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Escherichia coli Gene *ydeA* Encodes a Major Facilitator Pump Which Exports L-Arabinose and Isopropyl-β-D-Thiogalactopyranoside

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Inactivation of the *Escherichia coli* gene *ydeA*, which encodes a member of the major facilitator superfamily, decreased the efflux of L-arabinose, thereby affecting the expression of AraC-regulated genes. In addition, overexpression of *ydeA* decreased the expression of genes regulated by isopropyl-β-D-thiogalactopyranoside.

The major facilitator superfamily (MFS) is the main class of proteins that utilize protons as symporters or antiporters to transport a variety of molecules across the cytoplasmic membrane of bacteria and eukaryotes. Some MSF members, such as TetA, responsible for resistance to tetracycline, have a narrow substrate specificity. Others, such as EmrB or Bmr, can exclude from the cells a wider range of organic molecules (10, 13). The complete sequencing of bacterial genomes has revealed a large number of genes belonging to the MFS. In *Escherichia coli*, 64 such genes can be identified on the basis of sequence similarities (14), and the exact number may be somewhat higher (13). A majority of these genes were identified in the course of sequence annotation, and their transport capabilities have not been established yet. Here we report that an MFS transporter encoded by a gene in the terminus region of the *Escherichia coli* chromosome, *ydeA*, excludes L-arabinose (L-Ara) and isopropyl-β-D-thiogalactopyranoside (IPTG).

The bacterial strains used in this study are listed in Table 1.

The *yihA* gene codes for an essential GTPase (1). We have constructed a strain, JS1855 (Table 1), which expresses *yihA* from the L-Ara-regulated P_{BAD} promoter on a low-copy-number plasmid. Since JS1855 is deleted for the *ara-leu* region, *yihA* expression can be regulated by adjusting the extracellular L-Ara concentration. Strain JS1855 grew normally with 1 mg of L-Ara/ml or more but very slowly with 20 μg (133 μM) of L-Ara/ml. To isolate suppressors of this growth deficiency, we mutagenized JS1855 with mini-*tet* by infection with phage λ1098 (19) as described previously (12) and plated appropriate dilutions on Luria agar plates containing 20 μg of L-Ara/ml and 15 μg of tetracycline/ml. Fast-growing colonies were isolated, and this phenotype was ascertained by transducing the mini-*tet*-containing mutation into JS1855 and verifying the ability of the tetracycline-resistant transductants to grow rapidly with 20 μg of L-Ara/ml. DNA from appropriate clones was digested with the frequently cutting enzymes *Apo*I, *Bsa*HI, or *Hae*II, ligated, and subjected to reverse PCR by using primers MTL (AATAATCCAAATCCAGCCATCCC) and MTR (GATAA AAGGCACCTTTGGTCAACC). Then, the chromosomal DNA sequence at the junction with the mini-*tet* was established by using amplified DNA and MTR as template and primer, respectively. Two mini-*tet* insertions were within the same gene, *ydeA*. Insertion no. 4 was located before nucleotide 580 of the *ydeA* coding sequence (1615632 of the *E. coli* K-12 sequence, version M52), with *tetA* in the same orientation as the disrupted gene. Insertion no. 11 was after nucleotide 1115 (1616167 of genome sequence) and had the opposite orientation (Fig. 1).

Gene *ydeA* codes for a 396-amino-acid protein with 12 predicted transmembrane-spanning segments and closely resembles known MFS proteins, such as the chloramphenicol exclusion protein CmlR of *Streptomyces lividans*. Return to fast growth in the presence of a low extracellular L-Ara concentration might be explained by a higher intracellular L-Ara content resulting from *ydeA* disruption. To test this possibility, we determined the effect of the mutation on the sensitivity of P_{BAD} to added L-Ara. The *ydeA*::mini-*tet* no. 4 mutation was transduced into the Δ*lac* strain JS219, yielding JS1910. Then JS219 and JS1910 were transformed with pDAG92, a pBAD18 (7) derivative carrying *lacZ* under P_{BAD} control. Steady-state levels of β-galactosidase were measured in cultures grown in Luria broth containing high or low levels of L-Ara. In the presence of 2 mg of L-Ara/ml, β-galactosidase specific activity (in units/optical density at 600 nm [OD_{600}]) was essentially the same for both strains (21,000 and 23,000 U of JS219 and JS1910, respectively). In sharp contrast, the disruption strain expressed P_{BAD}-*lacZ* at a much higher rate (15,000 U) than the parent strain (2,400 U) in the presence of 5 μg (33 μM) of L-Ara/ml. This suggested that the disruption strain retained more intracellular arabinose than the wild-type parent.

To establish directly that YdeA expels L-arabinose from the cells, accumulation and efflux of L-Ara were determined in strains JS219 (*ydeA*$^+$) and JS1910 (*ydeA*::mini*tet*) (Fig. 2). The concentration of L-Ara (5 μM) used in these experiments was such that only the ATP-dependent high-affinity AraFGH system could contribute significantly to L-Ara uptake (9). As expected, accumulation of L-Ara was higher in the *ydeA* mutant than in the wild-type parent. Since the strains used lack *araC* and are therefore not L-Ara inducible, an indirect interference of YdeA with L-Ara uptake capabilities can be ruled out. In addition, the initial rate of efflux, 14.3%/min at 25°C, was higher in the wild type than in the mutant (6.2%/min) (Fig. 2). The efflux data strongly favor the hypothesis that YdeA promotes L-Ara export.

We have previously reported the inhibitory effect on cell division of a Φ(*malE-minE*) fusion gene overexpressed from the P_{TAC} promoter (15) and the isolation of multicopy suppressors of this effect (15, 16). Two suppressor plasmids, pMesBA5 and pMesBA24, were localized with respect to the Kohara library of ordered phage recombinants (8), as described for pMesJE11 (16), and aligned to Kohara's map by

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TABLE 1. Bacterial strains

Designation	Relevant genotype	Source
JS219	*araD* Δ(*ara-leu*) Δ(*lacIZYA*)*X74 malPp*::*lacI*^{q}	Our collection
JS1855	JS219 Δ*yihA*::*cat*/p(*araC*$^+$ P_{BAD}-*yihA*)	Our collection
JS1908	JS1855 *ydeA*::mini-*tet* no. 4	This work
JS1909	JS1855 *ydeA*::mini-*tet* no. 11	This work
JS1910	JS219 *ydeA*::mini-*tet* no. 4	This work
JS1921	JS219/pJPB274	This work

restriction analysis (Fig. 1). A *Pst*I deletion derivative of pMesBA5, pJPB274 (Fig. 1), still suppressed the filamentation phenotype of overexpressed Φ(*malE-minE*). The only complete gene contained in the plasmid insert was *ydeA*. *YdeA* is located distally in an operon also containing *yneJ* (a member of the *lysR* family) and *yneK*, of unknown function. In the pMesBA plasmids, transcription of *ydeA* may occur from the 5′ end of the operon and from a weak promoter in the *yneK-ydeA* intergenic region (3). In pJPB274, transcription can take place from the *ydeA* promoter and from resistance gene *aadA* in the vector.

The results indicating that *ydeA* excludes L-Ara prompted us to determine whether the suppression of *lac*-dependent filamentation by Φ(*malE-minE*) could be due to inducer exclusion. To test this possibility, strain JS219 was transformed with pJPB274, resulting in JS1921. Then, strains JS219 and JS1921, which express *ydeA* at normal and elevated levels, respectively, as well as JS1910 (*ydeA*::mini-*tet*) were transformed with pMLB1115, a pBR322 derivative carrying *lacI*^{q}, the *lac* regulatory region, and *lacZ* (5). The strains were compared for *lacZ* induction in the presence of different extracellular concentrations of IPTG (Table 2). The presence of the *ydeA*-overexpressing plasmid led to a significant reduction in *lac* activity at low or intermediate concentrations of inducer. These data suggest that *ydeA* is capable of also excluding IPTG and that this accounts for the MalE-MinE suppression phenotype. However, this activity seems to be weak compared to L-Ara exclusion, since no IPTG exclusion effect was observed with the wild-type strain compared to the *ydeA* deletion strain (Table 2).

Comparisons of sequences (13) and of hydropathy profiles (11) have revealed that YdeA belongs to a defined subfamily among the 12-transmembrane segment (12-TMS) efflux proteins. Paulsen et al. (13) defined it as subgroup d, which includes *S. lividans cml*, *Pseudomonas aeruginosa cmlB* and *opdE*, *Bacillus subtilis ywfA*, and *Synechocystis* sp. open reading frame Slr0616 gene products. Lolkema and Slotboom (11) proposed a subfamily including the products of *araJ*, *ydhP*, *ydeA*, and *yicM* of *E. coli* and of *ybcL*, *ydhL*, *ytbD*, and *yfhI* of *B. subtilis*. A BLAST search with YdeA as the query sequence indicated

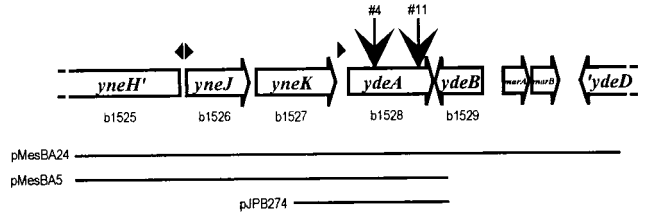

FIG. 1. Map of the chromosomal region carried by suppressing plasmids and location of mini-*tet* insertions. Gene b numbers (1a) are indicated below four-letter designations (18). Relevant putative promoters are shown above intergenic spaces. Mini-*tet* insertions in *ydeA* are indicated by vertical arrows. The size of the pMESBA24 insert is 7,460 bp.

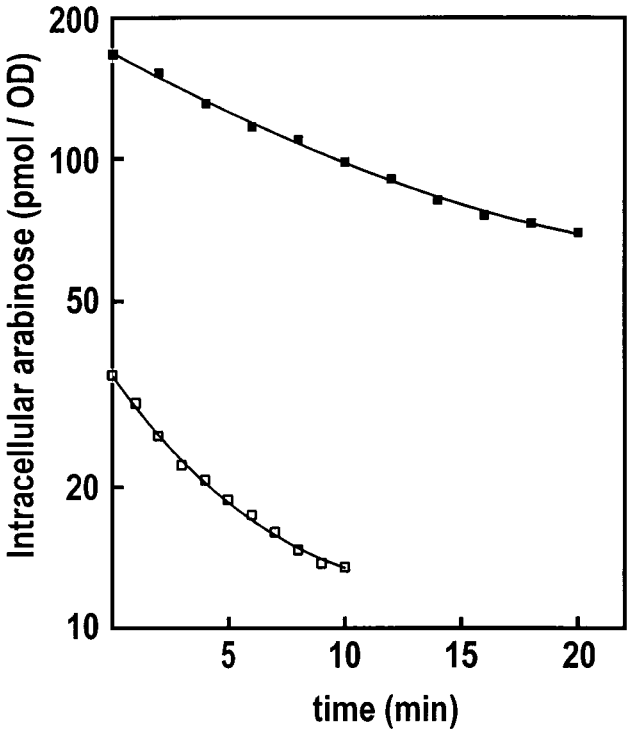

FIG. 2. Arabinose efflux in *ydeA*$^+$ and *ydeA*::mini-*tet* strains. Strains JS219 (*ydeA*$^+$) and JS1910 (*ydeA*::mini-*tet*) were grown in M9 medium supplemented with 1 μg of thiamine/ml, 0.2% Casamino Acids, and 1% glycerol until the OD_{600} was nearly 0.6. Cultures (5 ml) were transferred to 25°C, and ^{3}H-labeled L-Ara (5 μM [0.2 Ci/mmol]; Moravek Radiochemicals) was added for 15 min. The cells were filtered through a 48-mm-diameter 0.45-μm HAWP Millipore filter and washed briefly with and resuspended into the same volume of Ara-free medium. Then, 0.2-ml samples were taken at 1-min (JS219) or 2-min (JS1910) intervals, filtered through 25-mm-diameter Millipore filters, washed, and counted. Open squares, JS219; closed squares, JS1910.

that except for Slr0616, all these proteins have Expect values (number of expected matches by chance) of $<10^{-20}$.

Only a few proteins of this group have a known function. *cml* and *cmlA* confer resistance to chloramphenicol. We compared the wild-type, deletion, and YdeA-overproducing strains for their resistance to chloramphenicol. No difference was observed. Another gene of the subfamily, *araJ*, is positively regulated by *araC* and arabinose. Reeder and Schleif proposed that AraJ might be a transporter for arabinose-containing oligosaccharides (17). The resemblance of the *ydeA* gene product to AraJ suggested that the actual substrates for YdeA might be arabinose-containing antibiotics. To examine this possibility, strains JS1910, JS219, and JS1921 were transformed with plasmid pUT-DCK, a pBR322 derivative containing the gene for

TABLE 2. Induction of the *lac* promoter in different *ydeA* contexts

Strain and *ydeA* status	LacZ activity (U/OD_{600}) with indicated IPTG concn (μM)[a]		
	10	50	2,000
JS1910/pMLB1115 *ydeA*::mini-*tet*	30	439	7,854
JS219/pMLB1115 *ydeA*$^+$	37	602	9,325
JS219/pJPB274/pMLB1115 *ydeA*$^+$; p(*ydeA*$^+$)	8	70	5,772

[a] Values are the averages of two independent experiments.

human deoxycytidine kinase constitutively expressed from a strong synthetic promoter. This allowed phosphorylation of cytidine derivatives and made the cells sensitive to cytosine arabinoside (cytarabin, Ara-C) (4). Then the three derived strains were compared for susceptibility to Ara-C in Luria broth. In each case, the transition between normal cell morphology and a mixture of normal cells and filaments appeared between 10 and 20 μM Ara-C. Therefore, *ydeA* does not appear to confer resistance to arabinosyl nucleoside compounds.

Recently, Bohn and Bouloc reported that another MFS gene, *cmlA*/*mdfA*, was also capable of expelling IPTG from the cells (2). *cmlA* was first shown to confer resistance to chloramphenicol in *E. coli* and then to a variety of drugs, such as ethidium bromide, tetraphenylphosphonium, rhodamine, daunomycin, and puromycin (6). These findings suggest that the substrates most efficiently transported by YdeA may have only a remote relationship with the two compounds identified in this study.

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ADDENDUM

After this article was submitted for publication, Bost et al. (3) reported that YdeA interferes with the accumulation of L-Ara and thus with the induction of the P_{BAD} promoter.

REFERENCES

1. **Arigoni, F., F. Talabot, M. Peitsch, M. D. Edgerton, E. Meldrum, E. Allet, R. Fish, T. Jamotte, M. L. Curchod, and H. Loferer.** 1998. A genome-based approach for the identification of essential bacterial genes. Nat. Biotech. **16:**851–856.

1a. **Blattner, F. R.** 2 September 1997, revision date. Escherichia coli K-12 sequence, version M52. Department of Genetics, University of Wisconsin-Madison. http://www.genetics. wisc. edu. [25 June 1999, last accessed.]

2. **Bohn, C., and P. Bouloc.** 1998. The *Escherichia coli clmA* gene encodes the multidrug efflux pump Cmr/MdfA and is responsible for isopropyl-β-D-thiogalactopyranoside exclusion and spectinomycin sensitivity. J. Bacteriol. **180:** 6072–6075.

3. **Bost, S., F. Silva, and D. Belin.** 1999. Transcriptional activation of *ydeA*, which encodes a member of the major facilitator superfamily, interferes with arabinose accumulation and induction of the *Escherichia coli* arabinose P_{BAD} promoter. J. Bacteriol. **181:**2185–2191.

4. **Bouzon, M., and P. Marlière.** 1997. Human deoxycytidine kinase as a conditional mutator in *Escherichia coli*. C. R. Acad. Sci. III **320:**427–434.

5. **de Boer, P. A. J., R. E. Crossley, and L. I. Rothfield.** 1989. A division inhibitor and a topological specificity factor coded by the minicell locus determine proper placement of the division septum in *E. coli*. Cell **56:**641–649.

6. **Edgar, R., and E. Bibi.** 1997. MdfA, an *Escherichia coli* multidrug resistance protein with an extraordinary broad spectrum of drug recognition. J. Bacteriol. **179:**2274–2280.

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

8. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:**495–508.

9. **Kolodrubetz, D., and R. Schleif.** 1981. L-arabinose transport systems in *Escherichia coli*. J. Bacteriol. **148:**472–479.

10. **Lewis, K.** 1994. Multidrug resistance in bacteria: variations on a theme. Trends Biochem. Sci. **19:**119–123.

11. **Lolkema, J. S., and D.-J. Slotboom.** 1998. Hydropathy profile alignment: a tool to search for structural homologues of membrane proteins. FEMS Microbiol. Rev. **22:**305–322.

12. **Miller, J.** 1992. A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

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

14. **Paulsen, I. T., M. K. Sliwinski, and M. H. Saier, Jr.** 1998. Microbial genome analyses: global comparisons of transport capabilities based on phylogenies, bioenergetics and substrate specificities. J. Mol. Biol. **277:**573–592.

15. **Pichoff, S., B. Vollrath, and J.-P. Bouché.** 1997. MinCD-independent inhibition of cell division by a protein that fuses MalE to the topological specificity factor MinE. J. Bacteriol. **179:**4616–4619.

16. **Pichoff, S., L. Alibaud, A. Guédant, M. P. Castagné, and J. P. Bouché.** 1998. An *E. coli* gene (*yaeO*) suppresses temperature-sensitive mutations in essential genes by modulating Rho-dependent transcription termination. Mol. Microbiol. **29:**859–870.

17. **Reeder, T., and R. Schleif.** 1991. Mapping, sequence, and apparent lack of function of *araJ*, a gene of the *Escherichia coli* arabinose regulon. J. Bacteriol. **173:**7765–7771.

18. **Rudd, K. E.** 1998. Linkage map of *Escherichia coli* K-12, edition 10: the physical map. Microbiol. Mol. Biol. Rev. **62:**985–1019.

19. **Way, J. C., M. A. Davis, D. Morisato, D. E. Roberts, and N. Kleckner.** 1984. New Tn*10* derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. Gene **32:**369–379.