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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

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Induction of genes expressed from the arabinose P_{BAD} promoter is very rapid and maximal at low arabinose concentrations. We describe here two mutations that interfere with the expression of genes cloned under arabinose control. Both mutations map to the *ydeA* promoter and stimulate *ydeA* transcription; overexpression of YdeA from a multicopy plasmid confers the same phenotype. One mutation is a large deletion that creates a more efficient −35 region (ATCACA changed to TTCACA), whereas the other affects the initiation site (TTTT changed to TGTT). The *ydeA* gene is expressed at extremely low levels in exponentially growing wild-type cells and is not induced by arabinose. Disruption of *ydeA* has no detectable effect on cell growth. Thus, *ydeA* appears to be nonessential under usual laboratory growth conditions. The *ydeA* gene encodes a membrane protein with 12 putative transmembrane segments. YdeA belongs to the largest family of bacterial secondary active transporters, the major facilitator superfamily, which includes antibiotic resistance exporters, Lac permease, and the nonessential AraJ protein. Intracellular accumulation of arabinose is strongly decreased in mutant strains overexpressing YdeA, suggesting that YdeA facilitates arabinose export. Consistent with this interpretation, very high arabinose concentrations can compensate for the negative effect of *ydeA* transcriptional activation. Our studies (i) indicate that YdeA, when transcriptionally activated, contributes to the control of the arabinose regulon and (ii) demonstrate a new way to modulate the kinetics of induction of cloned genes.

The arabinose regulon of *Escherichia coli* consists of five operons scattered around the chromosome. AraC is the major transcriptional regulator of the regulon. AraC positively regulates transcription of the four other operons in the presence of arabinose and represses transcription in its absence (34, 35). Transcription of these operons is sensitive to catabolite repression and requires cyclic AMP and the catabolite repressor protein CRP. The interplay of these two transcriptional activators and the positions of their binding sites are slightly different for each promoter (10, 36). The *araBAD* operon encodes the three enzymes necessary for arabinose metabolism. The *araE* and *araFGH* operons encode two transport systems (14, 20, 21). AraE is a low-affinity sugar:proton symporter (23), while the periplasmic binding protein AraF and the two membrane proteins AraG and AraH constitute a high-affinity transport system (14, 15). All mutations which affect growth on arabinose as a carbon source or expression of the *araBAD* operon map to these eight genes.

A genetic search for arabinose-inducible promoters identified a fifth operon, which maps at 9 min and is now called the *araJ* operon (10, 22). The *araJ* gene encodes a nonessential membrane protein of unknown function (31). Disruption of *araJ* had no visible effect on growth in minimal arabinose medium, whether arabinose uptake was mediated by AraE or AraFGH. Furthermore, the kinetics of P_{BAD} induction were similar in wild-type and Δ*araJ* strains, indicating that AraJ is not involved in arabinose regulation. It has been proposed that AraJ can participate in the transport or processing of arabinose polymers, which are abundant nutrients in nature (31). When the sequence of AraJ became available, no homologs were detected in the databases. It is now known that AraJ belongs to a large class of multidrug resistance translocators (7), and in particular to the major facilitator superfamily (MFS), which includes AraE (24, 28, 29). These proteins have 12 transmembrane segments, and a number of them have been shown to export antibiotics and other small molecules (6, 7, 28).

The properties of the arabinose regulon have led to the development of a family of expression plasmids that are extensively used for physiological studies of null mutations in essential genes (9). These vectors encode the positive and negative regulator AraC, and they contain the intergenic control region and the P_{BAD} promoter. A number of features of the arabinose regulon contribute to the versatility of these vectors. Expression in the absence of inducer can be kept to very low levels in the presence of glucose, because of the repressor activity of AraC and the reduced concentration of cyclic AMP, allowing for the cloning of toxic genes. Expression levels can be modulated over a 1,000-fold range, and they are different in rich versus minimal medium. Finally, the kinetics of induction is very rapid, and the kinetics of repression upon removal of arabinose depends on the host Ara phenotype (9).

We have taken advantage of these properties to clone in pBAD24 a chimeric protein in which the signal sequence of a mammalian protein was fused to the mature portion of alkaline phosphatase (AP) (4). This chimeric protein is exported to some extent, but its expression is toxic when the P_{BAD} promoter is fully induced. We have shown that most suppressors of this toxic phenotype map to known *sec* genes, have a weak

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Sec phenotype, and selectively slow down export of the toxic protein (4). We report here the characterization of two suppressor mutations that do not directly affect protein export but interfere with induction of the P_{BAD} promoter by arabinose. These experiments led to the characterization of YdeA, a membrane protein that is homologous to AraJ and that interferes with the intracellular accumulation of arabinose.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* strains used in this study were DHB3 [F$^-$ *araD139* Δ(*ara-leu*)*7696* Δ(*lac*)*X74 rpsL150 galU galK thi malF*Δ*3 phoA*Δ(*Pvu*II) *phoR*] (5), SB0 (DHB3/pBAD72K) (4), JCB433 (MC1000 *recD1903*::mini-Tn*10*) (32; obtained from J. Bardwell), DB519 (JCB433/pSB13), and AD126 [F′ *lacI*^{q} Tn*5*/Δ*atpBC ilv*::Tn*10 malBF13 malT*(Con) *arg his*] (obtained from M. Ehrmann). Plasmid pBAD72K (4) is a derivative of pBAD18 (9) in which the sequence coding for the first 52 amino acids of murine PAI2 (2) was fused to a derivative of Tn*phoA* (8); the chimeric protein is expressed from the arabinose P_{BAD} promoter. A kanamycin resistance (Kan^r) cassette (from pUC4Kn; Pharmacia) was inserted downstream of the TnPhoA sequence. Suppressor strains are derivatives of SB0 isolated on NZ plates supplemented with 0.2% arabinose and 40 μg of 5-bromo-4-chloro-3-indolyl phosphate per ml (4). SB1 contains a suppressor of spontaneous origin, whereas SB34 was isolated after UV mutagenesis (26). Strains SB01 and SB034 are derivatives of SB0 in which the suppressor mutations of SB1 and SB34 were cotransduced with the *zdf-1*::Tn*10* transposon (see below). Arabinose uptake was measured in strains DB529, DB530, and DB533, derivatives of DHB3 containing the *zdf-1*::Tn*10* transposon and pBAD24 (9); DB529 contains the suppressor mutation of SB1, DB530 is *ydeA*$^+$, and DB533 contains the suppressor mutation of SB34. These strains are phenotypically Ara$^-$, although they express AraC from the plasmid.

Tn*10* transposons were linked to the suppressor mutations by infection with λ mini-Tet 1098 (37) and repeated cycles of P1 transduction and selection on plates containing arabinose and tetracycline. The closest Tn10 (*zdf-1*::Tn*10*) was 75% linked to the suppressor mutations of SB1 and SB34. This transposon was localized after PCR amplification of the chromosomal DNA directly flanking the IS*10* sequences (13). The amplified fragment was hybridized to a membrane on which an ordered library of genomic clones had been immobilized (19) (purchased from Takara Shuzo, Tokyo, Japan, through ITC Biotech, Heidelberg, Germany). The probe hybridized to Kohara's phages 306 and 307, at approximately 35.2 min on the *E. coli* chromosome.

AP assays. AP enzymatic activities were measured by determining the rate of *p*-nitrophenyl phosphate hydrolysis (25). Cultures grown in NZ medium to early log phase were induced with arabinose and collected on ice at the indicated times in the presence of 2 mM iodoacetamide.

RNA isolation, cRNA probes, and hybridizations. RNAs were isolated from cultures grown in NZ or LB medium to early log phase as described elsewhere (3, 30). The RNAs were digested with RNase-free DNase (Promega), and RNA integrity was verified by gel electrophoresis and Northern blot hybridization. cRNA synthesis, Northern blot hybridization, and RNase protection were performed as described elsewhere (1).

To make the PAI2 probe, a 250-bp *Kpn*I-*Bam*HI fragment from pBAD72K was subcloned into the cognate sites of pBSKS. The plasmid was linearized with *Sac*I and transcribed with T3 RNA polymerase in the presence of 50 to 100 μM unlabeled UTP and 10 to 50 μCi of [^{32}P]UTP. To make the *ydeA* probe, a 950-bp fragment of SB1 chromosomal DNA was PCR amplified with primers pSB1up (5′GATCACATTCTCAAGACGC) and pSB1don2 (5′GGCATGAGTGGTT GC) and digested with *Bcl*I and *Nsi*I; a 387-bp fragment, which is identical in wild-type and SB1 DNAs, was subcloned between the *Bam*HI and *Pst*I sites of pBSKS. The plasmid was linearized with *Eco*RI and transcribed with T7 RNA polymerase as described above. High-specific-activity probes were synthesized in the presence of 25 μM unlabeled UTP and 50 μCi of [^{32}P]UTP.

Cloning of *ydeA* and sequence of the suppressor mutations. The suppressors strains were crossed with F′ strains containing the episomes F500 and F506 (26), in which a Tn*10* was introduced by P1 transduction. All Tet^r Kan^r exconjugants were Ara^r, indicating that the suppressor mutations in SB1 and SB34 are dominant. DNA from the SB1 suppressor strain was partially digested with *Sau*3AI and fractionated on a 10 to 40% sucrose gradient, and fragments in the 4- to 10-kbp range were cloned into the *Bam*HI site of pACYC184. The library was screened for multicopy dominant inserts conferring the Ara^r phenotype to the parental strain (SB0), and four plasmids with overlapping inserts were isolated. A large 12-kbp insert (pSB11) was reduced to a 1.8-kbp insert (pSB13; up to the *Bsp*HI site in *ydeB*) and to a minimal 1.3-kbp insert (pSB16; up to the *Bsg*I site between *ydeA* and *ydeB*), both of which conferred the suppressor phenotype of the SB1 strain. DNA sequencing was performed with Sequenase version 2.0 (U.S. Biochemical).

Construction of a *ydeA* null allele. Plasmid pDB9722 is derived from pUC19 and contains a 1.3-kbp *Ava*I-*Nsi*I fragment derived from Kohara phage 304 (19), a Kan^r cassette (from pUC4Kn; Pharmacia), and a 1.3-kbp *Msc*I-*Sal*I fragment derived from plasmid pSB11. The left end of the deletion is located between the −35 and −10 regions of the *ydeA* promoter, and the right end is located 27 bp upstream of the *ydeA* UAG stop codon. The replacement cassette was excised with *Sal*I and *Kpn*I and electroporated into strains JCB433 and DB519, which express *ydeA* from pSB13. Kan^r recombinants were obtained with both strains, suggesting that *ydeA* is nonessential. The disruption was verified by Southern blot hybridization with two probes located on either side of the Kan^r cassette. The disrupted allele was introduced by P1 transduction with similar frequencies in DHB3 and in DHB3 carrying the *ydeA*-expressing plasmid pSB13.

Arabinose uptake. Cultures were grown in M63-glycerol medium supplemented with 18 amino acids to an A_{600} of 0.2 to 0.4, centrifuged, resuspended at an A_{600} of 0.6, and induced for 20 min with 2% arabinose. Under these conditions, wild-type and *ydeA*-expressing strains are induced to the same extent. AraFGH-mediated uptake was measured in strain AD126 containing the *araFGH*-expressing plasmid pKKATEB (14, 15) and either pSB13 or pACYC184. Cultures grown in LB medium were induced with 5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 30 min. The cells were washed four times with M63, resuspended at an A_{600} of 0.6, and incubated for 10 min at room temperature. To measure arabinose uptake in the absence of the proton motive force, AD126 cells were fed with 0.2% glucose and preincubated for 30 s with 16 μM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP); the same volume of dimethyl sulfoxide was added to control cells. [^{14}C]arabinose (250 mCi/mmol; CB-69; CEA-France; a generous gift from R. W. Hogg) was diluted with unlabeled arabinose. At the indicated times after the addition of [^{14}C]arabinose, 200 μl of cells was filtered through nitrocellulose (HAWP; 0.45-μm pore size; Millipore). The filters were immediately washed with 5 ml of M63 and dried, and the radioactivity was measured by liquid scintillation. An unfiltered aliquot was used to determine the radioactivity input and to calculate the intracellular arabinose levels.

RESULTS

Two mutations that interfere with transcriptional activation of the P_{BAD} promoter. A PAI2-AP chimeric protein was expressed under the control of the arabinose P_{BAD} promoter (4). Induction with arabinose was toxic and prevented colony formation on arabinose plates. A large collection of suppressors of this toxic phenotype have been isolated, and most of them map to one of the *secA*, *secY*, and *secG* genes, which encode components of the protein export machinery (27, 33, 38). These mutants slow down the export kinetics of the chimeric protein upon induction with arabinose (4).

Two strains, SB1 and SB34, contained suppressor mutations that did not map to known *sec* genes. They were localized between 30 and 45 min on the *E. coli* chromosome by Hfr mating. A transposon was linked to either of these two mutations, shown to be very closely linked, and mapped near 35 min (see Materials and Methods). These suppressors, like those in *sec* genes, slow down the accumulation of active AP upon induction, and this effect was more pronounced for strain SB01 than for SB034 (Fig. 1). This effect was particularly evident at early times, and by 60 min all three strains had similar levels of AP activity. The accumulation of active AP integrates transcription, translation, and protein export to the periplasm, and the suppressors could affect any of these processes. The two suppressor strains showed no apparent defect in export of MalE or β-lactamase (data not shown). It has been shown that synthesis of proteins expressed from the P_{BAD} promoter is maximally induced within 2 min upon addition of arabinose (9). In contrast, the synthesis of the PAI2-AP protein was induced much more slowly in the suppressor strains; a similar effect was observed with two unrelated proteins cloned in the same vector (an FtsQ-AP fusion protein and human Bcl-2 [data not shown]).

These results suggested that the suppressors could interfere with the activity of the P_{BAD} promoter. To test this hypothesis, we measured the kinetics of PAI2-AP mRNA induction in wild-type and suppressor strains (Fig. 2). Whereas induction of PAI2-AP transcription was maximal within 5 min in the parental strain, it was reduced approximately 2-fold in SB34 and at least 20-fold in SB1. Northern blot hybridizations showed that both suppressors did not affect the size distribution of PAI2-AP transcripts (data not shown). Thus, the decrease in transcriptional induction of the P_{BAD} promoter (Fig. 2) ac-

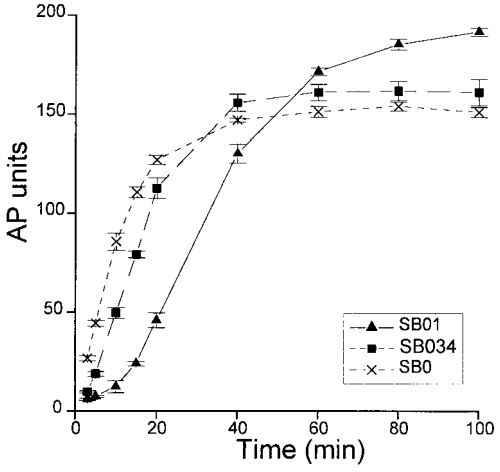

FIG. 1. Effects of suppressor mutations on kinetics of PAI2-AP export upon induction with arabinose. The two suppressor mutations of strains SB1 and SB34 were transduced into the parental strain SB0, generating suppressor strains SB01 and SB034, respectively. All strains express the chimeric PAI2-AP protein. Cells grown to an A_{600} of 0.1 in NZ medium at 37°C were induced with 0.2% arabinose at time zero. AP activity was assayed at the indicated times. Each curve represents the average of three independent cultures; error bars indicate standard deviations.

counts for the reduced kinetics of active AP accumulation (Fig. 1). These results indicated that both suppressors interfere with the transcriptional activation of the arabinose P_{BAD} promoter.

The kinetics of induction of a FtsQ-AP fusion protein (9) from the P_{BAD} promoter was decreased in the SB1 suppressor strain. In contrast, expression of the same chimeric protein from the P_{TAC} promoter was induced by IPTG with the same kinetics in the parental and SB1 strains (data not shown). Thus, decreased expression of cloned genes in the suppressor strains appeared specific for expression vectors derived from the arabinose regulon.

The suppressor mutations lead to increased expression of YdeA. To investigate the mechanism of action of these suppressors, we determined that the suppressor mutations are dominant (see Materials and Methods) and cloned the corresponding mutant genes. A genomic library prepared with DNA of strain SB1 was screened for inserts that suppress the toxicity of the PAI2-AP protein and confer an Ara^r phenotype. A 1.35-kbp fragment, derived from a large 12-kbp insert, was the minimal restriction fragment able to confer the Ara^r phenotype. This fragment contains one open reading frame (ORF), that of YdeA (SwissProt accession no. [AN] P31122). The sequence of the insert, isolated from strain SB1, was identical to that of *ydeA* in the *E. coli* genome sequence database throughout the coding region. However, the first 23 nucleotides (nt) of the insert were localized 8 kbp upstream of *ydeA*, suggesting that a large deletion is responsible for the mutant phenotype (Fig. 3). This deletion extends into the −35 region of the *ydeA* promoter and leads to the replacement of ATCACA by a TTCACA element. This change is expected to increase the activity of the promoter since the mutated element displays a much better match with the −35 consensus sequence (TTGACA). We also failed to detect a mutation in the *ydeA* coding region of strain SB34 and found a different mutation in the promoter. In this case, the change occurred in the putative transcription initiation site, where the sequence TTTT is changed to TGTT.

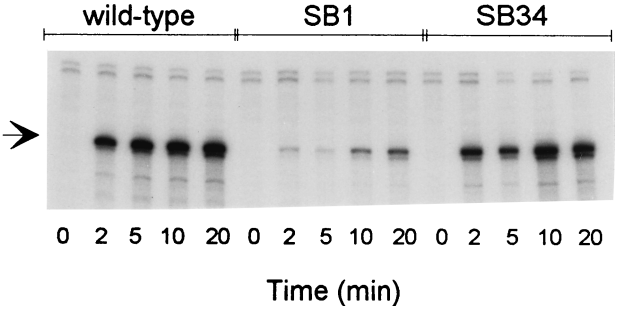

FIG. 2. Effects of suppressor mutations on kinetics of PAI2-AP transcription upon induction with arabinose. Cells grown to an A_{600} of 0.1 in NZ medium at 37°C were induced with 0.2% arabinose at time zero. Total RNA was extracted from samples collected at the indicated times. Two micrograms of RNA was hybridized to a ^{32}P-labeled PAI2 cRNA probe (290 nt). After digestion with pancreatic RNase, the hybrids were denatured and electrophoresed in a 6% urea-polyacrylamide gel. The protected cRNA fragment is 193 nt long (arrow); traces of undigested probe are visible in the upper portion of the gel. Lanes 1 to 5, parental strain SB0; lanes 6 to 10, suppressor strain SB1; lanes 11 to 15, suppressor strain SB34.

The identification of these two mutations strongly suggested that the suppressors act by increasing *ydeA* transcription. To test this hypothesis, we analyzed the level of *ydeA* mRNA by RNase protection in the wild-type and two mutant strains. Large amounts of transcripts were observed with RNA of strain SB1 (40 to 80 pg of mRNA per μg of total cellular RNA); lower levels were found with RNA of SB34. In contrast, only very low levels of *ydeA* mRNA were detected in RNA of the wild-type strain (Fig. 4A). To determine whether *ydeA* transcription is under arabinose control, we compared the level of *ydeA* mRNA in arabinose-induced cells to that in untreated cells (Fig. 4B). In the wild-type strain, the level of *ydeA* mRNA was only slightly elevated after addition of arabinose. A similar marginal increase was observed in strain SB1. This effect appears minimal compared to the more than 100-fold increase in transcription from the P_{BAD}, P_E, P_{FGH}, and P_J promoters upon induction with arabinose (Fig. 2; references 10, 20, and 23). These experiments indicate that the *ydeA* gene is poorly expressed in wild-type *E. coli*, at least under exponential growth conditions, and that it is not part of the arabinose regulon.

We have constructed a deletion allele of *ydeA* (see Materials and Methods). This disruption, which contains a Kan^r cassette, could be introduced at similar frequencies in strains expressing *ydeA* from a plasmid or in strains containing an empty plasmid, indicating that *ydeA* is a nonessential gene. The deletion strains grew normally at temperatures ranging from 23 to 42°C, in rich as well as in minimal media.

Increased expression of YdeA interferes with the intracellular accumulation of arabinose. A database search with the YdeA sequence showed that it belongs to the MFS, a large family of integral membrane proteins with 12 transmembrane domains (24, 28, 29). Since increased expression of YdeA interferes with induction of the P_{BAD} promoter by arabinose, it appeared possible that YdeA interferes with the intracellular accumulation of arabinose. To test this hypothesis, we have compared arabinose uptake in the different strains. Induction of the two known arabinose transporters requires the positive regulator AraC (10, 20), which was expressed from an empty pBAD24 plasmid. Cells were first treated with high concentrations of arabinose (see below) to allow induction of the *araE* and *araFGH* operons. We then measured arabinose uptake at two concentrations, to assay either mainly the high-affinity AraFGH transport system (K_m = 1 to 3 μM) or both the high- and low-affinity (AraE; K_m = 60 to 100 μM) systems (Fig. 5A) (15, 21). The amount of arabinose accumulated in cells carry-

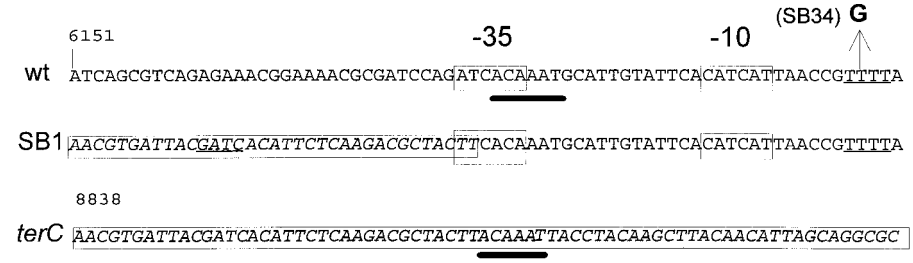

FIG. 3. Nucleotide sequences of mutations in the *ydeA* promoter. The nucleotide sequence of the wild-type (wt) *ydeA* promoter region is shown at the top; numbering corresponds to that of database entry ECAE000250. The putative −35 and −10 promoter regions are boxed, and the potential start sites are underlined. The mutation in strain SB34 introduces a G residue in the transcription initiation region. The large deletion in strain SB1 changes the −35 region, which now shows a five-of-six-position match with the TTGACA consensus sequence; the insert of pSB11 starts at the underlined *Sau*3AI site. The sequence upstream of the deletion is in italics and boxed, as is the wild-type sequence at the beginning of *terC*, shown at the bottom; numbering corresponds to that of database entry ECAE000249. The ACAAAT repeats (thick lines) upstream of *terC* (bottom) and *ydeA* (top) may have been involved in the spontaneous deletion of intervening sequences.

ing the SB1 deletion was much lower than that detected in wild-type cells. A similar reduction was observed at both arabinose concentrations, suggesting that YdeA overexpression exerts its effect independently of the arabinose import system. The low amounts of arabinose in strains carrying the SB1 deletion or the SB34 point mutation is probably accounted for by periplasmic binding to AraF (Fig. 5B). The levels of arabinose accumulated in the two mutant strains were too low to directly determine whether YdeA promotes arabinose export or whether it interferes with uptake. The latter hypothesis appears less likely, since YdeA exerts its effect on both the high-affinity and low-affinity transport systems.

Most members of the MFS are dependent on the proton motive force for substrate transport. To determine whether YdeA requires the proton motive force to interfere with arabinose uptake, we tested whether the effect of YdeA could be prevented by the protonophore CCCP (Fig. 5C). These experiments were performed in *unc* cells expressing the ATP-dependent AraFGH system from the P_{TAC} promoter. Under these conditions, arabinose uptake was identical in control and CCCP-treated *ydeA*$^+$ cells. In untreated cells, YdeA overexpression strongly reduced arabinose accumulation. Upon dissipation of the proton motive force, arabinose uptake was increased fourfold, although it did not reach the level measured with *ydeA*$^+$ cells. These results directly confirm that YdeA interferes with arabinose uptake by the AraFGH transport system and indicate that YdeA is a proton motive force-dependent arabinose export system.

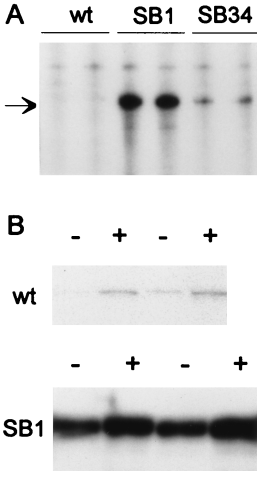

FIG. 4. *ydeA* mRNA levels in wild-type and mutant strains. For each strain, two independent cultures were grown at 37°C to an A_{600} of 0.4 in NZ medium, and total RNA was isolated. (A) Two micrograms of RNA was hybridized to a ^{32}P-labeled *ydeA* cRNA probe (450 nt). After digestion with pancreatic RNase, the hybrids were denatured and electrophoresed in a 5% polyacrylamide gel. The protected fragment is 360 nt long (arrow); traces of undigested probe are visible in the upper portion of the gel. The wild-type (wt) strain was SB0. (B) Two cultures of DB530 (*ydeA*$^+$) and DB529 (SB1 derivative) grown in LB medium were induced for 20 min with 2% arabinose (+) or not induced (−). Ten micrograms of RNA was hybridized to detect the very low levels of *ydeA* mRNA in wild-type cells. The gel was exposed for 4 days (wild type) and 20 h (SB1).

Increased expression of YdeA displaces the dose-response curve of induction by arabinose. Expression of genes cloned in pBAD vectors can be modulated over a wide range of arabinose concentrations (9). To determine whether it would still be possible to induce fully and rapidly the P_{BAD} promoter in cells overexpressing YdeA, we measured the arabinose dose-response curve in the wild-type and SB1 strains, 20 min after addition of the inducer. In the wild-type strain, the expression of PAI2-AP was already induced with low arabinose concentrations, and it was maximal at 0.2% arabinose (Fig. 6). In contrast, in the SB1 strain, expression of the chimeric protein was very low at arabinose concentrations ranging from 0.002 to 0.2% and reached wild-type levels only when the arabinose concentration was increased to 2%. Similar results were obtained with wild-type cells overexpressing YdeA from plasmid pSB11; in these cells, the level of *ydeA* mRNA was similar to that found in strain SB1 (data not shown). Thus, increasing the external arabinose concentration can compensate for the overexpression of YdeA and allows rapid and maximal activation of the P_{BAD} promoter.

DISCUSSION

The uptake and metabolism of arabinose are believed to involve eight proteins: AraE, a low-affinity transporter; AraF, AraG, and AraH, which constitute the high-affinity transport system; AraB, AraA, and AraD, the three enzymes necessary for arabinose metabolism; and AraC, the major transcriptional regulator of the regulon (34). Arabinose converts AraC from a repressor to an activator, and transcription of the three operons is rapidly and strongly induced (35). We show here that mutational activation of the *ydeA* promoter, which is essentially silent in wild-type cells, inhibits the transcriptional induction of the P_{BAD} promoter by lowering the intracellular concentration of arabinose. Overexpression of YdeA alone is sufficient for this effect, since the same phenotype was observed in wild-type cells carrying a plasmid with an insert that express only YdeA.

The extremely weak activity of the wild-type *ydeA* promoter

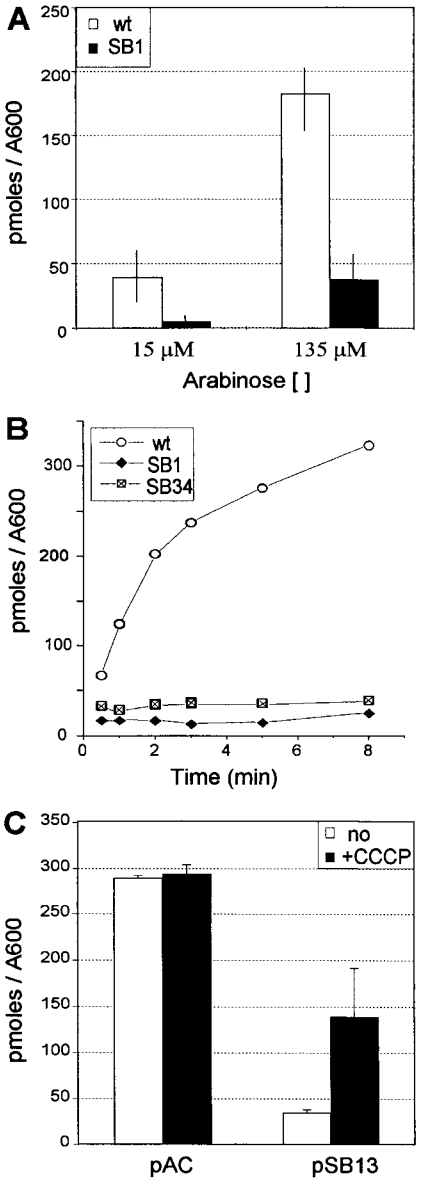

FIG. 5. Effect of the *ydeA* promoter mutations on arabinose uptake. (A) Two independent cultures of DB529 (SB1 derivative) and wild-type (wt) strain DB530 (*ydeA*$^+$) were assayed in duplicate for arabinose uptake during a 1-min pulse at the indicated arabinose concentrations. Intracellular arabinose levels (in picomoles/0.2 ml of cells) were normalized to the optical density of the cultures. Error bars indicate ranges. (B) The kinetics of arabinose uptake was measured with two independent cultures of DB529 (SB1 derivative), DB531 (SB34 derivative), and DB530 (*ydeA*$^+$ [wt]), the average of the two cultures is presented. The arabinose concentration was 62.5 μM. (C) Arabinose uptake by the AraFGH system was assayed in strain AD126 (*unc*) carrying pKKATEB and either pSB13 or pACYC184 (pAC). Cells were assayed in triplicate for arabinose uptake during a 2-min pulse at 14 μM arabinose. Glucose-fed cells were pretreated for 30 s with 16 μM CCCP (+CCCP) or with the same volume of solvent (no). Intracellular arabinose levels (in picomoles/0.2 ml of cells) were normalized to the optical density of the cultures. Error bars indicate standard deviations.

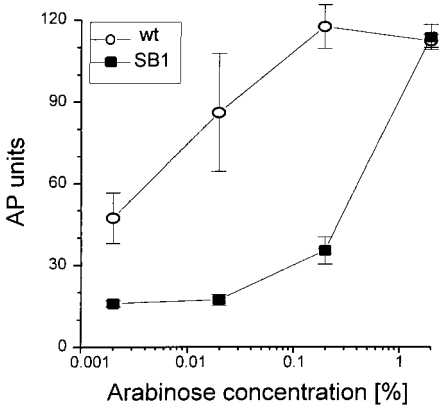

FIG. 6. Effect of YdeA on the induction of PAI2-AP by increasing arabinose concentrations. Cells were grown at 37°C in NZ medium to an A_{600} of 0.4, induced for 20 min with the indicated concentrations of arabinose, and assayed for AP activity. Each curve represents the average of three independent cultures. Error bars represent standard deviations. Both the wild-type (wt; SB0) and suppressor (SB1) strains carry pBAD72K and express the PAI2-AP chimeric protein.

is not surprising. Indeed, the putative −35 and −10 regions display poor matches with the consensus sequences. Furthermore, the initiation site lacks a properly positioned purine residue. Both mutations described here increase the activity of the *ydeA* promoter. In one case, SB1, a large deletion generated an improved −35 region. In the other case, SB34, a transversion introduced a G residue in the putative initiation site. The deletion provided a stronger stimulation than the initiation site mutation. The higher expression of *ydeA* in the SB1 strain probably accounts for its stronger effect on the kinetics of induction of genes expressed from the P_{BAD} promoter.

Most genetic studies of promoters have concentrated on the −35 and −10 regions, although initiation has been extensively studied in vitro (references 16 to 18 and references therein). The initiation site mutation of SB34 is almost the exact opposite of a mutation originally described in the phage P22 *sar* promoter (16). The TGTT-to-TTTT mutation at position +1 in the *sar* promoter was isolated in a genetic screen for strong promoter mutations and was shown in vitro to cause a defect in promoter clearance. In the *ydeA* promoter, the TTTT-to-TGTT mutation is at position +2. In strains SB1 and SB34, the *ydeA* transcription initiation sites appeared indistinguishable at the resolution of the RNase protection assay. Thus, initiation at the SB1 promoter may occur at either the −1 (G) or +1 (T) residue.

The *ydeA* gene, like many *E. coli* genes, appears nonessential, at least under the standard laboratory conditions tested. The large 7,856-bp deletion in strain SB1 shows that a number of adjacent genes and DNA elements are also nonessential. This large deletion, which is flanked by two ACAAAT repeats, removes a weak putative clockwise promoter separated by about 1 kbp from the *ydeA* gene and six ORFs of unknown function. The deletion also removes the *hotF* locus, a recombinational hot spot, and the *terC-terC3-psrA* locus, proposed to terminate clockwise replication (12). *terC* is in fact oriented opposite to what was originally proposed (12), and it is counterclockwise replication that does not require *terC*. It remains to be determined whether counterclockwise replication terminates only at the *terA* site (28.78 min) in wild-type cells or whether both sites are used to varying extents. Other large deletions near the terminus have been characterized (11), confirming the low density of essential genes in the *E. coli* 30- to 35-min map interval.

The experiments presented here are compatible with three types of hypotheses concerning the mode of action of YdeA.

YdeA could decrease arabinose entry into the cytoplasm, promote arabinose export, or stimulate arabinose degradation. Arabinose uptake experiments, which were performed with uniformly labeled [^{14}C]arabinose, show that the net accumulation is reduced to very low levels. Thus, if YdeA were to activate a catabolic pathway, all C-containing products would have to be rapidly exported from the cell. Furthermore, YdeA shows no homology to known enzymes. Arabinose import is mediated by two transport systems that are structurally and functionally independent (21, 34). AraE is a low-affinity proton:symporter that belongs to the MFS (23, 24), while AraFGH constitutes a high-affinity ATP-dependent transport system related to the ABC primary active transporters (15, 29). We have compared arabinose uptake in SB1 and wild-type cells at a low arabinose concentration, when only the AraFGH system is effective, and at a high arabinose concentration, when both systems contribute to arabinose uptake. Since the effect of YdeA was of the same magnitude, YdeA would have to act on both systems if it were to affect import. Finally, we have shown that YdeA interferes with arabinose uptake in cells that only express the AraFGH system and that this effect is partially abolished by dissipation of the proton motive force. Thus, these results strongly suggest that YdeA promotes arabinose export out of the cytoplasm. Because the amount of arabinose taken up by either SB1 or SB34 cells was so low, it is very difficult to definitively demonstrate, by isotope dilution, that YdeA is an arabinose exporter.

The notion that YdeA promotes arabinose export is supported by sequence comparison with related proteins. YdeA belongs to the very large group of integral membrane proteins with 12 transmembrane segments (24, 28). Within this group, the MFS constitutes one of the largest family, with 64 members in the *E. coli* genome (29). Approximately one-third of the *E. coli* members of the MFS have been studied genetically or biochemically, and the remaining are known only as putative proteins. They include sugars transporters (LacY, AraE, etc.), as well as multidrug resistance proteins (EmrB, MdfA, etc.). The closest homologs of YdeA (50% identity) are ORFs in the *Helicobacter pylori* and *Haemophilus influenzae* genomes, which most likely correspond to the orthologous genes. Three *E. coli* proteins show a relatively high homology to YdeA: AraJ, a member of the arabinose regulon (at 9 min, 24% identity; AN P23910) (31), Yicm/f451 (at 83 min, 26% identity; AN P31438), and f389 (at 37 min, 29% identity; AN D90809). The closest YdeA homologue that has been functionally characterized is a chloramphenicol resistance gene of *Streptomyces lividans* (30% identity; P31141), which exports the drug (6). YdeA overexpression fails to confer resistance to chloramphenicol or tetracycline; it also does not interfere with gene activation by IPTG (a FtsQ-AP fusion protein expressed from P_{TAC}) or maltose (MalE). Thus, the spectrum of molecules that can be exported by YdeA remains to be determined.

Although *ydeA* appears to be a nonessential gene, the presence of a set of related genes in *E. coli*, *H. influenzae*, and *H. pylori* suggests that they could provide or have provided some evolutionary advantage to these organisms. AraJ has been proposed to facilitate import of arabinose polymers (31). This appears now less likely, since no member of the MFS has been found to transport a substance larger than 1,000 Da (29), which corresponds to six to seven arabinose monomers. Another possibility is that YdeA, when expressed, and/or AraJ could promote the export of arabinose structural analogues that can be imported but not completely metabolized.

Expression vectors based on the arabinose P_{BAD} promoter are highly versatile and have been extensively used (9). Protein levels can be modulated over a wide range by using plasmids with various *ori* elements, by altering plasmid copy number, and by changing the extracellular arabinose concentration. YdeA overexpression now offers the means to modulate the kinetics of protein expression. For instance, shut off upon arabinose removal, which is slow in *ara* strains (9), could be accelerated by activation of *ydeA* gene expression. The most useful advantage is that the kinetics of induction can now be modulated: the effect of protein accumulation could be studied either in a short time frame (in wild-type cells) or over an extended time period (in cells overexpressing YdeA).

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