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Cloning of the *Klebsiella aerogenes nac* Gene, Which Encodes a Factor Required for Nitrogen Regulation of the Histidine Utilization (*hut*) Operons in *Salmonella typhimurium*

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The *nac* (nitrogen assimilation control) gene from *Klebsiella aerogenes*, cloned in a low-copy-number cloning vector, restored the ability of *K. aerogenes nac* mutants to activate histidase and repress glutamate dehydrogenase formation in response to nitrogen limitation and to limit the maximum expression of the *nac* promoter. When present in *Salmonella typhimurium*, the *K. aerogenes nac* gene allowed the *hut* genes to be activated during nitrogen-limited growth. Thus, the *nac* gene encodes a cytoplasmic factor required for activation of *hut* expression in *S. typhimurium* during nitrogen-limited growth.

The enteric bacteria *Klebsiella aerogenes* and *Salmonella typhimurium* can use the amino acid histidine as the sole source of carbon or nitrogen, using the enzymes encoded by the histidine utilization (*hut*) gene cluster (10, 16). In *S. typhimurium*, histidase formation (a convenient reporter for *hut* expression) is strongly repressed by glucose because activation of *hut* expression requires the catabolite gene activator protein-cyclic AMP complex (16). Thus, histidine can serve as the sole nitrogen source for *S. typhimurium* only in the absence of glucose (16). In contrast, *K. aerogenes* has an additional mode of activating *hut* expression, and this activation is dependent on the general nitrogen regulatory (NTR) system (17).

S. typhimurium has a fully functional NTR system (13), and replacement of the *K. aerogenes glnA ntrBC* (or *glnALG*) operon by the homologous *S. typhimurium* genes did not alter the regulation of hut_S (*hut* genes from *S. typhimurium*) in the *K. aerogenes* cytoplasm (4). Thus, it seemed logical to assume that the hut_S target for NTR regulation might be lacking. However, when F′ hut_S was transferred to *K. aerogenes*, hut_S was activated by nitrogen limitation just as hut_K (*hut* from *K. aerogenes*) was (9). As a result, Bloom and colleagues postulated a "cytoplasmic factor missing or defective in *S. typhimurium* and required for nitrogen regulation of *hut* expression by the NTR system" (4).

K. aerogenes strains with mutations in the *nac* gene have a phenotype very similar to that of wild-type *S. typhimurium*: both wild-type *S. typhimurium* and *K. aerogenes nac* mutants have normal nitrogen regulation of *glnA* expression but no nitrogen regulation of *hut*, *put* (proline utilization), or *gdh* (glutamate dehydrogenase) expression (2, 15). The phenotypic similarity between *K. aerogenes nac* mutants and wild-type *S. typhimurium* strains led us to suspect that NAC (the product of the *nac* gene) might be the factor required in *S. typhimurium* for the nitrogen regulation of histidase expression. We therefore cloned *nac* from *K. aerogenes* and tested whether the cloned *nac* would confer nitrogen regulation on the *hut* operons in *S. typhimurium*.

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MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used are listed in Table 1. All *E. coli* strains were derivatives of strain K-12, *K. aerogenes* strains were derived from KC1043, and *S. typhimurium* strains were derived from 15-59. Conditions for growth and enzyme assays have been described previously (15). Specific activities of enzymes are reported as units per milligram of total cell protein, where 1 U leads to the production of 1 nmol of product or consumption of 1 nmol of substrate per min at 37°C (except for β-galactosidase, which was measured at 30°C). All enzyme activities are the averages of the results for two or more independently grown cultures and vary less than 15% from the mean. Recombinant DNA techniques were essentially as described by Maniatis et al. (18).

DNA isolation. Plasmid DNA was prepared as described previously (3, 18). Chromosomal DNA from *K. aerogenes* was prepared from 100-ml cultures grown to saturation in TB (21) at 30°C. Cells collected by centrifugation were resuspended in 9 ml of STET (20% sucrose, 50 mM Tris [pH 7.6], 50 mM EDTA). A 10-mg amount of lysozyme in 1 ml of TE (10 mM Tris [pH 7.6], 1 mM EDTA) was added. After 30 min of incubation at 37°C, 1 ml 25% (wt/vol) sodium dodecyl sulfate in TE was added, followed by 1 ml of protease (1 mg of proteinase K per ml in TE), and the mixture was incubated for 60 min at 50°C with occasional gentle shaking. The mixture was extracted twice with phenol-Sevag (phenol-chloroform-isoamyl alcohol, 25:24:1). The aqueous phase was collected, and 0.5 volume of 7.5 M ammonium acetate and 2 volumes of 95% ethanol were added. The precipitated DNA at the interface was collected with a glass microcapillary pipette and dissolved overnight in 5 ml of TE. DNase-free RNase was added to a final concentration of 100 mg/ml; following incubation for 60 min at 37°C, the mixture was extracted, precipitated, and collected as described above. The DNA was dissolved in 4 ml of TE and stored at 4°C.

RESULTS

Cloning and characterization of the wild-type *nac* gene. The wild-type *nac* gene was isolated by using a modification of the marker exchange procedure described by Goss and Datta (11). Briefly, this involved an in vitro step in which a DNA

TABLE 1. Strains and plasmids

Strain or plasmid	Relevant characters	Source or reference
Strains		
E. coli		
DH5α	*hsdR17*	Bethesda Research Laboratories
EG48	*hsdR*(F′ *hut*$_S$)	9
K. aerogenes		
KC1043	*hutC515*	15
KC1047	Δ(*gal-hut-bio*)*401*	This laboratory
KC1611	*hutC515 nac-101*::Mu dX	15
KC1665	*hutC515 nac-112*::Mu dX	15
KC1786	*hutC515 nac-203*::Tn*5*	15
KC1836	Δ(*gal-hut-bio*)*401 nac-203*::Tn*5*	P1.KC1786 × KC1047[a]
KC1837	Δ(*gal-hut-bio*)*401*(pBR322)	
KC1839	*hutC515 nac-204*::λ *plac* Mu53	15
KC1855	Δ(*gal-hut-bio*)*401 nac-203*::Tn*5*(F′*hut*$_S$)	EG48→KC1836[b]
KC1864	Δ(*gal-hut-bio*)*401*(F′ *hut*$_S$)(pBR322)	EG48→KC1837
KC1921	KC1043(pEC201)	Transformation[c]
KC1967	KC1786(pEC205)	Transformation
KC2016	KC1611(pEC205)	Transformation
KC2208	KC1839(pEC216)	Transformation
KC2220	KC1043(pEC216)	Transformation
KC2222	KC1043(pEC216)	Transformation
KC2224	KC1786(pEC216)	Transformation
KC2225	KC1839(pEC216)	Transformation
KC2267	KC1611(pEC216)	Transformation
KC2268	KC1665(pEC216)	Transformation
KC2282	KC1839(pEC201)	Transformation
KC2623	*hutC515 nac-203*::Tn*5-131*(pCJ5)	Transformation
S. typhimurium		
NE7	*hutC7*	B. Magasanik
SB2269	NE7(pEC216)	Transformation
Plasmids		
F′*hut*$_S$	F′ *gal*$^+$ *hutC7 bio*$^+$	9
pBGS9	Very-high-copy-number cloning vector	19
pBR322	High-copy-number cloning vector	5
pCJ5	*nac*$^+$ cloned in pBGS9	This work
pEC201	*nac-203*::Tn*5* cloned in pBR322	This work
pEC205	*nac*$^+$ cloned in pBR322	This work
pEC216	*nac*$^+$ cloned in pLG338	This work
pLG338	Low-copy-number cloning vector	20

[a] Transduction (8) of strain KC1047 with phage P1 *vir* grown on strain KC1786.
[b] Conjugal transfer from strain EG48 to strain KC1836.
[c] Transformation (1) of strain KC1043 with plasmid pEC201.

fragment containing a mutant *nac*::Tn*5* allele was cloned, using the kanamycin resistance encoded by Tn*5* as the selection, and then an in vivo step in which the *nac*::Tn*5*-containing clone was used as a tool for rescuing the functional *nac*$^+$ gene by homologous recombination.

Chromosomal DNA from a *nac*::Tn*5* strain (KC1786) was cleaved with *Eco*RI, which does not cleave within Tn*5* (12), and ligated into the *Eco*RI site of plasmid pBR322. Two Km^r clones, isolated in independent experiments, were chosen for further analysis. Plasmid DNA was isolated from these two clones and digested with *Eco*RI. In addition to a 4.4-kb fragment, which corresponded to the pBR322 cloning vector, both clones contained a large (ca. 17.1-kb) fragment. One of the clones contained an additional *Eco*RI fragment of approximately 4.7 kb. The recombinant plasmid containing the single 17.1-kb *Eco*RI insert was designated pEC201 and was characterized in more detail. The restriction map of pEC201 is shown in Fig. 1.

The Tn*5* element that caused the *nac-203*::Tn*5* mutation was located approximately in the middle of the cloned *Eco*RI fragment, about 5 to 6 kb from each end (Fig. 1). Thus, it seemed likely that pEC201 would contain all of the sequences required for *nac* expression. The cloned *nac*::Tn*5* was then used in the in vivo step of rescuing the wild-type *nac* gene. For this purpose, it was important to note that the Tn*5* sequences contain three cleavage sites for *Xho*I and that no other *Xho*I sites exist in pEC201. Plasmid pEC201 was introduced into wild-type *K. aerogenes* KC1043 by transformation. The resulting strain, KC1921, was grown to stationary phase in L broth supplemented with tetracycline. Plasmid DNA isolated from strain KC1921 was expected to contain two classes of plasmid molecules. The majority class of plasmids would be expected to be parental molecules containing *nac*::Tn*5*, conferring resistance to ampicillin, tetracycline, and kanamycin, and containing cleavage sites for *Xho*I. A relatively small number of the plasmids isolated from KC1921 would be expected to be resistant to ampicillin and tetracycline, but not to kanamycin, and to lack cleavage sites for *Xho*I. This latter, minority class of plasmid would have either lost the Tn*5* element contained in pEC201 by excision or undergone recombinational events between sequences flanking the Tn*5* element on the plasmid and chromosomal sequences within and adjacent to the *nac*$^+$ gene. To select against the predominant, parental-type plasmids, plasmid DNA isolated from KC1921 was digested with *Xho*I and then exonuclease III and used to transform *Escherichia*

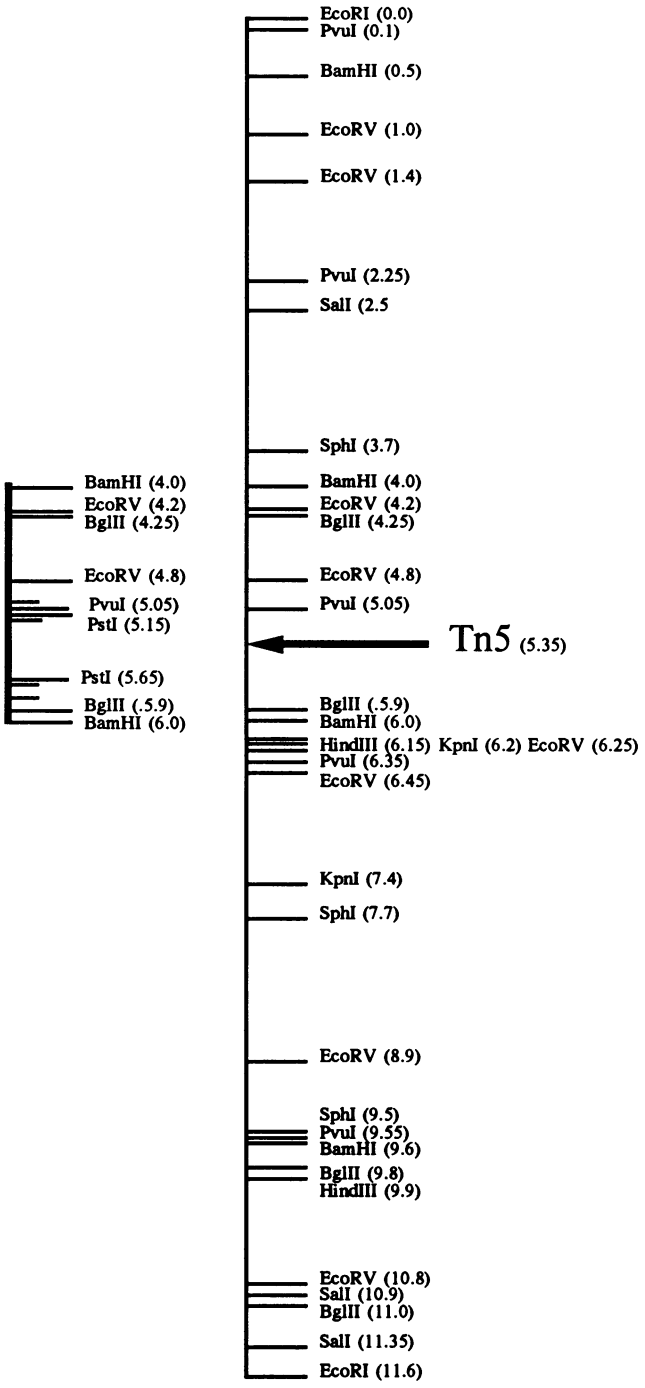

FIG. 1. Restriction map of the *nac* region from *K. aerogenes*. The upper line shows the restriction enzyme cleavage sites in the 11.6-kb *Eco*RI fragment cloned in pEC205. The arrow at position 5.35 (measured in kilobases from the left end) indicates the site of the Tn*5* insertion in *nac-203*::Tn*5*. The lower line indicates the restriction sites in the 2-kb *Bam*HI fragment cloned in pCJ5. The shorter, unlabeled vertical lines indicate cleavage sites for the enzyme *Pvu*II, located at positions 5.0, 5.15, 5.7, and 5.8. The relative positions of the *Pvu*II and *Pst*I cleavage sites at position 5.15 could not be determined.

coli DH5α to ampicillin and tetracycline resistance. From approximately 1 μg of *Xho*I-exonuclease III-digested pEC201 DNA obtained from KC1921, a total of 180 drug-resistant transformants were obtained. All 180 of these Ap^r Tc^r transformants were found to be kanamycin sensitive.

Thirty-six of these kanamycin-sensitive transformants were studied further. Digestion of plasmid DNA with *Eco*RI revealed the existence of three types of plasmids. One of the clones examined contained an *Eco*RI fragment of approximately 11.5 kb, consistent with the loss of a 5.7-kb Tn*5* element from the initial 17.3-kb cloned fragment. This clone, presumed to carry the intact wild-type *nac* region, was saved as pEC205. Thirty-three of the clones contained an approximately 7-kb *Eco*RI insert; one example was saved as pEC206. The remaining two clones contained an approximately 2-kb *Eco*RI insert; one example was saved as pEC207.

Verification. It was important to verify that the chromosomal DNA fragment contained in plasmid pEC201 was indeed from the *nac* region of the *K. aerogenes* chromosome and not a randomly cloned chromosomal fragment into which Tn*5* had subsequently transposed. If the cloned DNA fragment in pEC201 was from the *nac* region of the chromosome, then pEC205 should contain DNA sequences homologous to the *nac*::Tn*5* region in the well-characterized *K. aerogenes* mutant KC1786. Moreover, it should be possible for recombination to occur between sequences in pEC205 and in the *nac*::Tn*5* region of the chromosome of KC1786. Recombinant plasmids obtained would be expected to confer resistance to a high concentration of kanamycin, as a result of a gene dosage effect, and to have a physical structure identical to that of pEC201. Accordingly, we introduced plasmid pEC205 into strain KC1786 by transformation and then selected for a recombination event which would replace *nac* sequences on the plasmid with *nac*::Tn*5* by plating KC1967 in the presence of 500 μg of kanamycin per ml. Plasmid DNA isolated from six independently derived clones was found to be increased in size by approximately 5.6 kb, consistent with replacement of *nac* sequences on the plasmid by *nac*::Tn*5* sequences in the chromosome (data not shown). One highly kanamycin resistant clone was saved as pEC208. Digestion of plasmid pEC201 and pEC208 with restriction enzyme *Sal*I produced five DNA fragments of identical size, indicating that Tn*5* had been incorporated into the same *Sal*I fragment of pEC208 as of pEC201.

Finally, a 2-kb *Bam*HI fragment derived from pEC205 was cloned into pBGS9. This *Bam*HI fragment contained the site of the *nac-203*::Tn*5* insertion. The resulting plasmid, pCJ5, complemented the *nac-203* mutation fully both for activation of histidase expression and for repression of glutamate dehydrogenase expression (Table 2, line 5). Thus, the *nac* gene appeared to be wholly contained by the 2-kb *Bam*HI fragment that flanked the site of the Tn*5* insertion in *nac-203*::Tn*5*. DNA sequence analysis of the region (A. Schwacha and R. A. Bender, unpublished data) revealed an open reading frame, presumed to be *nac*, extending from about kb 4.8 to 5.8, consistent with *nac* being wholly contained within the 2-kb *Bam*HI fragment.

Complementation studies. To avoid the difficulties sometimes associated with high-copy-number plasmids, we recloned the *Eco*RI fragment from pEC205 in a low-copy-number cloning vector, pLG338, before testing its ability to complement *nac* mutations. The resulting low-copy-number *nac* clone, pEC216, was introduced into *K. aerogenes* KC1786 (*nac-203*::Tn*5*). The introduction of plasmid pEC216 into KC1786 (strain KC2224) restored the ability of

TABLE 2. Complementation of *nac* mutations by cloned *nac*[a]

Strain	Relevant genotype	Plasmid	Sp act							
			Histidase		Glutamate dehydrogenase		β-Galatosidase		Glutamine synthetase	
			−N	+N	−N	+N	−N	+N	−N	+N
1. KC1043	Wild type	—[c]	430	53	45	370	1	1	1,900	150
2. KC2220	Wild type	pEC216	370	42	36	280	ND[d]	ND	ND	ND
3. KC1786	*nac-203*::Tn*5*	—	43	33	460	360	ND	ND	1,800	190
4. KC2224	*nac-203*::Tn*5*	pEC216	330	47	28	250	ND	ND	ND	ND
5. KC2623	*nac-203*::Tn*5-131*	pCJ5	350	18	11	300	ND	ND	1,200	91
6. KC1864	*nac*$^+$ Δ*hut*	F′*hut*$_S$	72	11	33	440	ND	ND	ND	ND
7. KC1855	*nac-203*::Tn*5* Δ*hut*	F′*hut*$_S$	14	8	490	480	ND	ND	ND	ND
8. NE7	Wild type[b]	—	15	13	580	210	ND	ND	2,400	240
9. SB2269	Wild type[b]	pEC216	62	15	240	160	ND	ND	2,800	170
10. KC1665	*nac-101*::Mu dX	—	100	71	420	380	900	5	ND	ND
11. KC2222	*nac-101*::Mu dX	pEC205	110	57	500	440	1,000	6	ND	ND
12. KC2268	*nac-101*::Mu dX	pEC216	550	70	43	320	660	4	ND	ND
13. KC1611	*nac-112*::Mu dX	—	210	56	260	340	2,900	5	ND	ND
14. KB2016	*nac-112*::Mu dX	pEC205	200	31	260	360	1,300	6	ND	ND
15. KC2267	*nac-112*::Mu dX	pEC216	560	63	37	310	1,200	5	ND	ND
16. KC1839	*nac-204*λ *plac* Mu53	—	81	64	460	360	7,400	6	ND	ND
17. KC2208	*nac-204*λ *plac* Mu53	pEC205	400	32	42	230	660	4	ND	ND
18. KC2225	*nac-204*λ *plac* Mu53	pEC216	300	50	36	310	1,500	6	ND	ND
19. KC2282	*nac-204*λ *plac* Mu53	pEC201	160	53	220	270	5,200	6	ND	ND

[a] Strains were grown in glucose minimal medium with 0.025% (wt/vol) glutamine as the growth-rate-limiting nitrogen source in the presence (+N) or absence (−N) of 0.2% (wt/vol) ammonium sulfate. Specific activities were determined as described in Materials and Methods.
[b] *S. typhimurium*.
[c] —, Absence of a plasmid.
[d] ND, Not determined.

those cells to regulate expression of *hut* and *gdh*: histidase was activated to wild-type levels and *gdh* was repressed to wild-type levels in response to nitrogen limitation (Table 2, lines 3 and 4). In addition, the presence of plasmid pEC216 allowed strain KC2224 to use histidine and proline as sole sources of nitrogen, even in the presence of glucose. As expected, plasmid pEC216 had no obvious effect on regulation of *hut* or *gdh* in wild-type *K. aerogenes* cells (lines 1 and 2).

Regulation of the *hut* operons of *S. typhimurium*. As expected, wild-type *S. typhimurium* was capable of responding to nitrogen limitation in that glutamine synthetase formation was about 10-fold derepressed. Nevertheless, neither the derepression of histidase formation nor the repression of glutamate dehydrogenase formation normally seen in *K. aerogenes* was seen during nitrogen limitation of *S. typhimurium* (16; Table 2, line 8). Thus, wild-type *S. typhimurium* resembled the *nac* mutants of *K. aerogenes*. It had been shown previously that *hut*$_S$ was derepressed in response to nitrogen limitation if present in a *K. aerogenes* background (9). We therefore tested whether this derepression of *hut*$_S$ in a *K. aerogenes* background required a wild-type *nac* gene product.

F′ *hut*$_S$, an F′ plasmid carrying the *hut* genes from *S. typhimurium*, was introduced into isogenic *nac* and *nac*$^+$ strains of *K. aerogenes* deleted for *hut*$_K$. Derepression of *hut*$_S$ in response to nitrogen limitation required the *K. aerogenes nac* gene product. In the *nac*$^+$ strain, KC1864, histidase synthesis from *hut*$_S$ was sevenfold derepressed in response to nitrogen limitation. In the isogenic *nac* mutant, KC1855, histidase synthesis was not derepressed in response to nitrogen limitation (Table 2, lines 6 and 7).

We next examined the ability of the *K. aerogenes nac* gene to activate expression of the *S. typhimurium hut* genes when the *hut* genes were present in their native *S. typhimurium* background. Introduction of the *K. aerogenes nac* plasmid pEC216 into *S. typhimurium* (strain SB2269) resulted in a fourfold derepression of histidase formation in response to nitrogen limitation (Table 2, lines 8 and 9). Moreover, strain SB2269 (but not NE7) was able to use histidine as a sole nitrogen source even in the presence of glucose. Thus, *nac* was both necessary and sufficient for nitrogen-regulated activation of the *S. typhimurium hut* operons. The activation of histidase expression to 62 U/mg in strain SB2269 (NE7 with cloned *nac*) during nitrogen starvation was virtually identical to the activation to 57 U/mg reported for NE7 during carbon starvation (6). A derivative of *S. typhimurium* LT2 carrying the *hut*$_S$ genes from strain NE7 was also able to use histidine as sole nitrogen source even in the presence of glucose if plasmid pEC216 was present in the strain but not if it was absent (data not shown). Thus, *S. typhimurium* LT2 also lacked an active *nac* gene product.

We also examined the effect of plasmid pEC216 on regulation of glutamate dehydrogenase synthesis and, in an indirect manner, on regulation of the proline utilization genes in *S. typhimurium*. The regulation of glutamate dehydrogenase synthesis in *S. typhimurium* was at best only slightly affected by the presence of the *K. aerogenes nac* gene (Table 3, lines 8 and 9). Moreover, strain SB2269 was unable to utilize proline as a sole nitrogen source in the presence of glucose.

Regulation of *nac* expression. We had previously shown that *nac-lac* fusions were nitrogen regulated and that this nitrogen regulation required the components of the NTR system and the alternative sigma factor, σ^{54} (15). This regulation was seen even in the absence of a functional *nac* product, so it was concluded that *nac* was not strictly necessary for the regulation. Nevertheless, there were some differences in the degree of derepression of the various *nac-lac* fusions in response to nitrogen limitation. We therefore measured *nac-lac* expression in merodiploids with wild-

type *nac* present in low copy number. The three *nac-lac* fusions studied each responded differently to the presence of a copy of the wild-type *nac*. The *nac-101*::Mu dX *lacZ* fusion was unaffected by the presence of wild-type *nac* and showed about 100- to 200-fold derepression in response to nitrogen limitation in both the haploid and the merodiploid state (Table 3, lines 10 and 12). The *nac-112*::Mud X *lacZ* fusion showed about 600-fold derepression in the haploid state but only about 200-fold repression when wild-type *nac* was present in low copy number (lines 13 and 15). The *nac-204*::λ *plac* Mu53 *lacZ* fusion showed over 1,000-fold derepression in the haploid state but again only about 100- to 200-fold derepression when wild-type *nac* was present in low copy number (lines 16 and 18). In short, the differences in the derepression ratio of the *nac-lac* fusions seen in the haploids disappeared when a wild-type *nac* gene was present in *trans*. The defect in the regulation of histidase and glutamate dehydrogenase associated with the *nac-lac* mutations was fully complemented in each of the three cases.

The wild-type *nac* region cloned into the higher-copy-number vector (pEC205) was also used in complementation analysis against the three *nac-lac* fusions. Again the merodiploids showed about 100- to 200-fold derepression ratios; the *nac-204*::*lacZ* fusion was slightly higher but still reduced relative to the haploid (Table 2, lines 11, 14, and 17). Surprisingly, the high-copy-number *nac* was less effective than the low-copy-number clone in complementing the defective histidase and glutamate dehydrogenase regulation. Only the *nac-204*::λ *plac* Mu allele showed full complementation of histidase and glutamate dehydrogenase regulation with the high-copy-number *nac* clone (line 17). Both *nac-101* and *nac-112* showed no difference in regulation of histidase and glutamate dehydrogenase between the haploid and the high-copy-number merodiploid states (lines 10, 11, 13, and 14). As expected, pEC201, a high-copy-number clone with the *nac* gene inactivated (*nac-203*::Tn*5*), failed to complement even the *nac-204* allele in any assay (line 19).

DISCUSSION

Transfer of the cloned *K. aerogenes nac* gene into *S. typhimurium* resulted in nitrogen regulation of the resident *hut* operons. Thus, we conclude that the *K. aerogenes nac* gene provided the cytoplasmic factor (4) which, in concert with the NTR system, was necessary for activation of the *S. typhimurium hut* genes during nitrogen-limited growth. Our data did not allow us to distinguish whether *S. typhimurium* lacks a *nac* gene or encodes a defective *nac* gene product. Although *K. aerogenes nac* conferred nitrogen regulation on *S. typhimurium hut*, the *K. aerogenes nac* gene did not confer nitrogen regulation on the *S. typhimurium put* or *gdh* operon. This difference remains unexplained. Perhaps the *S. typhimurium put* and *gdh* operons have lost (or never had) target sites for the action of the *nac* product, or perhaps *K. aerogenes* and *S. typhimurium* have diverged sufficiently that the *K. aerogenes nac* can no longer recognize the target sites in *S. typhimurium put* or *gdh*.

The regulation of *nac* gene expression by the *nac* product was unexpected. NTR-mediated activation of *nac* expression in response to nitrogen limitation did not require an active *nac* product, but the presence of an active *nac* product somehow limited the maximum accumulation of *nac* product at about 100- to 200-fold above the uninduced level. We do not know what effect overexpression of *nac* product might have on the cell, but we note that the high-copy-number plasmid carrying *nac* was unable to complement two of the fusions for activation of *hut* or repression of *gdh* expression in *K. aerogenes*.

The *nac-lacZ* fusions studied here differed considerably in their response to cloned wild-type *nac*. The *nac-101* fusion was complemented for nitrogen regulation only by the low-copy-number *nac* clone, and the β-galactosidase expression was limited to a 100- to 200-fold induction with or without cloned *nac*. The *nac-204* fusion was complemented for nitrogen regulation by the low-copy-number *nac* clone (as was *nac-101*) but also by the high-copy-number *nac* clone. Moreover, the β-galactosidase expression was limited to 100- to 200-fold above uninduced levels only in the presence of cloned *nac*. The *nac-112* fusion resembled *nac-101* in that it was complemented for nitrogen regulation only by the low-copy-number clone of *nac* but resembled *nac-204* in that there was a reduction in maximum β-galactosidase expression in the presence of high- or low-copy-number *nac*. The differences among the three fusions suggest that *nac* may be a complex locus with several separable functions associated with the locus.

How universal is *nac*? Clearly *K. aerogenes* encodes a functional *nac* and *S. typhimurium* does not. Plasmid pRD1 (7), which carries the *his-nif* region from the chromosome of *K. pneumoniae* M5a1, complements *K. aerogenes nac* mutations for activation of *hut* expression and for repression of *gdh* expression (data not shown). Thus, *K. pneumoniae* carries an active *nac*, and it is located in the *his* region, similar to the site of *K. aerogenes nac* (2). *E. coli* lacks *hut* genes and cannot catabolize histidine. However, when the *nac*-dependent *hut* genes from *K. aerogenes* or *S. typhimurium* were transferred into *E. coli*, their expression was activated in response to nitrogen limitation (9), albeit only about 2- to 3-fold rather than the 10-fold seen in *K. aerogenes*. Thus, it seems likely that *E. coli* carries an active *nac*. Nitrogen regulation of *hut* expression is not restricted to the enteric bacteria; for example, pseudomonads also show this form of regulation (14). It remains to be seen whether nitrogen regulation of *hut* expression in pseudomonads is also *nac* dependent.

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LITERATURE CITED

1. **Bagdasarian, M., and K. N. Timmis.** 1981. Host vector systems for gene cloning in *Pseudomonas*. Curr. Top. Microbiol. Immunol. **96**:47–67.
2. **Bender, R. A., P. M. Snyder, R. Bueno, M. Quinto, and B. Magasanik.** 1983. Nitrogen regulation system of *Klebsiella aerogenes*: The *nac* gene. J. Bacteriol. **156**:444–446.
3. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
4. **Bloom, F. R., S. L. Streicher, and B. Tyler.** 1977. Regulation of enzyme synthesis by the glutamine synthetase of *Salmonella typhimurium*: a factor in addition to glutamine synthetase is required for activation of enzyme formation. J. Bacteriol. **130**: 983–990.
5. **Bolivar, F., R. Rodriguez, P. J. Greene, M. Betlach, H. L. Heyneker, H. W. Boyer, J. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
6. **Brill, W. J., and B. Magasanik.** 1969. Genetic and metabolic control of histidase and urocanase in *Salmonella typhimurium*, strain 15-59. J. Biol. Chem. **244**:5392–5402.

7. **Dixon, R. A., F. C. Cannon, and A. Kondorosi.** 1976. Construction of a P plasmid carrying nitrogen fixation genes from *Klebsiella pneumoniae*. Nature (London) **260**:268–271.
8. **Goldberg, R. B., R. A. Bender, and S. L. Streicher.** 1974. Direct selection for P1-sensitive mutants of enteric bacteria. J. Bacteriol. **118**:810–814.
9. **Goldberg, R. B., F. R. Bloom, and B. Magasanik.** 1976. Regulation of histidase synthesis in intergeneric hybrids of enteric bacteria. J. Bacteriol. **127**:114–119.
10. **Goldberg, R. B., and B. Magasanik.** 1975. Gene order of the histidine utilization (*hut*) operons in *Klebsiella aerogenes*. J. Bacteriol. **122**:1025–1031.
11. **Goss, T. J., and P. Datta.** 1985. Molecular cloning and expression of the biodegradative threonine dehydratase gene (*tdc*) of *Escherichia coli* K12. Mol. Gen. Genet. **201**:308–314.
12. **Jorgensen, R. A., S. J. Rothstein, and W. S. Reznikoff.** 1979. A restriction cleavage map of Tn*5* and location of a region encoding neomycin resistance. Mol. Gen. Genet. **177**:56–72.
13. **Kustu, A., K. Sei, and J. Keener.** 1986. Nitrogen regulation in enteric bacteria, p. 139–154. *In* I. R. Booth and C. R. Higgins (ed.), Regulation of gene expression. Symposium of the Society for General Microbiology. Cambridge University Press, Cambridge.
14. **Lessie, T. G., and F. C. Neidhardt.** 1967. Formation and operation of the histidine-degrading pathway in *Pseudomonas aeruginosa*. J. Bacteriol. **93**:1800–1810.
15. **Macaluso, A., E. A. Best, and R. A. Bender.** 1990 Role of the *nac* gene product in the nitrogen regulation of some NTR-regulated operons of *Klebsiella aerogenes*. J. Bacteriol. **172**:7249–7255.
16. **Magasanik, B.** 1978. Regulation in the *hut* system, p. 373–387. *In* J. H. Miller and W. S. Reznikoff (ed.), The operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Magasanik, B.** 1982. Genetic control of nitrogen assimilation in bacteria. Annu. Rev. Genet. **16**:135–168.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Spratt, B. G., P. J. Hedge, S. te Heesen, A. Edelman, and J. K. Broome-Smith.** 1986. Kanamycin-resistant vectors that are analogues of plasmids pUC8, pUC9, pEMBL8, and pEMBL9. Gene **41**:337–342.
20. **Stoker, N. G., N. F. Fairweather, and B. G. Spratt.** 1982. Versatile low-copy-number plasmid vectors for cloning in *Escherichia coli*. Gene **18**:335–341.
21. **Tartof, K. D., and C. A. Hobbs.** 1987. Improved media for growing plasmid and cosmid clones. Focus **9**:12.