A family of bacterial transporters, the SET (sugar efflux transporter) family, has been recently reported (Liu, J. Y., Miller, P. F., Gosink, M., and Olson, E. R. (1999) Mol. Microbiol. 31, 1845–1851). In this study, the biochemical and cell biological properties of the three _Escherichia coli_ members (SetA, SetB, and SetC) of the family are characterized. We show that both SetA and SetB can transport lactose and glucose. In addition, SetA has broad substrate specificity, with preferences for glucosides or galactosides with alkyl or aryl substituents. Consistent with the observed in vitro substrate specificities, strains that hyperexpress SetA or SetB are desensitized to lactose analogues as measured by induction of the _lac_ operon. In addition, strains that hyperexpress SetA are resistant to the growth inhibitory sugar analogue o-nitrophenyl-β-D-thiogalactoside. Strains disrupted for any one or all of the _set_ genes are viable and show no defects in lactose utilization nor increased sensitivity to inducers of the _lac_ operon and nonmetabolizable sugar analogues. The data suggest that the _set_ genes are either poorly expressed under normal laboratory growth conditions or are redundant with other cellular gene products.

The recently described bacterial SET (sugar efflux transporter) family of efflux pumps shares amino acid sequence similarity with the Major Facilitator Superfamily of transport proteins (1–3). Members of the Major Facilitator Superfamily perform diverse functions including the uptake of nutrients such as sugars and the secretion of noxious agents, including antibiotics (2, 3). The SET proteins were first identified in _Escherichia coli_, which encodes three members (SetA, SetB, and SetC) that share a high degree (at least 70%) of amino acid sequence similarity (1). Prior to this work, _setA_, _setB_, and _setC_ were given the generic names _yabM_, _yeiO_, and _yicK_, respectively, for _E. coli_ open reading frames of unknown function. It was shown that two of the proteins (SetA and SetB) could catalyze the secretion of lactose (1). Two additional members were identified as open reading frames in _Deinococcus radiodurans_ and _Yersinia pestis_. A recent examination of the sequenced microbial genomes data base yielded six additional, more distantly related, proteins. At present, the transport properties of these new family members have not been characterized. The _set_ proteins are not ubiquitously present in bacteria, suggesting an ecologically specialized role for this family of pumps.

Sugar efflux has been reported in many bacterial species (4, 5), including _E. coli_ (6–9). It was shown that sugar efflux is an integral part of the metabolism of lactose in _E. coli_ (7). In a strain constitutive for the _lac_ operon, the addition of lactose led to the immediate and stoichiometric appearance of the products (glucose, galactose, and allolactose) of β-galactosidase action in the medium (6). Consistent with this hypothesis, mutants defective in the uptake of glucose and galactose grow poorly on lactose as the sole carbon source (7).

Physiological evidence supports the hypothesis that efflux systems are involved in the detoxification of nonmetabolizable sugars in _E. coli_ (8–11). Methyl-α-glucoside (MG),2 a competitive inhibitor of glucose utilization (12, 13), enters the cell mainly by the transporter for glucose and mannose, the products of the genes _ptsG_ and _ptsM_, respectively, and accumulates to high levels as both the phosphorylated and the unmodified forms (9, 13). When glucose is the sole carbon source, growth inhibition by MG is due to both decreased uptake of glucose and interference with the utilization of intracellular glucose-6-phosphate, the latter because of the accumulation of MG and MG-6-phosphate (9, 12). It was shown that both MG and MG-6-phosphate are secreted from the cell by an uncharacterized mechanism, the latter being first dephosphorylated before secretion (9, 11, 12).

Many nonmetabolizable lactose analogues such as isopropyl-β-D-thiogalactoside (IPTG) and methyl-β-D-thiogalactoside are growth inhibitory when lactose is the only carbon source (10). These compounds enter the cell through the lactose permease, the product of the _lacY_ gene (14). To prevent the accumulation of IPTG and methyl-β-D-thiogalactoside, these sugar analogues are first acetylated by the LacA transacetylase and then secreted from the cell by an unknown transporter (8, 10). Entry into the cell is prevented because the acetylated sugar analogues are not substrates for the permease (8).

In this report, a role for the _set_ proteins (SetA, SetB, and SetC) in the metabolism of lactose or the detoxification of nonmetabolizable sugar analogues is investigated. As we show in this study, the range of sugars that are efflux substrates for the _E. coli_ SET proteins include selective monosaccharides and disaccharides, in addition to glycosides analogues such as IPTG. Because lactose and IPTG are both substrates for _set_ protein-catalyzed efflux, we also generated null mutations in _setA_, _setB_, and _setC_ and used these to help define the role of the _set_ proteins in _E. coli_ sugar metabolism.

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1 The abbreviations used are: MG, methyl-α-glucoside; IPTG, isopropyl-β-D-thiogalactoside; ONPTG, o-nitrophenyl-β-D-thiogalactoside; CCCP, carbonyl cyanide m-chlorophenylhydrazone; MOPS, 4-morpholinepropanesulfonic acid.

2 The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Table I  E. coli strains and plasmids

| Strains | Relevant genotype or phenotype | Plasmid | Source or reference |
|---------|--------------------------------|---------|--------------------|
| W3110   | F-λ-IN1 rph-l thyA36            | none    | K. Bertrand        |
| deoC2   |                                |         |                    |
| MN102   | W3110 Δ sacAB                  | none    | K. Bertrand        |
| MC4100  | F-λ araD139 λargF              | T. Silhavy |                    |
| lacU189 pl150 relA1 | fB5B301            |         |                    |
| ML308   | lacI                          | none    | ATCC 15224         |
| JLI72   | ML308 setA::TisM              | setA::TcM::setC::TetE | This study |
|         |                               | zih-207::Tc10   |                    |
| JLI86   | W3110 setI::TisM              | setB::TcM     |                    |
|         | setC::TcM                    | none     | Lab stock          |
| B359    | AB1157 recD::Tn10            | none    |                    |

MATERIALS AND METHODS

Bacterial Strains, Plasmids, Media, and Reagents—The bacterial strains and plasmids used in this study are listed in Table I. Cells were grown in LB broth (10 g tryptone/liter, 5 g yeast extract/liter, 5 g NaCl/liter) at 37 °C unless otherwise indicated. Plates contained 15 g agar/liter and top agar contained 7.5 g agar/liter. Antibiotics and arabinose, purchased from Sigma, were added to the following concentrations: 50 μg/ml ampicillin, 10 μg/ml tetracycline, 12.5 μg/ml chloramphenicol, 15 μg/ml streptomycin, 35 μg/ml kanamycin, 15 μg/ml amphenicol, or tetracycline, were inserted into the pBR-SetA, -SetB, or -SetC, previously referred to as interposons (denoted by an “I”), which conferred resistance to streptomycin, chloramphenicol, or tetracycline, were inserted into setA (at the 3′ end of setA), setB (at the 5′ end of setB), and setC (at the 5′ end of setC), respectively. The disruption markers (19), referred to as interposons (denoted by an “I”), were cloned downstream from the arabinose-inducible promoter, generating the plasmids pBAD-SetA, pBAD-SetB, and pBAD-SetC, respectively, and the resulting plasmids were transformed into E. coli strain MC4100. Inside-out membrane vesicles were prepared from cells that hyperpressed the plasmid-encoded Set proteins. (In this configuration, an efflux pump would be expected to transport a radiolabeled substrate into the inside of the vesicle, which can be centrifuged at 27,000 × g for 10 min to remove unlysed cells and debris. Protein concentrations were determined by the Bradford protein assay kit (Bio-Rad) using bovine serum albumin as the standard. The transport assay was performed at 21 °C. A 1.5-μl solution of 50 mM [14C]lactose, pH 7.5, 50 mM MgSO4, was added to 15 μl of a suspension of membrane vesicles (14.0 μg protein/ml) followed by incubation at 21 °C for 30 s. Transport was initiated by the addition of 60 μl of Buffer A (50 mM MOPS, pH 7.5, 10 mM MgSO4, 192 mM NaCl) containing the labeled sugar ([14C]lactose: 0.181 mM, 55.0 μCi/ml, or [14C]glucose: 0.57 mM, 8.36 μCi/ml). At the specified times, the entire solution was mixed with 50 μl of ice-cold stop buffer (50 mM MOPS, pH 7.5, 180 mM LiCl) followed by filtration through Whatman glass fiber filters. The filters were washed twice with 3 ml of the same buffer, and radioactivity was measured in a liquid scintillation counter. For the CCCP-treated samples, CCCP was added to 80 μM at the indicated time after the initiation of transport and allowed to incubate further before stopping the assay with stop buffer. For the kinetic studies of SetA catalyzed [14C]lactose transport, the stock [14C]lactose solution was diluted with unlabeled lactose so that the final concentrations in the assay were 0.33, 0.5, 1.0, 2.0, and 5.0 mM. In the assay for inhibitors of SetA catalyzed transport (of SetA)lactose, Buffer A contained the tested inhibitor at 31.25 mM and [14C]lactose at 0.181 mM (55.0 μCi/ml). The final concentrations of the test compound and lactose were 25 and 0.145 mM, respectively.

Induction of the lac Operon with IPTG and Lactose—β-Galactosidase Activity Assays—Log phase cultures (A600 = 0.2–0.4) were grown at the indicated concentration of IPTG or lactose for 1 h. Subsequently, β-galactosidase activity in the culture was determined as described (17). To follow the kinetics of IPTG induction of the lac operon, IPTG was added to early log phase cultures (A600 = 0.1) to final concentrations of 2.5 and 5.0 μM. At the indicated times, samples of the culture were removed and assayed for β-galactosidase activity.

Gradient Plate Assay—Gradient plates were prepared by the method described previously (18). Antibiotics were used at the following concentrations: 15 μg/ml kanamycin, 15 μg/ml neomycin, 0.008 μg/ml ciprofloxacin.

Asp-dependent Glucose Efflux—The quantitative assay for glucose is essentially that described previously (6). Log phase cells growing in M63 with 0.2% glycerol (6) were pelleted and washed once with buffer containing 0.1 M NaPO4, 1.0 mM MgSO4, pH 7.6. Cells were resuspended in assay buffer (0.1 M NaPO4, 7.0 mM MgSO4, 6.0 mM ATP, 0.3 units/ml hexokinase, 2.5 μg/ml glucose-6-phosphate dehydrogenase, 0.5 mg/ml NADP+, pH 7.6) to an A600 of 0.25. The assay was initiated by the addition of radioactive [1-14C]glucose to 1.0 μCi/ml and the absorbance recorded. The assay was followed. To inhibit lactose uptake by LacY, cells were preincubated in assay buffer with CCCP at 60 μM for 3.0 min before the addition of lactose. The glucose efflux activity was normalized to the β-galactosidase activity (17) in the solution.

Disruption of the set Genes and Construction of the Triple Disruption Strain—The disruption markers (19), referred to as interposons (denoted by the symbol Δ), which conferred resistance to streptomycin, chloramphenicol, or tetracycline, were inserted into setA (at the dIII site of setA), setB (at BglI), and setC (at BglI), respectively, which were cloned in pBAD18. The disrupted alleles were individually integrated into the chromosome by one of two independent methods: 1) homologous recombination following transformation with linearized DNA into a recA strain (20) and 2) cloning of the disrupted alleles into the temperature sensitive replicase plasmid pMK705 (21) to allow use of a two-step method (21) to obtain double cross-over recombinants. The disrupted alleles were confirmed both by polymerase chain reaction analysis and by F1 mapping studies. The triple mutant was assembled in both the W3110 and ML308 strain backgrounds by P1 transduction (22).

RESULTS

A study of the transport properties of the SET proteins was undertaken as a first step toward elucidating the physiological function of this newly identified family of efflux proteins. Both setA, setB, and setC were cloned downstream from the arabinose-inducible promoter, generating the plasmids pBAD-SetA, pBAD-SetB, and pBAD-SetC, respectively, and the resulting plasmids transformed into E. coli strain MC4100. Inside-out membrane vesicles were prepared from cells that hyperpressed the plasmid-encoded Set proteins. (In this configuration, an efflux pump would be expected to transport a radiolabeled substrate into the inside of the vesicle, which can be...
monitored as the accumulation of radioactivity in the vesicle interior.) It was shown previously and confirmed here that both SetA and SetB transport [14C]lactose (Fig. 1, A and B). Transport was sensitive to the addition of the protonophore CCCP, which caused the release of the accumulated radioactivity to near basal levels. The accumulation of the labeled lactose was also dependent on the presence of either ATP or NADH, which gave equivalent rates of labeled lactose or glucose and was not observed in vesicles prepared from cells that harbored the control plasmid pBAD18. Transport activity in the SetB-containing vesicles was higher than that of SetA. The SetB and more recent preparations of SetA vesicles were prepared from cells grown with increased aeration. The transport activity of recent preparations of SetA vesicles is comparable with SetB and is likely due to increased SetA protein expression (data not shown).

Fig. 1. SetA and SetB both transport [14C]lactose and [14C] glucose. A and B, [14C]lactose transport. C and D, [14C] glucose transport. Inside-out membrane vesicles were prepared from strain MC4100/pBAD-SetA (A and C, □, ■, and ▼), strain MC4100/pBAD18 (A and C, ○), and strain MC4100/pBAD-SetB (B and D) grown in the presence of arabinose. ATP was used to generate a proton gradient to energize transport. Partially filled square symbols (▼) represent transport in the absence of ATP. Uptake was initiated by the addition of radiolabeled lactose or glucose and was followed for 1 min. At the indicated time, CCCP was added to a final concentration of 80 μM (▼). Each time point represents the mean of duplicate determinations ± range.

The ability of SetA and SetB to transport [14C]glucose was also tested. In this assay, a higher concentration of [14C]glucose, which was of a lower specific activity than that of the [14C]lactose, was used. Both SetA and SetB promoted the transport of [14C]glucose, which was accumulated in the vesicle interior (Fig. 1, C and D). The accumulation of the radiolabeled glucose was also dependent on the presence of ATP, was sensitive to CCCP, and was not observed in vesicles prepared from cells that harbored the control plasmid pBAD18. We were unable to observe the transport of [14C]galactose by SetA or SetB (data not shown). In addition, vesicles prepared from the strain that harbored the setC expression plasmid was unable to transport any of the three sugars tested; however, the level of protein expressed was not determined.

Because our data from the SetA and SetB overexpression strains indicated that SetA has a broader substrate specificity than SetB (see below), an assay was developed to define the range of sugars and sugar analogues that could serve as SetA substrates. To facilitate the design of the assay, which was based on the inhibition of transport of radiolabeled lactose into inside-out vesicles, a study of the kinetic properties of lactose transport by SetA was conducted. Fig. 2A shows the kinetics of lactose transport at varying substrate concentrations. The rate of transport appears to be saturable (Fig. 2B) with an apparent \( K_m \) of 1.9 mM and \( V_{max} \) of 0.12 pmol lactose/µg protein/s (Fig. 2C). In the assay for possible substrates or inhibitors of lactose transport, the accumulation of radiolabeled lactose into inside-out vesicles was performed in the presence of unlabeled test compounds at 25 mM and [14C]lactose at 0.14 mM. (Note that this assay cannot distinguish between substrates that are effluxed by SetA and inhibitors that can compete with lactose for binding but that themselves are not transported.) The results of the competition assay are shown in Fig. 3 and are briefly summarized here. Of the tested sugars, trioses, tetroses, pentoses, and heptoses are poor inhibitors, whereas selective hexoses and disaccharides, the best being D-glucose (72% inhibi-
velocity versus 2.0 mM lactose; protein/s. M 0.50, 1.0, 2.0, and 5.0 mM [14C]lactose. Transport assayed with 0.33 mM were used for the transport studies. Transport was assayed at 0.33, time. Inside-out membrane vesicles prepared from MC4100/pBAD-SetA are plotted against the substrate concentration.

![Fig. 2](image)

**Fig. 2** Kinetic properties of SetA catalyzed [14C]lactose transport. A, SetA-dependent accumulation of [14C]lactose as a function of time. Inside-out membrane vesicles prepared from MC4100/pBAD-SetA were used for the transport studies. Transport was assayed at 0.33, 0.50, 1.0, 2.0, and 5.0 mM [14C]lactose. Transport assayed with 0.33 mM [14C]lactose in the absence of ATP is also shown. For clarity, the 5.0 mM substrate curve is omitted. Each time point represents the mean of duplicate determinations ± range. B, the relationship of the transport velocity versus substrate concentration. The slopes of the lines from A are plotted against the substrate concentration. C, Lineweaver-Burk plot of the data from A. Km = 1.9 mM, Vmax = 0.12 pmol lactose/µg protein/s. [●], 0.33 mM lactose; ○, 0.5 mM lactose; △, 1.0 mM lactose; ▲, 2.0 mM lactose; ◆, 0.33 mM lactose, no ATP.

When the alkyl substituent is a small methyl group, such as that found in the methyl-galactosides, there is a significant reduction in the inhibitory activity (methyl-α-galactoside 0%; methyl-β-galactoside 50%) in addition to a pronounced preference for a β-linkage of the aglycone to the sugar (Fig. 3D). Interestingly, α- and β-D-glucose-1-phosphate are both inactive inhibitors. The inactivity is likely due to the presence of the negative charge and not a steric effect of the phosphate group (Fig. 3D). The first position can be replaced with the larger and uncharged phenyl substituent found in phenyl-α- and phenyl-β-D-glucosides, which are both potent inhibitors (Fig. 3D).

A series of cell-based assays were designed to test whether SetA and SetB transport the substrates identified by the in vitro transport assays. A set of pBR322 derived plasmids were constructed that constitutively expressed each of the set genes by cloning them downstream from the promoter for the tetracycline resistance gene. The plasmids were transformed individually into E. coli strain W3110, which is wild type at the lac locus, and the resulting strains were each expected to hyper-express one of the Set proteins. These strains were tested for the ability of the individual Set proteins to transport either IPTG or lactose. Because IPTG and lactose are both inducers of the lac operon, the level of β-galactosidase activity should reflect the intracellular concentration of the inducer. If the inducer is effluxed from the cell by a Set protein, the level of β-galactosidase activity would be expected to be lower. Strains harboring individual set plasmids were titrated with either lactose or IPTG, and the level of β-galactosidase activity was measured. Fig. 4A shows that only SetA can efflux IPTG, because the level of β-galactosidase activity in the setA-containing plasmid was equal to that of the background at 0.1 mM IPTG. The level of β-galactosidase activity increased as the concentrations of IPTG rose from 1.0 to 100 mM, where it plateaued. This is the expected result if SetA is simply an efflux pump for the inducer. At high extracellular concentrations of the inducer, the rate of entry of the inducer exceeds the rate of efflux by SetA. In contrast, strains with either the setB or the setC plasmid, as well as the strain harboring the control plasmid pBR322, all showed high levels of β-galactosidase activity at 0.1 mM IPTG. The plateau level of β-galactosidase activity in the strain with the setB plasmid was lower than that of the control strain with pBR322, possibly reflecting the slow growth phenotype of this strain.

Fig. 4B shows that both SetA and SetB can efflux lactose. The β-galactosidase activity of the strain with the setA plasmid remained at background levels when titrated with lactose at up to 10 mM. Growth of this strain was not affected by the addition of lactose at the concentrations used. The β-galactosidase activity of the strain with the setB plasmid was equal to that of the background level at 0.1 mM lactose and was 22% of the level present in the control strain with pBR322 at 1 and 10 mM lactose. In contrast, the level of β-galactosidase activity in the strain with the setC plasmid was similar to that of the control strain with pBR322. Comparison of the lactose and IPTG titration curves indicates that IPTG is a better inducer of the lac operon, which is due to the fact that lactose is not active as an inducer until it is rearranged to allolactose by the action of β-galactosidase (23).

Because the in vitro transport studies indicated that aryl-glycosides may possibly serve as substrates for SetA, a cell-based assay was therefore designed to test this. It was previously reported that the intracellular accumulation of the toxic β-galactoside analogue, ONPTG, inhibits growth (24). Growth inhibition is dependent on the expression of lacY, because ONPTG is a substrate for the LacY permease (25). If ONPTG is also a substrate for SetA efflux, it would be expected that
strains that hyperexpress SetA would be more resistant to the sugar analogue. A strain constitutive for the lac operon (ML308) was transformed with plasmids pBAD-SetA, pBAD-SetB or the control plasmid pBAD18. Transformants were tested for resistance to ONPTG by following the growth curves of cultures in the absence or presence of arabinose; the latter condition is expected to induce expression of SetA or SetB in the corresponding transformants. In the pBAD18 transformant, the presence of ONPTG caused a decrease in the growth rate (Fig. 5A). The severity of the growth inhibition was dependent upon the concentration of ONPTG in the medium. The presence of arabinose slightly decreased the growth yield (compare Fig. 5, A and B without ONPTG) but did not change the pattern of growth inhibition seen with increasing concentrations of ONPTG (Fig. 5B). Similarly, the pBAD-SetB transformant was as sensitive to ONPTG as the control pBAD18 transformed strain in medium with or without arabinose (Fig. 5, E and F). In contrast, the growth of the pBAD-SetA transformant was uninhibited in arabinose medium at ONPTG concentrations up to 1.71 mM (Fig. 5D). However, in the absence of arabinose, this strain was as sensitive to ONPTG as the pBAD18 transformant (Fig. 5C). These results indicate that ONPTG and likely other aryl- or alkyl-β-glycosides are substrates for SetA efflux.

It was shown above with the in vitro SetA lactose transport assay that positively charged sugars such as glucosamine and aminophenyl-glucosides are good inhibitors of lactose accumulation (Fig. 3, B and D). This feature is reminiscent of the aminoglycoside family of antibiotics such as kanamycin and neomycin. A cell-based assay was used to determine whether kanamycin and neomycin are substrates for SetA efflux, which would become apparent as resistance to the antibiotics. An E. coli strain deleted for acrAB, which encodes a multiple antibiotic efflux pump, was transformed with either plasmid pBAD-SetA or control plasmid pBAD18. The resulting transformants were tested on antibiotic gradient plates with or without arabinose. Again, arabinose is expected to induce expression of setA in arabinose-containing medium, the strain with the setA plasmid was only slightly more resistant to kanamycin (31% growth across the gradient plate for pBAD-SetA versus 25% growth for pBAD18) and neomycin (63% growth for pBAD-SetA versus 53% growth for pBAD18) than the control strain with pBAD18 (Fig. 6). No difference in resistance to the tested antibiotics was observed in the absence of arabinose. As an additional control, both strains with either pBAD-SetA or pBAD18 showed identical sensitivity to the fluoroquinolone ciprofloxacin with or without arabinose in the medium. The data suggest that kanamycin and neomycin are poor substrates for SetA efflux.

It can be concluded from the in vitro and cell-based transport assays that SetA and SetB each can efflux glucose and lactose. Because the secretion of glucose was observed in the metabolism of lactose (6), the question emerged as to whether the set genes are involved in lactose utilization. To answer this question, all of the set genes (setA, setB, and setC) were disrupted in E. coli strains W3110 and ML308 by the insertion of different antibiotic resistance cassettes (see “Materials and Methods”). The mutant alleles were confirmed by polymerase chain reaction as well as by P1 mapping (data not shown). The growth rate of the triple mutant was identical to the parental strain in LB or minimal medium with lactose, glucose, glycerol, succinate, or lactate as the sole carbon source (data not shown). Also, the addition of lactose to log phase cells that were previously growing on glycerol did not cause any noticeable growth inhibition in the triple mutant compared with the parental strain. The triple mutant in the ML308 genetic background was further tested for glucose efflux in the presence of lactose. In this assay, washed log phase cells were placed in the coupled assay solution, which contained the enzymes hexokinase and glucose-6-phosphate dehydrogenase. The appearance of glucose was monitored as an increase in the absorbance at 340 nm because of the accumulation of NADPH as one of the final products of the sequential action of the two enzymes on glucose. In both the triple mutant and the parental strain, the addition of lactose led to the immediate appearance of glucose in the medium at a rate that reached steady state within 1.5 min.
(data not shown). No difference in the steady state rate of glucose efflux between the triple mutant and parental strains was observed (Fig. 7). As a control to show that the detected glucose was due to its secretion from the cell and not to the action of free β-galactosidase in the medium on extracellular lactose, preincubation of both the triple mutant and parental strains with the protonophore CCCP at 60 μM completely abolished the signal (Fig. 7). The complete inhibition of glucose efflux by treatment with CCCP indicated that there was no free β-galactosidase in the medium.

SetA and SetB can secrete inducers of the lac operon. The presence of efflux proteins for lactose or IPTG would be expected to lower the intracellular concentration of the inducer. To determine whether the triple mutant is more sensitive than the parental strain to induction of the lac operon by lactose or IPTG, the triple mutant in the W3110 strain background, which is wild type for the lac operon, along with the parental strain were titrated with IPTG or lactose and the levels of β-galactosidase activity in the cells after 1 h of exposure to the inducer was measured. No difference was observed between the triple mutant and the parental strain in this set of experiments (Figs. 8, A and B). Given that the setA hyperexpressing strain does not respond at all to lactose and is desensitized to IPTG, this finding suggested that the level of SetA, and possibly SetB, is low in wild type cells. In an effort to employ a more sensitive assay for SetA activity, the kinetics of lac operon induction at low concentrations of IPTG (2.5 and 5.0 mM) were examined in both the triple mutant and the parental strain. It was shown previously that induction of the lac operon, as measured by the level of β-galactosidase activity, is bi-phasic at low concentrations of IPTG (14). This is manifest as a lag in the rate of synthesis of β-galactosidase for 2–3 h before a high steady state rate is attained. This bi-phasic behavior is dependent on the lacY gene and can be explained as a positive feedback loop; leaky expression of the lac operon results in a low basal level of LacY permease activity, which catalyzes the uptake of the inducer IPTG, leading to higher rates of synthesis of the lac operon. If the SetA protein is present at an appreciable amount in the wild type cell, the triple mutant might be expected to
reach the high steady state rate of β-galactosidase synthesis sooner, because there would be no efflux protein to counteract the initial low levels of LacY. As shown in Fig. 8C, the kinetics of lac operon induction, with 2.5 and 5.0 μM IPTG, is the same for both the triple mutant and the parental strain. This result further supports the hypothesis that the expression of setA is low in the wild type cell. This conclusion is also consistent with our observation that the triple mutant is not more sensitive than the parental strain to growth inhibition by toxic sugar analogues such as IPTG, ONPTG, or methyl-α-glucoside (data not shown).

**DISCUSSION**

Biochemical characterization of the newly discovered SET proteins showed that SetA and SetB can transport selective monosaccharides and disaccharides. Using an inside-out vesicle system, glucose and lactose were demonstrated to be substrates for SetA and SetB efflux. In addition, as judged by the set of substrates considered here, SetA has broader substrate specificity than SetB. SetA also transports glucosides and galactosides with alkyl or aryl substituents. The results of these in vitro transport studies were confirmed using a series of cell-based assays for transport. Overexpression of SetA or SetB desensitized cells to the induction of the lac operon with lactose as the inducer but only SetA overexpression desensitized cells to IPTG. Transport of aryl-β-glycosides by SetA was confirmed by a growth sensitivity assay, because cells that overexpress SetA were more resistant to the toxic sugar analogue ONPTG. Although vesicles or cells with the setC expression constructs were unable to transport glucose, galactose, or lactose, the high degree of protein sequence identity of SetC to SetB (70%) suggests that the function is also conserved. It remains possible that setC may be poorly expressed in our plasmid constructs. However, each of the set genes, when present on a high copy number plasmid and expressed from the strong lac promoter, inhibited growth when induced with IPTG (data not shown), suggesting that a protein product was expressed in all cases. Additional transport studies with other sugars are warranted to identify substrates for SetC.

The range of substrates that are transported by SetA and SetB suggested possible roles for the SET proteins in lactose metabolism and in the secretion of nonmetabolizable sugar analogues. However, a mutant disrupted in all three set genes
showed no defect in lactose utilization; growth was normal in minimal medium supplemented with lactose as the sole carbon source, and glucose efflux was normal. In addition, no increase in sensitivity to the sugar analogues IPTG, methyl-β-D-thigalactoside, and ONPTG was observed. These results indicate that other transporters, possibly one of the newly identified \( E. coli \) members of the SET family,\(^3\) are responsible for the observed efflux of glucose in wild type cells. Interestingly, it was recently reported that the \( E. coli \) multidrug efflux pump \( cmlA \) may have IPTG efflux activity (26).

Our results suggest that the \( set \) genes are poorly expressed in \( E. coli \) under normal laboratory growth conditions. Consistent with this hypothesis, the triple \( setABC \) mutant has no observable phenotype. Because the expression of many bacterial efflux systems are induced by their substrates (3), it is likely that the true inducers or inducing conditions for the \( set \) genes have yet to be found. It is quite possible that the \( set \) genes function in a protective role, because hyperexpression of \( SetA \) was shown to protect cells against ONPTG. However, conditions that induce bacterial gene expression are frequently not obvious. Operons that were once thought to be poorly expressed (e.g. the \( β\)-glucoside operon (27, 28) and the chitose operon (29)) have been shown to be induced under conditions that were not apparent when these systems were first discovered. Indeed, methyl-β-glucoside and methyl-α-glucoside can modestly induce (about 4-fold) \( setA \) expression.\(^3\)

The growth inhibitory effects of toxic sugar analogues may result from either the inhibition of uptake of a \textit{bona fide} sugar substrate or the interference with normal metabolism by the intracellularly accumulated sugar analogues. The effectiveness of an efflux system to protect the cell from a toxic sugar analogue would be dependent on the rate of entry of the analogue into the cell and the activity of the efflux system. A sugar analogue that would otherwise be poisonous but have no pathway to enter the cell is harmless. As exemplified by ONPTG, sensitivity is dependent on the expression of \( lacY \), the transporter for \( β\)-galactosides (24, 25). On the other hand, it would be difficult to protect a cell from a toxic sugar analogue that enters the cell through a highly efficient and constitutively expressed uptake system. It is likely that the physiological substrates for \( Set \) protein efflux would also be expected to be substrates for \( E. coli \) uptake systems. Under this scenario, the role of the \( Set \) proteins would be to secrete toxic sugar analogues that are taken up by mistake. However, it remains possible that the \( Set \) proteins function to remove toxic sugar-like by products that are produced by normal \( E. coli \) metabolism.

High level expression of \( SetA \) alone would be expected to be sufficient to protect cells from selective sugar analogues that are not modified upon entry. Among the known sugar transporters in bacteria, one family of transporters, the PTS (phosphoenolpyruvate-sugar phosphotransferase system) family, modifies the sugars upon entry by the addition of a phosphate group (30, 31). Our biochemical studies showed that negatively charged sugars are not substrates for \( SetA \) efflux. This is reminiscent of an unidentified transporter for the removal of MG (9, 11, 12). In the case of MG, entry into the cells is mainly through \( ptsG \) and \( ptsM \), the PTS uptake proteins for glucose and mannose, respectively (13). MG accumulates in the cell predominately in the phosphorylated form as MG-6-phosphate (9). Presumably a phosphatase converts MG-6-phosphate to MG before secretion by the unidentified transporter. This detoxification system would rely on the unidentified phosphatase to discriminate between the phosphorylated sugar analogue and the \textit{bona fide} phosphorylated sugar, because only the uncharged sugar would be a substrate for efflux. We have not tested the \( set \) mutants for defects in MG secretion, although MG inhibits SET catalyzed \textit{in vitro} lactose transport (data not shown). However, the triple \( set \) mutant was not more sensitive to the growth inhibitory effect of MG as compared with the parental strain (data not shown).

The elucidation of the physiological function of the \( set \) genes would depend on finding growth conditions where the \( set \) mutants display a defective phenotype. Because the data suggest that the \( set \) genes are poorly expressed under normal laboratory growth conditions, a first step would be to identify conditions that induce \( set \) gene expression. Reporter strains have been constructed that carry chromosomal \( set \) fusions to \( lacZ \) or other reporter enzymes to screen for conditions or sugars that induce \( set \) gene expression. It may also be possible to design a genetic selection for chromosomal mutants that are up-regulated for \( setA \) expression based on its glycoside (such as IPTG) efflux activity.

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\(^3\) J. Y. Liu, unpublished observations.