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Identification of a Repressor Gene Involved in the Regulation of NAD De Novo Biosynthesis in *Salmonella typhimurium*

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Mutations at the *nadI* locus affect expression of the first two genes of NAD synthesis, *nadA* and *nadB*, which are unlinked. Genetic data imply that the regulatory effects of *nadI* mutations are not due to indirect consequences of physiological alterations. Two types of mutations map in the *nadI* region. Common null mutations (*nadI*) show constitutive high-level expression of the *nadB* and *nadA* genes. Rare *nadI*^{s} mutations cause constitutive low-level expression of *nadB* and *nadA*. Some *nadI*^{s} mutations shut off the expression of the biosynthetic genes sufficiently to cause a nicotinic acid auxotrophy. Spontaneous revertants of auxotrophic *nadI*^{s} mutants have a NadI$^-$ phenotype, including some with deletions of the *nadI* locus. The *nadI* locus encodes a repressor protein acting on the unlinked *nadA* and *nadB* genes.

NAD(H) and NADP(H) are the major electron carriers in cellular metabolism. In the enteric bacteria *Salmonella typhimurium* and *Escherichia coli*, NAD also serves as a substrate for DNA ligase (20, 23). The current understanding of the NAD metabolic pathways in *S. typhimurium* is summarized in Fig. 1 (for a review, see reference 11). The first two enzymes of the de novo pathway are encoded by the *nadB* gene at 55 min (11) and the *nadA* gene at 17 min (10, 19), respectively. When *E. coli* is grown in the presence of NAD precursors, the cells exhibit a decreased capacity for quinolinic acid synthesis (5, 6, 24). Also, studies with *nadA-lacZ* and *nadB-lacZ* operon fusions in *S. typhimurium* demonstrated transcriptional control of these genes in response to exogenous nicotinic acid (NA) (7, 12, 13).

Regulatory mutants have been isolated which express *nad*::*lac* fusions at a constitutively high level in the presence of a high concentration of NA or nicotinamide (7). Three kinds of mutants were obtained which are unlinked to the *nadA* and *nadB* genes; one, *nadI*, maps near the *serB* locus (7). The *nadI* mutations include ambers and insertions. Previously, Holley et al. (13) isolated similar mutants that were designated *nadR*; these mutations map at the same position as *nadI* mutations. Since the "*nadR*" nomenclature has been used previously to describe a class of mutations in the *nadB* region (27, 28), the regulatory mutations mapping near *serB* have been designated *nadI* (7).

Mutations causing constitutive high-level expression of a gene do not necessarily affect the regulatory mechanism of that gene. A mutation that alters the pool size of a relevant signal molecule may indirectly alter gene expression. Some constitutive mutations for the *nadA* and *nadB* genes do appear to cause derepression by indirect means. These mutations impair the biosynthetic genes *nadD* (7) and *nadE* (K. Hughes, B. M. Olivera, and J. R. Roth, unpublished results), which encode NA mononucleotide adenylyltransferase and NAD synthetase, respectively (15) (Fig. 1). Mutations that reduce the level of these enzymatic activities cause derepression of the *nadA* and *nadB* genes, presumably by limiting the level of NAD and NADP in the cell.

The *nadI* mutations map in or near a gene, *pnuA* (7, 13), involved in nicotinamide mononucleotide (NMN) transport (Fig. 1) (10, 18). The *nadI* and *pnuA* mutations are almost certainly in the same operon (7) or may even affect the same gene (N. Zhu, unpublished data). Genetic evidence is presented that the *nadI* locus encodes a repressor protein which is directly responsible for regulation of both the *nadA* and *nadB* genes.

MATERIALS AND METHODS

Bacterial strains. All strains used in this study and their sources are listed in Table 1. All strains used are derived from *S. typhimurium* LT2.

MudA refers to a conditional transposition-defective derivative of Casadaban's original Mu dl(Lac Amp^r) phage (1) which forms operon fusions (16).

Mu dJ refers to a transposition defective "mini Mu" bacteriophage, Mu dl-1734 (Lac Kan), constructed by Castilho et al. (2, 3). This phage is deleted for transposition functions and carries kanamycin resistance.

Media. The E medium of Vogel and Bonner (30), supplemented with 0.2% glucose, was used as minimal medium. Difco nutrient broth (NB; 8 g/liter), with NaCl added (5 g/liter), was used as rich medium. Difco agar was added at a final concentration of 1.5% for solid medium. Auxotrophic requirements were included in media at final concentrations described by Davis et al. (8), except as otherwise indicated in the text. Antibiotics were added to media at the following final concentrations: ampicillin (sodium salt), 30 μg/ml in NB and 15 μg/ml in E medium; tetracycline hydrochloride, 20 μg/ml in NB and 10 μg/ml in E medium; kanamycin sulfate, 50 μg/ml in NB and 125 μg/ml in E medium. All antibiotics were obtained from Sigma Chemical Co. Media containing ampicillin were always prepared fresh before use. 5-Bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) dissolved in *N*,*N*-dimethylformamide (20 mg/ml) was added to media at a final concentration of 25 μg/ml.

Transductional methods. The high-frequency, generalized transducing bacteriophage P22 mutant *HT105/1 int-201* was used for all transductional crosses. This phage was derived by G. Roberts (unpublished results) from the P22 *HT105/1* phage of Schmieger (26). To select for the inheritance of the Kan^r marker of Mu dJ, the transduction mixture of cell and phage was preincubated on NB plates overnight before being replica-printed to selective plates. In all other crosses, selective plates were spread directly with 2×10^8 cells and 10^8 to 10^9 phage. Transductants were purified, and phage-

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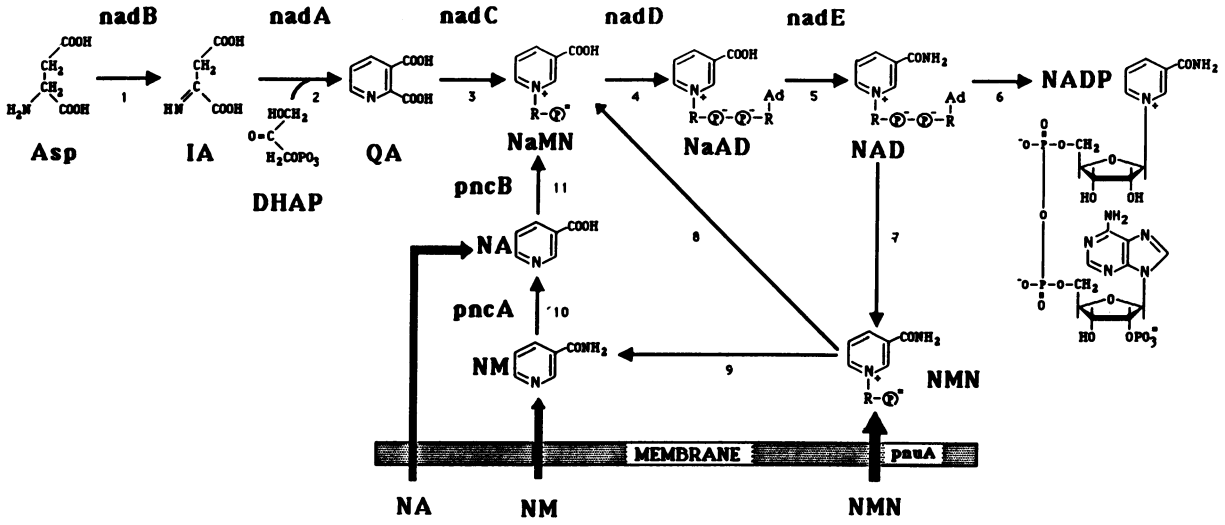

FIG. 1. NAD metabolic pathway of *S. typhimurium*. The enzymes included are 1, L-aspartate (Asp) oxidase; 2, quinolinic acid (QA) synthetase; 3, quinolinic acid phosphoribosyl transferase; 4, nicotinic acid mononucleotide (NaMN) adenylyltransferase; 5, NAD synthetase; 6, NAD kinase; 7, DNA ligase; 8, NMN deamidase; 9, NMN glycohydrolase; 10, nicotinamide (NM) deamidase; 11, NA phosphoribosyl transferase. Abbreviations: DHAP, dihydroxylacetone phosphate; IA, iminoaspartate; PRPP, 5-phosphoribosyl-1-pyrophosphate. Genetic loci corresponding to enzymatic steps are indicated above the reaction arrows.

free clones were isolated by being streaked nonselectively onto green indicator plates (4). Transductants containing plasmids were purified on NB plates containing ampicillin. Phage-free clones were then tested for phage sensitivity by cross-streaking with P22 H5 phage, a clear plaque mutant of P22.

Localized mutagenesis. Hydroxylamine mutagenesis of P22 transducing phage was done as described by Davis et al. (8). The general method is that of Hong and Ames (14).

Enzyme assay. β-Galactosidase activity was determined as described by Miller, with sodium dodecyl sulfate–chloroform-permeabilized cells (21). The β-galatosidase activity is reported as nanomoles per minute per optical density unit (at 650 nm) of cells.

Construction and manipulation of a tandem duplication. The procedure for constructing the tandem duplication that covers the *serB-nadI(pnuA)-thrA* region is presented in Fig. 2. It is one example of a general method of constructing duplications (15; M. Schmid and J. R. Roth, manuscript in preparation; D. Hillyard and J. R. Roth, manuscript in preparation).

The donor strain (TT11433) carries a wild-type *thr* operon and a Tn*10* insertion (*zaa-1868*) which is linked to the *thr* operon on the side clockwise from *thr* operon. The recipient strain (TT11434) carries a *pyrB692*::Tn*10* insertion and a *thr-470*::Mu dA insertion mutation. When the recipient is transduced to Thr^+ by using P22 transducing phage grown on the donor strain, one class of Thr^+ transductants is a merodiploid which carries a tandem duplication of the region from the *pyrB692*::Tn*10* to the *zaa-1868*::Tn*10* (see Fig. 2a). This merodiploid transductant class is identified by the following criteria. (i) The transductants are Thr^+ Amp^r Tc^r. (ii) The Tc^r phenotype is unstable, since the Tn*10* element is located at the novel join point between the two copies of the duplicated region; these two copies can recombine, resulting in the loss of the duplication and Tn*10* join point. (iii) The heterozygous state of the duplicated region (e.g. *thr*$^+$/*thr*::Mu dJ) is also unstable owing to the recombination between the two copies; therefore, these strains give rise to Thr^- Kan^r and Thr^+ Kan^s segregants. The merodiploid state can be moved into other strains by transducing the novel join point (Tn*10*) selectively into those strains (Fig. 2b). The merodiploid state can be maintained by selecting for Tc^r.

A general strategy for using these merodiploids in complementation tests is the following. First, the novel join point is transduced into a series of mutants to be tested, thus creating strains which are homozygous diploid for the recipient allele. The second mutation can be moved into these diploids by either directly selecting the second mutation, if it has a selectable phenotype, or using a linked selective marker. The existence of two different alleles in the strains constructed for the complementation test can be examined by a segregation test.

Cloning of *nadA* and *nadB* genes. Phage lambda clones of *nadA* and *nadB* genes were selected from an *Eco*RI bank of *S. typhimurium* DNA cloned into λgt7 (8). Clones carrying the *nadA* and *nadB* genes were selected by complementation of *E. coli nadA* and *nadB* mutants. These lambda clones also complement *S. typhimurium nadA* and *nadB* mutations. The initial genome inserts of *nadA* and *nadB* clones are about 7.5 and 5.5 kilobases, respectively. Both genes were subcloned into pBR322 plasmid as *Pst*I-*Eco*RI fragments. To further subclone these two genes, exonuclease BAL 31 was used to digest the cloned fragments sequentially from two ends; they were ligated into the multiple-cloning site of the pUC9 plasmid. The final subclones of the *nadA*(pZT352) and *nadB*(pZT349) genes used in this study are about 1.5 and 2.5 kilobases, respectively. They are able to complement *nadA* or *nadB* mutants of both *E. coli* and *S. typhimurium*.

RESULTS

Evidence for negative control of the *nadA* and *nadB* genes. Previous work has demonstrated that expression of *nadA* and *nadB* genes increases under conditions of pyridine limitation (5–7, 13). Both genes are expressed at a high constitutive level in *nadI* mutants, suggesting that the *nadI* gene might encode a repressor protein. To determine whether the existence of a repressor is likely, the effect of gene dosage on the expression of these genes was tested. If

TABLE 1. List of strains

Strain[a]	Genotype
TT8785	*nadA219*::Mu dA
TT8793	*nadB227*::Mu *dA*
TT10120	*serB1463*::Tn*10*
TT10738	*nadB499*::Mu dJ
TT11334	*nadA219*::Mu dJ
TT11335	*nadB499*::Mu dJ *srl-202*::Tn*10 recA1*
TT11336	*nadA219*::Mu dJ *srl-202*::Tn*10 recA1*
TT11347	*srl-202*::Tn*10 recA1 nadB499*::Mu dJ(pZT349)
TT11348	*srl-202*::Tn*10 recA1 nadB499*::Mu dJ(pZT352)
TT11349	*srl-202*::Tn*10 recA1 nadA219*::Mu dJ(pZT349)
TT11350	*srl-202*::Tn*10 recA1 nadA219*::Mu dJ(pZT352)
TT11356	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*507*
TT11357	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*508*
TT11358	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*509*
TT11359	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*510*
TT11360	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*511*
TT11379	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*513*
TT11385	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*519*
TT11389	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*523*
TT11392	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*526*
TT11394	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*528*
TT11395	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*511 nadB*^{c}*529*[b]
TT11396	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*511 nadB*^{c}*530*
TT11397	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*511 nadB*^{c}*531*
TT11398	*nadA219*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*511 nadA*^{c}*532*[b]
TT11399	*nadA219*::Mu dJ *serB1463*::Tn*10 nadI*^{s}*511 nadA*^{c}*533*
TT11400	*nadB499*::Mu dJ *nadB*^{c}*529*
TT11401	*nadB499*::Mu dJ *nadB*^{c}*530*
TT11402	*nadB499*::Mu dJ *nadB*^{c}*531*
TT11403	*nadA219*::Mu dJ *nadA*^{c}*532*
TT11404	*nadA219*::Mu dJ *nadA*^{c}*533*
TT11408	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI534*
TT11409	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI535*
TT11410	*nadB499*::Mu dJ *serB1463*::Tn*10 nadI536*
TT11411	*nadA219*::Mu dJ *serB1463*::Tn*10 nadI534*
TT11412	*nadA219*::Mu dJ *serB1463*::Tn*10 nadI535*
TT11413	*nadA219*::Mu dJ *serB1463*::Tn*10 nadI536*
TT11416	*nadB499*::Mu dJ *nadI*^{s}*511 srl-202*::Tn*10 recA1*
TT11418	*nadA219*::Mu dJ *nadI*^{s}*511 srl-202*::Tn*10 recA1*
TT11422	*nadB499*::Mu dJ *nadI*^{s}*511 srl-202*::Tn*10 recA1*(pZT349)
TT11424	*nadA219*::Mu dJ *nadI*^{s}*511 srl-202*::Tn*10 recA1*(pZT349)
TT11428	*nadB499*::Mu dJ *nadI*^{s}*511 srl-202*::Tn*10 recA1*(pZT352)
TT11430	*nadA219*::Mu dJ *nadI*^{s}*511 srl-202*::Tn*10 recA1*(pZT352)
TT11433	*zaa-1868*::Tn*10*
TT11434	*pyrB692*::Tn*10 thr-470*::Mu dA
TT11435	*DUP728*[(*zaa-1868*)Tn*10*(*pyrB692*)][c]
TT11441	*nadB227*::Mu dJ *serB9 pncA278*::Tn*10d* (Cam^r)[d]
TT11443	*nadB227*::Mu dJ *pncA278*::Tn*10d* (Cam^r) *DUP728*[(*serB9 zaa-1868*)Tn*10*(*pyrB692 serB9*)]
TT11449	*nadB227*::Mu dJ *pncA278*::Tn*10d* (Cam^r) *DUP728*[(*nadI*^{s}*511 zaa-1868*)Tn*10*(*pyrB692 serB9*)]
TT11450	*nadB227*::Mu dJ *pncA278*::Tn*10d* (Cam^r) *DUP728*[(*zaa-1868*)Tn*10*(*pyrB692 serB9*)]
TT11453	*nadB227*::Mu dJ *pncA278*::Tn*10d* (Cam^r) *DUP728*[(*nadI262 zaa-1868*)Tn*10*(*pyrB692 serB9*)]
TT11744	*nadB499*::Mu dJ *nadI262*
TT13239	*nadB499*::Mu dJ *srl-202*::Tn*10 recA1*(pNC9)
TT13240	*nadA219*::Mu dJ *srl-202*::Tn*10 recA1*(pNC9)
TT13242	*nadB499*::Mu dJ *nadI*^{s}*511 srl-202*::Tn*10 recA1*(pNC9)
TT13244	*nadA219*::Mu dJ *nadI*^{s}*511 srl-202*::Tn*10 recA1*(pNC9)

[a] All strains used in this study are derivatives of *S. typhimurium* LT2.
[b] *nadB*^{c} and *nadA*^{c} designate constitutive mutations linked to the *nadB* and *nadA* genes, respectively.
[c] Duplication nomenclature is as described by Schmid and Roth (25).
[d] Tn*10d* (Cam^r) designates a transposition-defective derivative of transposon Tn*10* (T. Elliott and J. R. Roth, personal communication).

a repressor exists, it should be possible to titrate the repressor in vivo by providing a high dosage of one of the regulated genes. As a consequence, the target gene, and other genes subject to the same repressor, would escape repression. We have used plasmids carrying either the *nadA* or *nadB* gene in these gene dosage tests. Both plasmids cause constitutive expression of the *nadA*::Mu dJ or *nadB*::Mu dJ fusion present in the host chromosome. This suggests that the *nadA* and *nadB* genes are negatively regulated by a common repressor molecule. These experiments are outlined below, and results are presented in Table 2.

The *S. typhimurium nadB* and *nadA* genes have been cloned into the multiple-cloning site of plasmid pUC9 (pZT349 and pZT352) (N. Zhu, L. Bossi, and R. Gesteland,

TABLE 2. Gene dosage effect of the expression of *nadA* and *nadB* genes in a *nadI*$^+$ or *nadI*^{s} background

Strain	Relevant genotype	Color[a] on X-Gal plate with:	
		10^{-6} M NA	2×10^{-4} M NA
TT11335	*nadB499*::Mu dJ	B	W
TT13239	*nadB499*::Mu dJ(pUC9)[b]	B	W
TT11347	*nadB499*::Mu dJ(pZT349)[c] (*nadB*)	B	B
TT11348	*nadB499*::Mu dJ(pZT352) (*nadA*)	B	B
TT11336	*nadA219*::Mu dJ	B	W
TT13240	*nadA219*::Mu dJ(pUC9)	B	W
TT11349	*nadA219*::Mu dJ(pZT349) (*nadB*)	B	B
TT11350	*nadA219*::Mu dJ(pZT352) (*nadA*)	B	B
TT11416	*nadB499*::Mu dJ *nadI*^{s}*511*	W	W
TT13242	*nadB499*::Mu dJ *nadI*^{s}*511*(pUC9)	W	W
TT11422	*nadB499*::Mu dJ *nadI*^{s}*511*(pZT349) (*nadB*)	B	B
TT11428	*nadB499*::Mu dJ *nadI*^{s}*511*(pZT352) (*nadA*)	B	B
TT11418	*nadA219*::Mu dJ *nadI*^{s}*511*	W	W
TT13244	*nadA219*::Mu dJ *nadI*^{s}*511*(pUC9)	W	W
TT11424	*nadA219*::Mu dJ *nadI*^{s}*511*(pZT349) (*nadB*)	B	B
TT11430	*nadA219*::Mu dJ *nadI*^{s}*511*(pZT352) (*nadA*)	B	B

[a] B, Blue on X-Gal plate; W, white on X-Gal plate.
[b] pUC9 is a plasmid used to clone the *nadA* and *nadB* genes.
[c] The plasmids pZT349 (*nadB* clone) and pZT352 (*nadA* clone) were transduced into the strains by transducing phage P22.

unpublished data). The pUC9 plasmid is a pBR322-derived cloning vector and exists in high copy number in cells (29; Zhu et al., unpublished data). These plasmids can be used to provide many copies of either *nadB* or *nadA* in vivo. The plasmids were introduced by P22-mediated transduction into hosts carrying in their chromosome either a *nadA*::Mu dJ or a *nadB*::Mu dJ fusion and a *recA* mutation (TT11338 and TT11341). The results were scored qualitatively by the color of colonies on media with X-Gal (Table 2). In each case, the presence of the plasmid causes derepression of the chromosomal *nad*::*lac* fusion. The simplest explanation of these results is that a repressor is involved in the regulation of the *nadA* and *nadB* genes. When either gene is present in high copy number, repressor is sequestered, resulting in derepression of the chromosomal fusions. We provide evidence that the *nadI* gene encodes this repressor protein.

Isolation of low-level constitutive mutants (*nadI*^{s}). If the *nadI* gene encodes a repressor, mutants with mutations analogous to the super repressor (*lacI*^{s}) of the lactose operon (22) might be recovered. Such *nadI*^{s} mutations, which would cause repression of *nadA* and *nadB* genes under all growth conditions, would be expected to be rare (compared with *nadI* null alleles) and dominant to the *nadI*$^+$ allele in complementation tests. In a search for such *nadI*^{s} mutants, we subjected the *nadI* region to localized mutagenesis and screened for mutants that express a *nadB*::Mu dJ fusion at a constitutive low level. Such mutants were isolated, and the mutations were mapped and characterized as described below.

To achieve local mutagenesis, a strain containing *serB1463*::Tn*10* (TT10120) was used as a donor. This Tn*10* insertion is about 85% linked to the *pnuA-nadI* locus. The recipient carries a *nadB499*::Mu dJ *lacZ* operon fusion (TT10738) far from the *nadI* region. The selection for tetracycline resistance was done on minimal X-Gal plates supplemented with a low concentration of NA (10^{-6} M); on this medium most transductants form blue colonies, but a few white colonies were found. These mutants express the *nadB*::*lac* fusion at very low level under either repressing (2×10^{-4} M NA) or derepressing (10^{-6} M NA) conditions.

TABLE 3. Expression of *nad*::*lac* at a low constitutive level by *nadI*^{s} mutants

Strain	Relevant genotype	β-Galactosidase activity (U) on medium[a] with:	
		10^{-6} M NA	2×10^{-4} M NA
TT10738	*nadB499*::Mu dJ	320	6
TT11356	*nadB499*::Mu dJ *nadI*^{s}*507*	5	3
TT11357	*nadB499*::Mu dJ *nadI*^{s}*508*	4	3
TT11358	*nadB499*::Mu dJ *nadI*^{s}*509*	4	3
TT11359	*nadB499*::Mu dJ *nadI*^{s}*510*	4	2
TT11360	*nadB499*::Mu dJ *nadI*^{s}*511*	4	3
TT11744	*nadB499*::Mu dJ *nadI-262*	373	372

[a] The strains were grown in minimal media supplemented with serine and the indicated concentrations of NA.

The putative *nadI*^{s} mutations show the expected linkage to the *serB* locus. When P22 phage grown on five *nadI*^{s} *serB*::Tn*10* double mutants (TT11351 to TT11355) was used to transduce *nadA*::*lac* and *nadB*::*lac* fusion strains (TT10738 and TT11334) on minimal X-Gal plates containing 10^{-6} M NA plus tetracycline and serine, 80 to 90% of the Tc^r transductants show the phenotype expected for *nadI*^{s} mutants and form white colonies on X-Gal plates with either high or low levels of NA.

The β-galactosidase activities of the putative *nadI*^{s} transductants are presented in Table 3. The results agree with those observed in the color test on X-Gal plates. Thus, these mutations are coinherited with *serB1463*::Tn*10* at about the same frequency as *nadI* mutations (7), but, in contrast to the derepression of *nadA* and *nadB* seen for *nadI* mutants, they express both *nadB-lac* and *nadA-lac* fusions at a constitutive low level.

Some *nadI*^{s} mutations cause pyridine auxotrophy. To test the phenotype of *nadI*^{s} mutations in a wild-type genetic background, without a *nadA*::*lac* or *nadB*::*lac* insertion, five *nadI*^{s} alleles (TT11356 to TT11360) were transduced into wild-type strain LT2 by selecting for inheritance of the linked *serB1463*::Tn*10* marker. Three of the five mutants (TT11357, TT11358, and TT11360 as donors) gave 80 to 90% Tc^r transductants that are NA auxotrophs (Table 4). The

TABLE 4. Transduction of *nadI*^{s} mutations into strain LT2

Donor	Relevant genotype	No. of Tc^r transductants[a]	No. of Nad$^-$ auxotrophs
TT11356	*serB1463*::Tn*10* *nadI*^{s}*507*	50	0
TT11357	*serB1463*::Tn*10* *nadI*^{s}*508*	49	44
TT11358	*serB1463*::Tn*10* *nadI*^{s}*509*	50	41
TT11359	*serB1463*::Tn*10* *nadI*^{s}*510*	50	0
TT11360	*serB1463*::Tn*10* *nadI*^{s}*511*	50	38

[a] Phages grown on these donors were used to transduce wild-type recipient strain LT2 selecting inheritance of Tc^r (conferred by the *serB1463*::Tn*10* insertion). The Tc^r transductants were picked and patched on NB plates containing tetracycline and then replica printed to minimal plates.

other two donors gave no auxotrophic transductants and have not been studied further. We presume that the super-repression phenotype of these three *nadI*^{s} alleles is sufficiently strong that de novo synthesis of NAD is prevented. No NA auxotrophic mutations have previously been isolated near *serB*; we presume that this is due to the rarity of *nadI*^{s} mutations (see below).

Frequency of *nadI*^{s} and *nadI* mutations. The *nadI*^{s} (constitutive low-level expression) mutations and the *nadI* (constitutive high-level expression) mutations show approximately the same linkage to *serB*, but have opposite phenotypes. The relative frequency of these two mutant types has been examined. The expectation is that the *nadI* class would include null mutations and thus be common, while the *nadI*^{s} mutations would produce a repressor with very specific properties and thus be rare.

Mutagenized P22 transducing phage, grown on the *serB1463*::Tn*10* strain (TT10120), was used to transduce *nadB499*::Mu dJ (TT10738), selecting for the inheritance of tetracycline resistance on nutrient agar medium. The Tc^r colonies were replica printed to minimal glucose plates containing X-Gal plus serine and tetracycline supplemented with a low level (10^{-6} M) or a high level (2×10^{-4} M) of NA. White colonies on the low-NA plates (*nadI*^{s}) and blue colonies on the high-NA plates (*nadI*) were scored. Putative *nadI*^{s} mutants were found at a frequency of 10^{-4} (17 from about 160,000 Tc^r transductants); *nadI* mutants were found at a frequency 2×10^{-2} (3,300 from 160,000 Tc^r transductants). The ratio of the former to the latter is 1:200. Thus, as predicted, the frequency of the constitutively high mutations (*nadI*) is significantly higher than that of the constitutively low mutations (*nadI*^{s}). The rarer *nadI*^{s} mutants recovered from this experiment all show constitutive low expression of *nadA* and *nadB* genes and are 80 to 90% linked to the *serB* locus (data not shown).

Effect of *nadA* and *nadB* copy number on *nadI*^{s} mutants. If the *nadI*^{s} mutations owe their phenotype to a mutant repressor active even under conditions of pyridine limitation, then these phenotypes ought to be corrected by providing, in high copy number, either of the target sequences (*nadA* or *nadB*) with which this protein is thought to interact. To test this, we introduced the *nadA* or *nadB* plasmid into strains carrying a *nad*::Mu dJ fusion, a *nadI*^{s} mutation, and a *recA* mutation (TT11415 to TT11418). This was done by comparing their relative level of *lacZ* expression on indicator media. In all cases, introduction of the high-copy-number plasmid relieved the low-constitutive phenotype of the *nadI*^{s} mutant and, as expected, led to a high constitutive expression of whichever fusion was present. Qualitative scoring of β-galactosidase for some of these strains is shown in Table 2.

Spontaneous Nad$^+$ revertants of *nadI*^{s} mutants. If *nadI*^{s} mutants owe their phenotype to production of an active super repressor, then this phenotype should be lost by the introduction of a null mutation in the *nadI* gene. Since some *nadI*^{s} mutants have an auxotrophic phenotype in an LT2 background, revertants can be positively selected. The frequency of Nad$^+$ revertants, their phenotype, and the linkage of the secondary mutation to the parental *nadI*^{s} mutation have been examined.

Overnight cultures of three auxotrophic *nadI*^{s} mutants (TT11366, TT11367, and TT11368) were washed and plated on the minimal-medium plates containing serine. Nad$^+$ revertant colonies were scored. The spontaneous revertant frequencies in these *nadI*^{s} mutants are high (3×10^{-5} to 5×10^{-6}).

Six revertants of each of the above *nadI*^{s} strains were examined. Several revertants are Ser$^-$, Tc^s, and PnuA$^-$, and when tested further, these phenotypes were not separable in genetic crosses, suggesting that they are deletions. Since the *pnuA* and *nadI* genes are known to map at the same genetic locus, the deletions probably extend from the *serB* locus to the *pnuA-nadI* region and remove the Tn*10* element inserted in *serB*. (These deletions may be generated by the Tn*10* element.) Similar deletions were also found in the spontaneous Nad$^+$ revertants of a *nadI*^{s} strain without *serB*::Tn*10*. The regulatory phenotypes of both Ser$^-$ Tc^s and Ser$^-$ Tc^r revertants were tested by transducing either a *nadB*::Mu dJ fusion (from TT10738) or a *nadA*::Mu dJ fusion (from TT11334) into these strains. All Nad$^+$ revertants of *nadI*^{s} mutants show constitutive high expression of *nadA* or *nadB*. These results suggest that the *nadI*^{s} auxotrophy is corrected by *nadI* null mutations.

Levels of β-galactosidase were assayed for several revertants of *nadI*^{s} mutants for which the *nadI* region had been transduced into a genetic background that includes a *nadA*::Mu dJ or *nadB*::Mu dJ fusion (TT10738 or TT11334). The results (Table 5) show that the revertants (which have mutations tightly linked to the parental *nadI*^{s} mutations) show constitutive high-level expression of both *nadB* and *nadA*, as do the *nadI* mutations described previously (7).

TABLE 5. Expression of *nadA*::*lac* and *nadB*::*lac* fusions in prototrophic revertants of *nadI*^{s}

Strain	Relevant genotype			β-Galactosidase activity (U) in medium with:	
	nad::*lac* allele	*nadI*^{s} allele	*nadI* revertant allele	10^{-6} M NA	2×10^{-4} M NA
TT11357	*nadB499*::Mu dJ	*nadI*^{s}*508*		4	3
TT11408	*nadB499*::Mu dJ	(*nadI*^{s}*508*)[a]	*nadI534*	295	256
TT11411	*nadA219*::Mu dJ	(*nadI*^{s}*508*)[a]	*nadI534*	370	316
TT11358	*nadB499*::Mu dJ	*nadI*^{s}*509*		4	3
TT11409	*nadB499*::Mu dJ	(*nadI*^{s}*509*)[a]	*nadI535*	328	335
TT11412	*nadA219*::Mu dJ	(*nadI*^{s}*509*)[a]	*nadI535*	450	473
TT11360	*nadB499*::Mu dJ	*nadI*^{s}*511*[a]		4	3
TT11410	*nadB499*::Mu dJ	(*nadI*^{s}*511*)[a]	*nadI536*	57	23
TT11413	*nadA219*::Mu dJ	(*nadI*^{s}*511*)[a]	*nadI536*	245	123
TT11744	*nadB499*::Mu dJ		*nadI262*	373	372

[a] The *nadI*^{s} allele indicated in parentheses is the parental *nadI*^{s} allele (auxotrophic) whose presence is inferred; the strain acquired the *nadI* mutation when it reverted to prototrophy.

These data suggest that the auxotrophic *nadI*^{s} mutants become prototrophic when they acquire a *nadI* mutation.

***nadI* mutations with different effects on *nadA* and *nadB* genes.** If *nadI* encodes a repressor that acts to control both the *nadA* the *nadB* genes, one might expect that some *nadI* mutants would produce a repressor that could distinguish between the two control regions. This is not expected if *nadI* mutations cause derepression indirectly (e.g., they might cause a reduction of the NAD pool and indirectly lead to derepression). Data in Table 5 describe a *nadI* mutant allele that distinguishes between *nadA* and *nadB* regions. Mutant *nadI536* (which was selected as a prototrophic revertant of *nadI*^{s}*511* and presumably carries both mutations) shows constitutive expression of both *nadA* and *nadB* fusions at a level higher than that shown by the parent *nadI*^{s} mutant. However, the constitutive level is fivefold higher for *nadA* than for *nadB*. Revertants of the other *nadI*^{s} mutants do not show this differential effect on the two target genes (Table 5).

Isolation of temperature-sensitive *nadI*^{s} mutants. Evidence presented thus far is consistent with, but does not directly address, the idea that *nadI* and *nadI*^{s} mutations are alleles of a single gene. This idea is directly supported by isolation of temperature-sensitive mutants that show a NadI^s phenotype at one temperature and a NadI$^-$ phenotype at another temperature.

Seventeen *nadI*^{s} mutants (TT11378 to TT11394), which form white colonies on X-Gal plates containing 10^{-6} M NA, were isolated at 30°C. The mutations were transduced into a unmutagenized background by crossing with the parental recipient strain TT10738 (*nadB*::Mu dJ) selecting for inheritance of mutation *serB1463*::Tn*10* (Tc^r). Transductants which formed white colonies at 30°C on X-Gal plates containing a low concentration of NA were tested further. The cotransduction frequency of these mutations to *serB1463*::Tn*10* is 80 to 90%. These *nadI*^{s} transductants can be divided into several classes on the basis of their phenotypes at elevated temperatures. The results of β-galactosidase assays for strains representative of each class are presented in Table 6.

The most informative class is represented by mutation *nadI*^{s}*526* in line 5 of Table 6. The strain carrying this mutation shows constitutive low-level expression of the *nadB* fusion at 30°C. At 37°C, expression is regulated in response to pyridine supplementation. At 40°C, the mutant shows constitutive high-level expression characteristic of a *nadI* null allele. Below we will show that the NadI^s phenotype (at 30°C) is dominant to NadI$^+$ and that the NadI$^-$ phenotype (at 40°C) is recessive to NadI$^+$.

Other classes of *nadI* mutants are also presented in Table 6. The most common class, including 6 of the 17 mutants, is shown in line 2. This class shows constitutive low-level expression at all temperatures. Line 3 presents a mutant class that shows a NadI^s phenotype at 30 and 37°C but regulates expression at 40°C. Another mutant type (line 4) regulates at both 37 and 40°C. Line 6 presents a mutant that shows no regulated expression, but increases expression in response to higher temperatures. As a control, a typical *nadI* null mutant is presented in line 7.

The existence of these mutants and the fact that at 40°C NadI$^-$ phenotypes are so frequent among mutants isolated as NadI^s types at 30°C (5 of 17) suggest that both phenotypes are caused by mutations in the same gene.

The dominance test. Results presented above show that *nadI*^{s} mutations are rare compared with *nadI* mutations and that these two kinds of mutations seem to be different alleles of the same gene. The results suggest that the *nadI*^{s} mutations might be dominant alleles that produce a functional but altered repressor, while the *nadI* type might be null mutations and therefore would be expected to be recessive. Dominance tests confirm these expectations: *nadI*^{s} mutations are dominant to the wild type, and *nadI* mutations are recessive.

Complementation tests were made by using tandem duplications to form the necessary merodiploids as discussed in Materials and Methods and as shown in Fig. 2. The duplicated segment starts within the *pyrB* gene (98 min) and extends to just beyond the *thr* locus (100 min).

A series of *nadI*^{s} and *nadI* mutations were transduced into this duplication strain by selecting for Ser$^+$ transductants which inherit the *ser*$^+$ locus (and very frequently the donor *nadI* allele) in one copy of the duplication. Thus, the strains are heterozygous for *serB* and for *nadI*, with the general structure (*serB*$^+$ *nadI*)Tn*10*(*serB nadI*$^+$). These merodiploids permit the testing of dominance and allow simple verification of the genotype of the diploid, since segregants which arise by recombination between the two copies are of two types: Tc^s Ser$^+$ Nad and Tc^s Ser$^-$ Nad$^+$.

The three *nadI*^{s} mutations tested (*nadI508*, *nadI509*, and *nadI511*) were each transduced into the Ser$^-$ merodiploid selecting Ser$^+$ transductants; the resulting strains (*nadI*^{s}/*nadI*$^+$) (TT11445, TT11447, and TT11449) form white colonies on X-Gal plates containing low concentrations of NA. This is a NadI^s phenotype, which demonstrates that in all cases tested, the *nadI*^{s} mutation is dominant to the *nadI*$^+$ allele carried in the other copy of the duplication. The structure of the merodiploids used for these tests was verified by isolating Tc^s segregants that had lost the duplication. Segregants from the strains used for the dominance tests fell into two classes: Ser$^+$ NadI^s and Ser$^-$ NadI$^+$. This verifies that both alleles of *nadI* were present in the diploid.

The three *nadI* mutations tested (*nadI260*, *nadI261*, and *nadI262*) were each transduced into the Ser$^-$ merodiploid

TABLE 6. Effect of *nadI*^{s}(Ts) mutations on *nadB*::*lac* fusion

nadI allele[a]	β-Galactosidase activity (U) at:					
	30°C		37°C		40°C	
	10^{-6} M NA	2×10^{-4} M NA	10^{-6} M NA	2×10^{-4} M NA	10^{-6} M NA	2×10^{-4} M NA
nadI$^+$	417	9	258	6	169	4
nadI^s*513*	4	3	3	3	3	3
nadI^s*528*	6	4	6	3	63	3
nadI^s*519*	8	6	31	9	120	26
nadI^{s}526	11	8	120	36	253	182
nadI^{s}523	18	11	126	128	251	276
nadI262	482	568	300	369	235	247

[a] All strains carry the fusion *nadB499*::Mu dJ.

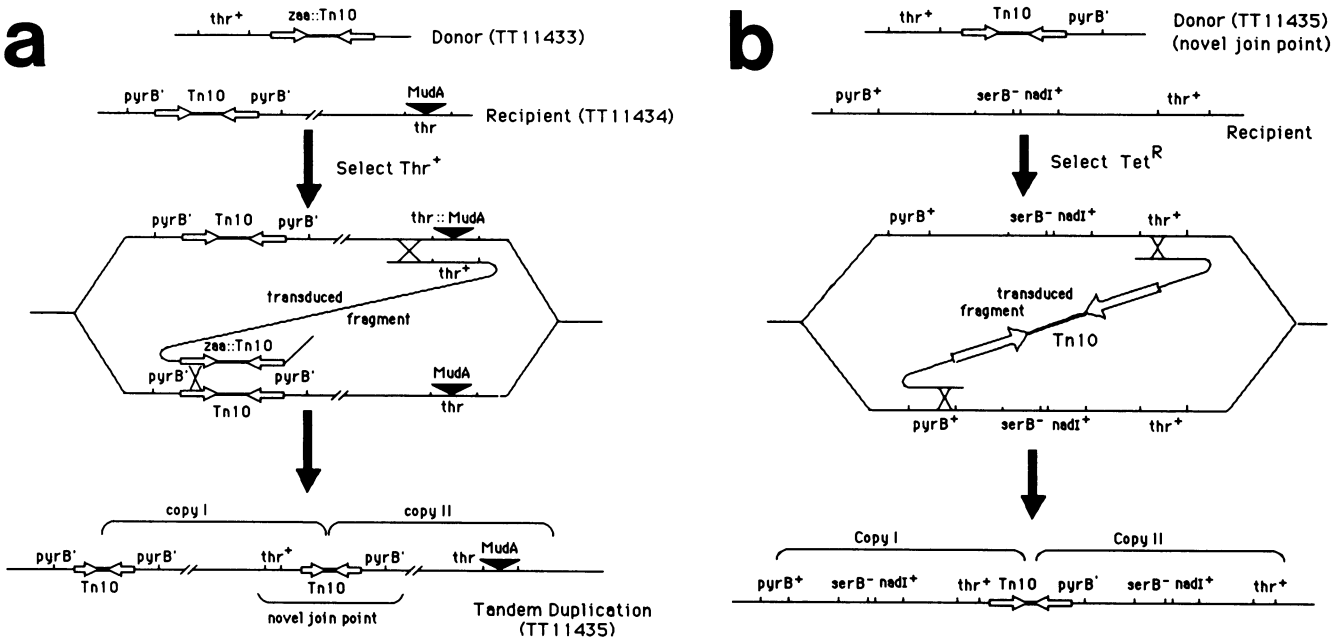

FIG. 2. Construction and transduction of a tandem duplication structure. (a) Construction of a tandem duplication which duplicates a region from *pyrB* to *thr* and has a Tn*10* transposon at the join point. (b) Transduction of the join point of the tandem duplication to a new strain. The structure of the tandem duplication is regenerated when inheritance of the join point is selected; all other duplicated material is of recipient origin.

selecting Ser$^+$ transductants. All transductants (*nadI*/*nadI*$^+$) (TT11451, TT11452, and TT11453) showed normal regulation of *nadB* as judged by colony color on X-Gal plates containing a high or low concentration of NA. Thus, the *nadI* mutations tested are all recessive to the wild-type allele (*nadI*$^+$) present in the other copy of the duplicated segment. The structure of the diploids on which this conclusion is based was demonstrated by allowing the duplication to segregate and testing the phenotypes of the haploid segregants. Some segregants show constitutive high-level expression of the *nadB*::Mu dJ fusion, whereas others show regulated expression. This demonstrates that the diploid did carry the mutant *nadI* allele as well as the wild-type *nadI*$^+$ allele. The β-galactosidase activities of some of the merodiploids and their segregants have been measured. The results (Table 7) agree with what was found with X-Gal plates.

Additional evidence on dominance of *nadI*^{s} mutations comes from the dominance test of mutation *nadI*^{s}*526*(Ts); this mutation has a NadI^s phenotype at 30°C, is NadI$^+$ at 37°C, and is NadI$^-$ at 42°C (Table 6, line 5). The diploid strain (TT11454) [*nadI*^{s}*526*(Ts)/*nadI*$^+$) shows constitutive low-level expression of the *nadB*::*lac* fusion at 30°C (the NadI^s phenotype at 30°C is dominant to NadI$^+$); at 40°C, expression of the fusion is regulated normally in response to NA concentration (the NadI$^-$ phenotype at 40°C is recessive to NadI$^+$).

Isolation of putative *cis*-regulatory mutations mapping near *nadA* and *nadB*. To further explore interactions between the *nadI* repressor and the genes regulated by this repressor, attempts have been made to isolate *cis*-acting regulatory mutants. The *cis*-acting regulatory or operator mutations are expected to map near the *nadA* and *nadB* genes and to be rare.

We used the Kan^r determinants of *nadB499*::Mu dJ and *nadA219*::Mu dJ as selectable markers and performed localized mutagenesis of each region. P22 transducing phage was

TABLE 7. Dominance test of *nadI*^{s} and *nadI* mutations

Strain	Relevent genotype of merodiploid [(Copy 1)*Tn*10*(Copy 2)]	β-Galactosidase activity (U) in medium with:	
		10^{-6} M NA	2×10^{-4} M NA
TT11443	*nadB227*::Mu dJ (*serB9 nadI*$^+$)*Tn*10*(*serB9 nadI*$^+$)	285	4
TT11449	*nadB227*::Mu dJ (*serB*$^+$ *nadI*^{s}*511*)*Tn*10*(*serB9 nadI*$^+$)	3	2
Tc^s segregant	*nadB227*::Mu dJ *serB*$^+$ *nadI*^{s}*511*	3	3
Tc^s segregant	*nadB227*::Mu dJ *serB9 nadI*$^+$	359	9
TT11450	*nadB227*::Mu dJ (*serB*$^+$ *nadI*$^+$)*Tn*10*(*serB9 nadI*$^+$)	301	4
Tc^s segregant	*nadB227*::Mu dJ *serB*$^+$ *nadI*$^+$	402	9
Tc^s segregant	*nadB227*::Mu dJ *serB9 nadI*$^+$	374	6
TT11453	*nadB227*::Mu dJ (*serB*$^+$ *nadI262*)*Tn*10*(*serB9 nadI*$^+$)	336	5
Tc^s segregant	*nadB227*::Mu dJ *serB*$^+$ *nadI262*	441	337
Tc^s segregant	*nadB227*::Mu dJ *serB9 nadI*$^+$	440	10

grown on *nadA*::Mu dJ and *nadB*::Mu dJ strains (TT10738 and TT11334); the free phage was mutagenized with hydroxylamine as described in Materials and Methods. The mutagenized lysate was used to transduce a *nadI*^{s} mutant strain (TT11368) selecting for kanamycin resistance on minimal X-Gal plates containing a low concentration (10^{-6} M) of NA. Most transductants form white colonies on this medium owing to the *nadI*^{s} (super-repressor) mutation present in the recipient. Rare blue colonies were picked as candidates for mutants that had become insensitive to the *nadI*^{s} repression; some of these show a constitutively high level of β-galactosidase.

Three putative *cis*-regulatory mutants from the *nad B499*::Mu dJ donor (TT11395, TT11396, and TT11397) and two from the *nadA219*::Mu dJ donor (TT11398 and TT11399) have been tested; each is highly linked (97 to 99% cotransduction) to the *nadA* or *nadB* fusion. The new regulatory mutations and their linked fusion were transduced into a wild-type (*nadI*$^+$) background (TT11400 to TT11404), and regulation was checked by assaying β-galactosidase (Table 8). The mutations linked to the fusion cause the escape of transcription from repression by both the *nadI*^{s} and the *nadI*$^+$ repressor.

Mutants shown in Table 8 have not lost all control of expression; small differences in the β-galactosidase levels are generated in response to changes in NA concentration. These data support the idea that these mutations have changed operator sites, which retain some affinity for repressor. Although these mutants are presumed to affect operator sites of *nadA* and *nadB* genes, we have not yet demonstrated the expected *cis*-dominance behavior.

TABLE 8. Effect of putative operator mutations on expression of the *nadA* and *nadB* genes

Strain	Relevent genotype	β-Galactosidase activity (U) in medium with:	
		10^{-6} M NA	2×20^{-4} M NA
TT10738	*nadB499*::Mu dJ	320	6
TT11360	*nadB499*::Mu dJ *nadI*^{s}*511*	4	3
TT11400	*nadB*^{c}*529 nadB499*::Mu dJ[a]	377	191
TT11395	*nadB*^{c}*529 nadB499*::Mu dJ *nadI*^{s}*511*	134	95
TT11401	*nadB*^{c}*530 nadB499*::Mu dJ	366	204
TT11396	*nadB*^{c}*530 nadB499*::Mu dJ *nadI*^{s}*511*	130	88
TT11402	*nadB*^{c}*531 nadB499*::Mu dJ	408	184
TT11397	*nadB*^{c}*531 nadB499*::Mu dJ *nadI*^{s}*511*	116	87
TT11403	*nadA*^{c}*532 nadA219*::Mu dJ[a]	511	279
TT11398	*nadA*^{c}*532 nadA219*::Mu dJ *nadI*^{s}*511*	236	154
TT11404	*nadA*^{c}*533 nadA219*::Mu dJ	493	266
TT11399	*nadA*^{c}*533 nadA219*::Mu dJ *nadI*^{s}*511*	198	153

[a] See Table 1, footnote *b*.

DISCUSSION

The results presented here provide genetic evidence for the existence of a repressor for the NAD biosynthetic pathway of *S. typhimurium*. This repressor, encoded by the *nadI* gene, appears to be directly responsible for the transcriptional control of the *nadB* and *nadA* genes, two biosynthetic genes that are not linked to each other or to the *nadI* gene.

The *nadI* gene was first discovered because mutations at that locus cause derepression of the *nadA* and *nadB* genes. Genetic analysis has revealed that *nadI* appears to be in an operon with the *pnuA* gene, which is known to encode a product involved in the transport system for NMN (7, 9, 13, 18). The mechanism by which mutations in this region cause derepression of the de novo pathway was not previously known; the relationship between *nadI* and *pnuA* was also unclear.

The first suggestion of a repressor has come from in vivo gene dosage experiments with a cloned *nadB* or *nadA* gene. The high copy number of either one of these two genes results in derepression of chromosomal *nadB* and *nadA* genes. We have pursued the possibility that a repressor is encoded in the *nadI* gene. A specific type of mutant which expresses *nadA* and *nadB* at a constitutively low level was isolated on the basis of the assumption that rare mutations in a repressor gene could result in a noninducible repressor like super-repressor mutations (*lacI*^{s}) in the *lac* system (17, 22). Because of this analogy, we have designated these mutations *nadI*^{s}.

Several lines of evidence suggest that *nadI*^{s} (super-repressor) and *nadI* (high constitutive) mutations affect the same gene. (i) Both *nadI*^{s} and *nadI* mutations show approximately the same cotransduction frequency with *serB1463*::Tn*10*. (ii) Selection for reversal of the NadI^s phenotype yields *nadI* mutations which are tightly linked to the original *nadI*^{s} mutations. (iii) Some temperature-sensitive *nadI*^{s} mutations isolated at 30°C show constitutive high-level (NadI$^-$) expression of *nadA-lac* and *nadB-lac* fusions at higher temperatures. The *nadI*^{s} mutations, which are rare, are dominant to *nadI*$^+$, and the common *nadI* mutations are recessive to the wild-type allele.

The data also suggest that the *nadI* protein interacts directly with *nadA* and *nadB* sequences. One of the spontaneous revertants of *nadI*^{s}*511 (nadI536)* causes constitutive expression of the *nadB-lac* and *nadA-lac* fusions at significantly different levels. This suggests that the mutant repressor distinguishes between the two normal control regions. Constitutive mutations linked to *nadB-lac* and *nadA-lac* appear to alter the site at which the regulatory protein acts; these mutants retain slight sensitivity to control, which is still affected by the difference between the *nadI*$^+$ and *nadI*^{s} backgrounds.

The results presented strongly support the idea that the *nadI* gene encodes a repressor affecting the transcription of the *nadA* and *nadB* genes. These results do not identify the corepressor that might act as a signal compound. The finding that leaky *nadE* (NAD synthetase) mutants grow with elevated transcription of *nadA* and *nadB* genes suggests that the final compounds in the pathway, NAD or NADP, may provide the regulatory signal (K. T. Hughes, B. M. Olivera, and J. R. Roth, unpublished results). Our results predict that a repressor binds a pyridine nucleotide and interacts with sequences near the *nadA* and *nadB* genes to control transcription of these biosynthetic genes. This protein may be bifunctional and play a direct role in NMN transport, which would explain the PnuA$^-$ phenotype of *nadI*^{s} mutants (unpublished data). Alternatively, the *nadI* repressor may be a monofunctional protein encoded in the same operon as the *pnuA* gene.

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ADDENDUM IN PROOF

The data included here were presented previously (Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, H-72, p. 139). Since this paper was submitted, similar data were published by Foster et al. (J. W. Foster, E. A. Holley-Guthrie, and F. Warren, Mol. Gen. Genet. **208**:279–287, 1987).

LITERATURE CITED

1. **Casadaban, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: *in vivo* probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA **76**:4530–4533.
2. **Castilho, B. A., P. Olfson, and M. J. Casadaban.** 1984. Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol. **158**:488–495.
3. **Chaconas, G., F. J. deBruijn, M. J. Casadaban, J. R. Lupski, T. J. Kwok, R. M. Harshey, M. S. DuBow, and A. I. Bukhari.** 1981. *In vitro* and *in vivo* manipulations of bacteriophage Mu DNA: cloning of Mu ends and construction of mini-Mu's carrying selectable markers. Gene **13**:37–46.
4. **Chan, R. K., D. Botstein, T. Watanabe, and Y. Ogata.** 1972. Specialized transduction of tetracycline resistance by phage P22 in *Salmonella typhimurium*. II. Properties of a high frequency transducing lysate. Virology **50**:883–898.
5. **Chandler, J. L. R., and R. K. Gholson.** 1972. De novo biosynthesis of nicotinamide in *Escherichia coli*: excretion of quinolinic acid by mutants lacking quinolinate phosphoribosyl transferase. J. Bacteriol. **111**:98–102.
6. **Chandler, J. L. R., and R. K. Gholson.** 1972. Studies on the biosynthesis of NAD in *Escherichia coli*. III. Precursors of quinolinic acid *in vitro*. Biochim. Biophys. Acta **264**:311–318.
7. **Cookson, B. T., B. M. Olivera, and J. R. Roth.** 1987. Genetic characterization and regulation of the *nadB* locus of *Salmonella typhimurium*. J. Bacteriol. **169**:4285–4293.
8. **Davis, R. W., D. Botstein, and J. R. Roth (ed.)** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Foster, J. W., D. M. Kinney, and A. G. Moat.** 1979. Pyridine nucleotide cycle of *Salmonella typhimurium*: isolation and characterization of *pncA*, *pncB*, and *pncC* mutations and utilization of exogenous nicotinamide adenine dinucleotide. J. Bacteriol. **137**:1165–1175.
10. **Foster, J. W., and A. G. Moat.** 1978. Mapping and characterization of the *nad* genes in *Salmonella typhimurium* LT-2. J. Bacteriol. **133**:775–779.
11. **Foster, J. W., and A. G. Moat.** 1980. Nicotinamide adenine dinucleotide biosynthesis and pyridine nucleotide cycle metabolism in microbiol systems. Microbiol. Rev. **44**:83–105.
12. **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.
13. **Holley, E. A., M. P. Spector, and J. W. Foster.** 1985. Regulation of NAD biosynthesis in *Salmonella typhimurium*: expression of *nad-lac* gene fusions and identification of a *nad* regulatory locus. J. Gen. Microbiol. **131**:2759–2770.
14. **Hong, J.-S., and B. N. Ames.** 1971. Localized mutagenesis of any specific small region of the bacterial chromosomes. Proc. Natl. Acad. Sci. USA **68**:3158–3162.
15. **Hughes, K., D. Ladika, J. R. Roth, and B. M. Olivera.** 1983. An indispensable gene for NAD biosynthesis in *Salmonella typhimurium*. J. Bacteriol. **155**:213–221.
16. **Hughes, K. T., and J. R. Roth.** 1984. Conditional transposition-defective derivative of Mu *dI* (Amp, Lac). J. Bacteriol. **159**:130–137.
17. **Jobe, A., A. D. Riggs, and S. Bourgeois.** 1972. *lac* repressor-operator interaction. J. Mol. Biol. **64**:181–199.
18. **Kinney, D. M., J. W. Foster, and A. G. Moat.** 1979. Pyridine nucleotide cycle of *Salmonella typhimurium*: in vitro demonstration of nicotinamide mononucleotide deamidase and characterization of *pnuA* mutants defective in nicotinamide mononucleotide transport. J. Bacteriol. **140**:607–611.
19. **Langley, D., and J. R. Guest.** 1974. Biochemical and genetic characteristics of deletion and other mutant strains of *Salmonella typhimurium* LT-2 lacking α-keto acid dehydrogenase complex activities. J. Gen. Microbiol. **82**:319–335.
20. **Lehman, I. R.** 1974. DNA ligase: structure, mechanism, function. Science **186**:790–797.
21. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Millson, C., D. Perrin, M. Cohn, F. Jacob, and J. Monod.** 1964. Non-inducible mutants of the regulatory gene in the "lactose" system of *Escherichia coli*. J. Mol. Biol. **8**:582–592.
23. **Olivera, B. M., and I. R. Lehman.** 1967. Diphosphopyridine nucleotide: a cofactor for the polynucleotide-joining enzyme from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **57**:1700–1704.
24. **Saxton, R. E., V. Rocha, R. J. Rosser, A. J. Andreoli, M. Shimoyoma, A. Sosska, J. L. R. Chandler, and R. K. Gholson.** 1968. A comparative study of the regulation of nicotinamide adenine dinucleotide biosynthesis. Biochim. Biophys. Acta **156**:77–84.
25. **Schmid, M. B., and J. R. Roth.** 1983. Genetic methods for analysis and manipulation of inversion mutations in bacteria. Genetics **105**:517–537.
26. **Schmieger, H.** 1971. A method for detection of phage mutants with altered transducing ability. Mol. Gen. Genet. **100**:378–381.
27. **Tritz, G. J.** 1974. Characterization of the *nadR* locus in *Escherichia coli*. Can. J. Microbiol. **20**:205–209.
28. **Tritz, G. J., and J. L. R. Chandler.** 1973. Recognition of a gene involved in the regulation of nicotinamide adenine dinucleotide biosynthesis. J. Bacteriol. **114**:128–136.
29. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
30. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.