Sequence similarity between multidrug resistance efflux pumps of the ABC and RND superfamilies

The LmrA multidrug resistance (MDR) efflux pump of *Lactococcus lactis* is a member of the ATP-binding cassette (ABC) superfamily (TC 3.A.1). LmrA catalyses drug export in a process driven by ATP hydrolysis. Since a primary form of energy (ATP) drives transport, such a system is called a primary active transporter.

Recently, Venter *et al.* (2003) used molecular genetic techniques to cleave the ATP-hydrolysing domain from the integral membrane transporter domain of LmrA and demonstrated that the latter could catalyse drug transport driven by the transmembrane proton electrochemical gradient. Substrate: proton symport or antiport is a general characteristic of simple carriers also called secondary active transporters. Their observations suggested that the primary active transporter, LmrA, was derived from a secondary carrier by superimposition of the ATP-hydrolysing subunit onto the carrier during evolution as suggested previously (Saier, 2000a).

There are four superfamilies of secondary active transporters that include members that catalyse drug export using a drug: cation antiport mechanism. These are the MF (TC 2.A.1), RND (TC 2.A.6), DMT (TC 2.A.7) and MOP (TC 2.A.66) superfamilies (Saier, 2000b; Saier & Paulsen, 2001). We were curious to know if LmrA could be shown to exhibit sufficient sequence similarity to the members of any one of these superfamilies to suggest a functional or an evolutionary connection.

Fig. 1 shows a binary alignment of an extended region of LmrA with a portion of the MexB MDR efflux pump of the RND superfamily (Tseng *et al.*, 1999). These two sequences exhibit 28% identity and 42% similarity for this 98 residue position alignment. A shorter segment including the first 63 residue positions gave 32% identity and 47% similarity. Using the GAP program with 500 random shuffles (Devereux *et al.*, 1984), comparison scores of 9.5 standard deviations (SD) and 11.6 SD were obtained for the longer and shorter binary comparisons, respectively. The probability of obtaining such a score by chance is less than $10^{-20}$. These comparison scores are sufficient to establish that the sequence similarity observed for these regions within the two proteins did not arise by chance.

As shown by the two shaded transmembrane segments (TMSs) depicted in Fig. 2(a), the region in LmrA which shows similarity to MexB includes the hairpin structure corresponding to TMSs 3 and 4. This region is homologous to the hairpin structure in MexB corresponding to TMSs 2 and 3 (Fig. 2b). Since both proteins have their N and C termini in the cell cytoplasm, this means that the regions of homology in the
Two proteins have opposite orientations in the bacterial cytoplasmic membrane. In order to determine if the residues conserved between LmrA and MexB were also conserved among other members of each of these two families, 10 homologues of each of these two proteins were selected for further analysis (see Table S1, available from Microbiology Online). The proteins listed include the well-characterized Escherichia coli AcrB protein of the RND superfamily and the E. coli MsbA protein of the ABC superfamily. The high-resolution structures of both of these proteins have been solved by X-ray crystallography (Chang, 2003; Chang & Roth, 2001; Murakami et al., 2002). A multiple alignment was generated as shown in Fig. S1 (available from Microbiology Online) and a phylogenetic tree based on this alignment revealed the relationships of these sequences to each other (Fig. S2, available from Microbiology Online). Only one residue, a proline, was fully conserved in all 22 proteins, but a glutamate was fully conserved in 21 of the 22 proteins, and many positions exhibited only conservative substitutions. In fact, most of the conserved positions between LmrA and MexB shown in Fig. 1 proved to be well-conserved between the 22 members of these two families arbitrarily selected for comparison, 11 from the ABC superfamily, 11 from the RND superfamily (see our website, http://www-biology.ucsd.edu/~msaier/supmat).

Fig. 1. Binary alignment of internal regions of LmrA of Lactococcus lactis (ABC superfamily) and MexB of Pseudomonas aeruginosa (RND superfamily). The GAP program was used to derive the alignment (Devereux et al., 1984). Symbols below the alignment have significance as follows: asterisk, identity; colon, close similarity; period, more distant similarity; a large dot (●) above the alignment signifies the exclusive occurrence of conservative substitutions in all of the 22 proteins, arbitrarily selected for comparison, 11 from the ABC superfamily, 11 from the RND superfamily (see our website, http://www-biology.ucsd.edu/~msaier/supmat).

Fig. 2. Schematic diagrams showing the topologies of LmrA (a) and the first half of the internally duplicated MexB (b). The hatched transmembrane segments are included within the regions showing the greatest degree of sequence similarity between the two proteins.
selected for analysis. All such residue positions are indicated by a large dot above the binary alignment in Fig. 1. We conclude that the many hydrophobic, hydrophilic and semi-polar residues that are conserved between LmrA and MexB are characteristic of both families of drug efflux pumps. It is probable that these conserved residues are important for structure and/or function in both protein families.

The fact that the two homologous hairpin structures are of opposite orientation in the membrane is worthy of comment. Many secondary carriers are built of duplicated units including uneven numbers of TMSs (3 or 5 TMSs duplicated to give 6 or 10 TMSs). In several such cases, opposite orientation of the two halves has been unequivocally demonstrated (for a review, see Saier, 2003). In addition, altering the phospholipid composition of the membrane can cause reversible inversion either of an entire transmembrane protein domain (Bogdanov et al., 2002; Wang et al., 2002) or of an integral membrane N-terminal α-helical hairpin structure within a larger protein domain (Zhang et al., 2003). It is therefore clear that the position of a transmembrane protein hairpin is determined not only by its amino acid sequence, but also by its position in the protein and its interaction with other membrane macromolecules.

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