at the AM1 quantum level[12].

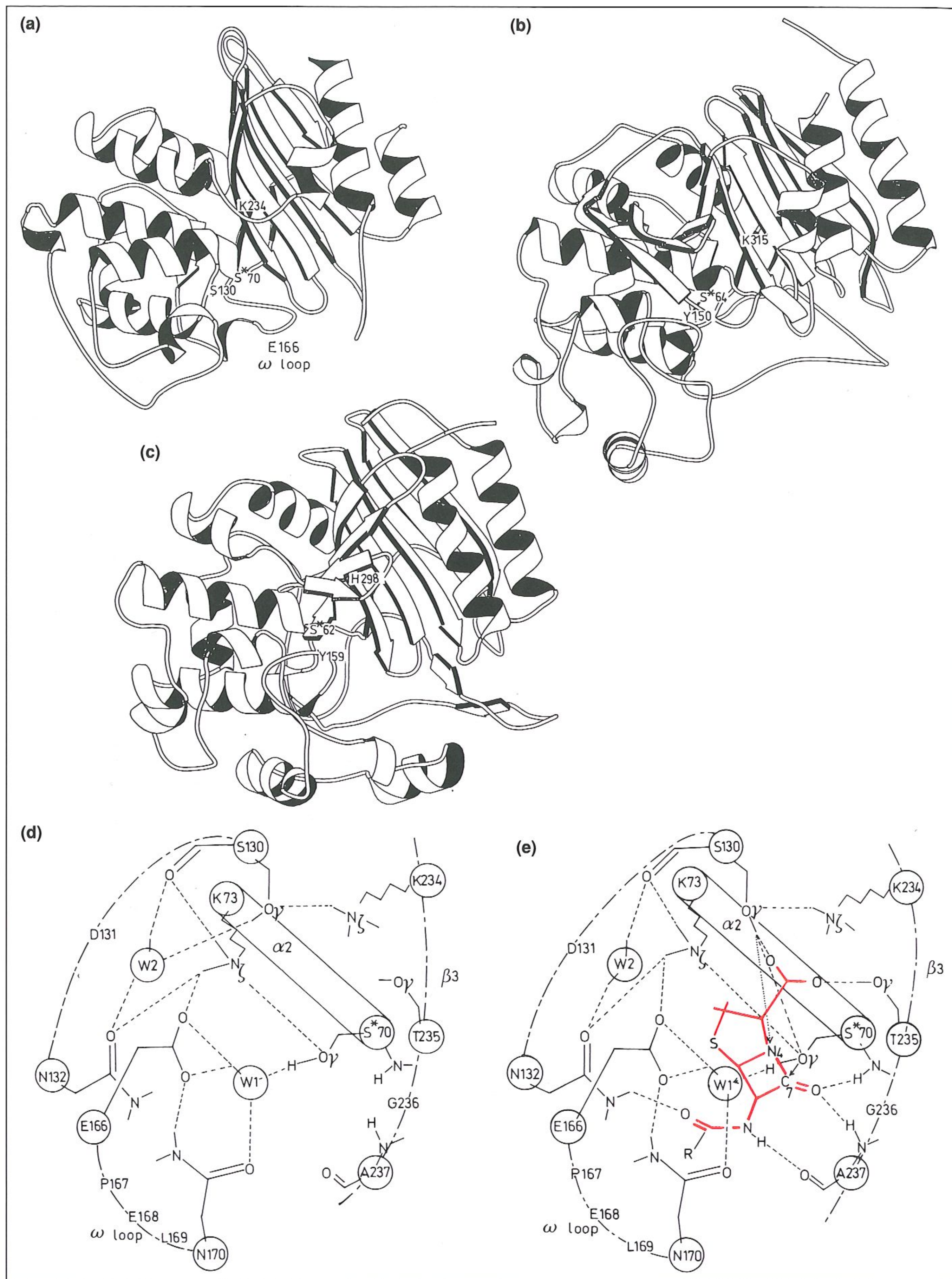
One important conclusion of this last study is that the charge-relay system is created by the interacting partners. It is the result of the combined effects of the active-site environment, the deformation undergone by the bound substrate molecule, the relaxation undergone by the polypeptide backbone and the freedom of a water molecule. Consequently, enzymes of the same family and even of the same class can use more than one proton-shuttle route, depending on minute structural variations in the active site and/or the bound ligand. Classes A and C β-lactamases, the R61 PBP and many β-lactamase[13] and PBP mutants (both site directed and naturally occurring) support this concept. At least 26 β-lactamases of varying specificities are known, all of which arose by alterations of a small number of amino acid residues in the TEM-1 β-lactamase[14] (Fig. 4). Drug resistance by the emergence of PBPs with a lower affinity for certain β-lactam antibiotics has occurred by interspecies recombinational events that have replaced parts of a PBP-encoding gene with the corresponding parts from homologous PBP-encoding genes of closely related species (see the review by Christopher Dowson, Tracey Coffey and Brian Spratt in this issue[15]). Evolution occurs before our eyes. The ability of the β-lactamase and PBP active sites to be remodelled appears to be endless.

Diverging evolution

The peptides of the translocated peptidoglycan precursors are either L-Ala-γ-D-Glu-L-Xaa-D-Ala-D-Ala pentapeptides or L-Ala-γ-D-Glu-L-Xaa tripeptides (where Xaa is a diamino acid residue, the ω-amino group of which is either free or substituted by one or several amino acid residues) (Fig. 1). Transfer of the carbonyl group of the penultimate D-alanine residue of a pentapeptide to the ω-amino group of another penta- or tripeptide (DD-transpeptidase activity) results in peptidoglycan crosslinking. Transfer to water (DD-carboxypeptidase activity) controls the extent of crosslinking. The notation 'DD' emphasizes that the reactions catalysed involve breaking a peptide bond (D-alanyl-D-Ala) that extends between two carbon atoms with the D configuration.

Divergent evolution implies that proteins of the same family evolved from a common ancestor. Assuming that cell-wall-peptidoglycan assembly in primitive Eubacteria was carried out by unlinked transglycosylases and transpeptidases, a plausible model of the transpeptidase ancestor is provided by the serine DD-transpeptidase/PBP of *Streptomyces* K15 (Ref. 16). This enzyme lacks membrane anchors, but interacts with the outer face of the plasma membrane. It catalyses transfer of the R-L-Xaa-D-alanyl moiety of R-L-Xaa-D-Ala-D-Ala peptides (analogous to the peptidoglycan precursors), via a serine-ester-linked R-L-Xaa-D-alanyl enzyme, to structured amino acceptors with a much higher efficiency than it does to water (activity is compatible with a wide range of substituents R on the α-amino side of the L-Xaa diamino acid). From a putative DD-transpeptidase/PBP ancestor of this type, three main evolutionary changes may have occurred (Fig. 5). (1) Conservation of the inertness of the penicilloyl enzyme, increased preference for water as the attacking nucleophile towards the serine-ester-linked acyl (R-L-Xaa-D-alanyl) enzyme and acquisition of carboxy-terminal membrane-associated amphiphilic α helices[31] may have given rise to the membrane-bound low-molecular-mass PBPs/DD-carboxypeptidases. (2) Catalysed hydrolysis of the penicilloyl enzyme, loss of peptidase activity and secretion of the expressed proteins may have given rise to the β-lactamases. (3) Fusion of PBPs to other polypeptides and membrane anchoring of the multimodule proteins at their amino-terminal ends may have given rise to the membrane-bound high-molecular-mass PBPs.

Acquisition of new functions, while retaining the basic three-dimensional structure, is achieved through local changes that do not affect the overall polypeptide folding. While the S*xxK and [K/H][T/S]Gx motifs occur in all existing penicilloyl serine transferases, the SxN motif is not ubiquitous: it is replaced by a YxN motif in the class C (and D) β-lactamases and in the R61 PBP (Figs 3a–3c and Fig. 5). The functional OH groups of the serine residue of the SxN motif and the tyrosine residue of the YxN motif have similar spatial dispositions within the active sites of the enzymes as a result of a considerable displacement of the corresponding motif-bearing loops. The E166xELN motif may be unique to the class A β-lactamases. Dicarboxylic acids fulfilling a similar function to that of E166, but attached to distinct structural elements, may be present in other penicilloyl serine transferases. The all-α domains of the class C β-lactamases and the R61 PBP are more complex than that of the class A β-lactamases (Figs 3a–3c and Fig. 5). Similarly, an insert of



(a)
K234
S*70
S130
E166
ω loop
(b)
K315
S*64
Y150
(c)
H298
S*62
Y159
(d)
S130
K73
K234
D131
W2
Oγ
Nζ
α2
β3
Oγ
T235
S*70
N132
W1
H
E166
G236
A237
P167
E168
ω loop
L169
N170
(e)
S130
K73
K234
D131
W2
α2
Nζ
Oγ
β3
T235
S*70
S
N4
C7
W1
N132
E166
G236
R
A237
P167
E168
ω loop
L169
N170

170–180 amino acid residues occurs in the presumed all-α domain of the PBP of *Actinomadura* R39 (Ref. 32) and of the PBP4 of *E. coli*[33].

Acquisition of new functions can also be achieved by fusion of the penicilloyl serine transferases to other polypeptides. While the β-lactamases and low-molecular-mass PBPs each have a single catalytic function, the multipartite high-molecular-mass PBPs have diverged so far that traces of similarity in the amino acid sequences, other than the S*xxK-SxN-K[T/S]Gx signature, have almost completely disappeared. They have also acquired unique properties.

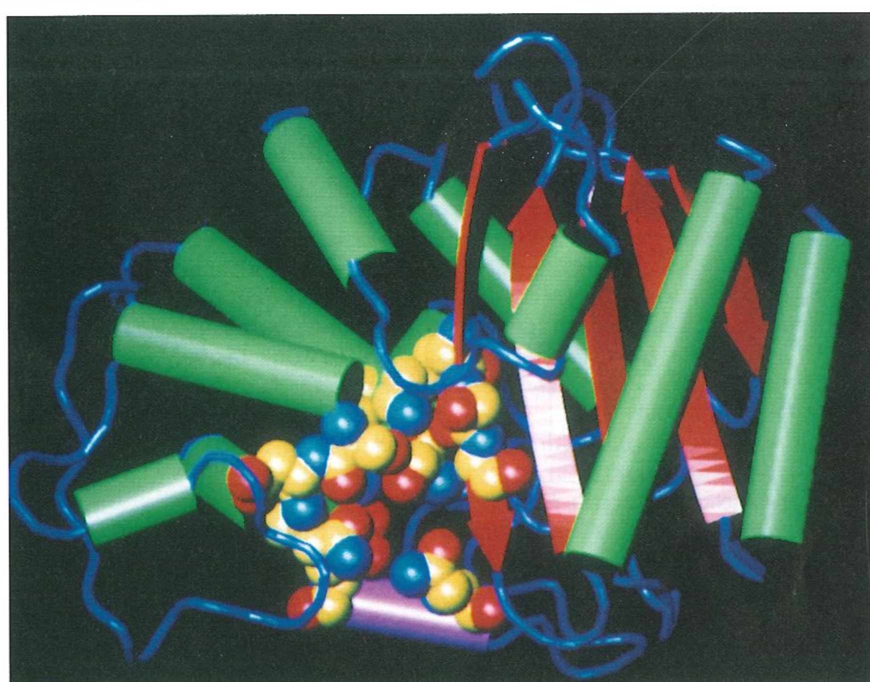


Fig. 4. Polypeptide scaffolding of the plasmid-borne TEM-1 β-lactamase and topology of the active site. Green cylinders: α helices; red arrows: five-stranded β sheet; blue wires: loops; violet cylinder: ω loop; spheres: nitrogen (blue), oxygen (red) and carbon (yellow) atoms of the active-site-defining amino acid residues: the active-site S*70 and K77; S130, D131 and N132; E166 and N170; and K234, S235 and G236. The structure has been resolved to 1.9 Å by molecular replacement using the known crystal structures of the *Streptomyces albus* G and *Bacillus licheniformis* β-lactamases as starting models. The protein is 50 Å×33 Å×30 Å. Courtesy of Eveline Fonzé, Paulette Charlier, Yves To'th and Marcel Vermeire, Centre d'Ingénierie des Protéines, Liège.

The bi(multi)functional high-molecular-mass PBPs

The high-molecular-mass PBPs are essentially constructed of two modules, one of which binds penicillin (PB module), linked to each other in a single polypeptide chain that folds on the outer side of the plasma membrane and is anchored into the membrane by an uncleaved amino-terminal signal peptide (Fig. 5). The high-molecular-mass PBPs fall into two classes depending on the motifs present in the non-penicillin-binding (n-PB) modules[10,34]: PBP1a of *Streptococcus pneumoniae* is the prototype of class A, and PBP3 of *E. coli* the prototype of class B. High-molecular-mass PBPs frequently have inserts and carboxy-terminal extensions that are large enough to form additional modules, each of which has a particular fold and performs a separate function. The PBP2′ (or PBP2a) of *Staphylococcus aureus* and PBP3^{r} and PBP5 of *Enterococcus hirae* have a very low affinity for all β-lactams. They each have a similar insert between the membrane anchor and the n-PB module.

The high-molecular-mass PBPs are the main enzymes responsible for cell-wall-peptidoglycan assembly. However, the high-molecular-mass PBPs are not equally important, and the widespread belief that all the high-molecular-mass PBPs are bifunctional, with transglycosylase (the n-PB modules) and transpeptidase (the PB modules) activities, is an unwarranted generalization. Bifunctionality of this form can be assigned only to *E. coli* PBP1a and PBP1b of class A (perhaps also, by extension, to other high-molecular-mass PBPs of the same class) on the basis of the reactions that they catalyse on lipid-linked disaccharide-peptide precursors *in vitro*. Moenomycin, which inhibits the transglycosylase of PBP1b, prevents peptide crosslinking. Penicillin, which inhibits peptide crosslinking, greatly enhances glycan-chain formation under certain conditions. Deletion of the genes encoding PBP1a and PBP1b is fatal in *E. coli*, but deletion of either the PBP1a- or the PBP1b-encoding gene is tolerated, suggesting that one can compensate for the other[35]. Inactivation of PBP1a of *S. pneumoniae* by elimination of the S*xxK penicillin-binding motif has little effect

Fig. 3. Three-dimensional structures of the penicilloyl serine transferases and disposition of a penicillin molecule bound to the active site of a class A β-lactamase. The figure shows the polypeptide folding of: **(a)** the class A β-lactamase of *Bacillus licheniformis*[3], **(b)** the class C β-lactamase of *Enterobacter cloacae* P99 (Ref. 7), and **(c)** the *Streptomyces* R61 penicillin-binding protein[9]. The active sites are located at the interface between the all-α helix and α-helix–β-sheet domains. The amino-terminal amino acid residues of each of the active-site-defining motifs (see text) are indicated. A more detailed comparison of these molecular structures will be published (J.R. Knox, pers. commun.). **(d)** Active site of the class A β-lactamase of *Streptomyces albus* G. Hydrogen-bonding network interconnecting the water molecules W1 and W2 and the side chains of the active-site S*70 and K73 (of the S*xxK motif on the α2 helix), S130 and N132 (of the SxN motif), E166 and N170 (of the ω loop), and K234 (of the K[T/S]Gx motif on the β3 sheet). **(e)** Michaelis (penicillin•β-lactamase active site) complex showing the interactions between the functional groups of the bound penicillin molecule and the side chains of S130 and T235 (at the top), and N132 and A237 (at the bottom). The carbonyl group C-7=O of penicillin interacts with the backbone NH groups of S*70 and A237. (Adapted from Ref. 4.)

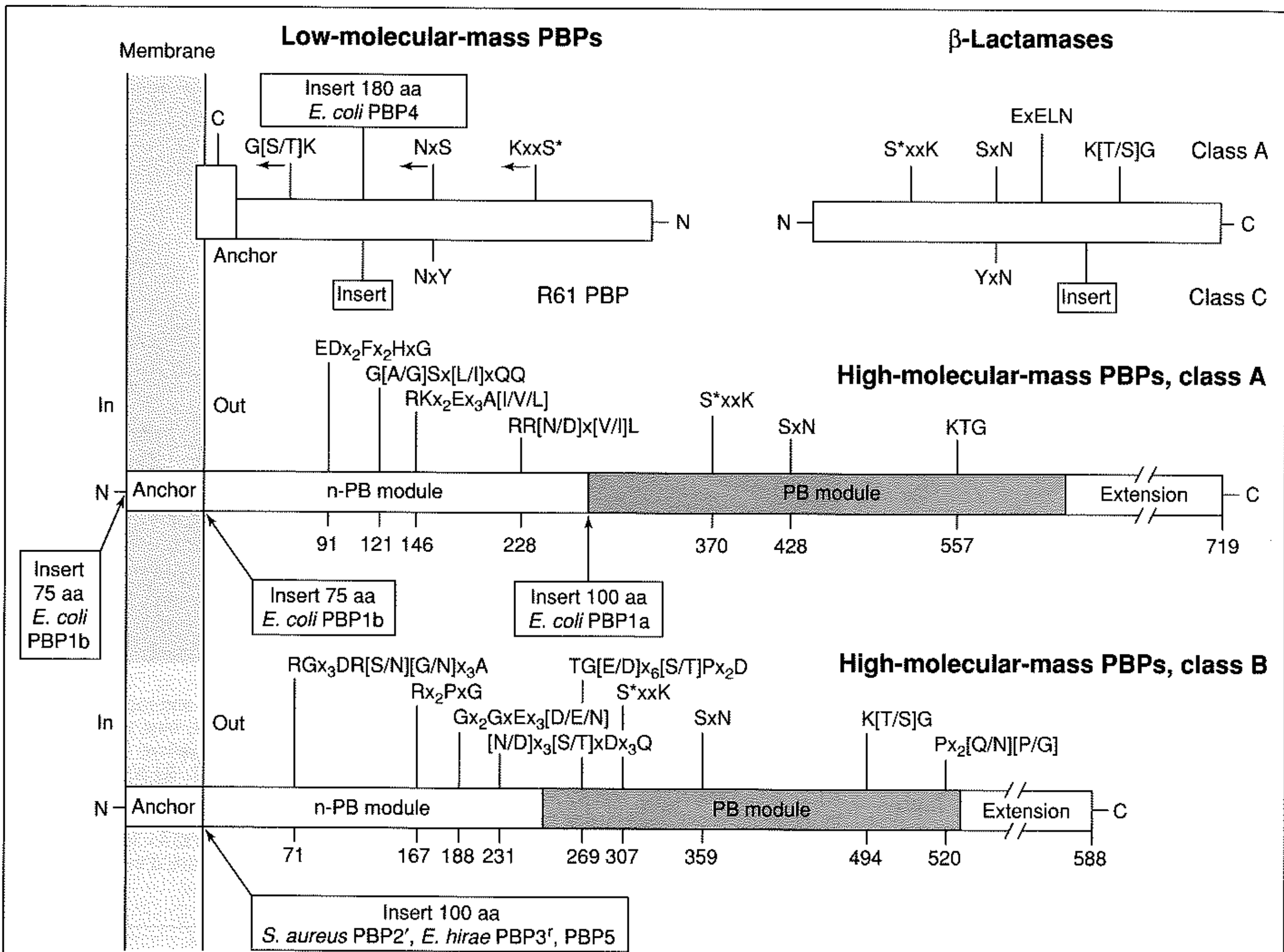


Fig. 5. Molecular organization of the penicilloyl serine transferases showing the modular design of the high-molecular-mass penicillin-binding proteins (PBPs) (PB, penicillin-binding module, n-PB non-penicillin-binding module). Conserved motifs are shown. S*, active-site serine. The amino acid numbering of the high-molecular-mass PBPs is that of the prototypic members, PBP1a of *Streptococcus pneumoniae* (class A) and PBP3 of *Escherichia coli* (class B). The high-molecular-mass PBPs of class A are PBP1as of *S. pneumoniae*[17], *E. coli*[18], *Bacillus subtilis*[19] and *Haemophilus influenzae*[20], and PBP1b of *E. coli*[18]. The high-molecular-mass PBPs of class B are PBP2 and PBP3 of *E. coli*[21,22], PBP2 of *Neisseria meningitidis*[23] and *Neisseria gonorrhoeae*[24], PBP2x of *S. pneumoniae*[25], PBP2b and SpoVD of *B. subtilis*[26], the low-affinity PBP2′ of *Staphylococcus aureus*[27], and PBP3ʳ and PBP5 of *Enterococcus hirae*[28,29]. PBP2b of *S. pneumoniae*[30] may be of class B, but lacks the Rx_2PxG motif. The amino acid sequences of the PBPs are given in the references cited.

on growth and susceptibility to penicillin[36]. Deletion of the PBP1a-encoding gene of *Bacillus subtilis* has no observable effect[19].

Indirect experimental evidence supports the view that the PB modules of the high-molecular-mass class B PBPs are transpeptidases. Although these PBPs have no activity towards peptide analogues of the peptidoglycan precursors, they have esterase and thiolesterase activities[37] *in vitro*. Analysis of the cell-wall peptidoglycans of methicillin-resistant staphylococci (synthesized by PBP2′)[38] and penicillin-resistant pneumococci (synthesized by other altered class B PBPs)[39] suggests that a PBP with a modified affinity for the drug synthesizes a cell-wall peptidoglycan with a different peptide moiety from that of the wild-type (see the review by Alexander Tomasz in this issue[40]).

The function of the n-PB modules of the high-molecular-mass class B PBPs is unclear. Given that these modules have a different signature from that of the n-PB modules of class A PBPs (Fig. 5), they may not have a transglycosylase activity. As suggested in Ref. 41, the transpeptidase activity of the PB modules of the high-molecular-mass class B PBPs might be coupled with the transglycosylase activity of the n-PB modules of some high-molecular-mass class A PBPs or with PBP-unlinked transglycosylases (detected in *E. coli*, *Micrococcus luteus*, *S. aureus* and *S. pneumoniae*[42]).

The high-molecular-mass class B PBPs appear to be essential. Inactivation of *E. coli* PBP2 and PBP3 results in growth as spherical cells and inhibition of cell septation, respectively, and causes cell death. Inactivation of either of the PBP2b- or PBP2x-encoding genes of *S. pneumoniae* is not tolerated[36], and alterations in either of these PBPs can increase the resistance to penicillin of the strain[43]. Carboxy-terminal truncation of *B. subtilis* PBP2b causes cells to grow as filaments[26]. The low-affinity PBP2′ of *S. aureus* and PBP3ʳ and PBP5 of *E. hirae* take over the functions needed for

cell-wall-peptidoglycan synthesis when the other PBPs are inactivated.

The n-PB modules of the high-molecular-mass class B PBPs, perhaps together with the carboxy-terminal regions of the proteins, confer an active, penicillin-binding conformation to the PB modules through interactions with specific recognition sites. Deletion of either of the carboxy-terminal Y540–V549 or F550–R559 segments of the *E. coli* membrane-bound PBP3 suppresses binding of penicillin[44]. Removal of the amino-terminal membrane anchor and truncation of the n-PB module up to the start of the first conserved motif are tolerated, but complete elimination of the n-PB module is not[45]. Similarly, soluble forms of the *S. aureus* PBP2′ in which the n-PB module has been truncated extensively do not bind penicillin[46]. This ability of the n-PB modules to interact with the PB modules to which they are fused is reminiscent of the β-lactamase inhibitory protein (BLIP)[47] of *Streptomyces clavuligerus*. BLIP has two domains, each with a helix–loop–helix motif packed against a four-stranded β sheet. BLIP has no effect on most PBPs tested and on certain class C β-lactamases; it stimulates the activity of other class C β-lactamases and inhibits the activity of class A β-lactamases, sometimes at picomolar concentrations.

Morphogenetic networks

The high-molecular-mass class B PBPs are members of tightly regulated 'morphogenetic' networks[48,49] (Fig. 1). Cell septation in *E. coli* depends on PBP3 and does not tolerate amino acid changes in or close to the membrane anchor of the protein[44]. Cell septation also requires FtsW, a protein that crosses the membrane several times and that may act as a receptor–transmitter of a cytosolic signal or may be involved in translocation of peptidoglycan precursors, as well as several cell-cycle proteins.

The 2 min region of the *E. coli* chromosome, which contains the genes encoding PBP3 and FtsW, also contains the morphogenes *ftsQ*, *ftsA* and *ftsZ* (Refs 42,48,49). These genes form a cluster, the expression of which is controlled by a gearbox and metabolic promoters. The function of FtsQ, a membrane-bound protein facing the periplasm, is unknown. FtsA, a membrane-associated protein facing the cytosol, is similar to the ATPase domain of kinases, heat-shock proteins and MreB (involved in the positive control of cell elongation and/or the negative control of cell division). FtsZ, a GTP-binding/GTPase cytosolic protein, self-assembles to form a ring at the future division site, and its GTP-binding site is analogous to that of the eukaryotic tubulins. FstZ may be a cytoskeleton element that interacts with the septal peptidoglycan-synthesizing machinery. It is the target of several endogenous cell-division inhibitors, and imbalance in the ratio of FtsA to FtsZ inhibits the initiation of cell division.

In *E. coli*, cell-wall elongation and maintenance of the rod shape depends on PBP2. The 14 min region of the chromosome, which contains the gene encoding PBP2, also contains the RodA-encoding gene, the product of which is very similar to FtsW. The PBP2–RodA complex and ribosomal activities appear to be coordinated by a chain of interacting elements, one of which is regulated by the nucleotide guanosine 5′-diphosphate, 3′-diphosphate (ppGpp, probably an RNA polymerase effector). Mecillinam inactivates PBP2 and blocks cell division, although PBP2 is not required for septation. This block can be overcome by a large pool of ppGpp or by the overproduction of FtsZ, suggesting that ppGpp is a positive regulator of *ftsZ* transcription[50,51].

Cell-cycle proteins are probably ubiquitous. The 133°–135° region of the *B. subtilis* chromosome contains the genes encoding PBP2b and SpoVB, which are analogous to *E. coli* PBP3. This region also contains genes that code for proteins that are similar to FtsW (called SpoVE), FtsQ (called DivIB), FtsA and FtsZ (Ref. 52). FtsZ-like proteins have been detected in many bacteria[53].

Understanding how the high-molecular-mass class B PBPs and the associated cell-cycle proteins function may lead to the discovery of new targets and to the development of novel 'bacterial-cell-wall inhibitors'. Future advances require an increased knowledge of the molecular structures of the interacting partners.

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Benefit and risk in the β-lactam antibiotic-resistance strategies of *Streptococcus pneumoniae* and *Staphylococcus aureus*

Alexander Tomasz

The radically altered cell-wall chemistry in penicillin-resistant pneumococci and the stringent requirement for specific cell-wall precursors for expression of high-level resistance to methicillin in staphylococci may represent compensating mechanisms to balance molecular risks produced by the strategies for antibiotic resistance in these bacteria.

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Since the late 1980s, the emergence and global spread of multidrug-resistant pathogens that apparently retain their ability to cause life-threatening disease has caught the world by surprise. Two of the most recent and most successful mechanisms of antibiotic resistance to emerge – resistance to penicillin in *Streptococcus pneumoniae* and resistance to methicillin in *Staphylococcus aureus* (see p. 421 for a glossary of antibiotics) – each involve modification of the molecular targets of the antibiotics in the bacterial cell. This is surprising because laboratory mutants with this type of drug-resistance mechanism are sluggish in growth and unstable, suggesting that the modification of antibiotic targets may exact an incapacitating price from the bacteria. Frequently, penicillin-resistant laboratory isolates of pneumococci and staphylococci in which resistance is based on alterations of the penicillin-binding proteins (PBPs) are unstable. It would also be expected that the genetic load of multidrug resistance would impair full virulence and thus limit the pathogenic potential (and clinical impact) of such bacteria.

Clearly, acquisition of antibiotic resistance must involve not only benefits, but also risks, for pathogenic

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