Staphylococcal Multidrug Efflux Protein QacA

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Abstract

The QacA multidrug exporter from Staphylococcus aureus mediates resistance to a wide array of monovalent or divalent cationic, lipophilic, antimicrobial compounds. QacA provides resistance to these various compounds via a proton motive force-dependent antiport mechanism that conforms to classical Michaelis-Menten kinetics. Fluorescent transport analyses have demonstrated that this QacA:substrate interaction occurs with high affinity and competition studies have shown that QacA-mediated ethidium export is competitively inhibited by other monovalent cations, and non-competitively inhibited by divalent cations, suggesting that monovalent and divalent cations bind at distinct sites on the QacA protein. The closely related export protein QacB, mediates lower levels of resistance to divalent cations, and lacks a high affinity-binding site for divalent cations. The cell membrane has been identified as the origin of QacA-mediated efflux; substrates are bound and expelled from within this hydrophobic environment.

Regulation of qacA expression is achieved via the transactivating repressor protein QacR. QacR belongs to the TetR family of transcriptional repressor proteins, which all possess a helix-turn-helix DNA-binding domain at their N-terminal ends, and have highly divergent C-termini postulated to be involved in the binding of inducing compounds. QacR specifically binds to an inverted repeat, IR1, which has been identified as the qacA operator region, and overlaps the identified promoter sequence for qacA. QacR, like the multidrug export protein whose expression it regulates, has been shown to interact directly with a number of structurally-dissimilar compounds.

Introduction

The Gram-positive bacterium, Staphylococcus aureus, has demonstrated a predilection to develop resistance to a wide range of antimicrobial compounds, most probably as a consequential response to the selective pressures incurred in the clinical environment. These agents include antibiotics, such as aminoglycosides, β-lactams, chloramphenicol, and tetracycline, and an extensive range of toxic organic cationic chemicals, which are commonly used as antiseptics and disinfectants. Frequently, resistance is conferred by determinants carried on mobile genetic elements, such as plasmids which facilitate their acquisition and dissemination.

Three plasmid-encoded determinants, qacA, qacB and smr, specify membrane proteins which mediate resistance to numerous structurally-dissimilar antimicrobial compounds via active transport, and have hence been termed multidrug resistance systems (Tennent et al., 1989; Littlejohn et al., 1992). Substrates of the QacA, QacB and Smr multidrug export proteins are monovalent cationic chemicals, such as quaternary ammonium compounds (Qacs; e.g., cetrimide, and benzalkonium) and dyes (e.g., acriflavine and ethidium). In addition, qacA confers resistance to divalent cationic compounds, such as the diamidines (e.g., propamidine) and the biguanidines (e.g., chlorhexidine) (Figure 1) (Tennent et al., 1989; Littlejohn et al., 1992; Mitchell et al., 1998).

QacA; a Multidrug Efflux Pump Belonging to the Major Facilitator Superfamily

The qacA determinant carried by the plasmid pSK1 from S. aureus was the first bacterial multidrug resistance gene to be described (Tennent et al., 1985). Sequence analysis and hydropathy studies showed that qacA encodes a 514 amino acid membrane protein, QacA, with a predicted size of 55 kDa (Tennent et al., 1988; Rouch et al., 1990). Membrane topological analysis, utilizing alkaline phosphatase and β-galactosidase fusions as reporters of subcellular location, demonstrated that the QacA protein is organized into 14 alpha-helical transmembrane segments (TMS) (Figure 2) (Paulsen et al., 1996a). QacA confers multidrug resistance via export of the compound driven by the proton motive force (PMF), which is generated by the transmembrane electrochemical proton gradient (Tennent et al., 1989; Rouch et al., 1990; Paulsen et al., 1996a; Mitchell et al., 1999).

Based on comparative amino acid analysis, QacA has been classified as a member of the major facilitator superfamily (MFS), also known as the uniporter-symporter-antiporter family, which is comprised of a large number of membrane-bound transport proteins that are present in all classes of living organisms (Griffith et al., 1992; Pao et al., 1998; Saier et al., 1999). These proteins perform a wide variety of cellular processes, including the uptake of essential ions and nutrients and the removal of toxic compounds, and are secondary transporters that typically utilize the PMF to drive the transport process. The MFS can be subdivided into more than 29 clusters or families of transporters based on sequence comparisons and topological predictions; a correlation exists between each phylogenetic family and the class of compound transported (Saier, 1999; Saier et al., 1999). Despite conveying substrates vastly different in structure, MFS proteins are alike in their predicted membrane topologies, possessing a secondary structure of 12 or 14 TMS. Three families within the MFS contain
Monovalent cations

Dyes
Acridine yellow
Acriflavine
Crystal violet
DiOC₃
Ethidium
Proflavine
Pyronin Y
Quinacrine red
Rhodamine 6G
Safranin O

Quaternary ammonium compounds
Benzyalkonium
Cetylpyridinium
Cetyltrimethylammonium
Dimethylaminostyryl-1-ethylpyridinium
Tetraphenylarsonium
Tetraphenylphosphonium
TMA-DPH
Triphenylmethylphosphonium

Divalent cations
Biguanidines
Chlorhexidine

Diamidines
Amicarbalide
DAPI
Dibromopropamidine
Diamidomethylphenylamine
Dimazene
Hexamidine
Pentamidine
Phenamidine
Propamidine
Stilbamidine

Guanylhydrazones
1i-39/JC-1-134
1a-62/JC-1-127

Quaternary ammonium compounds
Dequalinium

Figure 1. Substrates of the Staphylococcal Multidrug Efflux Protein QacA. QacA mediates resistance to an extensive range of monovalent and divalent lipophilic compounds, which can be broadly classified as dyes, quaternary ammonium compounds, biguanidines, diamidines and guanylhydrazones. A representative chemical structure from each class is displayed on the right.
Drug efflux systems, designated DHA1-3 for drug/H\(^+\) antiport, whose members contain 12 (DHA1 and 3) or 14 (DHA2) TMS (Saier et al., 1999). These families include exporters specific for a single substrate, such as the staphylococcal tetracycline resistance protein TetA(K), and multidrug resistance proteins, such as QacA, both of which belong to the DHA2 family (Paulsen et al., 1996a; Ginn et al., 1997; Saier et al., 1999).

A number of highly conserved amino acid motifs (A, B, C, D1/D2, E, F, and H; Figure 2) have been identified within MFS proteins which are likely to be essential for the structure and/or function of these transporters (Griffith et al., 1992; Paulsen et al., 1996b). These motifs are either ubiquitous within the MFS, such as motifs A and B, or family-specific, such as motif C, which is found only in the DHA families (Paulsen et al., 1996b; Ginn et al., 2000). Conservation of such motifs among proteins responsible for the transport of a wide variety of structurally-dissimilar compounds implies that they play some vital structural or functional role, viz., influencing the direction of transport (import versus export) and/or the vectorial mechanism (uniport versus symport versus antiport), rather than providing substrate specificity (Marger and Saier, 1993). For example, it has been postulated that the preponderance of glycine residues in motif C confers conformational plasticity to a TMS. Mutagenesis studies utilizing the TetA(K) exporter as a model DHA2 family member have confirmed the importance of these conserved glycine residues within motif C (gxxxGPxiGGxl) (Ginn et al., 2000).

**Molecular Mechanisms of QacA-Mediated Multidrug Resistance**

A major issue in the field of multidrug resistance is how a single protein recognizes and transports substrates which share properties such as hydrophobicity and charge, but are structurally-dissimilar. QacA mediates resistance to more than 30 cationic, lipophilic antimicrobials belonging to 12 distinct chemical families, including the aminoacridines, rhodamines, and pyronins (Figure 1) (Mitchell et al., 1998; Mitchell et al., 1999). However, no resistance was observed to trivalent cations or to anionic compounds, suggesting that the resistance spectrum of QacA is restricted to monovalent and divalent cationic substrates (Mitchell et al., 1998). A detailed analysis of a range of structural variants of divalent aromatic compounds, viz., diamidines, biguanidines and guanylhydrazones, revealed that QacA recognized these substrates irrespective of the interamidine linkage, the presence or type of side chain, or the position of the amidine group on the aromatic ring (Mitchell et al., 1998).

Fluorimetric transport studies employing monovalent and divalent substrates have demonstrated that QacA-mediated extrusion of these structurally-dissimilar organic cations is via a common PMF-dependent mechanism. Specifically, since QacA-mediated ethidium export was inhibited by the presence of either of the ionophores, valinomycin or nigericin, which dissipate the \( \Delta \psi \) and the \( \Delta \text{pH} \), respectively, the PMF-dependent export mechanism...
utilized by QacA requires both components of the Δψ + to energize transport; QacA-mediated resistance is via an electrionic drug/h+ (n ≥ 2) antiport mechanism (Mitchell et al., 1999). Additionally, QacA-mediated efflux was shown to be sensitive to the multidrug inhibitors reserpine and verapamil, although there was no observable resistance to these compounds (Mitchell et al., 1999). This is consistent with the action of these compounds in other transport systems, e.g., LmrP-mediated-efflux of tetrathenaiphosphonium in Lactococcus lactis is inhibited by the addition of reserpine (Bolhuis et al., 1996), and both reserpine and verapamil are potent reversing agents of multidrug resistance mediated by P-glycoprotein (Wigler and Patterson, 1993).

There are various hypotheses that attempt to explain the phenomenon of multidrug resistance. These include alteration of a biophysical parameter of the cell such that multiple compounds are excluded, or, alternatively, that the transport protein directly interacts with each of its specific substrates. Consistent with the latter hypothesis, determination of the kinetic parameters of transport (V_{max} and K_{m}) indicated that QacA-mediated export conforms to classical Michaelis-Menten kinetics and that the interactions with structurally-dissimilar substrates occur with high affinity, with K_{m} values in the low micromolar range (≤ 20 µM) (Mitchell et al., 1999). Similar findings have been obtained for other multidrug efflux pumps with their substrates; in S. aureus, Smr-mediated efflux of tetrathenaiphosphonium occurs with a K_{m} of 5 µM (Griniius and Goldberg, 1994) and NorA-mediated efflux of norfloxacin with a K_{m} of 6 µM (Ng et al., 1994).

In view of the large number of substrates that have been identified for QacA (Figure 1) (Tennent et al., 1989; Littlejohn et al., 1992; Mitchell et al., 1998) and for other multidrug transporters, it would seem unlikely that a single transporter possesses distinct binding and transport mechanisms for each individual substrate or even family of substrates. A more plausible scenario is that the transporter either contains a single flexible binding site enabling the protein to interact with each substrate differently, or alternatively, the transporter may possess a limited number of binding sites that interact with a specific component common to a group of substrates. Consistent with the latter proposition, the broad substrate specificity of QacA may be based on its ability to interact with one or more features shared by a large number of otherwise disparate structures; the substrates of QacA all contain a cationic moiety attached to a lipophilic structure. In support of this notion, fractional inhibitory concentration analyses have indicated that QacA confers resistance to two compounds at a rate proportional to the overall amount of substrate present, thereby implying that the presence of one compound inhibits the transport of another (Mitchell et al., 1998). Furthermore, QacA-mediated efflux of the monovalent cation ethidium was demonstrated by fluorescent competition assays to be inhibited competitively by other monovalent cations (e.g., benzalkonium) and non-competitively by divalent cations (e.g., propamidine). This suggests that monovalent substrates either share a common binding site or have unique but overlapping binding sites, and that divalent cations bind at a distinct site(s) in QacA (Mitchell et al., 1999). Therefore, QacA has at least two unique substrate-binding sites.

Bioenergetic analysis of accumulation of the membrane probe 1-(4-trimethyl-ammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) by QacA-containing bacterial strains indicated that QacA interacts with and expels this substrate from the inner leaflet of the cell membrane, thereby preventing it from entering the cytoplasm (Mitchell et al., 1999); similar findings have been reported for the related lactococcal multidrug transporter LmrP (Bolhuis et al., 1996). These data imply that critical sites/residues in QacA which play a role in transporter:substrate interactions are directly accessible from within the lipid phase, viz., amino acids that lie within the TMS of the QacA protein. Additionally, fluorescent efflux studies have also shown that QacA can extrude substrates from the cytoplasm since cells treated with the energy inhibitor carbonyl cyanide m-chlorophenylhydrazone, and therefore accumulate the substrate, can export it from the cytoplasm following the removal of the inhibitor and the addition of an energy source (Mitchell et al., 1999). However, efflux from the cytoplasm may require the substrate to re-enter the membrane where it can interact with the binding site or, alternatively, the substrate may be able to directly access the binding site from the cytoplasm.

The staphylococcal QacB multidrug efflux protein differs from QacA in that it confers little or no resistance to divalent cationic drugs, viz., diamidines and biguanidines, whereas both QacA and QacB confer high levels of resistance to a wide range of monovalent cationic dyes and Qacs (Littlejohn et al., 1992; Paulsen et al., 1996a; Mitchell et al., 1998). Consistent with their relative resistance profiles (Paulsen et al., 1996a; Mitchell et al., 1998), bioenergetic comparisons of efflux mediated by QacA and QacB has demonstrated that these two transporters share similar binding affinities for monovalent cations and are proposed to possess identical binding site(s) for monovalent substrates. In contrast, QacA utilizes an independent high affinity binding site(s) for the recognition of divalent cations, which QacB lacks; QacB is capable of binding some divalent cations, albeit with a lower affinity (Mitchell et al., 1999).

Sequence analysis has revealed that qacA and qacB differ from each other by only seven nucleotides (Paulsen et al., 1996a), and subsequent mutagenesis of qacA and qacB has provided evidence that the phenotypic differences resulting from the carriage of these genes are solely due to the presence of an acidic residue (aspartic acid, D) at residue 323 in TMS 10 in the QacA polypeptide (Figure 2), which is an uncharged alanine residue in QacB (Paulsen et al., 1996a). This implies that this region may form part of the high affinity binding site(s) for divalent cations in QacA. The qacB determinant has been identified on plasmids in S. aureus isolates dating back to the early 1950’s, whereas qacA has only been detected in staphylococcal strains isolated since 1980 (Littlejohn et al., 1992; Leelaporn et al., 1994; Paulsen et al., 1998). Thus, qacA is postulated to have evolved from qacB by acquiring a high affinity binding site for divalent ligands (Paulsen et al., 1998). Such an evolutionary process could have occurred either in staphylococci or in an unknown bacterial host, with subsequent transfer to a staphylococcal species. Regardless, the broader resistance profile of QacA may
well have been directly selected by pressures prevailing in the clinical environment (Paulsen et al., 1998; Mitchell et al., 1999).

Domains and residues critical to transporter:substrate interactions have been localised within the TMS of QacA. For example, in addition to the requirement of an acidic residue at position 323 for resistance to divalent cations, we have shown that an amino acid with a negative charge at position 34 in TMS 1 is crucial for QacA-mediated export, as is an arginine at position 114 in TMS 4, which is highly conserved within proteins of the DHA2 family (B. A. Mitchell, M. H. Brown, and R. A. Skurray, unpublished). Studies are in progress to identify further QacA residues required for multidrug recognition and export.

**Regulation of Multidrug Export Proteins**

Membrane transport proteins when overexpressed are frequently toxic to the host cell. This, combined with the metabolic cost of needless protein production, suggests that expression of drug transporter genes will be under some form of stringent regulatory control. The regulation of expression of the *Escherichia coli* tetracycline resistance gene, tetA, which encodes a protein, QacR (solid black circle), which is proposed to form a multimer that binds to IR1 to repress qacA transcription. Some QacA substrates (hatched rectangle), also termed inducers, are postulated to interact directly with QacR, inducing a conformational change in the protein which prevents QacR binding to its DNA target, contained within IR1, and dissociate previously bound protein, liberating PqacA and allowing transcription of qacA to proceed. The QacA polypeptide (grey oval) is subsequently produced, and after insertion into the cytoplasmic membrane, extrudes the substrate to the external environment, energized by the import of a proton (H⁺). The identity of the molecule that binds to IR2 is still unknown; see text for details.

![Figure 3. Proposed Model for the Regulation of qacA Expression by QacR. The qacA and qacR genes are represented by grey and black lines, respectively; arrowheads depict the direction of transcription. The promoters of qacA (PqacA) and qacR (PqacR), including their putative −10 and −35 regions are shown. The intergenic region containing the qacR operator site, a region of dyad symmetry known as IR1, and a second inverted repeat, IR2, which overlaps PqacR are displayed as two sets of converging arrows. qacR encodes a protein, QacR (solid black circle), which is proposed to form a multimer that binds to IR1 to repress qacA transcription. Some QacA substrates (hatched rectangle), also termed inducers, are postulated to interact directly with QacR, inducing a conformational change in the protein which prevents QacR binding to its DNA target, contained within IR1, and dissociate previously bound protein, liberating PqacA and allowing transcription of qacA to proceed. The QacA polypeptide (grey oval) is subsequently produced, and after insertion into the cytoplasmic membrane, extrudes the substrate to the external environment, energized by the import of a proton (H⁺). The identity of the molecule that binds to IR2 is still unknown; see text for details.](image-url)
actively transports them out of the cell, the intricate way by which these substrates interact with the side-chains of the amino acids within the binding pockets of both of these kinds of proteins may be similar.

**QacR: a Multidrug Export Regulatory Protein**

Regulation of expression of the qacA determinant carried by pSK1 is via the divergently encoded 188 amino acid QacR protein, which has been shown to be a trans-acting repressor protein (Rouch et al., 1990; Grkovic et al., 1998). Sequence analysis has also revealed a similar genetic organization for the qacB multidrug resistance determinant. There is a single amino acid difference between the QacR polypeptide encoded on pSK1 and that present on the QacB-carrying plasmid, pSK23; a tyrosine and a cysteine at position 104, respectively (Paulsen et al., 1998). QacR, belongs to a family of regulatory proteins, which includes TetR, that all possess an α-helix-turn-α-helix (HTH) DNA-binding domain at their N-terminal ends, but encode significantly different C-termini which are postulated to be involved in the binding of inducing compounds (Rouch et al., 1990; Aramaki et al., 1995).

The inverted repeat, IR1 (Figure 3), which overlaps the identified qacA promoter sequence, P_qacA, has been shown to contain the qacA operator region. IR1 is composed of an interrupted palindromic with two arms, each of 15 bp, separated by 6 bp. DNase I-footprinting studies have revealed that only 28 bp of IR1 is protected by QacR (Grkovic et al., 1998). Additionally, it has been demonstrated that deletion of one arm of the inverted repeat prevented QacR-mediated repression of P_qacA (Grkovic et al., 1998), whereas mutagenic substitution of the intervening 6 bp of IR1 had no effect (S. Grkovic, M. H. Brown and R. A. Skurray, unpublished). Recent evidence has suggested, that unlike TetR which binds to its DNA-target sequences with high affinity as a dimer (Hinrichs et al., 1994), the active DNA-bound form of QacR may be tetrameric (S. Grkovic, M. Schumacher, R. G. Brennan, M. H. Brown and R. A. Skurray, unpublished). The assembly of this multimeric form of QacR is consistent with the relatively large protected area identified from DNase I footprinting (Grkovic et al., 1998).

One of the most notable distinctions between QacR and the homologous TetR repressor lies in the fact that TetR only interacts with a limited range of structurally-similar tetracycline-based compounds. In contrast, the expression of qacA is induced by the addition of various substrates of QacA, e.g., proflavine, ethidium and benzalkonium; these compounds prevented the binding of QacR to the operator sequence resulting in transcription of qacA (Grkovic et al., 1998), most probably through a direct interaction between QacR and the inducing compound (Figure 3). Thus, like the multidrug export protein that it regulates, QacR interacts directly with a range of structurally-different toxic organic cations.

Unlike TetR, which binds to two operator sequences in the tet regulatory region, thereby preventing transcription of the resistance gene, tetA, and of its own gene, tetR (Hillen and Berens, 1994), gel-mobility shift and gene-fusion assays demonstrated that QacR does not bind to or autoregulate its own promoter, P_qacR (Grkovic et al., 1998).

Examination of the P_qacR region identified a palindromic sequence, termed IR2 (Figure 3). Intriguingly, this inverted repeat bears a very strong resemblance to the sequence of a tet operator (Wissmann et al., 1988); 6 of the 9 nucleotides are identical. Preliminary tests, where constitutively expressed TetR was supplied in trans, produced a marginal effect on the level of qacR expression from P_qacR (S. Grkovic, W. Hillen, M. H. Brown, and R. A. Skurray, unpublished), implying that TetR from *E. coli* does not bind to this inverted repeat. Furthermore, searches of the yet to be completed staphylococcal genome sequence databases have so far failed to identify a staphylococcal TetR protein homologue. Thus, the significance of IR2 is unknown, although it is tempting to speculate that the expression of qacR, and hence qacA, is under control of a global regulatory mechanism acting via IR2. Global regulatory proteins tend to modulate the expression of a number of different systems such as virulence determinants; recently, the Mta global activator protein has been identified in *B. subtilis* which regulates the expression of two different multidrug efflux pumps, Bmr and Blt (Baranova et al., 1999). Both of these export proteins have their own specific regulator, BmrR and BltR, respectively, but are also controlled at a higher level by one protein, Mta. Such systems may aid in coordinating the expression of multiple transport proteins possessing overlapping specificities in one organism (Baranova et al., 1999).

Although QacR shares homology with TetR in the N-terminal DNA-binding portion of the protein, particularly in the HTH domain, the overall mechanism of action of QacR may have more in common with other multi-ligand binding proteins viz., BmrR. In support of this proposition, preliminary molecular-modelling studies of QacR based on a TetR template, both with their respective cognate HTH regions deleted, failed to yield meaningful results, whereas threading the deleted QacR sequence through the coordinates of the crystal structure of the C-terminal ligand-binding portion of BmrR has enabled the identification of a number of QacR amino acids which may form a ligand-binding pocket in a similar manner to BmrR (S. Ranganathan, M. H. Brown, S. Grkovic, and R. A. Skurray, unpublished).

**Physiological Role of QacA**

Multidrug resistance transport systems have arisen from different evolutionary origins and are prevalent in all kingdoms of living organisms (Paulsen et al., 1996b; Pao et al., 1998). There has been much conjecture as to whether multidrug exporters have arisen as a specific mechanism by which cells protect themselves from a variety of structurally-disparate environmental toxic compounds or are a fortuitous side effect of a transporter whose primary function is the recognition and transport of an as yet unidentified “natural” substrate (Paulsen et al., 1996b; Neyfakh, 1997; Saier et al., 1998).

Consistent with the latter hypothesis, expression of the multidrug exporter Blt from *B. subtilis* has been shown to result in an increase in resistance to the natural polyanamine, spermidine (Woolridge et al., 1997), which may be the primary physiological substrate of the transporter since it is encoded by a gene, blt, which is cotranscribed
with another gene encoding an enzyme involved in spermidine degradation (Neyfakh, 1997; Woolridge et al., 1997). However, the identification of the global regulator Mta may have thrown some doubt on this putative “natural” function of Blt (Baranova et al., 1999). A chemoprotective role has also been demonstrated for the gonococcal multidrug efflux protein Mtr, which recognizes a wide range of structurally-diverse hydrophobic antimicrobials and has been shown to actively extrude an 18 amino acid broad-spectrum antimicrobial peptide, protegrin-1, isolated from porcine leukocytes (Shafer et al., 1998).

Recently, staphylococcal strains harboring the qacA-encoding plasmid pSK1 were shown to confer resistance to the cationic antimicrobial peptide, thombin-induced platelet microbicidal protein-1 (tPMP-1) (Yeaman et al., 1997), on an otherwise susceptible strain (Kupferwasser et al., 1999). Furthermore, there was no apparent cross-resistance to other tested cationic antimicrobial peptides, viz., protegrin-1; protamine, a basic polypeptide found in salmon sperm; the coagulase-negative staphylococcal lantibiotics, nisin and pepS; the human neutrophil defensin, hNP-1; or to CG 117-136, the 20-mer antimicrobial cationic peptide derived from human lysosomal cathepsin G (Kupferwasser et al., 1999). This suggested that the pSK1-mediated tPMP-1-resistance had relative specificity for tPMP-1.

tPMP-1 exerts a potent microbicidal activity against common bloodstream pathogens, such as S. aureus, whose cell membrane appears to be a principal target for the microbicidal action of tPMP-1 (Vu et al., 1994; Koo et al., 1997). To date, the mechanisms mediating tPMP-1 resistance in S. aureus are not fully delineated, although it is tempting to speculate that QacA confers tPMP-1 resistance via efflux of the peptide. However, studies have also raised the possibility that resistance to tPMP-1 could be due to more global changes, primarily in altering the fluidity of the staphylococcal cell membrane (Bayer et al., 2000). If the latter proves to be the case, resistance to tPMP-1 should probably be considered a “fortuitous” result of qacA expression, rather than an indication of the physiological role of the protein.

Concluding Remarks

Understanding the complexities presented by multidrug efflux pumps requires a comprehension of their mechanisms of action, the basis for their impressive substrate range, and their regulation (George, 1996; Paulsen et al., 1996b; Nikaido, 1998). It is only with such detailed knowledge that new chemotherapeutic agents can be rationally designed to either inhibit the activity of, or to evade recognition by, the multidrug efflux system. Continuing studies with the staphylococcal QacA multidrug export protein and its regulator, QacR, should provide fundamental molecular knowledge regarding the structure and function of both proteins, and will shed light on the manner by which a protein can recognize and bind an array of structurally-dissimilar compounds. The findings are also likely to be of relevance to other MFS drug exporters, since the sequence and secondary structure similarities of these proteins implies a commonality in their underlying mode of function.

Acknowledgements

We would like to thank Bernadette Mitchell and Steve Grkovic for their contributions to this project, and Neville Firth for critical reading of the manuscript. This work was supported in part by grants from the Australian Research Council and the National Health and Medical Research Council #980706 (Australia).

References


