Molecular Microbiology (1999) **31**(6), 1845–1851

The identification of a new family of sugar efflux pumps in *Escherichia coli*

Jia Yeu Liu,[1] Paul F. Miller,[1†] Mark Gosink[2] and Eric R. Olson[1]*

[1]Infectious Diseases Department and [2]Molecular Biology Department, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, MI 48106-1047, USA.

Summary

Using a functional cloning strategy with an *Escherichia coli* genomic plasmid library, we have identified a new family of sugar efflux proteins with three highly homologous members in the *E. coli* genome. In addition, two open reading frames, one present in *Yersinia pestis* and the other in *Deinococcus radiodurans*, appear to encode closely related proteins. An *in vitro* transport assay using inside-out membrane vesicles prepared from overproducing strains was used to demonstrate that members of this new family can efflux [^{14}C]-lactose. As sugar efflux phenomena have been reported previously in several bacterial species including *E. coli*, the identification of a new family of sugar efflux proteins may help to reveal the physiological role of sugar efflux in metabolism. It is proposed that the *E. coli* members of this family, whose functions were previously unknown, be given the gene family designation SET for sugar efflux transporter.

Introduction

There is good evidence supporting the existence of sugar efflux pumps in *Escherichia coli* (Wilson and Kashket, 1969; Winkler, 1971; Andrews and Lin, 1976; Huber *et al*., 1980a,b; Huber and Hurlburt, 1984). For example, a role for sugar efflux has been reported for lactose utilization: the secretion of the products of β-galactosidase action (glucose, galactose and allolactose) (Huber *et al*., 1980a,b; Huber and Hurlburt, 1984) and the secretion of non-metabolizable β-galactoside analogues, such as IPTG and methyl-β-D-thiogalactoside (TMG) (Winkler, 1971; Andrews and Lin, 1976). A role for sugar efflux in lactose metabolism is supported by the observation that mutants defective in the uptake of glucose and galactose grow poorly on lactose as the sole carbon source (Huber and Hurlburt, 1984). The efflux of IPTG and TMG presumably serves to prevent the intracellular accumulation of non-metabolizable sugar analogues (Herzengerg, 1959; Wilson and Kashket, 1969; Winkler, 1971; Andrews and Lin, 1976).

The work described here stemmed from our ongoing interest in the *E. coli* proteins AcrA, AcrB and TolC, which together comprise an efflux pump involved in intrinsic resistance to a structurally diverse set of antibiotics, dyes, detergents and organic solvents (Ma *et al*., 1994; 1995; White *et al*., 1997). In an effort to overexpress the individual components of the Acr pump for biochemical studies, we observed that the growth rate of wild-type *E. coli* was severely retarded when the rate of synthesis of AcrB, an inner membrane protein with multiple transmembrane domains (Ma *et al*., 1995), was increased. In our system, production of AcrB was under the control of the *lac* promoter and was affected by adding IPTG to the culture medium. In an effort to understand the growth inhibition phenomenon, we sought multicopy suppressors of IPTG-induced toxicity. Unexpectedly, this selection led to the identification of a previously uncharacterized gene, *yabM*, whose product prevented the intracellular accumulation of IPTG. We show here that *yabM* is a member of a new family of putative sugar efflux pump genes with five identified members from the available sequence databases. It is postulated that the products of these genes may function in the metabolism of lactose or other sugars, or in the removal of toxic sugars or sugar-like metabolites from the cell.

Results

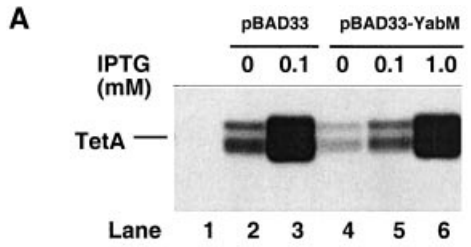
We observed that expression of either *acrB* or *tetA* from the strong IPTG-inducible promoter on plasmid pTrc-99a inhibited the growth of *E. coli* strain W3110. AcrB (Ma *et al*., 1995) and TetA (Yamaguchi *et al*., 1990a) are both inner membrane proteins with multiple transmembrane domains. The toxic effects of high levels of TetA have been reported previously (Eckert and Beck, 1989). One possible explanation for the observed IPTG-induced growth defect is that the overproduction of an integral membrane protein with multiple transmembrane domains, such as AcrB or TetA, results in the sequestration of a limiting factor involved in membrane protein biogenesis, as has

Received 2 October, 1998; revised 16 December, 1998; accepted 21 December, 1998. †Present address: Infectious Diseases Department, Pfizer Central Research, B118 Bin 2, Groton, CT 06340, USA. *For correspondence. E-mail Eric.Olson@wl.com; Tel. (+1) 734 622 5961; Fax (+1) 734 622 7158.

been suggested in a recent report (Ulbrandt *et al*., 1997). To test this hypothesis, an attempt was made to identify the gene encoding the limiting factor using a multicopy suppressor approach (see *Experimental procedures*). Plasmid clones that suppressed IPTG-induced toxicity associated with *acrB* overexpression were selected from our *E. coli* genomic library at a frequency of 1 per 1500 transformants. DNA sequence analysis of the ends of 21 cloned inserts and subsequent searching of the *E. coli* genome sequence database allowed us to make a presumptive identification of the genes present on the cloned DNA fragments. All were determined to be from the same locus at the 1.6 min position on the chromosome. Although most of the inserts were of different sizes and had different cloning junctions, four genes were present on all clones: *leuBCD* of the leucine biosynthetic operon and *yabM*, a previously uncharacterized open reading frame (ORF). Specific polymerase chain reaction (PCR) primers were then used to amplify *yabM* and *leuBCD* separately. The amplified genes were cloned into the arabinose-inducible expression plasmid pBAD33 and tested for their ability to suppress the toxic effect of IPTG-induced *acrB* or *tetA* expression in W3110. In strains that harboured either the *tetA* or the *acrB* expression plasmid, YabM, expressed from plasmid pBAD33-YabM, but not *leuBCD* could suppress IPTG-induced toxicity in an arabinose-dependent manner both on plates and in liquid cultures (data not shown), indicating that the expression of the cloned gene product was responsible for the suppressor activity. The DNA sequence of *yabM* predicted a 392-amino-acid protein with 12 putative transmembrane domains (from SWISSPROT annotation at http://www.ebi.ac.uk/htbin/swissfetch?P31675; accession number P31675). A BLAST search revealed that *YabM* exhibits amino-acid sequence similarity to the predicted products of two other previously uncharacterized *E. coli* ORFs, *yeiO* and *yicK*, located at 48.75 min and 82.64 min, respectively, on the chromosome. This similarity was also noted at the *E. coli* Database Collection (ECDC) web site (http://susi.bio.uni-giessen.de/ecdc.html). A check of the physical map of the *E. coli* chromosome indicated that *yabM* is probably not part of an operon, as both the upstream and the downstream adjacent genes are in opposite transcriptional orientations relative to that of *yabM*. The genes downstream of *yeiO* and *yicK* are in the same transcriptional orientation but do not encode proteins of known function. Two additional ORFs, one present in *Yersinia pestis* and the other in *Deinococcus radiodurans*, appear to encode closely related proteins. In pairwise comparisons between the three *E. coli* proteins, the identity and similarity scores are at least 45% and 70%, respectively, with YeiO and YicK showing the highest identity and similarity scores, indicating that these proteins are likely to be closely related (see *Experimental procedures*). The *D. radiodurans* ORF appears to be the most divergent among this set, with identity and similarity scores compared with the *E. coli* proteins in the range of 26–29% and 48–50% respectively.

One mechanism by which pBAD33-YabM could suppress IPTG-induced toxicity would be if it lowered the level of expression of genes driven by IPTG-inducible promoters. To test this, pBAD33-YabM was introduced into strain W3110/pTetA, which carries a plasmid containing the *tetA* gene under the control of the strong IPTG-inducible promoter of plasmid pTrc-99a. In addition, strain W3110/pTetA is wild type at the *lac* locus. The presence of IPTG is expected to induce high-level expression of both the chromosomal-encoded *lacZ* gene, encoding the gene for the soluble enzyme β-galactosidase, and the plasmid-encoded TetA, the inner membrane tetracycline efflux protein. W3110/pTetA was transformed with either pBAD33 or pBAD33-YabM, and membranes and soluble extracts were prepared from transformants grown in the presence of various concentrations of IPTG and arabinose, the latter to induce the expression of *yabM*. The levels of TetA in the membrane fractions were determined by Western blot analysis. In membranes from pBAD33-YabM-transformed cells, which overexpressed *yabM*, there was much less TetA protein at 0.1 mM IPTG than in membranes from vector (pBAD33)-transformed cells (Fig. 1A, compare lanes 3 and 5). At 1.0 mM IPTG, the amount of the TetA protein expressed in the pBAD33-YabM transformant was similar to that observed for the pBAD33 transformant at 0.1 mM IPTG (Fig. 1A, compare lanes 3 and 6). The TetA protein appeared as a doublet in the Western blot, with the lower band possibly a degradation product. The soluble fractions from the pBAD33-YabM- and pBAD33-transformed cells were also assayed for β-galactosidase activity. In the pBAD33-YabM transformant, there was a 128- and eightfold reduction in the β-galactosidase activity at 0.1 mM and 1.0 mM IPTG, respectively, compared with the vector (pBAD33)-transformed control strain (Fig. 1B). These results suggested that *yabM* suppressed IPTG-induced toxicity under these conditions by decreasing the expression of genes with IPTG-responsive promoters.

Based on these experimental results and given its predicted polytopic membrane structure, it seemed possible that YabM could prevent IPTG induction of target promoters by functioning as an IPTG efflux pump. We reasoned that, if YabM could transport IPTG, a lactose analogue, then it may transport lactose as well. To test this, we measured the ability of cells carrying either pBAD18 or pBAD-YabM, grown in the presence or absence of arabinose, to accumulate radiolabelled lactose. We found that the cells carrying pBAD-YabM accumulated less radiolabelled lactose than did control cells, and that the reduced accumulation was dependent on the presence of arabinose in the media, which lowered the intracellular lactose in the pBAD-YabM transformants to less than 40% of the vector-transformed



B

β -Gal Activity (Miller Units)

	IPTG (mM) 0	0.1	1.0
pBAD33	7.8+/- 1.7	836+/- 40	892+/- 9.4
pBAD33-YabM	8.1+/- 0.1	6.5+/-0.2	109+/- 0.3

Fig. 1. Increased expression of *yabM* decreases the amount of protein produced from IPTG-responsive promoters.
A. TetA protein analysis by immunoblot of membrane proteins from strains W3110/pTetA/pBAD33 (lanes 2 and 3) and W3110/pTetA/pBAD33-YabM (lanes 4–6). Lane 1 was from a control strain that does not express TetA. Cells were grown in media with the indicated concentrations of IPTG for 1 h before harvest. Each lane contained 0.4 μg of protein from the membrane fraction. The samples were separated by 12% SDS–PAGE and immunoblotted for the TetA protein with anti-TetA antibody.
B. The soluble fractions from cultures used in (A) were assayed for β-galactosidase activity, expressed in Miller units.

controls (data not shown). This suggested that YabM could act as an IPTG/lactose efflux pump. To confirm this, inside-out membrane vesicles were prepared from strains MC4100/pBAD-YabM and MC4100/pBAD18, both grown in the presence of arabinose. In this configuration, an efflux pump would be expected to transport substrates into the interior of the vesicle. Vesicles from vector-transformed control (pBAD18) cells showed little accumulation of radiolabelled lactose (Fig. 2A). In contrast, vesicles prepared from pBAD-YabM-transformed cells accumulated substantial amounts of lactose that was released upon dissipation of the protonmotive force with CCCP (Fig. 2A). We conclude that YabM is a proton-coupled β-galactoside efflux pump.

To determine whether YeiO and YicK also have lactose efflux activity, inside-out membrane vesicles were prepared from strains MC4100/pBAD-YeiO and MC4100/pBAD-YicK grown in the presence of arabinose to induce high-level expression of *yeiO* and *yicK* respectively. Vesicles from pBAD-YeiO-transformed cells accumulated substantial amounts of lactose that was released upon dissipation of the protonmotive force with CCCP (Fig. 2B). However, vesicles from pBAD-YicK-transformed cells failed to accumulate radiolabelled lactose. We conclude that YeiO, but not YicK, can also promote the proton-coupled efflux of lactose.

Discussion

Our results argue strongly that *yabM* encodes a β-galactoside efflux pump. As measured by the amount of protein expressed from IPTG-inducible promoters, cells that overexpressed *yabM* were less responsive to IPTG when compared with controls. In addition, inside-out membrane vesicles prepared from cells that overexpressed *yabM*, but not from controls, accumulated radiolabelled lactose in a manner that was sensitive to the proton ionophore CCCP. Therefore, we conclude that *yabM* encodes a proton/β-galactoside antiporter.

A BLAST search with *yabM* revealed that *E. coli* has two other genes, *yeiO* and *yicK*, with more than 70% sequence similarity to *yabM*. In addition, two additional ORFs, one present in *Yersinia pestis* and the other in *Deinococcus radiodurans*, appear to encode closely related proteins. However, no close homologues were found in the sequences of other completed bacterial genomes. The *Y. pestis* ORF is more closely related to *yabM* than to the other proteins. Similarly, *yeiO* and *yicK* are more closely related to each other than to other members of the family. The ORF

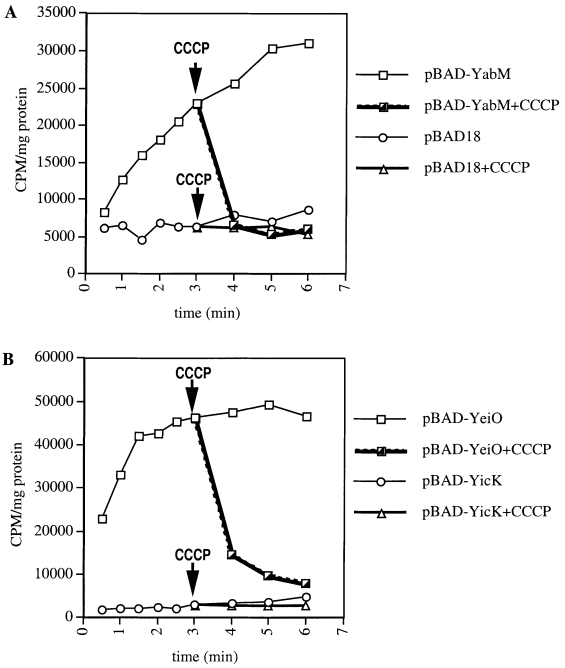


Fig. 2. YabM and YeiO have [^{14}C]-lactose efflux activity.
A. YabM and vector control vesicles. Inside-out membrane vesicles were prepared from strains MC4100/pBAD18 and MC4100/pBAD-YabM grown in the presence of arabinose. ATP was used to generate a proton gradient in the vesicles. Uptake was initiated by the addition of radiolabelled lactose and was followed for 6 min. At the indicated time, half of the uptake reaction was treated with CCCP to a final concentration of 80 μM.
B. YeiO and YicK vesicles. Inside-out membrane vesicles were prepared from strains MC4100/pBAD-YeiO and MC4100/pBAD-YicK grown in the presence of arabinose. [^{14}C]-Lactose uptake was performed as described in (A).

found in *D. radiodurans* is the most distant member of the family. Interestingly, four members of this family of presumed efflux pumps are from Gram-negative bacterial species (*E. coli* and *Y. pestis*), while the fifth is from a Gram-positive species (*D. radiodurans*).

A classification scheme was recently published for the major facilitator superfamily of membrane transporters (Paulsen *et al*., 1996; Pao *et al*., 1998). Members of this superfamily perform diverse transport functions, ranging from the uptake of nutrients to the efflux of noxious agents, and are presumed to have arisen from a tandem duplication of a primordial precursor, as these proteins can often be visualized as consisting of two homologous domains containing six transmembrane segments (Paulsen *et al*., 1996; Pao *et al*., 1998). A degenerate signature motif [GX_3(D/E)(R/K)XG[X](R/K)(R/K)], usually located between the second and the third and between the eighth and the ninth transmembrane domains, is characteristic of members of this superfamily (Paulsen *et al*., 1996). An examination of the sequences of YabM and its relatives, allowing for one mismatch in some cases, revealed that all five members carried this signature motif. As there are no closely related proteins in the existing classification scheme, we propose that a new family, the sugar efflux transporter (SET) family, be designated to encompass the five proteins described here, and that *yabM* and *yeiO* be designated *setA* and *setB*, respectively, as the current names for these proteins are generic and refer only to their locations in the chromosome. The high degree of sequence identity between the *E. coli* members of the SET family of transporters suggests similar physiological functions. As sequence conservation often predicts functional conservation (Paulsen *et al*., 1996; Saier *et al*., 1998), indeed YeiO was shown in an *in vitro* vesicle assay to have lactose efflux activity. While YicK was unable to transport lactose in similar experiments, the high degree of sequence similarity suggests that it, as well as the other members of the SET family, may also function as sugar efflux pumps whose substrates remain to be identified.

At present, the physiological role played by this family of sugar efflux pumps remains unknown. As YabM and YeiO can secrete lactose, a role in lactose metabolism is suspected. Interestingly, preliminary results indicate that YabM will also transport glucose (J. Y. Liu, unpublished data). It is important to note, however, that, while our findings demonstrate that YabM can efflux β-galactosides such as lactose and IPTG, we cannot at this point conclude that YabM plays a role in the normal metabolism of lactose. Another possible role for sugar efflux pumps is the removal of toxic sugars, sugar-containing antibiotics or sugar-like metabolites, as high intracellular concentrations of many sugars, sugar phosphates and non-metabolizable sugar analogues have growth-inhibitory effects (Hofsten, 1961; Ackerman *et al*., 1974; Andrews and Lin, 1976; Totemeyer *et al*., 1998). It has been reported previously that a sugar efflux system that transports TMG and IPTG exists in the Gram-positive bacterium *Streptococcus pyogenes* (Sutrina *et al*., 1988). Although the suspected mechanism of efflux in this bacterium is by facilitated diffusion (Sutrina *et al*., 1988), it remains possible that a homologue of the SET family is responsible for the observed efflux activity. In *E. coli*, it was estimated previously that lactose can accumulate intracellularly to concentrations that are several hundredfold above those present in the external environment (Winkler and Wilson, 1966; Huber *et al*., 1980a). As lactose uptake is the rate-limiting step in the utilization of lactose by β-galactosidase (Rickenberg *et al*., 1956), intracellular concentrations of the products (glucose, galactose and allolactose) of β-galactosidase action would be expected to rise as well. A sugar efflux system may act to address the anticipated rise in intracellular osmolarity when the cell is in a sugar-rich environment. Additionally, high intracellular concentrations of galactose may themselves be poisonous, as intermediates in galactose metabolism have growth-inhibitory or cell lytic effects (Yarmolinsky *et al*., 1959). Lastly, the presence of β-galactosides, under certain growth conditions, has unexpected growth-inhibitory effects that are dependent on the expression of β-galactosidase (Hofsten, 1961). Although the mechanism of growth inhibition is not understood, the presence of sugar efflux systems would clearly be expected to influence the cell's sensitivity to such compounds. Experiments are currently in progress to generate null mutants of *yabM*, *yeiO* and *yicK* that may be sensitized to sugars or unable to metabolize lactose. Additionally, the *in vitro* transport assay using inside-out vesicles from hyperexpression strains described here will allow a characterization of this new family of sugar efflux pumps in terms of substrate preferences and kinetic parameters. It is anticipated that these efforts will shed new light on the normal cellular roles played by the members of the SET family of transporters.

Experimental procedures

Bacterial strains, plasmids, media and reagents

The bacterial strains and plasmids used in this study are listed in Table 1. Cultures were grown in L broth (10 g l^{-1} tryptone, 5 g l^{-1} yeast extract, 5 g l^{-1} NaCl) at 37°C unless otherwise indicated. Antibiotics and arabinose, purchased from Sigma, were added so that final concentrations were: ampicillin, 50 μg ml^{-1}; tetracycline, 20 μg ml^{-1}; chloramphenicol, 12.5 μg ml^{-1}; arabinose, 15 mM. Radiolabelled lactose (catalogue no. CFA278; 55.0 mCi mM^{-1}) was from Amersham Life Sciences. Competent, library-efficient DH5α cells were purchased from Life Technologies.

Construction of plasmids

Standard DNA manipulation techniques were used (Sambrook

Table 1. *E. coli* strains and plasmids.

Strain	Relevant genotype or phenotype	Plasmid	Source or reference
W3110	F-λ-IN*1 rph-1 thyA*36 *deoC*2	None	K. Bertrand
B414	W3110 Δ *acrAB*	None	K. Bertrand
MC4100	F-*araD*139 Δ(*argF-lac*)U169 *rpsL*150 *relA*1 *flbB*5301	None	T. Silhavy
JL2	W3110 Δ *acrAB tolC*::Tn*10* tet^r	None	This study by P1 from MC4100 *tolC*::Tn*10* tet^r (O. Lomavskaya) into B414
Plasmid	**Relevant properties**		**Source or reference**
pBAD 18	amp^r		Guzman *et al*. (1995)
pBAD 33	Cm^r		Guzman *et al*. (1995)
pRB373	amp^r		Bruckner (1992)
pTrc99 A	amp^r		Pharmacia Biotech
pTrc-1	amp^r; derivative of pTrc99A; the *Nco*I site of pTrc99A is destroyed		This study
pBAD-YabM	amp^r; *yabM* cloned into SacI–*Hin*dIII site of pBAD18		This study
pBAD-YeiO	amp^r; *yeiO* cloned into SacI–*Hin*dIII site of pBAD18		This study
pBAD-YicK	amp^r; *yicK* cloned into SacI–*Hin*dIII site of pBAD18		This study
pBAD33-YabM	Cm^r; *yabM* cloned into SacI–*Hin*dIII site of pBAD33		This study
pTAcrB	amp^r; *acrB* cloned into SacI–*Hin*dIII site of pTrc-1		This study
pTetA	amp^r; *tetA* cloned into SacI–*Hin*dIII site of pTrc-1		This study
pLAcrB	Cm^r; *lacI^q*; *Sph*I–*Sca*I fragment from pTAcrB replaced the *Sph*I–*Cla*I fragment of pACY184		This study

et al., 1989). The plasmid pBXE1 (generously provided by K. Bertrand), which contains a 7 kb genomic insert spanning the *acrAB* locus, was used as the source of the *acrB* gene in the construction of other plasmids. The plasmid pTAcrB was constructed by inserting *acrB* into pTrc-1 at the *Sac*I–*Hin*dIII sites. pTrc-1 is a derivative of pTrc99A (Pharmacia Biotech), in which the *Nco*I site in pTrc99A was destroyed by digesting with *Nco*I and blunting with S1 nuclease. The plasmid pLAcrB was constructed by inserting the *Sph*I–*Sca*I fragment from pTAcrB, containing *acrB* with the promoter and terminator elements and *lacI^q* of pTrc99A, into the *Sph*I–*Cla*I site of pACYC184. The genes encoding *tetA*, *yabM*, *yeiO* and *yicK* were all obtained by PCR amplification from chromosomal DNA templates or, in the case of *yabM*, cloned fragments, using primers that added unique restriction sites at the 5′ and 3′ termini. All PCR products were sequenced and, except for three silent mutations in *yabM*, the products were identical to the sequences reported in the EMBL/GenBank database. For the construction of pBAD-YabM, pBAD-YeiO and pBAD-YicK, the PCR products encoding *yabM*, *yeiO* and *yicK*, respectively, were inserted into the *Sac*I–*Hin*dIII sites of pBAD18. For the construction of pBAD33-YabM and pTetA, the PCR products encoding *yabM* and *tetA* were inserted into the *Sac*I–*Hin*dIII sites of pBAD33 and pTrc-1 respectively.

Construction of a genomic library and isolation of genomic clones that suppress IPTG-induced toxicity

A genomic library prepared from strain MC4100 *tolC*::Tn*10* was constructed as follows. Chromosomal DNA was purified from a cell lysate using a chromosomal DNA isolation kit and procedure supplied by Qiagen. The purified DNA was partially digested with *Sau*3A, and DNA fragments estimated to range in size from 6 to 12 kbp were isolated from an agarose gel using a QIAquick kit (Qiagen). The resulting DNA was ligated to pRB373 (Bruckner, 1992), which had been digested with *Bam*HI and dephosphorylated with calf intestinal alkaline phosphatase. The resulting ligation mixture was introduced by transformation into strain DH5αF′*lacI^q*, and the resulting transformants were selected at 37°C on L agar plates containing ampicillin. Plasmid DNA was isolated from approximately 12 000 transformants (hereafter referred to as the genomic library). Plasmids that suppressed IPTG-induced toxicity were isolated by transforming strain JL2/pLAcrB with the genomic library and selecting for large colonies that grew on L plates with ampicillin, tetracycline, chloramphenicol and 0.1 mM IPTG at 37°C.

Isolation of inside-out vesicles, β-galactosidase assays and immunoblotting

Cells were grown in L broth containing ampicillin and arabinose to an OD_{600} of 0.6–1.0, harvested and inside-out vesicles made essentially as described previously (Yamaguchi *et al*., 1990b). For the IPTG titration experiment (Fig. 1B), a cell culture at mid-log phase was divided into three portions, and IPTG was added to two of the cultures to 0.1 mM and 1.0 mM. The cells were grown for an additional hour before harvesting. The cultures were chilled, and the cells were collected by centrifugation at 5000 × *g*. All subsequent manipulations were carried out at 4°C. Cell pellets were washed once with lysis buffer (50 mM MOPS-KOH, pH 6.6, 180 mM NaCl, 10 mM EDTA), resuspended in lysis buffer to an OD_{600} of 40–80 and lysed in a French press at 5000 psi. The lysate was centrifuged at 27 000 × *g* for 10 min to remove unlysed cells and debris. The supernatant was centrifuged for 1 h at 100 000 × *g* to pellet total membranes. The supernatant from the last centrifugation step contained the cytosolic fraction. The final membrane pellets, which were enriched in inside-out membrane vesicles, were washed once with 50 mM

MOPS-KOH, pH 6.6, 180 mM NaCl and resuspended in the same buffer. Protein concentrations were determined by the Bradford protein assay kit (Bio-Rad) using BSA as the standard. β-Galactosidase assays were performed on the soluble fraction with *O*-nitrophenyl-β-D-galactopyranoside (ONPG) as the substrate at 30°C essentially as described previously (Miller, 1972), but without the addition of chloroform and SDS. Immunoblot analysis of the membrane fractions was carried out according to the procedure described previously (Liu and Guidotti, 1997). The antiserum used (gift from Dr A. Yamaguchi; Yamaguchi *et al*., 1990a) recognized the C-terminal 14 amino acids of the TetA protein.

Uptake of [^{14}C]-lactose into inside-out vesicles

A sample of 180 μl of membrane vesicles (10.0 μg of protein ml^{-1}) was mixed with 540 μl of uptake buffer (50 mM MOPS-KOH, pH 7.5, 180 mM NaCl, 10 mM $MgSO_4$), followed by incubation at 21°C for 5 min. Subsequently, 14.4 μl of a 100 mM ATP solution was added, and the incubation was continued for an additional 10 s. Lactose uptake was initiated by mixing the suspension with 14.4 μl of 3.63 mM [^{14}C]-lactose. At the time indicated, one half of the suspension was removed, and carbonyl cyanide m-chlorophenylhydrazone (CCCP) was added to 80 μM. At specified time points, 60 μl aliquots were removed and mixed with 1 ml of ice-cold stop buffer (50 mM MOPS-KOH, pH 7.5, 180 mM LiCl), followed by filtration through Whatman glassfibre filters (catalogue no. 1825 024). The filters were washed twice with 3 ml of the same buffer, and radioactivity was measured in a liquid scintillation counter.

Comparison of YabM, YeiO, YicK, Y. pestis *ORF and* D. radiodurans *ORF protein sequences*

Sequence comparison was performed using the PILEUP program from the Genetic Computer Group suite of applications (Devereux *et al*., 1984). The analysis was performed using the default parameters except that the option (–endweight) was enabled. The proteins were aligned in their entirety. The *Y. pestis* ORF sequence was taken from the search results of the TBLASTN analysis on the Unfinished Microbial Genomes at the National Center for Biotechnology Information and is presumed to be incomplete, as additional N- and C-terminal sequences are missing. The *D. radiodurans* sequence was obtained by translating the reverse of bases 1475–2710 of contig gdr_196 from the *D. radiodurans* sequencing project at the Institute for Genomic Research (TIGR), and the N-terminal protein sequence is uncertain, as there was DNA sequence ambiguity in this region. The accession numbers for YabM, YeiO and YicK are SW: P31675, SW: P33026 and SW: P31436 respectively.

Acknowledgements

We thank J. Li, R. El-Gewely, T.-F. Wang and G. Guidotti for helpful comments on our manuscript, J. Willard for technical assistance, and A. Yamaguchi for antibodies. In addition, we are grateful for the encouragement and assistance received from members of our research group.

References

Ackerman, R.S., Cozzarelli, N.R., and Epstein, W. (1974) Accumulation of toxic concentrations of methylglyoxal by wild-type *Escherichia coli* K-12. *J Bacteriol* **119:** 357–362.

Andrews, K.J., and Lin, E.C. (1976) Thiogalactoside transacetylase of the lactose operon as an enzyme for detoxification. *J Bacteriol* **128:** 510–513.

Bruckner, R. (1992) A series of shuttle vectors for *Bacillus subtilis* and *Escherichia coli*. *Gene* **122:** 187–192.

Devereux, J., Haeberli, P., and Smithies, O. (1984) A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res* **12:** 387–395.

Eckert, B., and Beck, C.F. (1989) Overproduction of transposon Tn*10*-encoded tetracycline resistance protein results in cell death and loss of membrane potential. *J Bacteriol* **171:** 3557–3559.

Guzman, L.M., Belin, D., Carson, M.J., and Beckwith, J. (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J Bacteriol* **177:** 4121–4130.

Herzengerg, L. (1959) Studies on the induction of B-galactoside in a cryptic strain of *Escherichia coli*. *Biochim Biophys Acta* **31:** 525–538.

Hofsten, B.V. (1961) The inhibitory effect of galactosides on the growth of *Escherichia coli*. *Biochim Biophys Acta* **48:** 164–171.

Huber, R.E., and Hurlburt, K.L. (1984) Escherichia coli growth on lactose requires cycling of beta-galactosidase products into the medium. *Can J Microbiol* **30:** 411–415.

Huber, R.E., Lytton, J., and Fung, E.B. (1980a) Efflux of beta-galactosidase products from *Escherichia coli*. *J Bacteriol* **141:** 528–533.

Huber, R.E., Pisko Dubienski, R., and Hurlburt, K.L. (1980b) Immediate stoichiometric appearance of beta-galactosidase products in the medium of *Escherichia coli* cells incubated with lactose. *Biochem Biophys Res Commun* **96:** 656–661.

Liu, J.Y., and Guidotti, G. (1997) Biochemical characterization of the subunits of the Na^+/K^+ ATPase expressed in insect cells. *Biochim Biophys Acta* **1336:** 370–386.

Ma, D., Cook, D.N., Hearst, J.E., and Nikaido, H. (1994) Efflux pumps and drug resistance in Gram-negative bacteria. *Trends Microbiol* **2:** 489–493.

Ma, D., Cook, D.N., Alberti, M., Pon, N.G., Nikaido, H., and Hearst, J.E. (1995) Genes *acrA* and *acrB* encode a stress-induced efflux system of *Escherichia coli*. *Mol Microbiol* **16:** 45–55.

Miller, J.H. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Pao, S.S., Paulsen, I.T., and Saier, Jr, M.H. (1998) Major facilitator superfamily. *Microbiol Mol Biol Rev* **62:** 1–34.

Paulsen, I.T., Brown, M.H., and Skurray, R.A. (1996) Proton-dependent multidrug efflux systems. *Microbiol Rev* **60:** 575–608.

Rickenberg, H.V., Cohen, G.N., Buttin, G., and Monod, J. (1956) Galactoside-permease of *Escherichia coli*. *Ann Inst Pasteur* **91:** 829–857.

Saier, Jr, M.H., Paulsen, I.T., Sliwinski, M.K., Pao, S.S.,

Skurray, R.A., and Nikaido, H. (1998) Evolutionary origins of multidrug and drug-specific efflux pumps in bacteria. *FASEB J* **12:** 265–274.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sutrina, S.L., Reizer, J., and Saier, Jr, M.H. (1988) Inducer expulsion in *Streptococcus pyogenes*: properties and mechanism of the efflux reaction. *J Bacteriol* **170:** 1874–1877.

Totemeyer, S., Booth, N.A., Nichols, W.W., Dunbar, B., and Booth, I.R. (1998) From famine to feast: the role of methylglyoxal production in *Escherichia coli*. *Mol Microbiol* **27:** 553–562.

Ulbrandt, N.D., Newitt, J.A., and Bernstein, H.D. (1997) The *E. coli* signal recognition particle is required for the insertion of a subset of inner membrane proteins. *Cell* **88:** 187–196.

White, D.G., Goldman, J.D., Demple, B., and Levy, S.B. (1997) Role of the *acrAB* locus in organic solvent tolerance mediated by expression of *marA*, *soxS*, or *robA* in *Escherichia coli*. *J Bacteriol* **179:** 6122–6126.

Wilson, T.H., and Kashket, E.R. (1969) Isolation and properties of thiogalactoside transacetylase-negative mutants of *Escherichia coli*. *Biochim Biophys Acta* **173:** 501–508.

Winkler, H.H. (1971) Efflux and the steady state in alpha-methylglucoside transport in *Escherichia coli*. *J Bacteriol* **106:** 362–368.

Winkler, H.H., and Wilson, T.H. (1966) The role of energy coupling in the transport of beta-galactosides by *Escherichia coli*. *J Biol Chem* **241:** 2200–2211.

Yamaguchi, A., Adachi, K., and Sawai, T. (1990a) Orientation of the carboxyl terminus of the transposon Tn*10*-encoded tetracycline resistance protein in *Escherichia coli*. *FEBS Lett* **265:** 17–19.

Yamaguchi, A., Udagawa, T., and Sawai, T. (1990b) Transport of divalent cations with tetracycline as mediated by the transposon Tn*10*-encoded tetracycline resistance protein. *J Biol Chem* **265:** 4809–4813.

Yarmolinsky, M.B., Wiesmeyer, H., Kalcklar, H.M., and Jordan, E. (1959) Hereditary defects in galactose metabolism in *Escherichia coli* mutants. II. Galactose-induced sensitivity. *Proc Natl Acad Sci USA* **45:** 1786–1791.