JOURNAL OF BACTERIOLOGY, Jan. 1999, p. 689–694 Vol. 181, No. 2
0021-9193/99/$04.00+0

Cyclic AMP Receptor Protein and TyrR Are Required for Acid pH and Anaerobic Induction of *hyaB* and *aniC* in *Salmonella typhimurium*

KYEONG R. PARK,[1] JEAN-CHRISTOPHE GIARD,[2]† JUNO H. EOM,[3] SHAWN BEARSON,[2]‡ AND JOHN W. FOSTER[2]*

Department of Microbiology and Immunology, University of South Alabama, College of Medicine, Mobile, Alabama 38866,[2] and Department of Microbiology, Han Nam University, Taejon[1] and Department of Biology, Korea University, Seoul,[3] Korea

Received 10 September 1998/Accepted 3 November 1998

Two acid-inducible genes, *aniC* and *aciK*, that require anaerobiosis and tyrosine for expression were identified as *orf326a* encoding a potential amino acid/polyamine antiporter and *hyaB* encoding hydrogenase I, respectively. Cyclic AMP (cAMP) receptor protein, cAMP, and TyrR, regulator of aromatic amino acid metabolism, were strong positive regulators of both genes.

Salmonella typhimurium undergoes extensive molecular and physiological changes following both subtle and dramatic alterations in environmental pH (5, 12). Changes that occur in response to acid pH shifts include an adaptation to acid stress called the acid tolerance response (ATR), which helps protect the organism from potentially lethal acid environments (13). The ATR involves the induction of a series of polypeptides called acid shock proteins (ASPs), some of which are presumed to protect the cell from acid stress (4, 19, 22). While the ASPs discussed above were found by using two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis, other acid-inducible genes have been identified by using gene fusion technology. Many of these acid pH-inducible genes require medium components for induction in addition to low pH (14). Although these genes have no known role in acid tolerance, they are presumed to contribute in some way to survival in low-pH environments. Our estimates indicate that over 100 genes are transcriptionally sensitive to pH. In spite of the large number of genes that respond to environmental pH, the transcriptional mechanisms by which acidic pH alters gene expression are poorly understood.

The best-characterized acid-inducible gene systems are the lysine and arginine decarboxylases of *Escherichia coli*. The *cadBA* operon, encoding lysine decarboxylase (*cadA*) and a lysine/cadaverine antiporter (*cadB*), is controlled by acid pH through a membrane sensor protein called CadC (25). The arginine decarboxylase system (*adi*) is more complex than the *cad* operon in that *adi* requires anaerobiosis, acid, and a combination of amino acids for maximal induction (31). A positive regulator has been identified in *E. coli* (*adiY*), but an antiporter that would exchange arginine for its decarboxylation product, agmatine, has not (32).

Previously, *lacZ* gene fusion techniques were used to identify acid-inducible genes in *S. typhimurium*, or regulators of those genes, that may be involved in acid tolerance (11, 14). Most of the genes found were of unknown function. One regulatory locus, *atrE* (also called *oxrG*), had a clear effect on inducible acid tolerance. This regulator controlled the expression of three acid-inducible loci called *aniC*, *aniI*, and *aciK* that also required an anaerobic environment and tyrosine for induction. The *ani* designation reflected the original identification of these genes as being anaerobiosis induced, while the *aci* designation was based on an initial screen for acid induction. None of these genes were characterized further at the time. Since *atrE* participated in the acid tolerance response, the known targets of this regulator were identified and their regulation by tyrosine and pH was more clearly defined. The strains used throughout this study are listed in Table 1.

Identification of *aniC* and *aciK*. Transcriptional fusions of *aniC* and *aciK* with the reporter gene *lacZ* were originally created via Mu*d*J insertions. The left ends of Mu*d*J junctions were cloned from chromosomal digests by first identifying the sizes of *Sal*I restriction fragments containing the kanamycin resistance gene via Southern blot hybridization with a kanamycin gene probe. Fragments of the appropriate size were excised and extracted from an agarose gel and ligated to *Sal*I-digested pBluescript SK+ vector (Stratagene, La Jolla, Calif.). The ligated mixtures were transformed via $CaCl_2$ into XL1-Blue (EK112), with selection for resistance to ampicillin. Sequencing of the junction sites was performed by using an oligonucleotide specific to the left end of Mu (Oligo 47; 5′CCAA TGTCCTCCCGGTTTTT). The results of homology searches using the predicted translation product of *aniC* indicated that the gene from *S. typhimurium* is homologous to *orf326a* in the *adi* region of *E. coli* (100% identity over 15 amino acids), with the insertion having occurred after codon 137, based on the *E. coli* sequence (Fig. 1A). This identity was also consistent with respect to map position as both the *E. coli* and *S. typhimurium* genes map to similar locations (93 centisomes).

Combining *E. coli orf326a* with the downstream open reading frame, *orf122* (Fig. 1A), would produce a protein with striking homology to a specific class of amino acid antiporters such as CadB (lysine/cadaverine antiporter) and PotE (ornithine/putrescine antiporter) that exchange extracellular amino acids for their intracellular decarboxylation products. It should be noted that it is not clear whether the "split gene" found in the *E. coli* sequence is real or the result of a sequencing error.

* Corresponding author. Mailing address: Department of Microbiology and Immunology, University of South Alabama, College of Medicine, Mobile, AL 38866. Phone: (334) 460-6323. Fax: (334) 460-7931. E-mail: fosterj@sungcg.usouthal.edu.

† Present address: Laboratoire de Microbiologie de l'Environnement, IRBA, Université de Caen, 14032 Caen Cedex, France.

‡ Present address: Department of Microbiology and Molecular Genetics, University of California—Los Angeles, Los Angeles, CA 90095.

TABLE 1. Bacterial strains

Strain	Genotype	Source or reference
S. typhimurium		
SF430 (TT15239)	*putA1019*::Mu*d*Q	K. Sanderson
SF431 (TT15240)	*putA1019*::Mu*d*P	K. Sanderson
SF434 (TT15245)	*pyrF2690*::Mu*d*Q	K. Sanderson
SF435 (TT15246)	*pyrF2690*::Mu*d*P	K. Sanderson
SF436 (TT15625)	*tre-152*::Mu*d*Q	K. Sanderson
SF437 (TT15245)	*tre-152*::Mu*d*P	K. Sanderson
SF463 (TT10423)	LT2/pNK972 Tn*10* transposase	K. Sanderson
SF530 (UK1)	Wild type	R. Curtiss III (9)
JF2690	*UK1 rpoS*ΩpRR10(Ap)	22
JF3306	UK1 *aniC1052*::Mu*d*J	14
JF3307	UK1 *aciK*(*hyaB*)*2013*::Mu*d*J	14
JF3308	UK1 *atrE406*::Tn*10*	14
JF3330	UK1 *aniC1052*::Mu*d*J *atrE406*::Tn*10*	This paper
JF3331	UK1 *aciK*(*hyaB*)*2013*::Mu*d*J *atrE406*::Tn*10*	This paper
JF3335	UK1 *rpoS*::*Ap atrE406*::Tn*10*	This paper
JF3338	UK1 *aniC1052*::Mu*d*J *rpoS*ΩpRR10(Ap)	This paper
JF3339	UK1 *rpoS*ΩpRR10(Ap) *aciK*(*hyaB*)*2013*::MudJ	This paper
JF3343	LT2 *aniC1*::Tn*10*	This paper
JF3351	Δ(*aniC-adiA*)	This paper
JF3496	UK1 *aniC1052*::Mu*d*J *tyrR411*::Tn*10d*Tc (55% to *sapD*::Mu*d*J)	This paper
JF3586	UK1 *aniC1052*::Mu*d*J *crp-773*::Tn*10*	This paper
JF3587	UK1 *hyaB*(*aniK*)*1052*::Mu*d*J *crp-773*::Tn*10*	This paper
JF3602	UK1 *aniC1052*::Mu*d*J *atrE406*::Tn*10*/pJC100 $tyrR^+$ (Ap^r)	This paper
JF3603	UK1 *hyaB*(*aciK*)*1052*::Mu*d*J *atrE*::Tn*10*/pJC100 $tyrR^+$ (Ap^r)	This paper
JF3618	UK1 *aniC1052*::Mu*d*J *tyrR411*::Tn*10d*Tc/pJC100 $tyrR^+$ (Ap^r)	This paper
JF3633	UK1 *tyrR411*::Tn*10d*Tc	This paper
JF3634	UK1 *aniC1052*::Mu*d*J *fnr-2*::Tn*10*	This paper
JF3635	UK1 *hyaB*(*aniK*)*1052*::Mu*d*J *fnr-2*::Tn*10*	This paper
JF4053	UK1 *hyaB*(*aniK*)*1052*::Mu*d*J *tyrR411*::Tn*10d*Tc	This paper
E. coli		
EF346	pKPF242 (*aniC*-MuL junction)	This paper
EF348	pKPF243 [*aciK* (*hyaB*)-MuL junction]	This paper
EF422	pKPF276 (*tyrR*-Tn*10* junction)	This paper
EK112 (XL1-Blue)	*recA1 lac* mutant *endA1 gyrA96 thi hsdR17 supE44 relA1*/F′ *proAB*+*lacI*^{q} *lacZ*Δ(M15)	Stratagene, Inc.

Inducible amino acid decarboxylase systems typically include an amino acid decarboxylase enzyme and an amino acid/polyamine antiporter. In acidic environments, protons leaking into the cell across the cell membrane will be consumed by the amino acid decarboxylation reaction (e.g., lysine decarboxylase to form cadaverine). However, to efficiently consume protons there must be a means to rapidly transport additional substrate (e.g., lysine) into the cell. The inducible lysine and ornithine decarboxylase systems include antiporter systems that exchange intracellular product (e.g., cadaverine) for extracellular substrate (e.g., lysine). The result of this exchange is the gradual alkalinization of the medium as more protons leak into the cell and are consumed. Mutants lacking either the decarboxylase or the antiporter will not alkalinize the media. As is the case in *E. coli*, the *aniC* (*orf326a*)::Mu*d*J insertion in *Salmonella* maps near *adiA*. This is evident from mutant JF3351, which contains a deletion extending from an *aniC*::Tn*10* insertion (JF3343) that also results in complete loss of arginine decarboxylase activity (data not shown). The natural supposition based upon the proximity of *aniC* to *adiA* in *E. coli* was that AniC may be the antiporter for the arginine decarboxylation product (agmatine). However, the *aniC*::Mu*d*J mutant of *S. typhimurium* exhibited normal arginine decarboxylase activity as assayed by Moeller decarboxylase media (data not shown). It remains possible that AniC is an arginine/agmatine antiporter but that multiple antiporters exist such that eliminating one would not eliminate the ability to alkalinize media.

Homology analysis of the *aciK-Mu* junction indicated that *aciK* is homologous to *hyaB*, the second gene in the six-gene *hya* operon encoding hydrogenase I in *E. coli* (87% identity over 30 amino acids [23]). The insertion occurred after codon 340 based on the *E. coli* sequence (Fig. 1B). Based upon the map position of *hya* in *E. coli* (22 centisomes), one would predict that this gene should reside near 25 centisomes in *S. typhimurium*, yet previous reports indicated that it mapped between 33 and 36 min (37 to 40 centisomes [14]). To resolve this apparent discrepancy, the location of *aciK* (*hyaB*) was confirmed by Southern hybridization to be between 37 and 40 centisomes on the *S. typhimurium* linkage map. A biotin-labeled 320-bp *aciK* fragment prepared from pKPF243 hybridized to DNA from MuP22 lysates prepared from SF434 and SF436 (carrying DNA between 37 to 40 centisomes) but failed to hybridize to MuP22 lysates from SF430 or SF431 (carrying DNA from 22 to 28 centisomes) (data not shown). These results indicated that *hya* is located at a position in *S. typhimurium* different from that in *E. coli* and probably lies within the major chromosomal inversion that distinguishes these two organisms (27). The role of hydrogenase 1 (the product of the *hya* operon) in cellular physiology is not known, other than its suspected use for dihydrogen oxidation (28).

Regulation of *aciK* (*hyaB*) and *aniC*. Previous results indicated that *aciK* (*hyaB*) and *aniC* are best expressed under anaerobic, acidic conditions in complex media (1, 2). The reason these genes were poorly expressed in minimal media

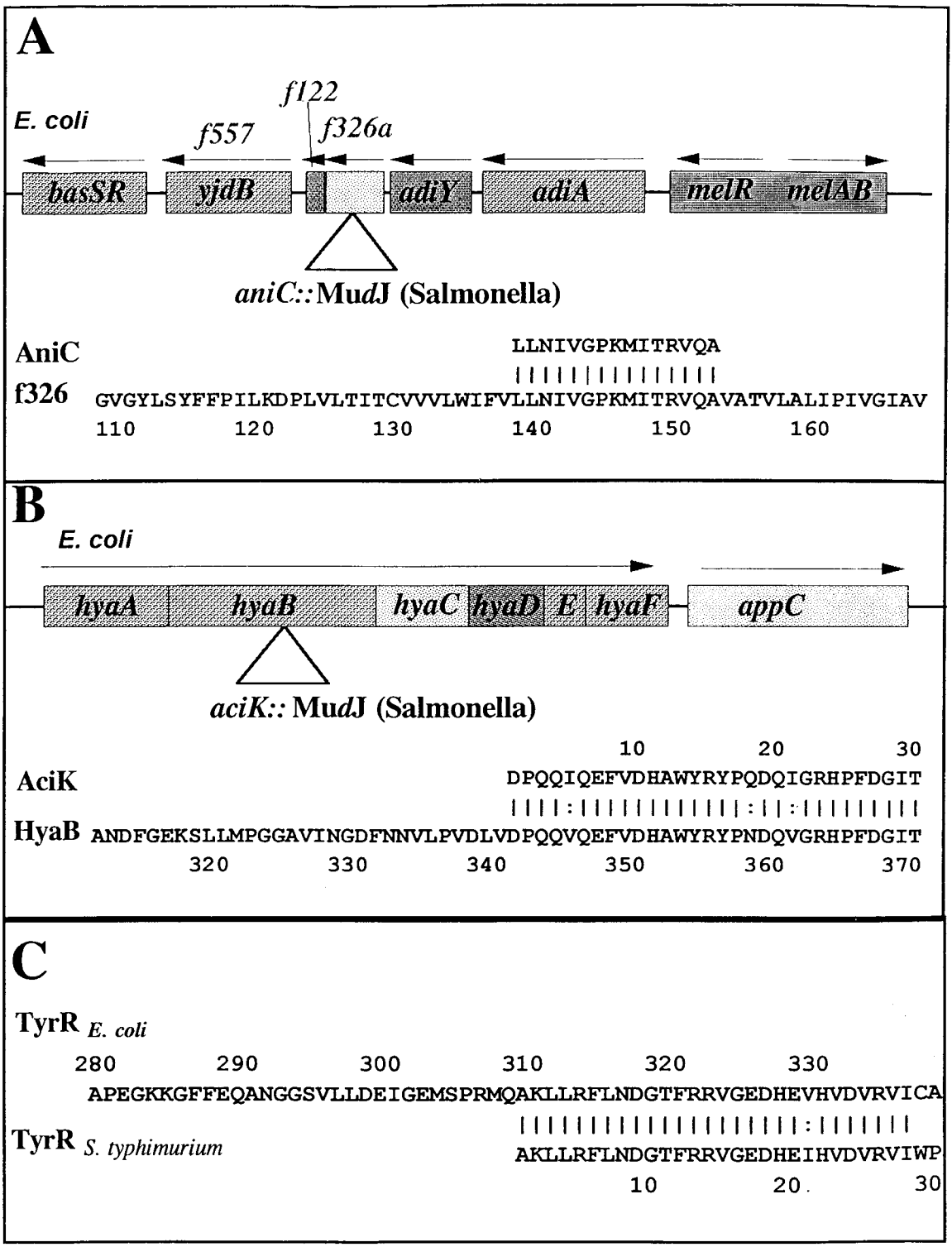

FIG. 1. Locations of Mu*d*J insertions and homologies. Locations of Mu*d*J insertions in the *S. typhimurium* homologs of *E. coli aniC* (*orf326a*) (A), *hyaB* (B), and *tyrR* (C). The upper diagrams in panels A and B illustrate gene organization in *E. coli* and the approximate site of the Mu*d*J insertion in *S. typhimurium* as determined by the sequence analysis shown at the bottom of each panel.

proved to be that they had a requirement for tyrosine as a coinducer (14). Since one of these genes is the *S. typhimurium* homolog of *hyaB*, we decided to examine the expression of both genes under conditions shown previously to affect *hya-lac* expression in *E. coli*. In these studies, β-galactosidase was measured according to the method of Miller (24) with cells grown to mid-log phase prior to assay. In *E. coli*, *hya* is repressed by nitrate and induced by formate under anaerobic

TABLE 2. Regulation of *aniC* and *aciK* (*hyaB*) by pH, anaerobiosis, tyrosine, nitrate, and formate

Strain	Genotype	β-Galactosidase activity (Miller units)[a]							
		Aerobic, pH 5.8, tyrosine[b]	Anaerobic						
			pH 7.7, tyrosine		pH 5.8				
					No tyrosine		Tyrosine		
			No KNO_3	KNO_3[c]	No formate	Formate[d]	No addition	Formate	KNO_3
JF3306	*aniC*::Mu*dJ*	0	6	21	0	0	333	350	172
JF3307	*hyaB*::Mu*dJ*	0	0	17	0	0	318	331	13

[a] Cells were grown in Vogel and Bonner E minimal medium supplemented with 0.4% glucose (EG media) (35). Anaerobiosis was achieved by paraffin oil overlay.
[b] Tyrosine was added at 100 μM.
[c] Potassium nitrate (KNO_3) was added at 0.5%.
[d] Sodium formate was added at 0.1%.

conditions (7). The results of studies with *S. typhimurium* are shown in Table 2. The data reaffirmed that maximum induction of *aniC* and *hyaB* requires anaerobiosis, acid pH, and tyrosine. As reported previously, nitrate stimulated expression of these genes at neutral to alkaline pH (2), contrary to what has been reported for *E. coli hya* (7). However, under optimally inducing acidic conditions (i.e., pH 5.8, anaerobic with tyrosine), nitrate clearly reduced expression of both genes. Nitrate repression was more dramatic for *hyaB* (24-fold) than for *aniC* (2-fold), indicating a major difference in the regulation of these genes. Repression of *hyaB* by nitrate was consistent with what has been reported for *hyaB* in *E. coli*, although tyrosine was not used in that study and the pH conditions were not clearly defined. Formate was then tested for its ability to induce *aniC* and *hyaB* in *S. typhimurium*. As noted above, growth with formate increased expression of *hya* in *E. coli*. However, when tested with *S. typhimurium*, formate did not increase expression of either *aniC* or *hyaB* (*aciK*), contrary to results with *E. coli hya* (3). Formate had no effect on *S. typhimurium hyaB* expression, even under anaerobic conditions with tyrosine (Table 2). The reason for this difference is unclear; however, we have moved *hyaB*::Mu*dJ* into several clinical strains of *S. enterica* and found similar results. Consequently, the phenotype is consistent among the salmonella strains tested.

Previous results with *E. coli hya-lac* fusions also indicated that induction in log phase requires the alternate sigma factor σ^S (3). The effects of an *rpoS*ΩpRR10(Ap) mutation on *aniC* and *hya* expression in *S. typhimurium* are shown in Table 3. In addition, the effect of the previously identified (14) positive regulator of these genes, *atrE*, is also shown. The results clearly indicated that both *aniC* and *hyaB* are under positive control by *atrE*. In addition, both genes were in some manner negatively, not positively, regulated by RpoS. RpoS control was modest, 2-fold for *aniC* and 1.5-fold for *hyaB* (*aciK*), suggesting that the effect could be indirect. Nevertheless, these results were once again the opposite of those reported for the *E. coli hya* operon, where RpoS was required for maximal activity.

We have previously observed that cyclic AMP (cAMP) receptor protein (CRP) and cAMP affected the expression of other acid pH-regulated genes (11). Consequently, the effects of *crp* and *cya* mutations on the expression of these genes were tested. Both *aniC* and *hyaB* exhibited an absolute requirement for the presence of CRP and cAMP for expression (Table 3). This also contrasts with the situation for the *E. coli hya* operon, which did not require CRP for induction (7). Again, several strains of salmonellae were tested for this phenomenon, with similar results. Consistent with the situation for *E. coli hya* (7), anaerobic control of *S. typhimurium hyaB* did not require Fnr

TABLE 3. Regulation of *aniC-lacZ* and *aciK* (*hyaB*)-*lacZ* by *rpoS*, *crp*, *atrE*, and *tyrR*

Strain	Genotype	β-Galactosidase activity (Miller units)			
		pH 7.7[a]		pH 5.8	
		− Tyrosine	+ Tyrosine	− Tyrosine	+ Tyrosine
JF3306	*aniC*::Mu*dJ*	0	3	2	358
JF3330	*aniC*::Mu*dJ atrE*::Tn*10*	1	2	3	13
JF3496	*aniC*::Mu*dJ tyrR*::Tn*10d*Tc	2	2	1	0
JF3338	*aniC*::Mu*dJ rpoS*ΩpRR10(Ap)	0	5	1	738
JF3307	*hyaB*::Mu*dJ*	0	0	0	318
JF4053	*hyaB*::Mu*dJ tyrR*::Tn*10d*Tc	0	0	0	0
JF3331	*hyaB*::Mu*dJ atrE*::Tn*10*	0	0	0	3
JF3339	*hyaB*::Mu*dJ rpoS*ΩpRR10(Ap)	0	0	0	476
JF3586	*aniC*::Mu*dJ crp*::Tn*10*	0	0	0	0
JF3587	*hyaB*::Mu*dJ crp*::Tn*10*	0	0	0	0
JF3591	*aniC*::Mu*dJ cya*::Tn*10*	0	0	0	6
JF3582	*hyaB* (*aciK*)::Mu*dJ cya*::Tn*10*	0	0	0	0
JF3602	*aniC*::Mu*dJ atrE*::Tn*10*/pJC100 (*tyrR*)	0	0	0	13
JF3603	*hyaB* (*aciK*)::Mu*dJ atrE*::Tn*10*	0	0	0	0
JF3618	*aniC*::Mu*dJ tyrR*::Tn*10*dTc/pJC100	0	6	95	697
JF3634	*aniC*::Mu*dJ fnr*::Tn*10*	0	0	0	422
JF3635	*hyaB* (*aciK*)::Mu*dJ fnr*::Tn*10*	0	0	0	248

[a] Cells were grown anaerobically in EG medium at pH 7.7 or pH 5.8 with or without tyrosine (100 μM).

(Table 3) or Arc (data not shown) (18, 33, 34). The reason for the different *hyaB* regulatory features noted when comparing *E. coli* and *S. typhimurium* is not apparent. Since *hyaB* is located at different map positions in the two organisms, it may be that the evolutionary process of moving the gene also altered its regulation.

The regulator of aromatic amino acid metabolism, TyrR, controls the tyrosine requirement for *aniC* and *hyaB* expression. A newly identified gene involved in the regulation of this system was identified following random Tn*10d*Tc transposition into an *aniC*::Mu*d*J strain. Tn*10d*Tc insertions were generated by first introducing pNK972, containing the Tn*10* transposase gene, into the strain targeted for Tn*10d*Tc transposition. Tn*10d*Tc does not contain the transposase gene. The strain containing pNK972 was then transduced (20) with P22 HT phage propagated on SF463 (TT10423). SF463 contains a Tn*10d*Tc insertion on an F factor. Because there is no homology between the F factor and the recipient chromosome, tetracycline-resistant transductants arise only through transposition. Transposon mutagenesis was performed on MacConkey lactose medium and screened under aerobic and anaerobic conditions (GasPak Systems; Becton Dickinson). Tc^r transductants were screened anaerobically on MacConkey lactose medium. Of approximately 10,000 insertion mutants screened, one produced a white colony under anaerobic conditions (JF3496). The insertion eliminated expression of both *aniC* (JF3496) and *hyaB* (JF4053; Table 3). Mapping of the Tn*10* insertion was accomplished by using the Mu*d*-P22 prophage system (6), which placed the insertion near *sapD* located at 33 centisomes (16, 17).

Identification of this regulatory gene as *tyrR* was made through sequencing the Tn*10d*Tc insertion site by a minicircle technique. The Tn*10d*Tc insertion with flanking DNA was identified from a *Sal*I digest from JF3496 via Southern hybridization with a biotin-labeled *tet* gene. *Sal*I does not cleave within the Tn*10d*Tc transposon. The DNA was excised and extracted from an agarose gel, diluted 1:20, and ligated. An oligonucleotide homologous to the inverted repeats at the ends of Tn*10* was used to PCR amplify DNA flanking Tn*10* (Oligo 51; 5′G ACAAGATGTGGATCCACCTTAAC). A 700-bp fragment was cloned into the pCRII TA cloning vector (Invitrogen, San Diego, Calif.) and used as a probe against *Sal*I-digested chromosomal DNA from JF3496 and SF530 ($tyrR^+$). The hybridization pattern indicated that the 700-bp fragment overlapped the insertion. The DNA sequence was then determined from the T7 primer site on pCRII. The results shown in Fig. 1C indicated that this gene is the *S. typhimurium* homolog of *tyrR* (90% identity over 30 amino acids), a regulator of aromatic amino acid biosynthesis and transport (15, 26).

The effects of the *tyrR*::Tn*10d*Tc insertion on gene expression are shown in Table 3. Confirmation that the insertion is in *tyrR* was obtained by showing that the function of the *S. typhimurium tyrR*::Tn*10d*Tc insertion could be complemented with a plasmid carrying *E. coli* $tyrR^+$ (Table 3, JF3618). The plasmid pJC100 was kindly provided by R. Somerville and does not express any *E. coli* genes other than *tyrR* (30). No previous report has suggested a role for TyrR in the regulation of any acid pH- or anaerobically controlled gene. Based upon these results, it is reasonable to predict that TyrR bound to tyrosine acts as a positive regulator of gene expression in this complex regulatory system.

An important feature of regulatory regions controlled by TyrR is the presence of one or more 18-bp TyrR boxes (TGT $AAAN_6$TTTACA [26]). A scan of the *E. coli* sequences revealed potential TyrR boxes upstream of *hyaA*, the first gene in the *hya* operon, and upstream of *adiY*, the gene immediately upstream of *orf326a* (the *aniC* homolog). These observations are consistent with a role for TyrR in the regulation of these genes, although no evidence that TyrR or tyrosine controls these genes in *E. coli* has been presented. Because *aniC* (encoding a potential amino acid antiporter) is located close to *adiA* (encoding arginine decarboxylase), we questioned whether *tyrR* might control *adiA* expression. However, tests in Moeller decarboxylase medium indicated that *tyrR*::Tn*10d*Tc did not affect arginine decarboxylase activity (data not shown).

Conclusions. We have identified two acid- and anaerobiosis-regulated genes in *S. typhimurium* as encoding hydrogenase I (*hyaB*, formerly *aciK*) and a potential amino acid antiporter (*aniC*). Regulatory factors required for their expression were also revealed. In addition to a previously identified gene designated *atrE*, we have now identified TyrR and CRP as essential regulators. TyrR, normally considered a regulator of aromatic amino acid biosynthesis and transport, plays an important role in regulating the expression of both genes and explains the requirement for tyrosine in their induction. TyrR, in *E. coli*, is a 53-kDa protein that represses the expression of *aroFGHLM*, *tyrB*, *tyrR*, and *aroP* (8). It activates *mtr* (a tryptophan-specific transport system) and can repress (in the presence of phenylalanine) or activate (in the presence of tyrosine) the tyrosine transport gene *tyrP* (26). Much is known about the function of TyrR in *E. coli*, but potential involvement in controlling low-pH- and anaerobiosis-inducible genes was not suspected. The function of the TyrR regulon under acidic and anaerobic conditions is a mystery. Experiments designed to reveal an anaerobic- or acid-medium phenotype related to tyrosine have so far failed. Tyrosine does not appear to provide an anaerobic growth advantage to wild-type versus *tyrR* mutant strains of *S. typhimurium* (data not shown).

A third essential positive regulator of these genes was determined to be the CRP which has previously been implicated in the pH control of another low-pH-regulated gene, *aniG* (now identified as *exu* [24a]). How pH controls the expression of these genes is unknown, but there are several possibilities. First, pH may affect DNA topology in the region of the target genes through alteration in DNA supercoiling (21). This has been suggested for several environmentally regulated genes (10). The alteration in DNA topology would influence the ability of TyrR and CRP to bind to their respective target DNA sequences. A second possibility is that there is another as yet unidentified pH sensor (possibly AtrE) that transmits a signal to the target genes. Finally, either TyrR or CRP might sense alterations in internal pH. Although one would not expect large differences in internal pH at external pH values between 6 and 8, the differences may be more significant under anaerobic conditions and in the presence of organic acid fermentation end products.

Many of the acid pH-induced genes identified thus far require coinducers, such as tyrosine, for expression (11, 14, 19, 25, 29). The mechanisms used to integrate acid pH and coregulator signals are not fully elucidated but clearly vary depending upon the system. It is apparent that *S. typhimurium* possesses specific genetic systems that sense and respond to encounters with acidic pH. Which pH response systems become engaged depends in large measure on the chemical composition of the environment. While the adaptive advantage of some of these systems (*cadBA*) is apparent (e.g., acid tolerance), the benefits of others (*aniC* and *hyaB*) remain enigmatic.

We thank R. Somerville, K. Sanderson, and R. Curtiss III for their generous gifts of various strains and plasmids. Various discussions with

M. Moreno and M. Spector were especially helpful and are gratefully acknowledged.

This work was supported by an award (GM48017) from the National Institutes of Health.

REFERENCES

1. **Aliabadi, Z., Y. K. Park, S. L. Slonczewski, and J. W. Foster.** 1988. Novel regulatory loci controlling oxygen and pH-regulated gene expression in *Salmonella typhimurium*. J. Bacteriol. **170:**842–851.
2. **Aliabadi, Z., F. Warren, S. Mya, and J. W. Foster.** 1986. Oxygen-regulated stimulons of *Salmonella typhimurium* identified by Mu*d*(A*plac*) operon fusions. J. Bacteriol. **165:**780–786.
3. **Atlung, T., K. Kundsen, L. Heerfordt, and L. Brondsted.** 1997. Effects of σ^s and the transcriptional activator AppY on induction of the *Escherichia coli hya* and *cbdAB-appA* operons in response to carbon and phosphate starvation. J. Bacteriol. **179:**2141–2146.
4. **Bearson, B. L., L. Wilson, and J. W. Foster.** 1998. A low pH-inducible, PhoPQ-dependent acid tolerance response protects *Salmonella typhimurium* against inorganic acid stress. J. Bacteriol. **180:**2409–2417.
5. **Bearson, S., B. Bearson, and J. W. Foster.** 1997. Acid stress responses in enterobacteria. FEMS Microbiol. Lett. **147:**173–180.
6. **Benson, N. R., and B. S. Goldman.** 1992. Rapid mapping in *Salmonella typhimurium* with Mu*d*-P22 prophages. J. Bacteriol. **174:**1673–1681.
7. **Brondsted, L., and T. Atlung.** 1994. Anaerobic regulation of the hydrogenase 1 (*hya*) operon of *Escherichia coli*. J. Bacteriol. **176:**5423–5428.
8. **Cui, J., L. Ni, and R. L. Somerville.** 1993. ATPase activity of TyrR, a transcriptional regulatory protein for sigma^{70} RNA polymerase. J. Biol. Chem. **268:**13023–13025.
9. **Curtiss, R., III, S. B. Porter, M. Munson, S. A. Tinge, J. O. Hassan, C. Gentry-Weeks, and S. M. Kelly.** 1981. Nonrecombinant and recombinant avirulent *Salmonella* vaccines for poultry, p. 169–198. *In* L. C. Blankenship, J. H. S. Bailey, N. A. Cox, N. J. Stern, and R. J. Meinersmann (ed.), Colonization control of human bacterial enteropathogens in poultry. Academic Press, New York, N.Y.
10. **Dorman, C. J.** 1995. DNA topology and the global control of bacterial gene expression: implications for the regulation of virulence gene expression. Microbiology **141:**1271–1280.
11. **Foster, J., and Z. Aliabadi.** 1989. pH-regulated gene expression in *Salmonella*: genetic analysis of *aniG* and cloning of the *earA* regulator. Mol. Microbiol. **3:**1605–1615.
12. **Foster, J. W.** 1995. Low pH adaptation and the acid tolerance response of *Salmonella typhimurium*. Crit. Rev. Microbiol. **21:**215–237.
13. **Foster, J. W., and H. K. Hall.** 1990. Adaptive acidification tolerance response of *Salmonella typhimurium*. J. Bacteriol. **172:**771–778.
14. **Foster, J. W., Y. K. Park, I. S. Bang, K. Karem, H. Betts, H. K. Hall, and E. Shaw.** 1994. Regulatory circuits involved with pH-regulated gene expression in *Salmonella typhimurium*. Microbiology **140:**341–352.
15. **Gollub, E. G., K. P. Liu, and D. B. Sprinson.** 1973. *tyrR*, a regulatory gene of tyrosine biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **115:**1094–1102.
16. **Groisman, E. A., and H. Ochman.** 1994. How to become a pathogen. Trends Microbiol. **2:**289–293.
17. **Groisman, E. A., C. Parra-Lopez, M. Salcedo, and C. J. Lipps.** 1992. Resistance to host antimicrobial peptides is necessary for *Salmonella* virulence. Proc. Natl. Acad. Sci. USA **89:**11939–11943.
18. **Gunsalus, R., and S. Park.** 1994. Aerobic-anaerobic gene regulation in *Escherichia coli*: control by the ArcAB and Fnr regulons. Res. Microbiol. **145:**437–450.
19. **Hall, H. K., and J. W. Foster.** 1996. The role of Fur in the acid tolerance response of *Salmonella typhimurium* is physiologically and genetically separable from its role in iron acquistion. J. Bacteriol. **178:**5683–5691.
20. **Holley, E. A., and J. W. Foster.** 1982. Bacteriophage P22 as a vector for Mu mutagenesis in *Salmonella typhimurium*: isolation of *nad-lac* and *pnc-lac* gene fusions. J. Bacteriol. **152:**959–962.
21. **Karem, K., and J. W. Foster.** 1993. The influence of DNA topology on the environmental regulation of a pH-regulated locus in *Salmonella typhimurium*. Mol. Microbiol. **10:**75–86.
22. **Lee, I. S., J. Lin, H. K. Hall, B. Bearson, and J. W. Foster.** 1995. The stationary-phase sigma factor σ^s(RpoS) is required for a sustained acid tolerance response in virulent *Salmonella typhimurium*. Mol. Microbiol. **17:**155–167.
23. **Menon, N. K., J. Robbins, H. D. J. Peck, C. Y. Chatelus, E.-S. Choi, and A. E. Przybyla.** 1990. Cloning and sequencing of a putative *Escherichia coli* [NiFe] hydrogenase-1 operon containing six open reading frames. J. Bacteriol. **172:**1969–1977.
24. **Miller, J. H.** 1992. A short course in bacterial genetics. A laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

24a. **Moreno, M., and J. W. Foster.** Unpublished data.

25. **Neeley, M. N., C. L. Dell, and E. R. Olsen.** 1994. Roles of LysP and CadC in mediating the lysine requirement for acid induction of the *Escherichia coli cad* operon. J. Bacteriol. **176:**3278–3285.
26. **Pittard, A., and B. Davidson.** 1991. TyrR protein of *Escherichia coli* and its role as repressor and activator. Mol. Microbiol. **5:**1585–1592.
27. **Sanderson, K. E., and J. R. Roth.** 1988. Linkage map of *Salmonella typhimurium*, edition VII. Microbiol. Rev. **52:**485–532.
28. **Sawers, R. G., D. J. Jamieson, C. F. Higgins, and D. H. Boxer.** 1986. Characterization and physiological roles of membrane-bound hydrogenase isoenzymes from *Salmonella typhimurium*. J. Bacteriol. **168:**398–404.
29. **Schlensog, V., and A. Bock.** 1990. Identification and sequence analysis of the gene encoding the transcriptional activator of the formate hydrogenlyase system of *Escherichia coli*. Mol. Microbiol. **4:**1319–1326.
30. **Somerville, R. L., T.-L. N. Shieh, B. Hagewood, and J. Cui.** 1991. Gene expression from multicopy T7 promoter vectors proceeds at single copy rates in the absence of T7 RNA polymerase. Biochem. Biophys. Res. Commun. **181:**1056–1062.
31. **Stim, K. P., and G. N. Bennett.** 1993. Nucleotide sequence of the *adi* gene, which encodes the biodegradative acid-induced arginine decarboxylase of *Escherichia coli*. J. Bacteriol. **175:**1221–1234.
32. **Stim-Herndon, K. P., T. M. Flores, and G. N. Bennett.** 1996. Molecular characterization of *adiY*, a regulatory gene which affects expression of the biodegradative acid-induced arginine decarboxylase (*adiA*) of *Escherichia coli*. Microbiology. **142:**1311–1320.
33. **Unden, G., and J. Schirawski.** 1997. The oxygen-responsive transcriptional regulator FNR of *Escherichia coli*: the search for signals and reactions. Mol. Microbiol. **25:**205–210.
34. **Unden, G., and M. Trageser.** 1991. Oxygen regulated gene expression in *Escherichia coli*: control of anaerobic respiration by the FNR protein. Antonie Leeuwenhoek **59:**65–76.
35. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218:**97–106.