Lipase LipA from *Serratia marcescens* is a 613-amino acid enzyme belonging to family I.3 of lipolytic enzymes that has an important biotechnological application in the production of a chiral precursor for the coronary vasodilator diltiazem. Like other family I.3 lipases, LipA is secreted by Gram-negative bacteria via a type I secretion system and possesses 13 copies of a calcium binding tandem repeat motif, GGGXGXDXUX (U, hydrophobic amino acids), in the C-terminal part of the polypeptide chain. The 1.8-Å crystal structure of LipA reveals a close relation to eukaryotic lipases, whereas family I.1 and I.2 enzymes appear to be more distantly related. Interestingly, the structure shows for the N-terminal lipase domain a variation on the canonical α/β hydrolase fold in an open conformation, where the putative lid helix is anchored by a Ca$^{2+}$ ion essential for activity. Another novel feature observed in this lipase structure is the presence of a helical hairpin additional to the putative lid helix that exposes a hydrophobic surface to the aqueous medium and might function as an additional lid. The tandem repeats form two separated parallel β-roll domains that pack tightly against each other. Variations of the consensus sequence of the tandem repeats within the second β-roll result in an asymmetric Ca$^{2+}$ binding on only one side of the roll. The analysis of the properties of the β-roll domains suggests an intramolecular chaperone function.

Lipases (EC 3.1.1.3) hydrolyze the ester bonds of long-chain acylglycerols (1). Biotechnologically, they have gained considerable importance as biocatalysts that are able to catalyze not only hydrolysis but also synthesis reactions, with the latter occurring in nearly water-free organic solvents (see Refs. 2 and 3 for detailed reviews) and often with high regio- and enantioselectivity (4). Virtually all of the technologically employed lipases are of microbial origin, because the respective genes are relatively easy to access and can efficiently be expressed. Consequently, it is a lipase produced by the Gram-negative bacterium *Pseudomonas aeruginosa* that currently constitutes the best studied example of creating an enantioselective enzyme by directed evolution (5–7). A characteristic feature to distinguish lipases from esterases is called interfacial activation and describes the observation that lipase activity sharply increases as soon as the monomolecular substrate starts to form a micellar emulsion (8). An obvious explanation was provided by several lipase crystal structures that revealed the presence of a surface-exposed α-helical polypeptide chain termed the “lid” which covers the active site and moves away upon contact with the micellar substrate interface (9, 10). However, exceptions have also been observed of lipases that possess a lid and still do not show interfacial activation. Therefore, lipases are presently defined as carboxylesterases catalyzing the hydrolysis of long-chain (>10 carbon atoms) acylglycerols (11).

A classification of bacterial lipases was first put forward by Arpigny and Jaeger (12), who defined eight families (I–VIII) based on amino acid sequences and biological properties. The largest family I is further subdivided in seven subfamilies (I.1–I.7) with the first three subfamilies comprising true lipases from Gram-negative bacteria. All lipases are members of the α/β hydrolase superfamily, where the canonical fold comprises a mostly parallel, central eight-stranded β-sheet. The active site carries the Ser-His-Asp/Glu catalytic triad, where the serine residues are usually located within a characteristic GXXSG motif, which forms a sharp γ-like turn between a β-strand and the following α-helix (13).

Although lipases of families I.1 and I.2 are clearly homologous with amino acid sequence identities above 30%, the family I.3 enzymes exhibit only a very low sequence similarity to the former two families. Also, they are translocated to the extracellular medium by a different mechanism. Whereas family I.1 and I.2 lipases are secreted by a type II secretion system (T2SS, also named general secretory pathway) (14–16), the family I.3 lipases are transported by a type I secretion system (T1SS) (16–18). This secretion pathway utilizes a C-terminal, non-cleavable secretion signal, and translocation occurs in one step from the cytoplasm to the extracellular medium without the occurrence of periplasmic intermediates. The *Escherichia coli* hemolysin secretion system Hly constitutes the paradigm of a T1SS (19), which like the other T1SSs consists of three membrane-associated proteins; that is, an inner membrane ABC
transporter (HlyB), an outer membrane protein (ToIC), and a periplasmic membrane fusion protein (HlyD). T1SS passenger proteins possess as a characteristic feature a variable number of tandem repeats of a glycine-rich nine-residue motif (GGXGXDX(U)Y)ₙ (U, apolar residue) that precedes the C-terminal secretion signal. The number n of the repeats correlates positively with the molecular weight of the protein. This so-called RTX signature (repeats in toxins) (20) is responsible for Ca²⁺ binding. X-ray crystallographic analyses of serralysin-type metalloproteases showed that these repeats fold into a parallel β-ribbon, where the first six amino acids build a turn that forms two half-sites for Ca²⁺ binding, and the remaining three residues fold into a short β-strand (21–23). The calcium ions bridge the spatially adjacent aspartic acids (position 6 in the motif) from two sequence motifs 18 residues apart. The nonpolar amino acids (abbreviated U) at position 8 build the hydrophobic core of the β-ribbon. This peculiar structure is unstable in the absence of Ca²⁺ but does fold spontaneously in the presence of calcium concentrations in the mM range (24, 25).

*Serratia marcescens* SM6 produces an extracellular lipase LipA that has been a subject of research for more than 30 years. Its production was found to be stimulated up to 100-fold when the standard growth medium was supplemented with various polysaccharides like glycogen or hyaluronate (26). Later it was shown that this enzyme is a family I.3 lipase of 613 amino acids in length (27). The catalytic residue Ser²⁰⁷ is located in a conserved GHSLGG motif. From inspection of the sequence, *S. marcescens* LipA possesses about 12–14 repeats of the glycine-rich sequence motif that come in two blocks spanning residues 369–418 and 489–564. Furthermore, *S. marcescens* LipA is a biotechnologically very important enzyme as it serves for the large-scale kinetic resolution of racemic 3-(4′-methoxyphe-nyl)glycidic acid methyl ester. LipA catalyzes the enantioselective hydrolysis of the (2S,3R) enantiomer, which is then easily separated from the desired (2S,3R) enantiomer (28). This biocatalytic reaction reduces by 4 steps the original 9-step chemical synthesis to yield diltiazem, a calcium-channel blocker and coronary vasodilator produced worldwide in an excess of 100 t a⁻¹ (29).

Presently, crystal structures have not been reported for *S. marcescens* LipA nor for any other lipase of family I.3 despite ongoing work (30). Furthermore, homology modeling would be unreliable because the lipase domain of LipA shows only a very limited sequence identity (<20%) to other known lipase structures. Therefore, we have determined the high resolution crystal structure of LipA from *S. marcescens*.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—The LipA expression construct was made by cloning the PCR product derived from *S. marcescens* genomic library into the Ndel and HindIII cloning sites of the pET28a vector (Novagen) using the primers 5′-GGAATTC-CATATGGGATCTTATAGCTTAAAGG-3′ and 5′-GGCGGAGGTTTATGAGG-3′. The respective restriction sites within the primer sequences are underlined.

LipA was expressed in *E. coli* BL21 (DE3) cells (Stratagene). Cells were grown at 37 °C to an A₅₆₀ of 1.2 and then induced with 1 mM isopropylthiogalactopyranoside. 5 h after induction, cells were harvested and resuspended in 20 mM Tris-HCl, pH 8.0, 300 mM NaCl and disrupted using a French press. Inclusion bodies were isolated from the crude cell lysate by centrifugation and washed twice with 100 mM Tris-HCl, pH 7.5, 100 mM EDTA, 10% (v/v) Triton X-100. Purified inclusion bodies were stirred for 40 min in 100 mM NaH₂PO₄, 10 mM Tris, pH 8.0, 8 M urea. The solution of solubilized inclusion bodies was clarified by centrifugation at 10,000 × g for 15 min at room temperature. 20 ml of solubilized LipA inclusion bodies was then diluted into 180 ml of 250 mM Tris, pH 8.0, 50 mM CaCl₂, 800 mM L-arginine, 8% glycerol. Refolded protein solution was concentrated in an Amicon ultrafiltration cell (Millipore) to 80 ml. A gel filtration on a Superdex 75 column (Amersham Biosciences) in GPC buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 20 mM CaCl₂, 0.05% (v/v) Triton X-100) was performed with 5 ml of the concentrated refolded LipA. One major peak representing the LipA fraction was pooled and concentrated to 18 mg/ml and −0.2% (v/v) Triton X-100 (about 3 mM).

**Activity Assays**—LipA was dialyzed overnight at 4 °C against buffer 20 mM Tris, 100 mM NaCl, 0.05% (v/v) Triton X-100, pH 8.0, to remove excess of calcium deriving from the gel filtration buffer. After dialysis, LipA was incubated with 1, 2, and 5 mM EDTA for 1 h at room temperature. The enzymatic activity of the hydrolysis of p-nitrophenyl palmitate was determined at a concentration of 0.1 μM in 1000 μl of 25 mM Tris-HCl, pH 8.0, 10% acetonitrile, and 50 μM p-nitrophenol palmitate at room temperature. Protein concentrations were determined by a bicinchoninic acid assay (Pierce). The absorption of the p-nitrophenolate produced by the reaction was determined at 412 nm using an Ultrospec 2100 pro (Amersham Biosciences) spectrophotometer.

**Site-directed Mutagenesis**—The D157A point mutation was introduced by a modified extension-overlap protocol (31) using the pET28a-LipA vector as template and the primers 5′-CGGGCGCTGTATTATACGGATCTGATCGCCG-GTTCGGGCCG-3′ and 5′-CCGGGCTAGCAGATCGTATCAGCGCCG-3′. The solution of solubilized inclusion bodies was clarified by centrifugation at 18 °C using a hanging drop vapor diffusion method. Drops were set up by mixing 3–6 μl of reservoir solution (0.1M sodium acetate, pH 4.6, 22–28% (v/v) 2-methyl-pentane-2,4-diol) and equilibrated against 500 μl of reservoir solution at 18 °C. Hexagonal-shaped crystals were observed after 2 days and grew to an average size of 100 × 100 × 25 μm. These crystals belong to space group P321 with one molecule per asymmetric unit were obtained at 18 °C using a hanging drop vapor diffusion method. Drops were set up by mixing 3–6 μl of protein solution (18 mg/ml) with 1 μl of reservoir solution (0.1M sodium acetate, pH 4.6, 22–28% (v/v) 2-methyl-pentane-2,4-diol) and equilibrated against 500 μl of reservoir solution at 18 °C. Hexagonal-shaped crystals were observed after 2 days and grew to an average size of 100 × 100 × 25 μm. These crystals belong to space group P321 with cell parameters of a = 115.1 Å and c = 104.6 Å. Heavy atom derivative crystals were prepared by soaking crystals in the mother solution described above containing additional 5 mM Pb(II) acetate for 30 and 1 h. After soaking, crystals were back-soaked to mother liquor containing 30% (v/v) 2-methyl-pentane-2,4-diol.

A second crystal form appeared in the same drops. These crystals belong to the space group R3 with cell constants in the hexagonal setting of a = 202.4 Å and c = 317.7 Å. This crystal form contains six molecules in the asymmetric unit. “Interme-
**RESULTS**

**Overall Structure**

The structure of LipA was determined and refined in two crystal forms at 2.0 and 1.8 Å of resolution (Table 1). The two crystal forms grew under the same conditions. Virtually all amino acids are defined in the electron density map in both crystal forms. The seven crystallographically independent molecules totally present in the two crystal forms showed no significant deviations. The root mean square pairwise difference between the structures is 0.366 and 0.451 Å for backbone atoms and all protein atoms, respectively, whereas the root mean square difference from the mean structure is 0.240 and 0.295 Å for protein atoms, respectively.

LipA forms an ellipsoid-shaped molecule with overall dimensions of 80 × 45 × 30 Å (Fig. 1); 8 Ca2+ ions were identified per molecule. The N-terminal domain comprising amino acids 1–320 (supplemental Fig. S1) shows a modified α/β hydrolase fold (supplemental Fig. S2). Compared with the canonical fold, strand β1 is split, β3 and αA are missing, and αD has been replaced by a loop (supplemental Fig. S2). Furthermore, the eight β strands are now split into six-stranded mixed and a two-stranded parallel sheet.

A DALI search (42) was performed, revealing significant similarity of *S. marcescens* LipA to the fungal *Thermomyces lanuginosa* lipase (Z-score 15), whereas family 1.1 and 1.2 lipases proved to be much more distantly related (Z-score 3.8 for *P. aeruginosa* lipase). Hence, *S. marcescens* LipA resembles more closely eukaryotic than prokaryotic lipases (see supplemental Table S1).

Comparison to the most similar lipase structure from *T. lanuginosa* (PDB entries 1EIN and 1DT3) (43) revealed a number of large insertions in LipA, most notably amino acids 33–74, which form an α-helix hairpin, i.e. two antiparallel helices connected by a short loop. This element is located at one side of the active-site cleft (Figs.
S. marcescens LipA Crystal Structure

2 and 3A). At residue 343 the polypeptide chain leaves the lipase domain and enters the first β-roll domain at amino acid 369. After 2.5 turns of the β-roll, at residue 417, the polypeptide chain winds through an antiparallel β-structure which extends the first β-roll domain. Eventually, the second β-roll domain starts at residue 489. This domain is displaced laterally from the major long axis of the molecule and packs to the first β-roll as well as to the antiparallel β structure succeeding it. The interface is hydrophobic, consisting mainly of leucines and aromatic residues. After 4.5 turns the polypeptide chain extends into an antiparallel β-structure enlarging the second β-roll domain and placing the C terminus in the neighborhood of the N terminus. This last bit of the structure is very similar to the C-terminal segments of the structurally characterized β-roll domain proteins, namely the serralysins.

Active Site

Catalytic Triad—The active site of lipases is defined by a canonical catalytic triad, which in LipA consists of Ser207, Asp256, and His314. These residues superimpose quite well with the corresponding amino acids from other lipases, e.g. for T. lanuginosa or P. aeruginosa the average distance difference is 0.5 and 1.5 Å, respectively.

Lid Structures—Inspection of the lipase active site cleft and comparison with structures in closed and open conformation from the fungus T. lanuginosa (PDB entries 1EIN and 1DT3 (43)) shows that S. marcescens LipA is in the open conformation in both crystal forms (Figs. 2 and 3) and identifies helix α6 (supplemental Fig. S1) as lid helix. This lid, an α-helical surface-exposed segment, which presents a very hydrophobic face toward the active site cleft, is characteristic for many lipases and explains interfacial activation; it is assumed that this hydrophobic patch interacts with a micellar substrate surface (Fig. 3A) and opens the active site. Interestingly, in LipA the putative lid helix is anchored in its position by a Ca\(^{2+}\) ion (termed Ca1), which is coordinated by aspartic acids 153 (monodentate) and 157 (bidentate), the carbonyl oxygens of Thr118 and Ser144, and the side chain of Gln120 (Fig. 3B). The coordination sphere is completed by a water molecule. This binding site is strongly conserved in lipases of family I.3 but absent in the structurally similar T. lanuginosa lipase. The calcium ion is deeply buried and at the opposite site of the active site cleft compared with the Ca\(^{2+}\) ions found in lipases from P. aeruginosa (PDB entry 1EX9) (44) and Pseudomonas cepacia (PDB entry 1OIL) (45). For the homologous Pseudomonas sp. MIS38 lipase it was shown that calcium is essential for activity, since dialysis against calcium-free buffer reduced the activity by about 75% and dialysis against EDTA abolishes activity completely (46). Therefore, we have determined the influence of EDTA on the activity of S. marcescens LipA (supplemental Fig. S3), showing that calcium is strictly required. Mutation of Asp153 to alanine abolished activity completely even in the presence of 20 mM Ca\(^{2+}\) (data not shown). Another striking feature of S. marcescens lipase is the helix hairpin mentioned before, comprising amino acids 33–74 (Figs. 2 and 3). This element presents a conspicuous hydrophobic patch interacting with the aqueous medium and may well act as a second lid.

The Parallel β-Roll Domains

The first β-roll domain spans residues 368–418 and contains five GGXGXDUXX motifs. These conserved sequences
interrupted in the tertiary structure of the respective protein making it difficult to determine by counting the exact number of repeats.

**DISCUSSION**

We have determined for the first time the atomic structure of a lipase belonging to family I.3. As expected, this enzyme consists of an N-terminal lipase domain and a C-terminal RTX domain involved in secretion of this enzyme. Additionally, our high resolution structure revealed several unusual and interesting features that deserve a more detailed discussion.

**A New Type of Ca^{2+}-dependent Lipase**—*S. marcescens* LipA possesses an N-terminal α/β hydrolase domain with some peculiar features. It is a calcium-dependent lipase for which interfacial activation has not been demonstrated yet. In the structure presented here, this lipase exhibits the open conformation that may have resulted from the presence of detergent in the crystallization medium, although the electron density map does not indicate the presence of detergent molecules. As a novel feature within microbial lipases, we have identified a Ca^{2+} ion, which binds the putative lid helix and is, therefore, crucial for enzymatic activity. Consequently, treatment with EDTA resulted in inactivation of LipA.

Besides the Ca^{2+} ion discussed above, seven additional calcium ions were identified; six bind to the GGXGDXUX motifs and a further one (designated Ca2 in Figs. 2 and 3) is coordinated by the side chains of Glu^{254} (monodentate), Asp^{276} (bidentate), Asn^{285}, the carbonyl O of Asn^{284}, and two water molecules. This site is also strongly conserved; however, it is located remote from the active site, and its function remains elusive. Calcium ions bound within β-roll domains are usually too tightly bound to be removed by dialysis or even by chelators (24, 47). Hence, Ca1 (Fig. 2) must be the essential Ca^{2+} ion, which is bound to the lid helix, and its removal will result in a permanent closure or even the complete distortion of the active site cleft. The inactivity of the D157A mutant strongly corroborates this hypothesis.

**The Parallel β-Roll as Intramolecular Chaperone**—The structure of *S. marcescens* lipase revealed a novel and unique feature that we have termed “β-roll sandwich” consisting of tandem repeats split into two groups and separated by a 70-residue spacer. The first β-roll domain is located at a position equivalent to the serralysin-type metalloproteases, whereas the
second one, which is unique for *S. marcescens* lipase, packs from the side tightly against the first domain and other parts of the molecule. The tandem repeats, which separate the C-terminal secretion signal from the catalytic part of the molecule, consist mainly of highly hydrophilic amino acids, thus making them soluble even in the absence of calcium, where they adopt a random coil structure (24).

This has implications for the translocation mechanism and folding of the passenger protein; contrary to T2SS, secretion by T1SS is independent of chaperones, with the exception of HasA, the *Serratia marcescens* heme binding protein (48, 49), which is also the only one not to possess a tandem repeat domain. HasA secretion is SecB-dependent. This cytoplasmic chaperone possibly prevents spontaneous folding of HasA, and folded HasA inhibits its own secretion (50).

The absence of chaperones poses a 2-fold challenge for the passenger protein; first, it must not fold into its native structure inside the cell because this would block at least its passage through the outer membrane protein tunnel (e.g. TolC) since crystallographic studies on TolC revealed a channel of only about 30 Å in width (51). Second, because the passenger protein is translocated into the chaperone-free external medium, it must be able to fold efficiently into its native conformation. Consequently, a characteristic feature of T1SS passenger proteins is the absence of disulfide bonds, presumably because there are no disulfide-bond isomerases or similar helper proteins present that could rescue "scrambled" proteins. It is tempting to speculate that the parallel β-roll structure may act as an intramolecular chaperone that keeps the polypeptide chain unfolded in the cytoplasm and provides a nucleus for the folding of the entire polypeptide domain along the helical axis but pack laterally against it. This 31482 JOURNAL OF BIOLOGICAL CHEMISTRY possibly prevents spontaneous folding of HasA, and folded HasA inhibits its own secretion (50).

The absence of chaperones poses a 2-fold challenge for the passenger protein; first, it must not fold into its native structure inside the cell because this would block at least its passage through the outer membrane protein tunnel (e.g. TolC) since crystallographic studies on TolC revealed a channel of only about 30 Å in width (51). Second, because the passenger protein is translocated into the chaperone-free external medium, it must be able to fold efficiently into its native conformation. Consequently, a characteristic feature of T1SS passenger proteins is the absence of disulfide bonds, presumably because there are no disulfide-bond isomerases or similar helper proteins present that could rescue "scrambled" proteins. It is tempting to speculate that the parallel β-roll structure may act as an intramolecular chaperone that keeps the polypeptide chain unfolded in the cytoplasm where Ca²⁺ concentrations are about 0.1 μM, although in the external medium with Ca²⁺ concentrations in the mM range the glycine-rich repeats would fold spontaneously (24, 25, 47) and provide a nucleus for the folding of the entire polypeptide chain.

Such a chaperone-like function would imply that the number of β-roll domains positively correlates with the size of the respective passenger proteins. Furthermore, this assumption is nicely supported by our recent finding that *S. marcescens* LipA can be autodisplayed on the surface of *E. coli* cells by fusing it to an autotransporter protein from *P. aeruginosa*. The autotransporter domain efficiently inserts into the bacterial outer membrane with the lipase domain facing the extracellular medium, where it folds into its enzymatically active conformation (52).

Studies on the homologous *Pseudomonas* sp. MIS38 lipase have shown that partial or complete knock-out of the tandem repeats leads to strongly reduced protein and secretion levels. Furthermore, knock-out of all repeats resulted in a virtually inactive enzyme (53). The intracellular stability is also affected less than the wild type. This can be explained by rapid degradation of non-secreted protein rather than by a lower stability because a mutant lacking just the 19 C-terminal amino acids accumulates to an amount 100 times less than the wild type.

Another new and unexpected feature revealed by the structure of *S. marcescens* LipA is related to the second set of tandem repeats. These repeats do not simply extend the first β-roll domain along the helical axis but pack laterally against it. This appears to be a general feature of all RTX proteins possessing more than eight nine-residue motifs, e.g. *E. coli* hemolysin (28 repeats) or *Bordetella pertussis* cycloolysin, where the ~40 repeats come in blocks separated by linker sequences. In this way the β-roll domains become integral parts of the whole structure and can act as folding nuclei, stabilizing different parts of the catalytic N-terminal domains. The larger those N-terminal domains, the more β-rolls are needed. The Ca²⁺-induced folding of the N-terminal passenger domains by the β-roll has been demonstrated directly in some cases (47, 54, 55).

Acknowledgments—We are grateful to Santina Russo, Clemens Schulze-Briese, and Takashi Tomizaki from the Swiss Light Source, Paul-Scherrer-Institute, Villigen, for help during data collection. Furthermore, we want to thank Michael Werner for developing a protocol for *S. marcescens* lipase purification and refolding. Ulrich K. Winkler, professor emeritus at Ruhr-University Bochum, Germany, is greatly acknowledged for encouragement and support during the early days of research on *Serratia marcescens* lipase.

REFERENCES

1. Jaeger, K. E., Ransac, S., Dijkstra, B. W., Colson, C., van Heuvel, M., and Misset, O. (1994) *FEMS Microbiol. Rev.* 15, 29–63
2. Jaeger, K. E., Dijkstra, B. W., and Reetz, M. T. (1999) *Annu. Rev. Microbiol.* 53, 315–351
3. Jaeger, K. E., and Eggert, T. (2002) *Curr. Opin. Biotechnol.* 13, 390–397
4. Reetz, M. T., and Jaeger, K. E. (2000) *Chem. Eur. J.* 6, 407–412
5. Reetz, M. T., Zonta, A., Schimossek, K., Liebeton, K., and Jaeger, K.-E. (1997) *Angew. Chem. Int. Ed. Engl.* 36, 2830–2832
6. Liebeton, K., Zonta, A., Schimossek, K., Liebeton, K., and Jaeger, K. E. (1997) *Angew. Chem. Int. Ed. Engl.* 36, 2833–2836
7. Reetz, M. T., Pulz, M., Carballeira, J. D., Vogel, A., Jaeger, K. E., Eggert, T., Thiel, W., Bocula, M., and Otte, N. (2007) *ChemBiochem* 8, 106–112
8. Sarda, L., and Desnuelle, P. (1958) *Biochim. Biophys. Acta* 30, 513–521
9. Brzozowski, A. M., Derewenda, U., Derewenda, Z. S., Dodson, G. G., Law- son, D. M., Turkenburg, J. P., Bjorkling, F., Hauge-Jensen, B., Patkar, S. A., and Thin, L. (1991) *Nature* 351, 491–494
10. Cygler, M., and Schrag, J. D. (1997) *Methods Enzymol.* 284, 3–27
11. Verger, R. (1997) *Trends Biotechnol.* 15, 32–38
12. Arpinay, J. L., and Jaeger, K. E. (1999) *Biochem. J.* 343, 177–183
13. Schrag, J. D., and Cygler, M. (1997) *Methods Enzymol.* 284, 85–107
14. Filloux, A. (2004) *Biochim. Biophys. Acta* 1694, 163–179
15. Johnson, T. L., Abendroth, J., Hol, W. G., and Sandkvist, M. (2006) *FEMS Microbiol. Lett.* 255, 175–186
16. Vandersanden, C. (1992) *Trends Genet.* 8, 317–322
17. Angkawidjaja, C., and Kanaya, S. (2006) *Cell. Mol. Life Sci.* 63, 2804–2817
18. Thanassi, D. G., and Hultgren, S. J. (2000) *Curr. Opin. Cell Biol.* 12, 420–430
19. Gentschev, L., Dietrich, G., and Goebel, W. (2002) *Trends Microbiol.* 10, 39–45
20. Welch, R. A. (2001) *Curr. Top. Microbiol. Immunol.* 257, 85–111
21. Baumann, U. (1994) *J. Mol. Biol.* 242, 244–251
22. Baumann, U., Wu, S., Flaherty, K. M., and McKay, D. B. (1993) *EMBO J.* 12, 3357–3364
23. Hege, T., and Baumann, U. (2001) *J. Mol. Biol.* 314, 187–193
24. Lüle, H., Haehnel, W., Rudolph, R., and Baumann, U. (2000) *FEBS Lett.* 470, 173–177
25. Rose, T., Sebo, P., Bellalou, J., and Ladant, D. (1995) *J. Biol. Chem.* 270, 26370–26376
26. Winkler, U. K., and Stuckmann, M. (1979) *J. Bacteriol.* 138, 663–670
27. Li, X., Tetling, S., Winkler, U. K., Jaeger, K. E., and Benedik, M. J. (1995) *Appl. Environ. Microbiol.* 61, 2674–2680
28. Matsunae, H., Furui, M., and Shibata, T. (1993) *J. Ferment. Bioeng.* 76, 93–98
29. Liese, A., Seebach, K., and Wandrey, C. (eds) (2006) *Industrial Biotransfor-
30. Angkawidjaja, C., You, D. J., Matsumura, H., Koga, Y., Takano, K., and Kanaya, S. (2007) Acta Crystallogr. F Struct. Biol. Crystalliz. Comm. 63, 187–189
31. Zheng, L., Baumann, U., and Reymond, J. L. (2004) Nucleic Acids Res. 32, 1115
32. Kabsch, W. (2001) in International Tables for Crystallography (Rossmann, M. G., and Arnold, E., eds) Vol. F, Kluwer Academic Publisher, Dordrecht, Germany
33. Leslie, A. G. W. (1996) Joint CCP4/ESF-EACBM Newsletter Protein Crystallogr. 32, 7–8
34. Schneider, T. R., and Sheldrick, G. M. (2002) Acta Crystallogr. D Biol. Crystallogr. 58, 1772–1779
35. Brzozowski, A. M., Savage, H., Verma, C. S., Turkenburg, J. P., Lawson, D. M., Svendsen, A., and Patkar, S. (2000) Biochemistry 39, 15071–15082
36. Kleywegt, G. J., and Jones, T. A. (1996) Structure 4, 1395–1400