Penicillin Binding Proteins: key players in bacterial cell cycle and drug resistance processes

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Abstract

Bacterial cell division and daughter cell formation are complex mechanisms whose details are orchestrated by at least a dozen different proteins. Penicillin-binding proteins (PBPs), membrane-associated macromolecules which play key roles in the cell wall synthesis process, have been exploited for over 70 years as the targets of the highly successful β-lactam antibiotics. The increasing incidence of β-lactam resistant microorganisms, coupled to progress made in genomics, genetics and immunofluorescence microscopy techniques, have encouraged the intensive study of PBPs from a variety of bacterial species. In addition, the recent publication of high-resolution structures of PBPs from pathogenic organisms have shed light on the complex intertwining of drug resistance and cell division processes. In this review, we discuss structural, functional and biological features of such enzymes which, albeit having initially been identified several decades ago, are now being aggressively pursued as highly attractive targets for the development of novel antibiotherapies.

Introduction

The bacterial peptidoglycan, a three-dimensional, net-like mesh which lines the exterior of the cell membrane, not only must be synthesized in a timely manner during the cell cycle but also protects bacteria from osmotic shock. Peptidoglycan determines the overall cellular shape, serves as attachment site for virulence factors and adhesins, aids bacteria in undergoing morphological transformations in response to different stress-related factors, and its eventual fragility or instability may lead to cell lysis and death (Höltje, 1998; Nanninga, 1998). Many Gram-positive bacteria produce a multi-layered peptidoglycan which is incorporated within the inner cell wall surface, moves outward allowing cellular expansion, and is eventually shed into the medium in the form of its building blocks; Gram-negative organisms, on the other hand, carry a thin layer of peptidoglycan, 50% of which is recycled during each cell cycle (Höltje, 1998; Nanninga, 1998).

The peptidoglycan itself is composed of β-linked N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) polysaccharide chains, which are cross-linked through peptide chains on alternating strands (van Heijenoort, 2001). Peptidoglycan synthesis is initiated in the bacterial cytoplasm by six Mur enzymes (named MurA to MurF; Fig. 1), which catalyze the formation of uridine diphosphate (UDP)-MurNAc from a UDP-GlcNAc precursor (MurA and MurB) and subsequently add five amino acids onto the d-lactoyl group of UDP-MurNAC (MurC to MurF). The peptidic sequence may vary according to bacterial species, but is inevitably terminated by a d-alanine, D-alanine moiety (except in certain vancomycin-resistant enterococcal strains, which carry D-alanine, D-lactate (Arthur et al., 1998; Lessard & Walsh, 1999), but this is rather an exception). This soluble molecule, UDP-MurNAc-pentapeptide, becomes associated to the bacterial membrane through the action of MrAY, a ten transmembrane helix protein, which catalyzes its linkage to a 55-carbon-long phospholipid, harbouring a membrane-embedded undecaprenyl diphosphate group (van Heijenoort, 2001; Bouhss et al., 2004). This molecule, now called Lipid I, is subsequently acted upon by MurG, an N-acetylgucosaminyl transferase which adds a soluble UDP-GlcNAc group to it. These reactions thus finalize the synthesis of Lipid II, the disaccharidic precursor for peptidoglycan biosynthesis and substrate for penicillin-binding proteins (PBPs) (Goffin & Ghuysen, 2002). Cytosolic steps leading to the synthesis of Lipid II are well understood due to the fact that most substrates and enzymes are soluble, and X-ray structures are available for all seven Mur enzymes, albeit from different bacterial species. At this point, however, Lipid II is still within the cytoplasmic compartment, and must be ‘flipped’ over to the exterior of the membrane (by a yet undetermined ‘flippase’ activity, which could involve either one or both of the integral
membrane proteins FtsW and RodA (Höltje, 1998; Nanninga, 1998). Once on the outer side of the membrane, Lipid II is polymerized and cross-linked by PBPs, membrane-associated enzymes present in all peptidoglycan-containing organisms.

The term ‘PBP’ has been at times loosely employed in the literature to refer to any enzyme which recognizes and/or metabolizes β-lactams, independently of its function in the cell. The Streptomyces K15 and R61 transpeptidases, for example, have often been referred to as PBPs, but, although

Fig. 1. Peptidoglycan synthesis in a nutshell. Peptidoglycan building blocks are synthesized and assembled in a series of cytoplasmic steps; subsequently they are linked to a membrane-associated undecaprenyl group (generating Lipid I) which is further modified with a GlcNAc group (to form Lipid II) and subsequently ‘flipped’ to the external side of the membrane. Lipid II located on the external side of the bacterial membrane is the substrate for glycosyltransfer and transpeptidation reactions catalyzed by PBPs. Fig. 1b shows details of these two reactions, which are catalyzed on distinct chains.
they bind to penicillin and are able to catalyze transpeptidation/carboxypeptidation reactions with dipeptides in solution, (Kelly & Kuzin, 1995; Foné et al., 1999; Rhazi et al., 2003; Rhazi et al., 2005), they have not been reported to participate in peptidoglycan biosynthesis, and their precise cellular function to date remains unknown. Our goal, in this review, is to highlight the structure and function of the PBP enzymes which are associated to the bacterial membrane and play essential roles in peptidoglycan biosynthesis.

Bacteria have multiple PBPs, some of which have started to reveal their clear roles during the cell division cycle. High molecular mass (HMM) PBPs are classified according to the number of reactions a single polypeptide is able to catalyze: bifunctional (class A) enzymes catalyze both the polymerization of the GlcNAc–MurNAc chains (glycosyltransfer, GT) and cross-linking of adjacent stem peptide (transpeptidation, TP) reactions, while monofunctional (class B) proteins present only transpeptidase activity (Goffin & Ghuysen, 2002). The glycosyltransferase reaction catalyzes the association between MurNAc and GlcNAc groups, generating free undecaprenyl pyrophosphate, which is again ‘flipped’ back towards the cytosolic side of the membrane where it is hydrolyzed to undecaprenyl phosphate and is recycled in a new reaction (Hölter, 1998; van Heijenoort, 2001). Transpeptidation, on the other hand, generally requires that the enzyme recognize the terminal d-alanine, d-alanine moiety of the stem peptide and catalyze the attack of the carbonyl group of the penultimate d-alanine by the carbonyl group of the stem peptide lysine; in staphylococci, the same feature in Gram-positive organisms. In streptococci, for example, MurM and MurN catalyze the addition of short dipeptides (serine-alanine or di-alanine) onto the ε amino group of the stem peptide lysine; in staphylococci, the same residue is decorated with a pentaglycine group, in reactions catalyzed by the FemX, FemA and FemB enzymes; and in enterococci, the stem peptide lysine harbors either an asparagine or an aspartic acid side chain (van Heijenoort, 2001). Interestingly, these features seem to be linked to an increased level of drug resistance. In streptococci, many penicillin-resistant strains carry branched muropeptides, and inactivation of the murM and murN genes reverts the drug resistance phenotype and supports synthesis of un-branched peptidoglycan (Filipe & Tomasz, 2000). Laboratory-selected drug-resistant Enterococcus faecium strains bypass D,D-transpeptidation by carrying a L, D transpeptidase which is able to generate cross-links between L-Lys (3) and D-Asn/Asp–L-Lys(3), thus overcoming the 4–3 cross-linking reaction (Mainardi et al., 2002).

In addition, the acceptor in the transpeptidation reaction can also be a water molecule, which will thus finalize a D-alanine, D-alanine (or D, D-) carboxypeptidation reaction, catalyzed by low molecular mass (LMM) PBPs. In this case, stem pentapeptides are hydrolyzed into tetrapeptides (with d-alanine being released), preventing further peptidoglycan crosslinking (Goffin & Ghuysen, 2002). Thus, LMM PBPs are involved in the regulation of the level of peptidoglycan reticulation (Morlot et al., 2004).

Blocking of either the transpeptidation or carboxypeptidation reactions by β-lactam antibiotics, which structurally resemble the D, D- stem peptide moiety, weakens the peptidoglycan and may engender cell death (Ghuysen, 1994). This powerful mechanism, which has made penicillin and its analogs the most widely employed antibiotics for any infectious malady worldwide for the past 70 years, has been challenged by the propagation of drug-resistant strains, underlining the need for novel antibiotic therapies (discussed below). Inhibition of the glycosyltransferase reaction by the natural product moenomycin also weakens the peptidoglycan and kills bacterial cells, but moenomycin’s toxicity, a direct consequence of its long half-life, has precluded it from human use (Goldman & Gange, 2000). It is however clear that the glycosyltransferase activity of PBPs is a potential, yet unexplored antibiotherapy target, which awaits solution of its three-dimensional structure as well as elucidation of further biochemical and mechanistic details (Walsh, 2003).

**Peptidoglycan synthesis by PBPs: cell division vs. wall elongation**

During the bacterial cell cycle, new peptidoglycan must be synthesized at the cell division site (the septum); in rod-shaped cells, such as E. coli and Bacillus subtilis, peptidoglycan must also be synthesized along the sidewalls (thus ensuring wall elongation), a process also present in Streptococcus pneumoniae. Cell division and wall elongation are processes which require timely and precise localization of a variety of essential proteins, including PBPs, on specific sites on the cell wall, and they have been recently described in other reviews (Giesbrecht et al., 1998; Foster & Popham, 2001; Errington et al., 2003; Popham & Young, 2003; Young, 2003; Cabeen & Jacobs-Wagner, 2005; Scheffers & Pinho, 2005). Elegant genetic, immunoprecipitation, biochemical and immunofluorescence experiments performed in E. coli, B. subtilis, Caulobacter crescentus, Staphylococcus aureus and...
Streptococcus pneumoniae have shown that cell division and elongation processes each employ at least one class A and one class B PBP, themselves possibly possessed to lytic transglycosylases and peptidoglycan-scaffolding proteins (Romeis & Holtje, 1994; Vollmer et al., 1999; Morlot et al., 2003; Poham & Young, 2003; Figge et al., 2004). Cell division is initiated by the polymerization of FtsZ (the bacterial homolog of tubulin) at the septum, underneath the cytoplasmic membrane, into a ring-like structure (Bi & Lutkenhaus, 1991; Errington et al., 2003). In the absence of FtsZ, rod-shaped cells such as E. coli are incapable of septation and only elongate, forming long filaments (Bi & Lutkenhaus, 1992); in cocci, such as Staphylococcus aureus, FtsZ depletion causes cell wall synthesis to be delocalized over the entire cell surface, rather than localized to the future division site (Pinho & Errington, 2003). Interestingly, FtsZ has been shown to display a GTP hydrolysis-related dynamic behavior, causing the septal ring to remodel itself within a 30-s time frame (Anderson et al., 2004); in addition, it has also recently been shown to localize outside the septal ring, as a member of a potential helical cytoskeleton (Thanedar & Margolin, 2004).

Once FtsZ is positioned at the septum, other members of the septal peptidoglycan formation machinery (described here using E. coli nomenclature) start to assemble at the division site in a specific and interdependent fashion: FtsA, ZipA, and ZapA, whose main function may be to provide stability to the FtsZ ring (Den Blaauwen et al., 1999; Gueiros-Filho & Losick, 2002; Pichoff & Lutkenhaus, 2002). Subsequently, FtzK, FtsQ, FtsL, FtsB, FtsW, FtsI (a class B PBP), FtsN and AmiC localize at the same site (Errington et al., 2003); FtsL and FtsB are interdependent for proper localization, and their complex also only localizes in the presence of FtsQ (Buddelmeijer & Beckwith, 2004; Noirclerc-Savoye et al., 2005). It is of note that, at least in E. coli, there is a time lag of approximately 20 min between FtsZ polymerization and septal constriction; it is conceivable that this time difference is necessary to allow cell division proteins to assemble onto the ring itself (Den Blaauwen et al., 1999; Aarsman et al., 2005). In addition, the rate of peptidoglycan synthesis is increased during constriction, a phenomenon which is independent of PBP3 (or FtsI (Wientjes & Nanninga, 1989)). Another class B PBP, PBP2, directs peptidoglycan elongation and insertion of strands into the wall, but is also present at the division site (Spratt, 1975; de Pedro et al., 1997; Den Blaauwen et al., 2003). It is of interest that in E. coli, the inhibition of FtsZ in mutants which lack the PBP5 D-, D-carboxypeptidase creates morphologically aberrant cells which are twisted into spirals, suggesting that a concerted relationship between FtsZ and low molecular weight PBPs is necessary not only for cell division but also for the determination of cell shape (Varma & Young, 2004).

The participation of class A PBPs in division, although more elusive, has been demonstrated by co-localization with FtsZ during the pneumococcal cell cycle (Morlot et al., 2003). This pathogen contains six PBPs, three of which (PBP1a, PBP2a, PBP1b) are class A (bifunctional) enzymes. Immunofluorescence studies showed that PBP1a co-localized at the septum with FtsZ (although de-localization required a 5-min time lag); PBP1b, on the other hand, localized either septally or equatorially, but never at both places in the same cell, leaving open the question of the function of the latter macromolecule (Morlot et al., 2003). Bacteria which have had specific class A PBP-encoding genes deleted have provided a wealth of information regarding the function of these enzymes in cell division. Mutants of B. subtilis PBP1, a septally-located protein (Scheffers & Errington, 2004), display average cell length increase, with additional increases in mutants lacking other bifunctional PBPs (Poham & Setlow, 1995; Poham & Setlow, 1996). In E. coli, individual deletion of genes coding for bifunctional enzymes PBP1a and PBP1b is tolerated, but the suppression of both is lethal (Denome et al., 1999); a similar phenomenon was observed in Streptococcus pneumoniae cells, which tolerated deletion of either PBP1a, PBP1b, or PBP2a, but double mutations of pbp1a/pbp1b or pbp2a/pbp1b genes presented abnormal septum positioning. Interestingly, the double pbp1a/pbp2a knockout was not viable (Hoskins et al., 1999; Paik et al., 1999), suggesting that pneumococcal viability requires PBP1a and PBP2a for division and elongation, and that PBP1b can substitute for either one of the enzymes, but not for both.

PBPs are multi-domain assemblies

A genomics overview

Our analysis of 213 unique eubacterial genomes (i.e. excluding sequences from drug-resistant variants and those from mycoplasmal species, which do not have peptidoglycan) revealed more than 430 class A and 350 class B PBPs, as well as 250 low molecular mass D, D-carboxypeptidases (Fig. 2). Since PBPs are membrane-associated molecules, most of the HMM enzymes display a short cytoplasmic region at the N-terminus, followed by a single transmembrane region; class A molecules then carry sequences coding for both GT and TP domains, while class B molecules display only a TP domain preceded by an N-terminal unit of unknown function.

Class A PBPs can be found in two main forms (Fig. 2a): the most common arrangement displays GT and TP domains interconnected by a linker of variable length, followed by a short C-terminal sequence. In a second group, the C-terminal region is followed by different structural elements, such as a fibronectin type 3 domain. In general, there are
two class A PBPs per genome, reflecting the importance of these enzymes in bacterial metabolism. Bacteria may also carry monofunctional GT enzymes (MGTs) which are characterized by a unique GT unit associated to the transmembrane region (Fig. 2b). Although the in vivo function of these proteins is yet to be determined with precision, the demonstrated glycosyltransfer activity of the purified molecules (Di Berardino et al., 1996; Wang et al., 2001) suggests that they may play complementary roles to bifunctional enzymes in the bacterial cell cycle.

Class B PBPs can be divided into four main groups (Fig. 2c). The largest and by far most representative one harbors the simplest arrangement, which involves an N-terminal unit followed by the transpeptidase domain; this group includes well-studied proteins such as PBP3 from E. coli. Two other groups are represented by approximately 60 PBPs which display either one or two short sequences corresponding to an $\alpha/\beta$ unit positioned after the TP domain; streptococcal PBP2x harbors two of these $\alpha/\beta$ units at its C-terminus (Parès et al., 1996; Gordon et al., 2000). In several databases this $\alpha/\beta$ unit is identified as a PASTA ('PBP and Ser/Thr kinase attached') domain, since its homologs are harbored by over 100 PBP and kinase sequences (Young et al., 2003). Although the PASTA domain has been
suggested as being a β-lactam binding, peptidoglycan-sensing unit (Yeats et al., 2002), no biochemical evidence regarding this fact is available to date. The last group of class B enzymes harbors an NTF2 (‘nuclear transport factor 2’) -like domain upstream from the N-terminal domain; although the function of this insertion is unclear, this group of enzymes is represented by the well-studied staphylococcal PBP2a and enterococcal PBP5fm (Lim & Strynadka, 2002; Sauvage et al., 2002), whose structures are discussed below. There are approximately 1.7 class B PBPs per eubacterial genome.

LMM PBPs, namely D, D-carboxypeptidases, are unusual in the sense that the TP domain is encoded after a signal peptide and their membrane association is accomplished by either a transmembrane or an amphipathic helix, both C-terminally located (Fig. 2d). D, D- endopeptidases are also present within some genomes, but at 0.07 examples per genome they are far less statistically representative of classical PBPs than the ones shown in Fig. 2.

**High molecular mass PBPs – class A and class B enzymes**

Class A PBPs are some of the most fascinating, and intractable, macromolecules being explored as potential or de facto drug targets today. Several laboratories (academic and industrial alike) have attempted, for many years, to express the glycosyltransferase domain of class A PBPs either in the context of the full-length enzyme or as an independent unit, only to encounter aggregation and instability problems. These results are probably related to the proximity of the glycosyltransferase domain to the membrane, giving it a highly hydrophobic character; in addition, in the absence of the stabilizing bilayer, it is conceivable that it shows signs of instability. Only recently have there three reports of successful purifications of soluble forms of class A PBPs (in the absence of detergent) been made available: that of the bifunctional form of PBP1b from *Streptococcus pneumoniae* (which lacks the cytoplasmic region and transmembrane...
helix; (Di Guilmi et al., 2003)), and the isolated glycosyltransferase domains of PBP1b from E. coli and PBP2 from Staphylococcus aureus (Barrett et al., 2004; Barrett et al., 2005).

To date, structural information for class A PBPs is only available for the protease-resistant cores of PBPs 1a and 1b from Streptococcus pneumoniae (Contreras-Martel et al., 2005; Macheboeuf et al., 2005). Difficulties in crystallizing the full-length molecules led the authors to identify a trypsin-resistant region, which, in both enzymes, lacks most of the GT domain but reveals, nevertheless, a very similar three domain organization: the interdomain linker, the transpeptidase domain and a C-terminal region (Fig. 3a, with the interdomain linker shown in dark blue). In both proteins, the interdomain linker represents the region which bridges GT and TP domains in the full-length molecules, and is mostly composed of a small β-sheet (βa–βe), and one helix (Ha). Notably, βa–βe harbors two strands (βa, βb) formed by a short peptide from the GT domain that remained associated to both PBP1a and PBP1b in precisely the same orientation even after proteolysis and protein purification (yellow in Fig. 3a). It is of interest that sequences of the GT peptides in the two different PBPs point to the same region at the N-terminus of the GT unit (Contreras-Martel et al., 2005; Macheboeuf et al., 2005). The almost identical fold of the PBP1a and 1b interdomain linker region suggests that, in the full-length molecules, GT and TP domains may be associated in a very similar fashion. If one considers that class A PBPs must catalyze two concerted reactions during precisely orchestrated cell cycle events, possibly requiring that polymerization of Lipid II glycan chains be initiated prior to transpeptidation (Terrak et al., 1999; Schwartz et al., 2001; Bertsche et al., 2005), a common positioning for GT and TP active sites in all class A molecules could represent a major advantage within a complex network of partner molecules and substrates, all arranged in a three-dimensional mesh.

The TP domain of class A PBPs is highly reminiscent of the three-dimensional fold of the same domain in class B enzymes, as well as other transpeptidases and serine β-lactamases. It is composed of a central, five-stranded β-sheet (β1–β5) surrounded by α-helices, with α1 and α11 always in front of the sheet, and α8 behind; this fold is colored in red in Fig. 3 (PBP structural nomenclature follows that for β-lactamases). The high conservation of this domain within all enzymes which bind β-lactam antibiotics is evident from the analysis of PBPs and other β-lactam recognizing proteins, such as PBP2x from Streptococcus pneumoniae (Parés et al., 1996), PBP2a from Staphylococcus aureus (Lim & Strynadka, 2002), PBP5fm from Enterococcus faecium (Sauvage et al., 2002), the Streptomyces R61 and K15 transpeptidases (Kelly et al., 1985; Fonzé et al., 1999), and β-lactamases (i.e. TEM-1, AmpC and Oxa-2; note the commonality of the red α/β fold in Fig. 3). Interestingly, although superposition of the TP domains of these enzymes reveals considerable structural deviations (with Cα root mean square (r.m.s.) values ranging between 1.4 and 2.9 Å) and little sequence identity (in the range of 15%), the fold itself is common to all, with the positioning of the α-helices around the central sheet showing some angular differences.

The first structure of a class B PBP, that of PBP2x from Streptococcus pneumoniae (Parés et al., 1996), was only obtained after the protein was solubilized by expression in the absence of its cytoplasmic region and transmembrane helix. PBP2x is a three-domain molecule where the central region, which harbors the transpeptidation active site, is flanked by an elongated N-terminal region and a helical-rich C-terminal domain (Fig. 3b). The N-terminal region resembles a sugar tong, but neither it nor the small C-terminal region possess any structural homologs in available databases. The TP domain of PBP2x, however, is reminiscent of that of other PBPs (discussed above).

Structural and sequence comparisons of class B PBPs calls into question the function of the N-terminal domain, which can be highly variable, ranging from 60 amino acids (as in Deinococcus radiodurans; UNIPROT Q9RT97) to 400 (Clostridium tetani; UNIPROT Q892M5). In Fig. 3b, this domain is shown in light and dark blue. Notably, the class A interdomain linker region identified in the structures of PBP1a and PBP1b (Contreras-Martel et al., 2005; Macheboeuf et al., 2005) has structural counterparts in class B enzymes, located with precisely the same orientation (with respect to the TP domain) as in class A PBPs (note all dark blue regions in Fig. 3a–3b). In class A enzymes, the interdomain linker involves a five-stranded β-sheet (βα–βe) and either two (PBP1b) or one (PBP1a) α-helix; in class B PBPs, only one helix is present (here called Ha), while the
specific structural observations. It is of note that within the tionary relationship between such molecules based on classes now at hand, it is tempting to propose an evolu-
cycle-related proteins. Sharing catalytic responsibilities or contacting other cell parts (compare domain organizations in Fig. 2), possibly from the cellular membrane as their bifunctional counter-
from the cell membrane and towards the peptidoglycan. It is thus more probable that, in all class B PBPs, the N-
(H¨oltje, 1998), but this function was never demonstrated. It was originally suggested as serving as a possible association work which can serve as an anchor point which stabilizes the interdomain linker region and the N-terminal domain. A fourth motif also identified by the Ghuysen group (Goffin & Ghuysen, 2002; GXDX3TXDX3Q) is present on strand β6 but is much less well conserved and does contribute any residues to the anchor point.

Hence, the size disparity amongst N-terminal domains of class B PBPs seems to be mostly a consequence of the different lengths of regions which are interconnected by βa–βe and Ha (and are shown in light blue in Fig. 3b). In order to highlight this point, a ‘mini-PBP2x’, in which the abovementioned interconnecting loops were shortened to between two and three residues, was cloned, overexpressed and crystallized. Its three-dimensional structure revealed a substantial stabilization of the N-terminal domain, including the formation of a considerably longer helix in the vicinity of the β-strands (compare the dark blue region in PBP2x views in Fig. 3b), revealing that the presence of extensions from the anchor point is dispensable for protein stability (Dideberg et al., 2003).

Interestingly, the N-terminal domain of class B PBPs was originally suggested as serving as a possible association domain for other proteins involved in the bacterial cell cycle (H¨oltje, 1998), but this function was never demonstrated. It is thus more probable that, in all class B PBPs, the N-terminal domain serves as a pedestal or connector, which points and places the catalytic region of the protein away from the cell membrane and towards the peptidoglycan. It is conceivable that class B PBPs have developed a mechanism by which they can be positioned at a comparable distance from the cellular membrane as their bifunctional counterparts (compare domain organizations in Fig. 2), possibly sharing catalytic responsibilities or contacting other cell cycle-related proteins.

With three-dimensional folds of PBPs from both HMM classes now at hand, it is tempting to propose an evolutionary relationship between such molecules based on specific structural observations. It is of note that within the structures of the interdomain linker β-sheets present in class B structures, the class A GT peptide is replaced by a peptide from the N-terminal domain. Thus, the interdomain linker may function as a harness, whose role is to provide structural connectivity for whichever domain may be N-terminally located to the TP unit: the GT domain, in class A enzymes, or the pedestal, in class B PBPs. In light of the commonality of the interdomain linker in all high molecular mass PBPs, we propose that class B enzymes evolved either from an ancestral class A molecule or both class A and class B PBPs evolved from a common, bifunctional ancestor whose domains were already connected by the interdomain linker. Solution of other class A and class B structures will shed light on this proposal.

Low molecular mass PBPs
The penicilloyl serine transferase fold is also shared by the LMM PBPs (such as PBP3 from Streptococcus pneumoniae, PBP5 from E. coli and PBP4 from Staphylococcus aureus (Nicholas et al., 2003; Rajashankar et al., 2004; Morlot et al., 2005), which are D-, D-carboxypeptidases and, albeit able to recognize the C-terminal end of stem peptides as substrates, do not catalyze a transpeptidation reaction. Interestingly, these molecules possess an elongated, β-strand rich C-terminal domain which separates the catalytic region from the amphipathic helix (the latter not included in the crystal structures; Figs 2 and 3c). This domain has been proposed to act as a pedestal which could position the catalytic region of LMM PBPs in close apposition to the peptidoglycan (Davies et al., 2001; Morlot et al., 2005). If this were the case, the amphipathic helix could permit the enzymes to skid on the membrane surface without being associated within it, while the pedestal could place the catalytic domain at the required height for optimal substrate recognition (Morlot et al., 2005).

Sharing fold, but not function: other transpeptidases and β-lactamas
Soluble transpeptidases such as Streptomyces K15 and R61 are some of the most extensively studied enzymes, both from the biochemical and the structural point of view. Although they have not been shown to be involved in bacterial peptidoglycan metabolism, the fact that they are sensitive to β-lactams, have D-, D-carboxypeptidase and transpeptidase activities, and their crystals in apo and complexed forms diffract X-rays to high resolution has allowed the study of transpeptidation and β-lactam active site recognition in atomic detail. Like PBPs and β-lactama-
rances, these proteins also carry the penicilloyl serine trans-
ferase fold characterized by a five-stranded β-sheet and two or three angled helices (Fig. 3d); the active site lies between the conserved α/β region and the C-terminal domains (Kelly
Interestingly, the R39 \textit{Actinomadura} transpeptidase, as well as PBP4 from \textit{E. coli}, in addition to the aforementioned domains, harbor an additional unit which has been described as corresponding to one half of a Rossmann fold (green in Fig. 3d). Although its function seems to be unclear, a highly charged surface has been identified as a possible protein interaction platform (Sauvage \textit{et al.}, 2005; Kishida \textit{et al.}, 2006).

\( \beta \)-lactamases are secreted enzymes which hydrolyze the lactam bond of \( \beta \)-lactam antibiotics with great efficiency, and have been identified in a variety of bacterial species. \( \beta \)-lactamases are divided into class A to D based on primary structures; three-dimensional structures of representative enzymes of all four classes are now available, providing a plethora of data on catalysis and mechanisms of drug resistance (Dideberg \textit{et al.}, 1987; Herzberg & Moult, 1987; Lobkovsky \textit{et al.}, 1993; Carfi \textit{et al.}, 1995; Paetzel \textit{et al.}, 2000; Garau \textit{et al.}, 2005). Active site serine-\( \beta \)-lactamases, direct structural descendants of PBPs (Parès \textit{et al.}, 1996), also belong to the superfamily of penicillin-recognizing enzymes and thus harbor the conserved \( \alpha/\beta \) fold described above (Fig. 3d). Complex epidemiologic analyses of \( \beta \)-lactamase-producing pathogens performed by laboratories worldwide, coupled to extensive chemical synthesis efforts and detailed studies on

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig5}
\caption{The active sites of PBPs and \( \beta \)-lactamases. (a) The active site of pneumococcal PBP2x and (b) TEM-1 \( \beta \)-lactamase share a very similar three-dimensional arrangement, with the catalytic serine (SXXK) in hydrogen-bonding distance to residues from the SXN and KT(S)G motifs. (c) The surface representation of PBP2x shows that the active site (in green) is nestled within an elongated cleft, while that of TEM-1 \( \beta \)-lactamase (d) is a pocket on the surface of the molecule. (e) The superposition of open (yellow) and closed (brown) forms of PBP1b from \textit{Streptococcus pneumoniae} reveals that active site closure separates the C-terminus of \( \beta \)3 and the N-terminus of \( \beta \)4, and only when the cleft is open (by ligand binding) do the two \( \beta \)-strands recover their antiparallel nature.}
\end{figure}
enzyme action and inhibition have been the driving forces in the development of multi-generational β-lactams which have, in part, aided in circumventing, or at least delaying, an expansive drug-resistance problem (discussed below).

**Mechanism, active sites and β-lactam action**

PBPs are serine acyltransferases whose TP-associated catalytic activity, in addition to catalyzing the formation of cross-linked peptidoglycan, is also the target of β-lactam antibiotics. Upon binding to the PBP catalytic cleft, β-lactams become covalently associated to the active site serine; the resulting acyl-enzyme can only be hydrolyzed at a very slow rate, thus reducing the amount of peptidoglycan cross-linking. β-lactamases, however, are able to hydrolyze the β-lactam ring with extremely high turnover rates (Goffin & Ghysen, 2002). In both cases, these activities require three fingerprint sequence motifs: Ser-X-X-Lys, where Ser is the catalytic nucleophile and is located at the N-terminus of β2; Ser(Tyr)-X-Asn(Cys), which lies on the loop between residue 24 and 25, and Lys(His)-Thr(Ser)-Gly, which lines β3 (where X is any amino acid; Figs 5a and 5b). Interestingly, in PBPs, the active site is located at the bottom of an elongated cleft, while in β-lactamases it is positioned within a surface pocket (Figs 5c and 5d) (Gordon et al., 2000; Contreras-Martel et al., 2005). This localization, rather intuitive, clearly reflects the fact that, in *in vivo*, PBPs must accommodate two stem peptides within their cleft, while β-lactamases only have to recognize β-lactam antibiotics.

It is of note that the active site of pneumococcal PBP1b, a class A enzyme, was recently shown to vary between open (active) and closed (inactive) states (Macheboeuf et al., 2005). In the absence of ligand, the interactions between the C-terminus of β3 and the N-terminal region of β4 are disrupted by the movement of the intervening loop into the active site region, which blocks entry into the active site cleft and forms an interaction with the left side of the cavity. The strength of this interaction is such that the C-terminus of β3 is separated from β4, affecting the anti-parallel nature of the two strands. This closed active site configuration, which had not been observed for any other peptidoglycan or β-lactam-recognizing enzyme, is modified into an open form in the presence of the antibiotics nitrocefin or cefotaxime (notice the conformational modification required for ligand recognition in Fig. 5e). Interestingly, the open form of the enzyme was only obtained after authors soaked PBP1b crystals (containing the ‘closed’ form of the enzyme) in S2d, a thio-analog of the stem peptide, subsequently washing away the molecule and re-soaking the crystals in antibiotic solutions. The observation of this unusual active site restructuring mechanism led to the suggestion that within a dormant cell or during phases of the cell cycle, when their activity is not necessary, PBPs may be inactive, and thus have their active sites closed. As their catalytic activity becomes necessary, such as during peptidoglycan elongation and cell division phases, they are activated, bringing about active site opening either by contact with other proteins involved in cell division or the presence of the substrate itself (Macheboeuf et al., 2005). It is of interest that the latter possibility is supported by *in vivo* observations made on PBPs from different organisms. In *Staphylococcus aureus*, PBP2, a class A enzyme, requires its substrate in order to localize to the septum and participate in division; in experiments where either the active site was blocked or the peptidoglycan was modified, PBP2 no longer localized to the division site, being present around the entire cell surface (Pinho & Errington, 2005). *Escherichia coli* PBP3 (this a class B enzyme) becomes acylated at a faster rate in dividing cells than in non-dividing cells, and it is conceivable that the enzyme’s transpeptidase activity may become activated during septation (Eberhardt et al., 2003). Thus, *in vivo* and structural observations all point to the possibility that PBPs may exist in both closed and open conformations in the cell, the former switching into the latter depending on substrate availability or localization (Macheboeuf et al., 2005).

*Escherichia coli* PBP1b has been the enzyme of choice for the study of transpeptidation and glycosyltransfer reactions catalyzed by PBPs. In a recent article, Vollmer and colleagues (Bertsche et al., 2005) have shown that the full-length *E. coli* PBP1b (thus containing the transmembrane segment) is a dimer, and catalyzes both reactions on the Lipid II substrate simultaneously; subsequently, a secondary transpeptidation activity could also be identified, confirming the previously reported delayed transpeptidation event for this enzyme (Terrak et al., 1999; Schwartz et al., 2001). Although these studies prove that the TP domain of PBPs is capable of transpeptidation, crystal structures of PBPs complexed to substrates, products or catalysis intermediates are still not available. Hence, detailed information regarding the TP-catalyzed mechanism can only be inferred from studies performed with antibiotics, pentapeptide pseudosubstrates or peptidic boronic acid inhibitors (Jamin et al., 1993; Gordon et al., 2000; Lim & Strynadka, 2002; Pechenov et al., 2003; Contreras-Martel et al., 2005; Macheboeuf et al., 2005; Nicola et al., 2005), as well as from high-resolution complexes and kinetic studies performed on the R61 transpeptidase (Anderson & Pratt, 2000; McDonough et al., 2002; Rhazi et al., 2005) and β-lactamases (Strynadka et al., 1992; Pratt, 2002; Chen et al., 2004). Based on results from these authors and numerous others who have painstakingly delved into details of PBP catalysis, a general route for PBP-catalyzed transpeptidation has been widely discussed in the literature. In brief, after the stem pentapeptide has been accommodated in the elongated active site cleft, a non-covalent complex is formed involving the penultimate
d-alanine group and the nucleophilic serine. In PBPs and R61 β, d-transpeptidase, the methyl group of the penultimate d-alanine of the substrate is buried in a hydrophobic pocket at the bottom of the cleft, explaining why the requirement for a d-alanine at the penultimate position is more stringent than at the C-terminus of the stem peptide. Interestingly, the absence of a pocket at the same position in β-lactamases guarantees that they do not recognize peptidoglycan (Pratt, 2002).

Binding is followed by the nucleophilic attack of a deprotonated active site serine on the penultimate d-alanine moiety, which gives rise to a stable acyl-enzyme intermediate. The withdrawal of a proton from the active site serine has been proposed to be accomplished by either the lysine residue of the Ser-X-X-Lys motif (located on α2) or the carboxylate group of the substrate (Damblon et al., 1996; Ishiguro & Imajo, 1996; Gordon et al., 2000; Lim & Strynadka, 2002; Nicola et al., 2005). Subsequently, the nucleophilicity of the active site serine hydroxyl group must be increased so that deacylation can proceed; for PBPs, details of this step are still a matter of controversy.

In class A β-lactamases, Glu166, located on the omega loop and hence at the bottom of the active site cleft (Fig. 5b), abstracts a proton from the catalytic serine via a water molecule. The absence of such a residue in PBPs precludes this mechanism, and may explain the drastic difference in deacylation rate constants between these enzymes (9 s⁻¹ and 10⁻⁵ s⁻¹; (Gordon et al., 2000; Goffin & Ghuysen, 2002)); because the half-life on a PBP-β-lactam complex is in the range of 10 h, and the bacterial doubling time is approximately 30 min, it becomes clear how a long-lived blocked PBP active site can be disadvantageous for the cell. Both the catalytic lysine and the serine residue of the SXN motif have been suggested as possible general bases in the deacylation reaction (Nicola et al., 2005). It is of interest that, in the high resolution crystal structures of the CTX-M class A β-lactamases complexed to different transition state analogs, the catalytic lysine group changes conformation as the reaction proceeds, suggesting that it may act both by activating the catalytic serine for nucleophilic attack and stabilizing the negative charge which is created in the deacylation transition state (Chen et al., 2004).

PBPs and drug resistance

Once a PBP is acylated by a β-lactam antibiotic, it is unable to catalyze hydrolysis of the covalent acyl-enzyme intermediate and is inactivated; peptidoglycan transpeptidation cannot occur, and the cell wall is weakened. Although this fact has been the stronghold of the action of β-lactam antibiotics for decades, the emergence of drug-resistant strains has proven to be a worldwide problem. Gram-negative pathogens such as Pseudomonas aeruginosa and E. coli evade β-lactam action by excreting β-lactamases into the periplasm. In another mechanism, the antibiotic cannot access its macromolecular target due to its forced efflux from the bacterium via an antibiotic efflux pump, such as the MexA,B-OprM pump in Pseudomonas strains (Nehme & Poole, 2005). In addition, certain Gram-positive bacteria, such as streptococci, which do not secrete β-lactamases, produce highly mutated, drug-insensitive PBPs. Staphylococcus aureus strains circumvent β-lactam action by acquiring a novel, highly drug resistant class B PBP (PBP2a) through horizontal transfer of the meca gene from a yet unidentified species (Wu et al., 2001; Pinho et al., 2001). Enterococci, some of which are major nosocomial pathogens, are naturally resistant to β-lactams due to the presence of one PBP (PBP5fm in E. faecium) with low level affinity for penicillin and its analogs; point mutations in or overproduction of PBP5fm can lead to very high levels of resistance (Zorzi et al., 1996; Sauvage et al., 2002). Interestingly, albeit the incorporation of these key PBP mutations, resistant bacteria still seem to divide and turn over peptidoglycan at comparable rates; the complex mechanisms underlying this enduring catalytic efficiency are unknown.

Pneumococcal PB2x and PBP1a

Streptococcus pneumoniae is naturally highly transformable microorganism which, through interspecies homologous recombination, is able to acquire blocks of genes from drug-resistant species and thus develop mosaic PBPs which may have up to 100 mutations (Spratt, 1994). In the pneumococcus, a low-level β-lactam resistance is accomplished by mutations in PB2x and PB2b; high-level resistance, however, requires additional mutations in a class A enzyme, PBP1a (Reichmann et al., 1996; Hakenbeck et al., 1998; Hakenbeck & Coyette, 1998). Although the structure of PBP1a from a drug-resistant strain is not yet available, the crystal structure of the wild type enzyme upon which all of the mutations found in PBP1a from a highly drug resistant clinical isolate were mapped reveals a ‘mutational hotspot’ in the form of a semi-circle around the catalytic cleft. An analysis of sequences of PBP1a molecules from resistant pneumococcal isolates from different countries reveals a very similar pattern of mutations, suggesting that acquisition of the hotspot in the active site area could be a universal mechanism of PBP1a-related drug resistance (Contreras-Martel et al., 2005).

Structural and biochemical studies of both clinical and laboratory PB2x mutants have revealed at least two mechanisms of pneumococcal resistance to β-lactam-type drugs. The first one was evidenced by the analysis of PB2x from highly penicillin resistant clinical isolate Sp328 [minimal inhibitory concentration (MIC) for
penicillin = 4 μg mL⁻¹; MIC for a drug-sensitive strain is of approximately 0.03 μg mL⁻¹ (Chalkley et al., 1991). This molecule, which contains 92 mutations when compared to PBP2x from the sensitive strain, possesses an active site where the Ser-X-Asn motif, which is located on the loop between α4 and α5, is displaced away from the active site (Fig. 6a) (Dessen et al., 2001). Recent elegant mutagenesis and kinetic studies performed on PBP2x from another highly drug-resistant pneumococcal strain (5204) showed this loop to be indeed highly susceptible to protease digestion (and thus flexible/unstructured), thus defining the loop between α4 and α5 in PBP2x to be an important resistance determinant (Carapito et al., 2005). In addition, a mutation of the residue that immediately follows the catalytic Ser from Thr to Ala affects the local hydrogen bonding pattern, and together with the mutations described above, plays a key role in lowering the acylation efficiency for β-lactams (Mouz et al., 1998; Mouz et al., 1999; Dessen et al., 2001). It is of note that a mutation in the Thr residue which follows the active site serine is also present in PBP1a from most drug-resistant pneumococcal strains; in this case, the Thr residue is burrowed in a hydrophobic pocket, and its mutation could affect non-polar interactions occurring within the region (Contreras-Martel et al., 2005).

The second resistance mechanism which involves pneumococcal PBP2x was evidenced by the study of mutant molecules from low drug resistance strain 5259 (MIC penicillin = 0.19 μg mL⁻¹). In this case, although the active site is in a closed conformation, there is a charge modification at the entry of the catalytic gorge as a result of a mutation from Gln552 to Glu and the entire β3 strand shows a slight closure movement when compared to the molecule from the sensitive strain. Since antibiotics are negatively charged, the inclusion of an acidic residue at the entry of the catalytic groove could hinder drug accessibility to the site, thus participating in the resistance mechanism (Pernot et al., 2004). Interestingly, the crystal structure of a laboratory PBP2x mutant which contains the two key mutations Thr338Ala/Met339Phe in the immediate vicinity of the active site residue Ser337 reveals a major distortion of α2, which is probably responsible for the marked decrease in acylation efficiencies with a variety of β-lactams (Chesnel et al., 2003). Thus, through the introduction of mutations which can affect active site stability and charge in an essential enzyme, the pneumococcus is capable of avoiding β-lactam recognition and/or stabilization within the cleft, while still being able to process peptidoglycan.
Methicillin resistance in *Staphylococcus aureus*

*Staphylococcus aureus* is a major cause of hospital-acquired, multi-drug resistant infections. Its methicillin-resistant variant (MRSA) appeared in 1961, one year after the introduction of methicillin into the market, and now is prevalent in hospital settings worldwide (Chambers, 2001). *Staphylococcus aureus* employs a distinct method for the development of resistance to β-lactams than the pneumococcus; instead of acquiring blocks of mutations that will affect PBP structure and function, it produces an exogenous, class B PBP (PBP2′ or PBP2a). PBP2a is encoded by the mecA gene, and it is acquired through the horizontal transfer of a genetic element (SCCmec) from a yet unidentified donor (Wu et al., 2001; Pinho et al., 2001b; Enright et al., 2002). Since the other four PBPs in *Staphylococcus aureus* are β-lactam sensitive, PBP2a can take over their functions even after their inactivation, thus conferring broad resistance by displaying low affinity to a variety of β-lactams, albeit continuing to catalyze the transpeptidation reaction (Pinho et al., 2001), as is the case for pneumococcal PBP2x (Mouz et al., 1998, 1999).

PBP2a synthesis is inducible through a signal transduction system encoded by the genes on the SCCmec genetic element, and involves an integral membrane protein sensor (MecR1) and a transcriptional repressor (MecI). De-repression of mecA transcription by MecI is accomplished through a signaling cascade involving the sensing of β-lactams in the extracellular environment by MecR1 and the transmission of a signal, which involves its conformational change, proteolysis of its cytoplasmically-located domain, and interaction with MecI (Garcia-Castellanos et al., 2004; Sharma et al., 1998; Zhang et al., 2001). The expressed PBP2a is a 78 kDa enzyme which acts as a transpeptidase only when other PBPs have been inactivated (Pinho et al., 2001). The crystal structure of PBP2a, both in apo form as well as complexed to β-lactams, reveals that drug resistance is achieved through a distorted active site, necessitating movement of β3 for drug binding (Fig. 6b). This unusual conformation would be required only upon the catalytic transition from the enzyme-drug Michaelis complex to the covalent intermediate, a costly energetic step highly likely to be responsible for the enzyme’s recalcitrance to blockage by such drugs (Lim & Strynadka, 2002). A close look at both liganded and unliganded structures reveals that antibiotic binding could require rotation of the entire TP domain (or ‘opening’ between the TP and C-terminal domains). It is of interest that staphylococcal strains displaying very high levels of drug resistance and with mutations within PBP2a have also already been identified (Katayama et al., 2004).

Drug resistance in *Enterococcus faecium*: PBP5fm

Enterococci are generally 10 to 1000-fold less susceptible to β-lactams than streptococci (Murray, 1990). Such natural resistance is linked to production of a class B PBP with low affinity for β-lactams, PBP5fm, which takes over the catalytic role of other PBPs when these are inhibited by antibiotics. In fact, overproduction of PBP5fm is linked to an initial, low level of resistance (Rybkine et al., 1998). However, acquisition of mutations within the PBP5fm sequence are responsible for the development of even higher levels of resistance which may reach MIC values of 512 μg mL⁻¹ (al-Obeid et al., 1990; Murray, 1990; Fontana et al., 1994; Zorzi et al., 1996). The crystal structure of PBP5fm reveals that it contains a classical transpeptidase domain preceded by an elongated N-terminal region (Sauvage et al., 2002). Within its active site (Fig. 6c), Met485 is located three residues after the Ser480-X-Asn482 catalytic triad, and its mutation into Thr...
or Ala is responsible for MIC values which are 16-fold higher than for wild type. It is conceivable that the absence of the large Met side chain allows the catalytic Lys425, located nearby, to have more degrees of freedom, thus participating less efficiently in the acylation mechanism (Rybkin et al., 1998; Sauvage et al., 2002). It is also of note that, in certain resistant enterococcal strains, a single serine residue (Ser466') is introduced in the PBP5fm sequence (thus in between residues 466 and 467 (Zorzi et al., 1996)). Indeed, the left side of the cavity of PBP5fm is already stabilized by a salt bridge between Arg464 and Asp481, and Val465 plays a key role in β-lactam recognition; insertion of a residue at position 466' could affect this recognition pattern, and thus β-lactam binding (Rybkin et al., 1998; Sauvage et al., 2002).

**PBPs as targets for novel antibacterial development**

The bacterial cell wall biosynthetic machinery remains one of the most promising niches for antibiotic targets. Many proteins which participate in its metabolism are essential for bacterial viability, and a similar macromolecular framework is absent in humans, two important conditions for the development of antibiotherapy. Although PBPs have been known and studied for over 40 years, it is only now that a plethora of genetic, biochemical, immunofluorescence and structural data are coming together to reveal the full potentiality of PBPs for the development of novel antibiotics.

Since the introduction of penicillin into the market, several other β-lactams (naturally occurring or synthesized) have been made available (Fig. 7). Carbapenems are penicillin analogs which, instead of the classical β-lactam ring, carry an unsaturated five-membered ring which lacks the sulfur atom; they are broad-spectrum molecules which display strong bactericidal action against enterobacteria, *P. aeruginosa*, *Streptococcus pneumoniae* and even *Mycobacterium tuberculosis*, the latter of which is recalcitrant to treatment with most β-lactam antibiotics (Chambers et al., 2005; Coulthurst et al., 2005; Kobayashi et al., 2005). Carbapenems are not hydrolyzed by class A or C β-lactamases, but can be targeted by class B metallo-β-lactamas; although the production of this type of β-lactamase by pathogens in clinical settings is rare, some outbreaks have already been recorded (Peleg et al., 2005). Doripenem, a parenteral carbapenem, is in clinical trials in both Japan and the United States for the treatment of respiratory and urinary tract infections, respectively, and has shown high efficacy even against pathogenic strains which are resistant to other β-lactams (Bush et al., 2004; Fritsche et al., 2005). In addition, tebipenem, an oral carbapenem, has also shown efficacy against drug-resistant *Streptococcus pneumoniae* strains; it targets PBP1a and PBP2b, high molecular mass enzymes, as well as PBP3, a D, D-carboxypeptidase. Tebipenem is currently in Phase II clinical trials in Japan (Kobayashi et al., 2005).

Ceftobiprole, a broad-spectrum cephalosporin which is active against methicillin-resistant and vancomycin-resistant *Staphylococcus aureus* strains as well as penicillin-resistant *Streptococcus pneumoniae* bacteria, derives its efficacy from its ability to bind to PBP2a, which is naturally recalcitrant to binding of other β-lactams (see above; (Hebeisen et al., 2001; Chambers, 2005)). Ceftobiprole was developed by Basilea Pharmaceutica AG and is currently being tested in Phase III clinical trials for complicated skin infections and pneumonia (see http://www.basilea.com).

In addition to targeting the PBP TP active site with novel β-lactams, two additional strategies are currently being tackled, largely by academic and small biotech groups. The first one includes searching for transpeptidation inhibitors whose chemical structures do not resemble those of β-lactams and thus will be unlikely to display cross-resistance. In this respect, two novel classes of aryalkylidene-related molecules seem to affect cell wall synthesis in a variety of different pathogens by inhibiting key PBPs, including PBP2x in *Streptococcus pneumoniae* and PBP2a in *Staphylococcus aureus* (Zervosen et al., 2004), and could be interesting starting points for the development of new non-β-lactams. A second strategy includes the search for inhibitors of the GT activity of PBPs which, in many cases, is essential for cell survival. GT domains share little or no sequence similarity with other glycosyltransferases and have yet to be studied from a structural point of view; it is conceivable, thus, that they may display a novel fold which could represent a very interesting potential antibiotic development target.

**Concluding remarks**

The central involvement of PBPs in bacterial cell cycle processes has been recognized for over 30 years, and the key roles that they play in antibiotic resistance mechanisms in a variety of pathogens has also been documented by laboratories worldwide. However, it is only in the past few years that groups employing techniques ranging from immunofluorescence localization to structure solution, chemical synthesis and membrane biochemistry, have started to converge upon a detailed functional and structural study of PBPs. Certainly, the near future should answer questions relating to their specific and timely functions in the cell, their macromolecular partners, and most importantly, how the scientific community can continue to exploit their well-studied active sites for the development of novel antibiotherapies which can presently circumvent widespread drug resistance problems.
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