Molecular characterization of adiY, a regulatory gene which affects expression of the biodegradative acid-induced arginine decarboxylase gene (adiA) of Escherichia coli

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A transcriptional regulator gene, designated adiY, was found downstream of the biodegradative arginine decarboxylase (adiA) gene (previously known as adi) of Escherichia coli. The arginine decarboxylase system is maximally induced under conditions of acidic pH, anaerobiosis and rich medium, and AdiY was found to increase the expression of adiA. The DNA sequence of adiY encodes a protein of 253 amino acids. Primer extension analysis defined the promoter. The amino acid sequence of AdiY showed homology to the XylS/AraC family of transcriptional regulators, which includes EnvY and AppY. Studies suggested that sequences required for acid induction were also necessary to observe the stimulation by AdiY. An examination of the substitution of AdiY, AppY and EnvY showed that these three proteins can, to some extent, stimulate the other systems.

Keywords: regulation, envY, appY, acid response, virulence

INTRODUCTION

In Escherichia coli, the biodegradative arginine (adiA) and lysine (cadA) decarboxylases are strongly induced in rich medium at low pH in the presence of excess substrate under anaerobic conditions (Auger et al., 1989; Gale, 1940) and appear to play a role in pH homeostasis by consuming protons and neutralizing the acidic by-products produced during carbohydrate fermentation (Gale, 1946; Rescei & Snell, 1972). AdiA and CadBA have several properties in common. Both are produced under similar conditions (although CadBA is also produced under aerobic conditions) and both are major cellular proteins, representing over 2% of the total cell protein under maximal induction. Arginine and lysine decarboxylases are capable of increasing the surrounding pH by removing acidic carboxyl groups and releasing CO₂ from their substrates (arginine and lysine, respectively), and both utilize pyridoxal 5’-phosphate as a cofactor (Blethen et al., 1968).

AdiA and CadBA, however, exhibit differences in induction. The lysine decarboxylase gene, cadA, is part of an operon including cadB, the gene for a proposed lysine-cadaverine membrane-bound transport protein (Meng & Bennett, 1992a). Immediately adjacent to the cadBA operon is cadC, a gene encoding a protein with homology to toxR and other prokaryotic transcriptional activators involved in environmental sensing (Watson et al., 1992). The nucleotide sequences of the adiA gene, the cadBA operon, the cadC gene and their promoter regions have been determined (Meng & Bennett, 1992a; Stim & Bennett, 1993; Watson et al., 1992), a region essential for acid induction of the cad system has been identified (Meng & Bennett, 1992b), and the role of regulators has also been further defined (Neely et al., 1994). Through analysis of mutations that affect acid induction and complementation studies, several genes have been identified that affect the expression of adiA. Mutations in H-NS, a histone-like DNA-binding protein, derepress the expression of both adiA and cadBA (Shi et al., 1993). The effects of various rpoA alleles were tested and rpoA341 was found to abolish acid induction of adiA but not cad (Shi & Bennett, 1994b). Plasmids containing the hfg gene, which encodes the host factor HF-1 for bacteriophage Qₐ or stpA, which encodes a protein with more than 60% identity with the protein sequence of H-NS, can comp-
lement hns mutants of adiA (Shi & Bennett, 1994a). The regulator of the cysteine biosynthetic genes, CysB, was also found to be required for effective expression of adiA (Shi & Bennett, 1994b). Integration host factor (IHF) is a DNA-binding protein involved in the bending of DNA and the regulation of gene expression, and a consensus binding sequence has been proposed (Friedman, 1988). A potential IHF consensus sequence was identified in the adiA promoter, and the absence of induction of adiA in IHF mutants was observed (Stim et al., 1993).

In this report, an open reading frame (ORF) downstream of adiA has been sequenced and it exhibits similarity to the XylS/AraC class of transcriptional regulators which includes EnvY and AppY. Accordingly, it has been the XylS/AraC class of transcriptional regulators which also found to be required for effective expression of IHF mutants was observed (Stim adiA et al., 1989). DNA fragments from pKERR10 were cloned into phagemid pEMBL8 to prepare subclones for sequencing (pKS4 and pKS11). To create pKSO15, adiY was amplified from pKS4 by PCR with primers PCR6 and PCR7. PCR6 incorporates an EcoRI restriction site at nucleotides 239-256 (Fig. 2) was labelled with [α-32P]dATP as described by Kingston (1987). After heat-inactivation of the T4 DNA polymerase, the PCR fragment was purified by use of the Millipore Ultrafree-MC filter unit or by the method of Heery et al. (1990). Deoxyribonucleoside triphosphates, avian myeloblastosis virus reverse transcriptase, and placental RNase inhibitor were from Promega. Site-directed mutagenesis was performed by using the Muta-Gene M13 in vitro mutagenesis kit, version 2, from Bio-Rad. Plasmids were prepared by use of the QIAGEN plasmid kit in accordance with the manufacturer’s instructions. All other chemicals were purchased from Sigma or Research Organics.

**METHODS**

**Materials.** Restriction enzymes were purchased from Promega, and T4 DNA ligase and its buffer were purchased from US Biochemical; enzymes were used in accordance with the manufacturer’s recommendations. Subclones were sequenced by using the Sequenase version 2.0 sequencing kit from US Biochemical and [35S]dATP from NEN Research Products. The -40 primer was provided by the manufacturer; other oligomers (18-mers) for DNA sequencing (or other procedures) were synthesized on a Biosearch 8600 DNA synthesizer in the Department of Biochemistry and Cell Biology, Rice University. The polymerase chain reaction (PCR) was performed using the GeneAmp PCR Reagent kit and instrument from Perkin Elmer Cetus. DNA fragments were purified by use of the Millipore Ultrafree-MC filter unit or by the method of Heery et al. (1988). Primer extension was performed by use of a primer labelled with [γ-32P]dATP from ICN Biomedicals. Deoxyribonucleoside triphosphates, avian myeloblastosis virus reverse transcriptase, and placental RNase inhibitor were from Promega. Site-directed mutagenesis was performed by using the Muta-Gene M13 in vitro mutagenesis kit, version 2, from Bio-Rad. Plasmids were prepared by use of the QIAGEN plasmid kit in accordance with the manufacturer’s instructions. All other chemicals were purchased from Sigma or Research Organics.

**Bacterial strains, plasmids, and media.** The bacterial strains and plasmids used are listed in Table 1. Modified Falkow arginine or lysine decarboxylase medium was as described previously (Stim & Bennett, 1993). Cultures were grown in Luria broth (LB) as described previously (Stim & Bennett, 1993) or on MacConkey lactose plates (40 g MacConkey agar base and 10 g lactose per litre). Ampicillin (50 mg l⁻¹) and spectinomycin (100 mg l⁻¹) were included in the media at all times to retain plasmids during growth. Pl crosses were done as described earlier (Auger et al., 1989).

**Recombinant DNA techniques.** Cloning experiments were conducted in accordance with standard procedures (Sambrook et al., 1989). The construction of the mini-Mu-derived plasmid pKERR10 (mini adiA') was described previously (Auger et al., 1989). DNA fragments from pKERR10 were cloned into phagemid pEMBL8 to prepare subclones for sequencing (pKS4 and pKS11). To create pKSO15, adiY was amplified from pKS4 by PCR with primers PCR6 and PCR7. PCR6 incorporates an EcoRI restriction site at nucleotides 239-256 (Fig. 2) was labelled with [γ-32P]dATP as described by Kingston (1987). After heat-inactivation of the T4 polynucleotide kinase, labelled primer KPS-P was purified by use of a Sephadex G25-150 chromatography column as described by Sambrook et al. (1989). The primer extension
Table 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>E. coli strain or plasmid</th>
<th>Relevant genotype or marker</th>
<th>Reference</th>
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<tr>
<td>E. coli strains</td>
<td></td>
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<tr>
<td>71-18</td>
<td>supE thi b(lac-pro-AB) F' (pro-AB lacI&lt;sup&gt;+&lt;/sup&gt; lacZ ΔM15)</td>
<td>Dente et al. (1983)</td>
</tr>
<tr>
<td>GNB7145K</td>
<td>MC4100 adi:A&lt;sup临时&lt;/sup&gt; - Mu dl 1734 (Km&lt;sup&gt;+&lt;/sup&gt; lac)</td>
<td>Auger et al. (1989)</td>
</tr>
<tr>
<td>GNB8385K</td>
<td>MC4100 αcd:B&lt;sup临时&lt;/sup&gt; - Mu dl 1734 (Km&lt;sup&gt;+&lt;/sup&gt; lac)</td>
<td>Auger et al. (1989)</td>
</tr>
<tr>
<td>GNB725</td>
<td>GNB7145K hns-25: Tn5 (Cm&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>Shi et al. (1993)</td>
</tr>
<tr>
<td>MC4100</td>
<td>araD139 A(argF-lac)U169 rpsL150 relA1 ββB5301 dec1 pif25301 &lt;br&gt;U169 rpsL150 relA1 flbB5301</td>
<td>Casadaban &amp; Cohen (1979)</td>
</tr>
<tr>
<td>TC3594</td>
<td>C600 lac trp&lt;sup&gt;+&lt;/sup&gt; Δ(appY-ent)</td>
<td>Atlung &amp; Brondsted (1994)</td>
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<tr>
<td>E534</td>
<td>N99 Δ[hip]:Cm&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Flamm &amp; Weisberg (1985)</td>
</tr>
<tr>
<td>GNB7145K5</td>
<td>GNB7145K Δ[hip]:Cm&lt;sup&gt;+&lt;/sup&gt;</td>
<td>This work</td>
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<tr>
<td>Plasmids</td>
<td></td>
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<tr>
<td>pBR322</td>
<td>Ap&lt;sup&gt;+&lt;/sup&gt; Tc&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Sutcliffe (1978)</td>
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<tr>
<td>pEMBL8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Ap&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Dente et al. (1983)</td>
</tr>
<tr>
<td>pKER110</td>
<td>Mu d5005 mel&lt;sup&gt;+&lt;/sup&gt; adi:A&lt;sup&gt;+&lt;/sup&gt; Km&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Auger et al. (1989)</td>
</tr>
<tr>
<td>pKPS7</td>
<td>pX52 with two mutations in the IHF consensus site in the adiA promoter</td>
<td>This work</td>
</tr>
<tr>
<td>pKS4</td>
<td>4.3 kb SaI fragment from pKER110 cloned in pEMBL8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Stim &amp; Bennett (1993)</td>
</tr>
<tr>
<td>pKS11</td>
<td>Same as pKS4 but cloned in the opposite orientation</td>
<td>This work</td>
</tr>
<tr>
<td>pKSO15</td>
<td>1.23 kb EcoRI PCR fragment containing adiY cloned in pEMBL8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>This work</td>
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<tr>
<td>pML22</td>
<td>0.945 kb EcoRI-PstI fragment containing envY cloned in pBR322</td>
<td>Lundrigan &amp; Earhart (1984)</td>
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<tr>
<td>pTAC3303</td>
<td>2 kb HindIII-Smal fragment containing appY cloned in pUN121</td>
<td>Atlung et al. (1989)</td>
</tr>
<tr>
<td>pUN121</td>
<td>Ap&lt;sup&gt;+&lt;/sup&gt; Tc&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Nilsson et al. (1983)</td>
</tr>
<tr>
<td>pXA</td>
<td>Sm&lt;sup&gt;+&lt;/sup&gt; Sp&lt;sup&gt;+&lt;/sup&gt;</td>
<td>de Lorenzo et al. (1988)</td>
</tr>
<tr>
<td>pX1/4</td>
<td>166 bp EcoRI-BamHI PCR fragment of the adiA promoter cloned in pXA</td>
<td>This work</td>
</tr>
<tr>
<td>pX1/5</td>
<td>144 bp EcoRI-BamHI PCR fragment of the adiA promoter cloned in pXA</td>
<td>This work</td>
</tr>
<tr>
<td>pX52</td>
<td>274 bp EcoRI-BamHI PCR fragment of the adiA promoter cloned in pXA</td>
<td>This work</td>
</tr>
<tr>
<td>pX-B/S-1</td>
<td>124 bp BamHI-SspI PCR fragment of the adiA promoter cloned in pXA</td>
<td>Stim &amp; Bennett (1993)</td>
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</table>

procedure of Kingston (1987) was used, except that annealing was carried out at 25 °C. The products were separated on a sequencing gel and the sequence generated from pKSO15 and primer KPS-P was used as a reference.

**Site-directed mutagenesis of the adiA promoter region.** To investigate the promoter region, a primer was designed to alter bases in the proposed IHF binding site of the adiA promoter. Primer SDM7 (5'-CACATAACGTAggGTT TATAAAACT-3' [the changes are represented by lower-case letters]), corresponding to positions 66-90 of the adiA sequence (Stim & Bennett, 1993), was used for the mutagenesis with uracil-containing single-stranded pX52 as described previously (Stim & Bennett, 1993) along with the Mutagen-Gene M13 in vitro mutagenesis kit, version 2. The product was transformed into E. coli 71-18. Single colonies were isolated, and single-stranded DNA was prepared. The DNA was then sequenced with primer X (de Lorenzo et al., 1988) to verify the correct mutations. A QIAgen plasmid preparation was made from an isolate (pKPS7) containing the desired mutations.

**β-Galactosidase assays.** Cultures to be analysed for β-galactosidase activity were grown anaerobically in test tubes in pH-buffered modified Falkow arginine or lysine decarboxylase medium as described by Auger et al. (1989). The cells were grown to an OD<sub>600</sub> of 0.4-0.7 for anaerobically grown cultures, and harvested by centrifugation. β-Galactosidase assays were performed by the SDS-chloroform permeabilization method, and the units were calculated as described by Miller (1972).

**Acid phosphatase assays.** Quantitative measurements were carried out on cells with or without recombinant plasmids grown to OD<sub>600</sub> of ~0.6 in 5 ml LB by a modification of the method described by Atlung et al. (1989). Briefly, after growth, the OD<sub>600</sub> was determined, and 0.5 ml culture was centrifuged in an Eppendorf tube at 8000 r.p.m. for 2 min. The supernatant was decanted, and the remainder was removed with a pipette. The cell pellet was resuspended in 0.5 ml 25 mM p-nitrophenyl phosphate in 250 mM glycine/HCl (pH 2.5), and incubated at 37 °C for 15 min. The reaction was stopped by the addition of 1 ml 1 M NaOH, and the reaction mixture was centrifuged at
14000 r.p.m. for 30 s. The $A_{410}$ of the supernatant was then determined. One unit of acid phosphatase was defined as $1000 \times A_{410}/15 \times 0.5 \times OD_{600}$.

RESULTS

DNA sequence analysis

The location at 93-4 min on the E. coli chromosome (Auger et al., 1989) and sequence (Stim & Bennett, 1993) of $adiA$ had been determined previously. pKER110 ($mel^+$ $adiA^+$) contained this region of the chromosome and was used to create subclones for DNA sequencing of $adiA$. Fig. 1 shows the restriction map of pKER110. Two of these subclones (pKS4 and pKS11) contained a 4.3 kb Sall fragment cloned in pEMBL8* (in opposite orientations) which included the 3' end of $adiA$. Sequencing downstream of $adiA$ and analysis by use of MacVector release 3.5 revealed another ORF in the same orientation as $adiA$. This ORF was named $adiY$, due to its homology to other genes and its effect on acid phosphatase (Atlung et al., 1989). Amino acid sequence of AdiY and comparison with other regulatory proteins

To detect and analyse the 5' end of the mRNA transcript of $adiY$, we performed a primer extension analysis with RNA isolated from E. coli 71-18(pKSO15). Primer KPS-P, corresponding to nucleotides 239–256 (Fig. 2), was used in the extension. The extension product ended at position 71 of the $adiY$ sequence and is indicated by +1 in Fig. 2. The sequence of the promoter region was sent to Dr John Anderson at Purdue University for analysis of DNA curvature, and a bend was found with the centre located between the –10 and –35 regions. The centre of the bend is denoted in Fig. 2 by the double-underlined base at position 47. An inverted repeat was found in the $adiY$ promoter region and is indicated by the inverted arrows in Fig. 2. A possible Shine–Dalgarno ribosome-binding site is indicated. The C-terminal amino acid of $adiY$ is not followed by a region containing any obvious transcription-termination structures, suggesting that it may be part of an operon with ORF f326a cited in GenBank U14003 (see Fig. 1).

Amino acid sequence of $adiY$ and comparison with other regulatory proteins

The amino acid sequence of AdiY is also presented in Fig. 2. The ORF encoding $adiY$ extends from a methionine codon at nucleotide 184 to nucleotide 945 and encodes a 253-amino-acid protein of 29 kDa.

A search of the GenBank and EMBL databases by use of the TFASTA program of the GCG package revealed that the encoded amino acid sequence of AdiY is most similar to EnvY, a regulator of temperature-dependent expression of the outer-membrane porins OmpF and OmpC (Lundrigan et al. 1989) (56% identity), and shares between 20 and 30% identity with seven other proteins of the XylS/AraC family. These proteins include: CfdD, a regulator of fimbria production (Savelkoul et al., 1990); Rns, a regulator of colonization factor antigens and adhesin production of enterotoxigenic E. coli (Caron et al., 1986); AppY, a regulator of the 987P operon in enterotoxigenic E. coli (Klaasen & de Graaf, 1990); and UreR, a regulator of urease expression present in many urinary tract pathogens (D’Orazio & Collins, 1993). The protein VirF from Shigella dysenteriae, a regulator of virulence, is also homologous (Dorman, 1992). An alignment of the most similar of these proteins by the PILEUP program of the GCG package is presented in Fig. 3. A consensus sequence has been found in the C terminus in a comparison of 27 members of the XylS/AraC family of regulators (Gallegos et al., 1993) which is rich in z-helix and could be involved in DNA binding. The alignment presented in Fig. 3 generally supports this consensus sequence. Secondary structure prediction programs for the aligned sequences also indicate some correlation of structural features. All of these proteins have a similar molecular size. They are all basic proteins with high pIs, a property consistent with DNA binding.

Analysis using Kyte & Doolittle (1982) hydrophathy plots of AdiY and EnvY analysed with the TopPred II program showed that both proteins have very similar overall patterns and indicate a putative membrane-spanning region between amino acid residues 150 and 190. The TMH and KKD programs of the MacProt package also indicated a putative transmembrane helical region from amino acid residue 154 to 180. This region is underlined in the alignment of AdiY and EnvY shown in Fig. 3. At the end of the putative transmembrane regions of both

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**Fig. 1.** Chromosomal region cloned in pKER110. [ ], Coding region for the $mel$ operon; [ ] coding regions for $adiA$ and $adiY$; [ ] coding regions for f326a, f122 and f557 (yjdB)(GenBank U14003); [ ] coding region for basSR (Nagasawa et al., 1993). The direction of transcription of these genes is indicated by an arrow above the gene. The solid bars represent vector sequences. DNAs subcloned to produce pKSO15, pKS4, pKS11, pKS2, pX1/4, pX1/5 and pX-B/S-1 are denoted by cross-hatched rectangles below pKER110 (see Table 1). Relevant restriction sites are as follows: B, BamHI; D, DraI; E, EcoRI; G, BglII; H, HindIII; RV, EcoRV; S, SalI.
AdiY and EnvY are three lysine residues which produce a positive charge typical of the inside terminus of a transmembrane region (von Heijne, 1986). EnvY has been located in the membrane fraction of minicells containing envY on a plasmid (pML22) and labelled with [35S]methionine (Lundrigan et al., 1984). Taken together, these analyses suggest that AdiY may also be a membrane-associated protein. A Kyte–Doolittle hydropathy plot of the related protein, AppY, does not reveal an obvious membrane-spanning region between amino acids 150 and 190.

**Function of AdiY and effect on adiA gene expression**

Initial β-galactosidase assays of the adiA::lacZ strain GNB7145K containing the sequencing plasmids pKS4 or pKS11 indicated elevated production of β-galactosidase in comparison to GNB7145K. To determine whether AdiY was acting on adiA to cause its increased expression, PCR was used to obtain a 1·23 kb fragment containing adiY only. This PCR product was cloned into pEMBL8+ to create pKSO15 (adiY+). Table 2 shows the β-galactosidase activity of GNB7145K (adiA::lacZ), GNB8385K (cad::lacZ), and GNB725 (adiA::lacZ hns) with and without pKSO15. This experiment shows the increased expression of adiA when adiY is provided on a plasmid; the effect is similar to the observation of increased appY expression when appY was supplied in a multicopy system (Atlung et al., 1989). The effect is specific for adiA, as no effect of AdiY is seen in the cad system (GNB8385K results). The effect of AdiY on adiA expression is most readily detected at pH 8 in GNB7145K, where AdiY causes a 65-fold increase in β-galactosidase production; at pH 5·5, the increase is only threefold. Mutations in H-NS, a histone-like DNA-binding protein, have been found to derepress the expression of adiA (Shi et al., 1993). Although adiA expression is already elevated in the hns mutant GNB725, an effect of AdiY (a threefold increase) is still observed at either pH, so it apparently does not act solely by relieving H-NS repression, a result

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**Fig. 2.** Nucleotide and deduced amino acid sequences of adiY. The total sequence shown starts 141 nucleotides downstream of the adiA stop codon and ends 155 nucleotides downstream of the adiY stop codon. The numbers on the left indicate the first base pair at the beginning of each row of the DNA sequence; a sequence from the region of bp 1 to 404 was reported previously (Stim & Bennett, 1993). The transcription start site (+1) and the −10 and −35 sequences of adiY, and the Shine–Dalgarno sequences (SD) of adiY and f326a are underlined. The centre of the bend in the promoter region is denoted by a double underline. An inverted repeat (IR) is noted by inverted arrows under the DNA sequence. The AdiY amino acid sequence is translated from the top strand and is positioned below each codon.
The short amino acid sequence below the line in the C-terminal region of the proteins of the alignment is a consensus sequence found in at least 50% of 27 AraC-like proteins analysed by Miller (1972) and represent the means of several assays of independent colonies. The reproducibility of assays was ±10-15%.

### Table 2. Effect of increased copy number of adiY on gene expression of adiA and cad

<table>
<thead>
<tr>
<th>Plasmid(s)</th>
<th>β-Galactosidase activity at pH:</th>
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<tbody>
<tr>
<td></td>
<td>5.5</td>
</tr>
<tr>
<td>pX52</td>
<td>4440</td>
</tr>
<tr>
<td>pX52+pKSO15</td>
<td>3830</td>
</tr>
<tr>
<td>pKP7</td>
<td>970</td>
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<tr>
<td>pKP7+pKSO15</td>
<td>340</td>
</tr>
<tr>
<td>pX1/4</td>
<td>170</td>
</tr>
<tr>
<td>pX1/4+pKSO15</td>
<td>20</td>
</tr>
<tr>
<td>pX1/5</td>
<td>440</td>
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<td>pX1/5+pKSO15</td>
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<tr>
<td>pX-B/S-1</td>
<td>450</td>
</tr>
<tr>
<td>pX-B/S-1+pKSO15</td>
<td>130</td>
</tr>
</tbody>
</table>

similar to that found with the related virulence regulator CfaD (Jordi et al., 1992).

To further ascertain which region of the adiA promoter is required for the stimulation by AdiY, plasmids were constructed which contained various portions of the adiA promoter region, or alterations in the adiA promoter. The plasmids with various lengths of the adiA promoter were: pX52 (274 bp), pX1/4 (166 bp), pX1/5 (144 bp), and pX-B/S-1 (124 bp). pKP7 has the same length as pX52 (274 bp) but contains an alteration within the IHF consensus sequence, ATTGAAAATCAA (Goodrich et al., 1990). The putative IHF binding site in the adiA promoter is ATTGCATAAACAAT (from bp 70 to 82; Gürtler & Bennett, 1993) and has been changed to ATTGATCAAAAC in pKP7 (the changes are underlined), which is less like the IHF consensus sequence. These five plasmids encode β-galactosidase protein fusions which join the eighth codon of adiA to lacZ, and allow the monitoring of adiA expression by β-galactosidase production. They were combined with pKSO15 in E. coli MC4100 to determine if the increased adiA expression caused by AdiY could still occur. The results of this experiment are shown in Table 3. The first point of interest is the fact that most of these five modified adiA::lacZ constructs exhibit a considerable reduction in the acid expression level of adiA (compare the β-galactosidase production at pH 8 versus pH 5.5). As the adiA promoter region becomes smaller, the relative acid induction drops from a high of 49-fold to 1-2-fold, indicating the loss of sequences important for acid induction. The second point of interest is that an intact IHF site with the proper length of DNA on either side of the IHF consensus sequence is necessary to detect the effect of AdiY. pX52 has an intact IHF site with a complete wild-type DNA...
strain did not increase when the adiY plasmid, pKSO15, was introduced, further confirming the need for IHF action and suggesting that AdiY may not act independently of other factors. Whether AdiY acts through a direct or indirect mechanism is still uncertain.

Overlapping interactions between AdiY, AppY and EnvY

AdiY has sequence similarity to the XylS/AraC family of transcriptional activators which includes AppY and EnvY. This family is based upon the presence of a helix-turn-helix structure found in the C-terminus of these proteins. A hypothesis proposes that these basic proteins use this helix-turn-helix motif to bind DNA and alter gene expression in their respective systems. Since AdiY was most similar to AppY and EnvY, these three systems were used to investigate whether these proteins could substitute for one another and cause altered gene expression in the other two systems. AppY has been found to increase the synthesis of more than 36 proteins by two-dimensional gel analysis (Atlung et al., 1989) and experiments were undertaken to investigate possible effects on this system.

The first of these experiments determined whether AppY and EnvY had an effect on adiA gene expression. Plasmids and their respective vectors (pUN121 and pBR322) were obtained which contained either appY (pTAC3303) or envY (pML22). These plasmids, along with pKSO15 (adiY), and its vector (pEMBL8+) were transformed into the adiA::lacZ fusion strains GNB7145K and GNB725 (adiA::lacZ hns) and the cad::lacZ fusion strain GNB8385K. β-Galactosidase production was then used to determine whether AppY and/or EnvY caused an increase in adiA gene expression. These results are presented in Fig. 4. The increased expression of adiA is most notable at pH 8. Both AppY and EnvY cause an increase in adiA expression at both pH 5.5 and pH 8 in GNB7145K. AppY seems more effective than EnvY, even though EnvY is more similar to AdiY in its amino acid sequence. GNB8385K, the cad::lacZ fusion, does not show an effect of increased AdiY, AppY or EnvY concentration at either pH. adiA expression in the hns mutant GNB725 also displays modest induction by AppY, most notably at pH 8.

The other system used to check for substitution of the three proteins for one another was the acid phosphatase (appA) system. To quantify acid phosphatase production, cultures were grown and harvested in the exponential phase. The quantitative acid phosphatase assay was performed as described in Methods, and the results are shown in Fig. 5. Plasmids bearing the genes adiY and envY are capable of enhancing acid phosphatase activity, but not to the levels produced by appY. Taken together, these results indicate that AdiY, AppY and EnvY can substitute for one another in some actions. Substitution among transcriptional regulators has also been found in other systems. Savelkoul et al. (1990) reported that sfaD and rmt (two members of the XylS/AraC family which are also similar to AdiY) could functionally substitute for one
another in the regulation of fimbrial synthesis. Those two proteins, however, are very similar in amino acid sequence. In the case reported here, the interaction among these proteins and expressed genes could be through direct protein–promoter interactions as is typical for the XylS/AraC class of proteins, or it could be indirect through affecting other systems capable of modulating gene expression.

**DISCUSSION**

Several genes involved in the regulation of the *adi* system have now been identified. The subject of this report, *adiY*, is a member of the XylS/AraC family of transcriptional regulators which now contains more than 30 members, including AppY and EnvY. All of these regulators possess a helix-turn-helix structure in their C-terminus and are thought to act by binding the DNA of promoters of genes which they regulate. The N-terminus and central region of the proteins in the XylS/AraC family are thought to include a region which recognizes chemical signals and contains binding sites for activator molecules that confer specificity (Ramos et al., 1990). For example, the removal of the N-terminus of AraC relieves the requirement for arabinose in the induction of three *ara* operons (Menon & Lee, 1990). The effector molecule active in the *adi* system is unknown at this time, although one could speculate an involvement of arginine, pH, or oxygen/redundant factors.

From preliminary experiments of *adiY::lacZ* operon fusion constructs, it appears that *adiY* is expressed under both aerobic and anaerobic conditions. It would be interesting to determine if it is involved in the regulation of other genes in a fashion similar to AppY, which can affect the synthesis of more than 30 cellular proteins (Atlung et al., 1989; Atlung & Brøndsted, 1994).

The finding that plasmids overexpressing AdiY, EnvY and AppY can affect the expression of other genes might suggest that genes sensitive to these environmental parameters may respond to additive effects acting through these diverse regulators. Further testing of the ‘cross-talk’ effect by examination of *lac* fusions to the appropriate genes in chromosomal mutants of the regulators may give additional insight into the connections between these systems. The three features – growth deceleration, anaerobic condition, and low pH due to accumulation of metabolites – which accompany the post-exponential phase of growth may have overlapping effects on the expression of proteins induced in this environmental situation. Different genes induced during this time may have a particular pattern of induction (and mechanism) for responding to each of the factors.

Previous reports on members of the XylS/AraC regulator family have noted the use of unusual codons and a G + C content different from the bulk *E. coli* G + C content of 50 mol%. *rns* was found to use three rare codons for arginine, isoleucine and glycine, which is typical of genes that are not highly expressed (Caron et al., 1989). The use of these codons in *adiY* was not noteworthy. The G + C content of *virF* from *Shigella flexneri* and that of *aggR* from *E. coli* is 30 mol% (Dorman, 1992; Nataro et al., 1994) while that of *rus* from *E. coli* is 28 mol% (Caron et al., 1989). Since these G + C contents are so different from the 50 mol% of *E. coli*, the suggestion has been made by Caron et al. (1989) that *rus* was acquired from another micro-organism. The G + C content of *adiY* is 41 mol%, a value below that of the normal 50 mol% of *E. coli*, but not as low as that found in *rus* or *virF*. The G + C content of *appY* is 32 mol%, similar to the lower values obtained for *rus* and *virF*, and in line with the suggestion of Caron et al. (1989). This region is apparently contained on a remnant lambdoid phage (Nakata et al., 1993).

A recent search of the GenBank database revealed that the DNA sequence downstream of *adiY* has been completed as part of the *E. coli* Genome Project at the University of Wisconsin-Madison (GenBank accession no. U14003). The ORF and ribosome-binding site sequenced and shown in Fig. 2 also fit the database entry. Several genes and ORFs exist in this region. These include *bus*RD, f557 (*yjB*), f122, and f326a noted in Fig. 1. ORF f326a is 45% identical to *cadB*, a 444-amino-acid protein which has been suggested to be a lysine/cadaverine antiporter of the lysine decarboxylase (*cad*) system (Meng & Bennett, 1992a; GenBank accession no. M76411). ORF f326a is also 36% identical to *potE*, a putrescine transport protein of *E. coli* (Kashiwagi et al., 1991; GenBank accession no. M64495). ORF 326a is only 137 bp downstream of *adiY* and a transcription-termination structure is not evident in this region, suggesting the possibility that the two genes are part of an operon. If ORF 326a acts in the *ad* system in an analogous fashion to the *cad* system, it could possibly have an antiport function in exchanging arginine and agmatine (the product of arginine decarboxylation).

The *adi* and *cad* systems have several properties in common. These include (i) their maximal induction at low pH under anaerobic conditions in the presence of excess substrate, (ii) the fact that they are major cellular proteins, (iii) the use of pyridoxal 5'-phosphate as a cofactor, (iv) their proposed role in pH homeostasis, and (v) the involvement of H-NS, which reduces expression of both systems. These two systems, however, have several
differences in their induction. $adiA$ is not part of an operon, while $cadA$ is part of an operon with $cadB$. There are no acid-responding regulatory genes encoded upstream of $adiA$, while immediately upstream of $cadB$ is the membrane-bound activator gene $cadC$ which is involved in environmental sensing (Watson et al., 1992; Neely et al., 1994). Titration of an essential activator by the presence of the $cadBA$ regulatory region has been demonstrated (Meng & Bennett, 1992b), while no titration of the $adiA$ activator was detected using the $adiA$ promoter region (Stim & Bennett, 1993). A possible role for DNA gyrase and, consequently, DNA supercoiling in $adiA$ activation has been reported, while none was found in the $cad$ system (Stim & Bennett, 1993).

Some similarity in the regulation of $adiA$ to that of certain virulence genes seems to exist. A recent example is that reported with $virB$. Negative supercoiling in the $virB$ promoter (which is involved in Shigella invasion), plus the presence of VirF (a transcriptional activator similar to AdiY) were sufficient to activate $virB$ (Tobe et al., 1995). In addition, H-NS is a repressor of $virB$ (Tobe et al., 1993). The overall information available shows that although the $adi$ and $cad$ systems share several similarities, their mechanisms of acid induction are quite different, with the arginine decarboxylase system revealing features in common with various virulence systems. An investigation of the acid defence systems of $E. coli$ indicates that $adiA$ functions in extreme acid survival (Lin et al., 1995), and this work may lead to a better understanding of the role of amino acid decarboxylases in stress responses.

At this point, several proteins involved in the induction of the $adi$ system have been identified and it is possible to speculate on a model for the activation of $adiA$. A protein or proteins must be present to sense the change of the external pH environment and transmit this signal to the promoter involved in activating $adiA$. When the $adi$ system is induced, AdiY could function directly or indirectly with CysB. The small histone-like DNA binding proteins IHF and H-NS could modulate the bending and necessary access of the $adiA$ promoter for activation.

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REFERENCES


Dorman, C. J. (1992). The VirF protein from Shigella flexneri is a member of the AraC transcription factor superfamily and is highly homologous to Rns, a positive regulator of virulence genes in enterotoxigenic Escherichia coli. Mol Microbiol 6, 1575.


von Heijne, G. (1986). The distribution of positively charged
residues in bacterial inner membrane proteins correlates with the transmembrane topology. EMBO J 5, 3021–3027.


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