A Genetic Characterization of the *nadC* Gene of *Salmonella typhimurium*

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ABSTRACT

The *nadC* gene of Salmonella encodes the pyridine biosynthetic enzyme PRPP-quinolinate phosphoribosyltransferase. Using a combination of genetic techniques, a deletion map for the Salmonella *nadC* gene has been generated which includes over 100 point mutants and 18 deletion intervals. The *nadC* alleles obtained by hydroxylamine mutagenesis include those suppressed by either amber, ochre, or UGA nonsense suppressors as well as alleles suppressed by the missense suppressor, *sumA*. Deletions were obtained by three separate protocols including spontaneous selection for loss of the nearby *aroP* gene, recombination between *aroP*::MudA and *nadC*::MudA insertion alleles, and selection for spontaneous loss of tetracycline resistance in a nearby *guaC*::Tn*10*dTc insertion mutant allele. The *nadC* mutants comprise one complementation group and the *nadC*$^+$ allele is dominant to simple, *nadC* auxotrophic mutant alleles. Intragenic complementation of two *nadC* alleles, *nadC493* and *nadC494*, mapping to deletion intervals 17 and 18, respectively, suggests that *nadC* encodes a multimeric enzyme. Both *nadC* and the nearby *aroP* locus are transcribed counterclockwise on the standard genetic map of Salmonella, in opposite orientation to the direction of chromosome replication.

NICOTINAMIDE adenine dinucleotide (NAD), NAD-phosphate (NADP) and their reduced forms NADH and NADPH, are the major donors and acceptors of electrons in cellular metabolism. NAD is synthesized by *Salmonella typhimurium* by either a *de novo* pathway starting with aspartate and dihydroxyacetone phosphate, or from exogenous pyridines using the salvage pathways (Figure 1) (FOSTER and MOAT 1980). Exogenously supplied NAD precursors are taken up primarily through the Priess-Handler pathway in which the precursors are eventually converted to nicotinic acid (ANDREOLI *et al.* 1972; FOSTER, KINNEY and MOAT 1979; GHOLSON *et al.* 1969; LIU *et al.* 1982; SUNDARAM 1967). Both the *de novo* biosynthetic pathway and the exogenous utilization pathway converge to the key metabolite, nicotinic acid mononucleotide (NaMN). The enzymatic step that precedes NaMN in the *de novo* pathway is very similar to the reaction preceding NaMN in the pathway used to assimilate exogenous pryidines. Both reactions are phosphoribosyl transferase reactions, one utilizing quinolinic acid (*nadC*) and the other utilizing nicotinic acid (*pncB*) as substrates. The *pncB* gene product, nicotinic acid phosphoribosyl transferase (NAPRTase), catalyzes the formation of NaMN from nicotinic acid and phosphoribosyl pyrophosphate (PRPP) (IMSANDE and HANDLER 1961). This reaction also requires ATP hydrolysis. The *nadC* gene product, quinolinic acid phosphoribosyl transferase (QAPRTase), catalyzes the formation of NaMN and CO_2 from quinolinic acid and 5-phosphoribosyl-1-pyrophosphate (PRPP) (PACKMAN and JACKOBY 1967). This step does not require ATP hydrolysis although extra energy is derived from decarboxylation of quinolinic acid. It is not directly obvious why the *pncB* step would require ATP hydrolysis. It may allow the cell to take up nicotinic acid when present in low concentrations, or the equilibrium of the reaction may actually require ATP hydrolysis in making the high energy glycosidic bond in NaMN.

Recently, quinolinic acid has been found to accumulate in patients suffering from Huntington's chorea (BRUYN and STOOF 1990). Quinolinate serves as an agonist for certain excitatory amino acid receptors in the vertebrate central nervous system, most notably the NMDA (*N*-methyl D-aspartate) receptor. The NMDA receptor controls an ion channel which allows the entry of Ca^{2+} into neurons; thus it has been suggested that the accumulation of quinolinate leads to inappropriate and excessive entry of Ca^{2+}, and the subsequent neuronal cell death characteristic of Huntington's chorea. One presumptive cause for quinolinic acid accumulation is a dysfunction in quinolinic acid phosphoribosyl transferase.

The *nadC* gene of *S. typhimurium* is one of three nonessential genes involved in *de novo* NAD biosynthesis. None of the genes whose products are required for *de novo* NAD biosynthesis are closely linked on the Salmonella chromosome (see Figure 2) (SANDERSON and ROTH 1983). The *de novo* NAD biosynthetic pathway is transcriptionally regulated by the product of the *nadI* gene (COOKSON, OLIVERA and ROTH 1987; HOLLEY, SPECTOR and FOSTER 1985; FOSTER, HOL-

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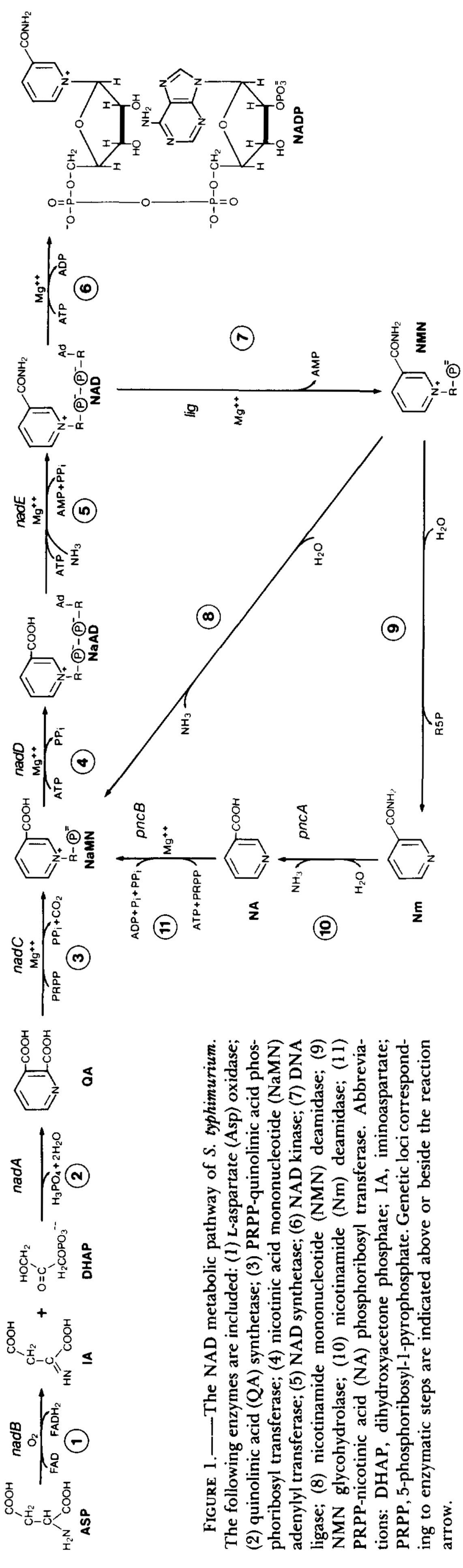

FIGURE 1.——The NAD metabolic pathway of *S. typhimurium*. The following enzymes are included: (1) L-aspartate (Asp) oxidase; (2) quinolinic acid (QA) synthetase; (3) PRPP-quinolinic acid phosphoribosyl transferase; (4) nicotinic acid mononucleotide (NaMN) adenylyl transferase; (5) NAD synthetase; (6)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

LEY-GUTHRIE and WARREN 1987). The *nadI* gene product is a transcriptional repressor of the *nadB* and *nadA* genes when cells are grown in the presence of exogenous NAD precursors (FOSTER *et al.* 1990; ZHU, OLIVERA and ROTH 1988, 1991; ZHU and ROTH 1991). When internal NAD levels drop, the *nadI* product activates the *pnuC* gene product which provides for transport of exogenously supplied nicotinamide mononucleotide (NMN) and nicotinic acid mononucleotide (NaMN) (SPECTOR *et al.* 1985; ZHU, OLIVERA and ROTH 1989; FOSTER *et al.* 1990). Of the three nonessential genes, *nadB, nadA* and *nadC,* only *nadC* is not known to be under any form of genetic regulation (HOLLEY and FOSTER 1982; SAXTON *et al.* 1968). It is unclear why both *nadB* and *nadA* are transcriptionally regulated while *nadC,* the third step in the nonessential branch of the *de novo* pathway, is under no known mechanism of genetic regulation.

MATERIALS AND METHODS

Bacterial strains: All strains used in this study are listed in Table 1. All *S. typhimurium* strains were derived from *S. typhimurium* strain LT2. Several derivatives of the Mu *d(lac)* phage described by CASADABAN and COHEN (1979) were used in this work. MudA refers to Mu *d1–8,* a transposition-defective derivative of the original Mu *d1*(Ap *lac*) phage of Casadaban and Cohen which forms *lac* operon fusions (HUGHES and ROTH 1984). This fusion vector transposes readily in amber suppressor strains and only rarely in strains lacking an amber suppressor mutation. MudJ refers to the transposition-defective *lac* operon fusion vector Mu *d1734*(Km *lac*) described by CASTILHO, OLFSON and CASADABAN (1984). This phage is deleted for transposition functions and carries kanamycin resistance in place of ampicillin resistance. MudJ insertion mutants are isolated by providing transposition functions in *cis* on a single P22-transduced fragment (HUGHES and ROTH 1988). During this process the MudJ insertion transposes from the fragment into the recipient chromosome while the remaining transposition genes are degraded leaving a MudJ insertion lacking transposition functions.

Media: The E medium of VOGEL and BONNER (1956), supplemented with 0.2% glucose, was used as minimal medium. Difco nutrient broth (NB; 8 g/liter) with NaCl (5 g/liter) added was used as rich medium for growing cells. Luria-Bertani medium (LB) (DAVIS, BOTSTEIN and ROTH 1980), supplemented with E salts and 0.2% glucose, was used as rich medium for growing P22 phage lysates. Difco agar was added to a final concentration of 1.5% for solid medium. Auxotrophic supplements were included in media at final concentrations suggested by DAVIS, BOTSTEIN and ROTH (1980). The following additives were included in media as needed (final concentrations given): azaserine (1 mM), tetracycline hydrochloride (25 μg/ml in rich medium or 10 μg/ml in minimal medium), kanamycin sulfate (50 μg/ml in rich medium or 125 μg/ml in minimal medium), ampicillin (30 μg/ml in rich medium or 15 μg/ml in minimal medium), and chloramphenicol (25 μg/ml in rich medium or 5 μg/ml in minimal medium). Exogenous pyridines were included in media as needed (final concentrations given): nicotinamide (2 μg/ml), nicotinic acid (2 μg/ml), 6-aminonicotinamide (50 μg/ml), 6-aminonicotinic acid (50 μg/ml) and quinolinic acid (10 mM) which was recrystallized in cold 40% acetic acid.

TABLE 1

List of strains

Strain	Genotype	Source[a]
LT2		Strain collection
purC7		Strain collection
TH1688	*purC882*::Tn*10* DEL644(*guaC568*::Tn*10*dTc-*nadC-aroP-pgn-ace*)	
TH1692	*proA692*::MudA (Lac$^-$)	
TH1693	*purC882*::Tn*10* DUP1107[(DEL644 *proA692*)*MudA*(leuA1179 DEL644)]	
TH1696	*guaC568*::Tn*10*dTc *nadC436*	
TH1697	*guaC568*::Tn*10*dTc *nadC437*	
TH1698	*guaC568*::Tn*10*dTc *nadC444*	
TH1699	*guaC568*::Tn*10*dTc *nadC445*	
TH1700	*guaC568*::Tn*10*dTc *nadC447*	
TH1701	*guaC568*::Tn*10*dTc *nadC493*	
TH1702	*guaC568*::Tn*10*dTc *nadC494*	
TH1703	*guaC568*::Tn*10*dTc *nadC495*	
TR6720	DEL603(*ace-aroP-nadC*)	
TT287	*purC882*::Tn*10*	Strain collection
TT8046	Δ*proAB47 pyrB64*/F′128 Lac$^+$Pro$^+$ *zzf-1066*::MudA	Hughes and Roth (1985)
TT8269	*leuA1179*::MudA (Lac$^+$)	Hughes and Roth (1985)
TT8370	*thr-458*::MudA (Lac$^-$)	Hughes and Roth (1985)
TT8371	*thr-469*::MudA (Lac$^+$)	Hughes and Roth (1985)
TT8784	*nadC218*::MudA (Lac$^-$)	Hughes and Roth (1984)
TT8786	*nadC220*::MudA (Lac$^+$)	Hughes and Roth (1984)
TT8787	*nadC221*::MudA (Lac$^-$)	Hughes and Roth (1984)
TT8788	*nadC222*::MudA (Lac$^-$)	Hughes and Roth (1984)
TT10205	*nadC351*::MudA (Lac$^-$)	
TT10206	*nadC352*::MudA (Lac$^+$)	
TT10209	*nadC355*::MudA (Lac$^+$)	
TT10210	*nadC356*::MudA (Lac$^+$)	
TT10288	*hisD9953*::MudJ *hisA9944*::MudI	
TT10423	LT7 Δ*proAB47*/F′Pro$^+$Lac$^+$*zzf-1831*::Tn*10*dTc	T. Elliott
TT10427	LT2/pNK972	T. Elliott
TT10492	*nadC367*::MudJ (Lac$^-$)	
TT01493	*nadC368*::MudJ (Lac$^-$)	
TT10547	*guaC568*::Tn*10*dTc	
TT10705	*aroP578*::MudA (Lac$^+$)	
TT10706	*aroP579*::MudA (Lac$^+$)	

[a] Unless indicated otherwise, all strains were constructed during the course of this work.

Transductional methods: For all transductional crosses, the high frequency generalized transducing mutant of bacteriophage P22 (*HT105/1 int-201*) was used (Sanderson and Roth 1983). Selective plates were spread directly with 2×10^8 cells and 10^8–10^9 phage. For transduction of MudA, 10^9–10^{10} phage were used per 2×10^8 cells. The MudA prophages are inherited by a two-fragment transductional event and therefore require a higher phage input (Hughes and Roth 1985; Hughes, Olivera and Roth 1987). Transductants were purified and phage-free clones were isolated by streaking nonselectively onto green indicator plates (Chan *et al.* 1972). P22 lysates were titered according to the method of Davis, Botstein and Roth (1980).

For transductional crosses used in the construction of the *nadC* deletion map, P22 transducing phage was first single plaque isolated on a large deletion mutant, *nadC644*, which covers the entire region (Δ(*ace-aroP-nadC-guaC*)). A single plaque was then used to inoculate a 1 ml overnight culture of the *nadC644* deletion mutant followed by the addition of a 4-ml portion of L broth supplemented with E salts and 0.2% glucose. After 4 hr of incubation at 37° with vigorous shaking, a liquid P22 lysate was obtained. This lysate, prepared on the *nadC644* deletion mutant was used to prepare all the transducing lysates on all the *nadC* point insertion and deletion mutants used to generate the deletion map. This procedure eliminated any possible transducing particles that might carry a *nadC*$^+$ allele from a previous phage lysate. The *nadC* point mutants were first roughly mapped with respect to the deletion mutants by spot tests. A 0.1 ml aliquot of each recipient culture was spread on a selective plate. A drop of four different donor lysates was transferred by pasteur pipette onto each quadrant of the recipient lawn and allowed to soak in. Crosses that failed to yield prototrophic transductants were then retested by a full plate cross. This was done by plating 0.1 ml of a 10^{11} pfu/ml donor lysate with 0.1 ml of an overnight culture of the *nadC* deletion mutants whose endpoints flanked the point mutant. If less than 10 colonies arose, then the transduction was repeated five more times.

Isolation of Tn*10*dTc, MudA and MudJ insertion mutants: Techniques for the isolation of MudA and MudJ insertions in the Salmonella chromosome have been described (Hughes and Roth 1984, 1988). Four MudA insertion mutants in the *nadC* gene were found among previously isolated nicotinamide-requiring MudA insertion mutants by genetic mapping (Hughes and Roth 1984). Another four *nadC*::MudA insertion mutants were isolated by growing P22 transducing phage on strain TT8046 and transducing an amber suppressor recipient to MudA-encoded ampicillin resistance (Amp^{r}) on NB-ampicillin plates. The plates were replica printed to minimal E-ampicillin plates with and without nicotinamide. Of 11 MudA insertions which resulted in nicotinamide auxotrophy, 4 mapped to the *nadC* locus.

Insertions of MudJ in the *nadC* and *aroP* genes were isolated by growing P22 transducing phage on strain TT10288 and using this phage stock as a donor to transduce LT2 to MudJ-encoded kanamycin resistance on NB + kan plates supplemented with 0.2% acetate. The plates were replica-printed to minimal E plates with 1 mM azaserine to screen for insertions in the *aroP* locus as well as minimal E plates with and without acetate and nicotinamide. One acetate-requiring auxotroph was isolated; 12 nicotinamide auxotrophs were also identified. The acetate auxotroph was found to be unlinked to the *aceEF* genes. Of the 12 *nad*::MudJ insertion mutants isolated four were unable to utilize quinolinic acid as a sole pyridine source and mapped to *nadC*.

Tn*10*dTc refers to Tn*10* Del16 Del17 Tet^{r}, a 3 kb transposition-defective derivative of Tn*10* constructed by Way *et al.* (1984). Insertions of Tn*10*dTc were isolated by growing P22 transducing phage on strain TT10423 and using this phage stock as a donor to transduce the *purC7* strain carrying the Tn*10* transposase-producing plasmid, pNK972, to tetracycline-resistance on NB + tet plates. These plates were replica printed to minimal E plates supplemented with thiamine, adenine and nicotinamide and minimal E plates supplemented with thiamine and guanine. Among the nicotinamide-requiring auxotrophs, one was unable to utilize quinolinic acid as an exogenous pyridine source and mapped to the *nadC* locus. All insertions which rendered the cells unable to utilize guanine as a sole purine source were found linked to *nadC* and are presumed to be in the *guaC* gene.

Selection of spontaneous deletions of the *nadC* region: Two methods were employed to select for spontaneous deletions that yielded deletion endpoints within the *nadC* gene. The first method used a positive selection for mutants in the nearby *aroP* locus. Mutants defective in the *aroP* gene are resistant to the amino acid analog azaserine (Ames and Roth 1968). *Escherichia coli aroP* mutants are selected for as resistant to the combination of amino acid analogs *p*-fluorophenylalanine, 5-methyltryptophan and β-thienylalanine (Langley and Guest 1977). While *S. typhimurium* strain LT2 is reportedly sensitive to fluorophenylalanine (Ames 1964), the LT2 strain used in this study was found to be resistant to fluorophenylalanine. We also found that inhibition of our LT2 strain required much higher doses of the other two analogs than that reported for *E. coli*. The *S. typhimurium* LT2 strain used in this study is very sensitive to the glutamine analog, azaserine, and this analog proved extremely useful in the isolation of *aroP* mutants in Salmonella (Ames and Roth 1968).

In the initial experiment, a 0.1-ml portion of an LT2 culture grown overnight in NB medium was plated onto an E plate containing 1 mM azaserine, nicotinamide, acetate and succinate. After replica plating to the same plate and to an E + azaserine plate without supplements, 1 auxotroph was isolated which required both acetate and nicotinamide for growth. To isolate larger numbers of *nadC* deletion mutants, a 0.1-ml portion from each of 200 independent cultures of strain *purC7* was plated on minimal E medium containing 1 mM azaserine and supplemented with acetate (0.2%), succinate (0.2%), nicotinamide (2 μg/ml), adenine and thiamine. After overnight incubation at 37°, an average of 250 azaserine-resistant (Aza) colonies per plate grew up. These plates were replica printed to E + azaserine plates supplemented with adenine and thiamine, and E− azaserine plates supplemented with adenine, thiamine, nicotinamine acetate and succinate to screen for deletions of the *aroP* region which extend into either the *nadC* gene (nicotinamide auxotrophs) or the *aceEF* operon (acetate plus succinate auxotrophs). Those found to require nicotinamide for growth were further screened for the ability to use guanine as the sole purine source to screen for deletions which extend into the *guaC* gene. The *guaC* gene product, GMP reductase, is required for growth on guanine as the sole source of purine (Magasanik and Karibian 1960). From the 200 independent plating experiments, 2 deletion mutant required acetate for growth (in addition to the purine and thiamine requirement), 7 required both acetate and nicotinamide, and 20 required nicotinamide alone. One mutant, auxotrophic for nicotinamide, was found which could not utilize guanine as sole source of purine. The deletion mutants which required only nicotinamide (in addition to adenine and thiamine due to the *purC7* allele) fell into two classes: one class of 4 mutants grew poorly on nutrient broth plates while the remaining 22 mutants grew normally on nutrient broth. Since both classes include deletions later shown to end within the *nadC* gene, the defect for growth on broth must be due to the lack of a gene lying on the *aceEF* side of *aroP*. We have designated a locus, *pgn*, for poor growth on nutrient plates which maps between *aroP* and the *aceEF* operon. We presume that deletions which extend into this region result in the Pgn phenotype.

A second series of experiments was devised to obtain spontaneous deletions which extend into the *nadC* gene from the *guaC* side. A *guaC*::Tn*10*dTc insertion mutant was obtained as described above. A 0.1-ml aliquot from each of 100 independent cultures of strain TT10547 grown overnight in NB media at 37° was plated on modified Bochner tetracycline-sensitive plates (Bochner *et al.* 1980; Maloy and Nunn 1981) and incubated at 42°C. After overnight incubation, the colonies were replica printed to minimal tetracycline-sensitive plates (Bochner *et al.* 1980) and the modified (complex medium) tetracycline-sensitive plates. Putative auxotrophs were picked and screened for nicotinamide and acetate auxotrophy as well as azaserine resistance. Of the 100 independent cultures, 10 auxotrophic mutants were found. Of these, 9 required nicotinamide and were Aza^{s}, while 1 required both acetate and nicotinamide and was Aza^{r}. Thus, using spontaneous selections a total of 39 independent auxotrophic deletions mapping to the *nadC* region of the chromosome were isolated. After deletion mapping with *nadC* point mutants (see below), 11 of these spontaneous deletions were found to end within the *nadC* gene.

Transcriptional orientation of the *nadC* and *aroP* operons: The chromosomal orientation of Lac^{+} and Lac^{-} MudA insertion mutants in the *aroP* and *nadC* genes were determined as previously described (Hughes and Roth 1985). P22 transducing phage was grown on Lac^{+} and Lac^{-} *thr*::MudA insertion mutants and Lac^{+} and Lac^{-} *nadC*::MudA insertion mutants. P22-mediated transduction of the Mud prophage by homologous recombination involves two simultaneously transduced fragments (Hughes and Roth 1985; Hughes, Olivera and Roth 1987). When P22 transducing lysates grown on *thr*::MudA and *nadC*::MudA insertion mutants are mixed, then fragments from the two donor Mud insertions can enter a single recipient and recombine to generate a hybrid Mud with *nadC* material flanking one side of the hybrid element and *thr* material flanking the other end. Provided that the parental insertions are in the same orientation on the chromosome, integration of the hybrid fragments will yield either a deletion recombinant (in which the genetic material between the parental insertion points is removed) or a duplication recombinant in which the genetic material between the parental insertion points is duplicated upon integration of the hybrid element. The resulting hybrid element is at the join point of the duplication. Since essential genes map between the parental insertion locations, then integration of the deletion recombinant is inviable. If the donor insertions are in the opposite orientation on the chromosome, integration of the hybrid elements will break the chromosome and form an inviable recombinant. In short, if equal titers of P22 transducing phage grown on *nadC*::MudA and *thr*::MudA insertion mutants are mixed and used to transduce LT2 to MudA-encoded Ap^r, the Ap^r transductants will include only the auxotrophic *thr*::MudA and *nadC*::MudA parental recombinants if the parental insertions are in the opposite orientation on the chromosome. If the donor insertions are in the same orientation on the chromosome, prototrophic duplication recombinants will form which segregate Ap^s haploids (see Figure 3); these will be present in addition to the parental auxotrophic *thr*::MudA and *nadC*::MudA recombinants.

The transcriptional orientation of *aroP* was similarly shown to be the same as that of the *nadC* gene. Equal titers of P22 transducing phage grown on *nadC*::MudA and *aroP*::MudA insertion mutants were mixed and used to transduce LT2 to MudA-encoded Ap^r. The Ap^r transductants were then screened for the parental donor insertion phenotypes of Aza^r for *aroP*::MudA insertion recombinants and Nad$^-$ for *nadC*::MudA insertion recombinants as well as hybrid recombinants which were either protrophic Aza^s duplication recombinants or double mutant Aza^r Nad$^-$ deletion recombinants. An *aroP*::MudA insertion that gave deletion and duplication recombinants with a given *nadC*::MudA insertion was determined to be in the same orientation on the chromosome as the *nadC*::MudA insertion. By knowing the transcriptional orientation of the *nadC*::MudA element, one could assign a transcriptional orientation to *aroP*.

Isolation of site-directed deletions with endpoints within *nadC*: The transcriptional orientation of *nadC* and *aroP* was found to be counterclockwise on the Salmonella standard linkage map for both operons. Thus, a Lac$^+$ *nadC*::MudA and a Lac$^+$ *aroP*::MudA are in the same orientation on the chromosome. P22 transducing lysates were grown on both Lac$^+$ and Lac$^-$ MudA insertions in *aroP* and *nadC*. P22 lysates from the Lac$^+$ *aroP*::MudA were mixed separately with P22 lysates from the different Lac$^+$ *nadC*::MudA insertions and used to transduce LT2 to Mud-encoded ampicillin resistance. The Ap^r transductants which became simultaneously NadC$^-$ and aroP$^-$ were found to be deletions of the genetic material between the *nadC*::MudA and *aroP*::MudA donor insertion elements due to inheritance of a hydrid MudA transduced fragment. The same experiments were performed with Lac$^-$ MudA insertions in *aroP* and *nadC* to obtain deletion recombinants between those insertion elements.

Recombination between Mud insertions was also used to generate an internal *nadC* deletion, DEL1109. Transducing phage grown on a *nadC352*::MudJ allele was used to transduce a *nadC220*::MudA allele to MudJ-encoded kanamycin resistance. Most of the Kan^r transductants lost the ampicillin resistance of the *nadC220*::MudA allele. Four such Kan^r Amp^s transductants were screened for the ability to rescue *nadC* alleles which mapped between *nadC352* and *nadC220*. All four could not be used as donor material to transduce *nadC* point mutants, mapping between *nadC352* and *nadC220*, to NadC$^+$ and presumably have lost the genetic material between *nadC352* and *nadC220*. These are presumed to be internal deletions of *nadC* resulting from recombination between the Mud elements in the donor and recipient.

Isolation of *nadC* point mutants by hydroxylamine mutagenesis: P22 transducing phage grown on wild-type strain LT2 was mutagenized with hydroxylamine by the method of Hong and Ames (HONG and AMES 1971; DAVIS, BOTSTEIN and ROTH 1980). This mutagenized phage stock was used to transduce strain TR6720 (DEL603(*ace-aroP-nadC*)) to growth in the absence of acetate (Ace$^+$) but in the presence of nicotinamide. This cross demands that recombinants inherit *nadC* from the donor phage since the deletion in the recipient cells included both *ace* and *nadC*. The resulting Ace$^+$ transductants were screened for nicotinamide auxotrophs. Of ~25,000 Ace$^+$ transductants, 117 *nadC* mutants were isolated and used in the construction of the *nadC* deletion map.

Informational suppression of *nadC* mutant alleles: The *nadC* alleles obtained by hydroxylamine mutagenesis were tested for suppression by amber suppressor alleles of *supD, supE, supF* and *supJ* amber suppressors, which insert serine, glutamine, tyrosine and leucine, respectively, ochre suppressor alleles of *supC* and *supI,* UGA suppressor alleles of *supK* and *supU* and an uncharacterized suppressor thought to suppress missense alleles, *sumA* (SANDERSON and ROTH 1988). Strains carrying the different suppressor alleles were transduced to azaserine resistance with P22 transducing phage grown on the *nadC644* deletion which lacks the *ace, aroP, nadC* and *guaC* genes. Aza^r transductants which acquired acetate and nicotinamide auxotrophies were presumed to have inherited the donor insertion of the *nadC* region. P22 lysates were then grown on each suppressor strain which was deleted for the *nadC* region and used to transduce each of the *nadC* point mutants selecting for NadC$^+$. The ability to yield NadC$^+$ transductants was taken as an indication of suppression. NadC$^+$ transductants arose after 2 days incubation at 37° for amber suppressors, 3 days for UGA suppressors, 4 days for ochre suppressors and 5 days for the *sumA* suppressor transductants. All the *sumA*-suppressed alleles gave rise to mucoid colonies on selective plates.

Complementation and dominance experiments: In order to carry out complementation studies with different *nadC* alleles, chromosome duplications of the *nadC* region were constructed between the *leuA* gene at 2.8 min and the *proA* gene at 7.0 min on the *S. typhimurium* linkage map. This chromosomal duplication was constructed in a strain carrying the *nadC644* deletion, which covers the *aceEF, aroP, nadC* and *guaC* genes. This duplication was transduced to AceEF$^+$ with transducing phage grown on eight strains, each with different *nadC* point mutant alleles linked to a *guaC*::Tn*10*dTc insertion mutation. Four of the eight *nadC* alleles mapped to deletion intervals 2, 3 and 4, at the amino terminus of *nadC*, while the other four alleles mapped to deletion intervals 17 and 18 at the carboxy terminus of the *nadC* gene. The resulting eight strains carried the *nadC644* deletion [Δ(*ace-aroP-nadC-guaC*)] in one duplicated interval and each of the eight different *nadC* alleles linked to a *guaC*::Tn*10*dTc insertion in the other duplicated interval. These eight duplication strains were then transduced to GuaC$^+$ with phage grown on the same eight *nadC* alleles and the wild-type *nadC*$^+$ allele linked to a wild-type *guaC*$^+$ gene. The GuaC$^+$ transductants were screened for tetracycline resistance (Tc^r) to identify transductants in which the *nadC* alleles linked to the *guaC*$^+$ gene had replaced the *nadC644* deletion and not the *guaC*::Tn*10*dTc insertion. The resulting GuaC$^+$, Tc^r transductants were diploid for the eight *nadC* auxotrophic alleles in all 64 possible combinations to be used in complementation experiments. In

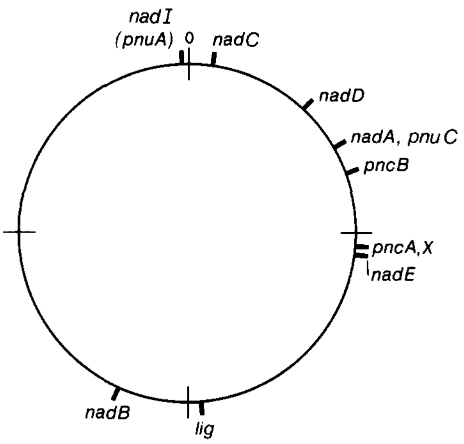

FIGURE 2.—Chromosome of *S. typhimurium* showing positions of the known genes of the NAD metabolic pathway.

addition, eight strains were constructed which were diploid for each of the *nadC* auxotrophic alleles and the *nadC*$^+$ gene to be used in dominance experiments.

RESULTS

Spontaneous deletions of the *nadC* region in Salmonella: The *nadC* gene is unlinked to all other known genes involved in NAD metabolism in Salmonella (Figure 2). The *nadC* gene maps to the 3-minute region of the chromosome. The order of known genes on the chromosome is *guaC-nadC-aroP-aceEF* (LANGLEY and GUEST 1974, 1977). This entire region covers 15 kb of the chromosome (ROBERTS *et al.* 1988) and deletions of this region have been isolated in both *E. coli* and *S. typhimurium*.

Deletions of the *nadC* region were generated by three different techniques. The first method relied on the fact that *aroP* mutants are resistant to the amino acid analog azaserine. The *aroP* gene encodes a general aromatic permease protein which is presumably necessary for azaserine to enter the cell. In one experiment, 200 independent cultures of a *purC* mutant were plated on minimal medium containing azaserine, nicotinic acid, and acetate. Of the 200 plates, 29 yielded azaserine resistant revertants which had acquired a requirement for acetate, nicotinate or both acetate and nicotinate. An additional 11 auxotrophs were isolated which were unlinked to the *nadC* region. Of these 11 mutants, one required tryptophan for growth, one required either cysteine or methionine, one required serine and the remaining eight were biotin auxotrophs. The predominance of biotin auxotrophs suggests that another locus conferring Aza^r lies near genes involved in biotin synthesis.

The selection for azaserine resistance proved useful for isolating deletions extending into the *nadC* gene from the *aroP* side. A second experiment was set up in order to isolate deletions extending into the *nadC* gene from the *guaC* side. The second experiment involved selection for tetracycline sensitivity in a *guaC*::Tn*10*dTc mutant strain. Unlike intact Tn*10*, Tn*10*dTc lacks Tn*10* transposase (WAY *et al.* 1984). Thus, tetracycline-sensitive (Tc^s) revertants of a Tn*10*dTc insertion mutant arise as spontaneous deletion events, occurring 10 to 100 times less frequently than Tn*10* transposase-generated Tc^s revertants. The Tc^s selection worked much better for the *guaC*::Tn*10*dTc insertion mutant at 42° than it did at 37°. Using this selection at 42°, 13 independent deletions were isolated which acquired a nicotinic acid auxotrophy, including two deletions whose endpoints were within the *nadC* gene.

Site-directed deletions of the *nadC* region: Deletions ending within the *nadC* gene were generated by recombination between Mud insertions in the *nadC* gene and Mud insertions in the *aroP* locus. We have shown previously that recombination between Mud insertions in different genes can be used in the formation of directed deletions and duplications of the genetic material between the insertion mutants (HUGHES and ROTH 1985). The basic strategy is outlined in MATERIALS AND METHODS. Figure 4 diagrams such recombinants obtained when P22 grown on mutants with MudA insertions in the *aroP* and *nadC* genes is used in such mixed lysate transductions. An internal *nadC* deletion (DEL1109) was generated by recombination between different Mud insertions in the *nadC* gene. Using the technique of recombination between donor Mud alleles, an additional seven deletions with endpoints within the *nadC* gene were generated. In total, 18 independent deletion mutants were isolated with deletion endpoints within the *nadC* gene and were used in recombination assays with *nadC* point mutants to generate the deletion map presented in Figure 5.

Fine structure map of the *nadC* gene: The *nadC* fine structure map was constructed by P22-mediated transductional crosses between all the deletion mutants covering the *nadC* region and the various point mutants and insertion mutants isolated in the *nadC* gene. Many of the *nadC* deletions that extended through the *aroP* gene resulted in a poor growth phenotype on nutrient plates which was not corrected by the addition of acetate. Since all deletions that included both *aroP* and *ace* loci exhibited the poor growth phenotype as well as some deletions that extended through *nadC* and *aroP*, and since other deletions that extended through *nadC* and *aroP* did not exhibit the poor growth phenotype, the gene responsible for this phenotype must lie between the *aroP* and *ace* loci and is designated *pgn* on the map. The deletion map of the *S. typhimurium nadC* gene reported here was constructed using spontaneous deletions, site-directed deletions, Mud insertions, Tn*10*dTc insertion, and point mutants generated by hydroxylamine mutagenesis. The *nadC* deletion map is depicted in Figure 5 and is the result of crosses between the deletion mutants and all of the point or insertion mutants using

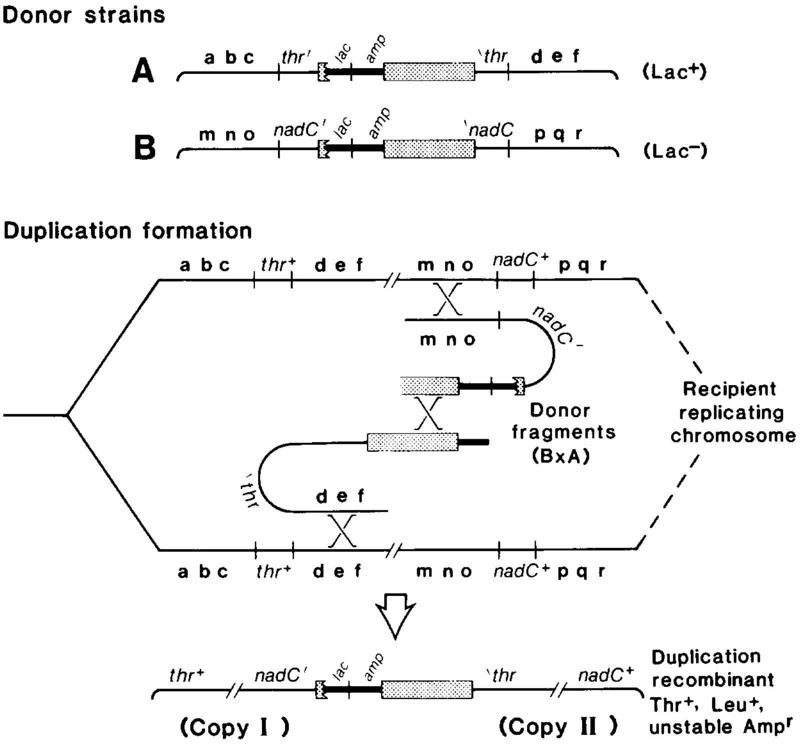

FIGURE 3.—Transcription orientation of *nadC* using Mud-generated chromosomal duplication formation. Recombination between *nadC*::MudA and thr::MudA donor MudA-transducing fragments and a replicating recipient chromosome leading to the formation of a duplication recombinant. Donor strain A is TT8371, a Lac$^+$ MudA insertion in the thr operon. Donor strain B is TT8788, a Lac$^-$ MudA insertion in *nadC*. Both insertions are in the same orientation on the chromosome. When P22 lysates grown on strain A and strain B are mixed and the resulting mixed lysate is used to transduce a recipient to Mud-encoded Ap^r, four different recombinants can arise. Two recombinant types are the parental *thr* and *nadC* recombinants. Two additional recombinant types result from transduction of two Mud fragments, one from each of the different parent insertion mutants. Depending on which combination of parent fragments are transduced into a recipient, recombination of the hybrid Mud element into the recipient chromosome will lead to duplication or deletion events of the chromosomal material between the points of insertion; however, a deletion of the region between *nadC* and *thr* would not be recovered due to the deletion of essential genes.

P22-mediated generalized transductional crosses. Such crosses have been demonstrated to distinguish between mutant alleles that are separated by less than 10 base pairs of DNA (JOHNSTON and ROTH 1981). The result of these crosses is a deletion map which divides the *nadC* gene into 18 deletion intervals. The *nadC* point mutants isolated by hydroxylamine mutagenesis were screened for informational suppression by various nonsense suppressors and the *sumA* missense suppressor. Of 117 *nadC* point mutants tested, 22 were suppressed by amber suppressors, 13 were suppressed by ochre suppressors, 10 were suppressed by UGA suppressors and 11 were suppressed by *sumA* (Table 2).

Transcriptional orientation of the *nadC* and *aroP* genes: The ability of MudA insertions in the same orientation on the chromosome to form duplications has provided a simple method for determining the transcription orientation for any gene to which MudA fusions are available. During the course of this work we have constructed 8 MudA insertions in *nadC* and 2 MudA insertions in the *aroP* gene. By generating deletions through recombination between MudA insertions in *nadC* and MudA insertions in *aroP* we have already determined that the *nadC* and *aroP* genes are transcribed in the same orientation on the *S. typhimurium* chromosome: only Lac$^+$ *nadC*::MudA insertions recombine with Lac$^+$ *aroP*::MudA insertions to yield deletion recombinants, and only Lac$^-$ *nadC*::MudA insertions recombine with Lac$^-$ *aroP*::MudA insertions to yield deletion recombinants (Table 3). In order to know the transcriptional orientation of *nadC* and *aroP* on the chromosome, duplication studies were performed with MudA insertions in the *nadC* gene and MudA insertions in the threonine biosynthetic operon (*thr*). This cross is diagrammed in Figure 3 and described in more detail in MATERIALS AND METHODS. The results of these crosses are presented in Table 4. We have found that only Lac$^-$ *nadC*::MudA insertions are capable of forming duplications with Lac$^+$ *thr*::MudA insertions and vice versa. Therefore, the *nadC* gene and the *thr* genes are transcribed in opposite orientations. It has already been established that the threonine operon is transcribed in the clockwise direction on the standard *S. typhimurium* genetic map (SANDERSON and ROTH 1988). Thus, *nadC* and *aroP* are transcribed in a counterclockwise orientation on the standard Salmonella genetic map.

Complementation and dominance studies: Complementation and dominance studies with different *nadC* alleles were carried using tandem duplication between MudA insertions in the *leuA* and *proA* genes at 2.8 and 7.0 min on the *S. typhimurium* standard linkage map. To test dominance, the *nadC*$^+$ allele was introduced into the diploid strain which already carried each of the eight *nadC* alleles tested below in the complementation experiments (Figure 6). All of the diploids were phenotypically NadC$^+$ (Table 5). Selection for duplication was removed by omitting ampicillin in the growth medium, and Ap^s segregants were obtained for all eight duplicated strains. These segregants included both the *nadC*$^+$ and *nadC* parent alleles. These results suggest that the *nadC*$^+$ allele is dominant to simple, auxotrophic *nadC* alleles. To test for complementation, an 8 × 8 matrix was generated

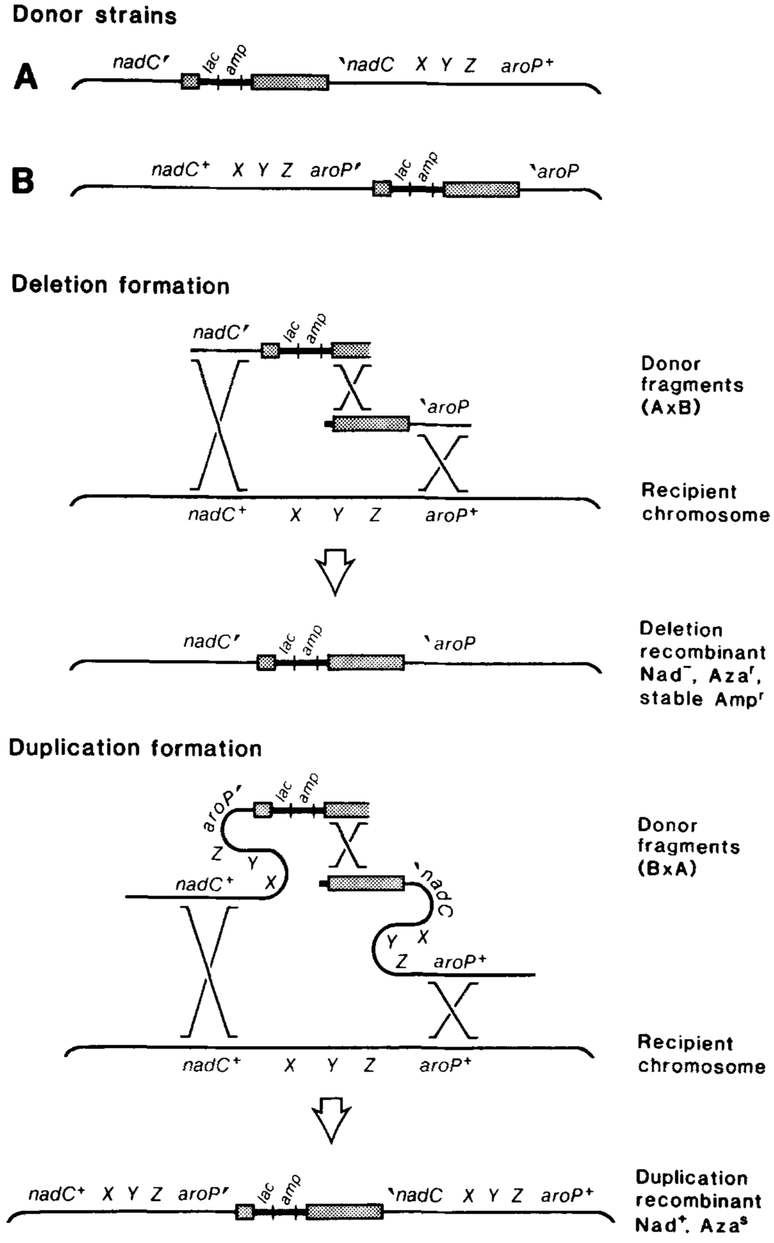

FIGURE 4.—Recombination between fragments of MudA inserts in the *aroP* and *nadC* genes. Recombination events between different Mud-transducing fragments leading to the formation of hybrid deletion and duplication recombinants are shown. Donor strain A is a Lac$^-$ MudA insertion in the *nadC* gene. Donor strain B is a Lac$^-$ MudA insertion in the *aroP* gene. The insertions are in the same orientation on the chromosome.

for four *nadC* alleles mapping to deletion intervals 3, 4 and 5 at the amino-terminal portion of *nadC*, and four other *nadC* alleles mapping to deletion intervals 17 and 18 at the carboxy-terminal portion of *nadC*. Alleles presumed to be due to missence mutations (those not suppressed by nonsense suppressors) were chosen for the complementation analysis. The results of this complementation analysis, presented in Table 5, demonstrate that *nadC* is in a single complementation group since all the diploids except one pair were NadC$^-$. All of the heteroallelic diploids segregated NadC$^+$ recombinants when ampicillin selection for duplication maintenance was removed (Table 6). Although the frequency of NadC$^+$ recombinants was higher between alleles that mapped to opposite ends of *nadC*, the frequency of NadC$^+$ recombinants was sufficiently high for alleles in the same deletion interval to propose that this could be used to separate alleles which map very close to each other, possibly to within one base of each other. One anomally occurred in the strains diploid for the *nadC493* and *nadC494* alleles. These diploids were NadC$^+$ at 37° and NadC$^-$ at 42°, and yielded NadC$^+$ recombinants at 42° when selection for the duplication was removed. Tested individually, neither mutation caused a temperature-sensitive auxotrophy. The *nadC493* and *nadC494* alleles map to deletion intervals 17 and 18, respectively. The fact that the diploids are NadC$^+$ at 37° suggests that these alleles are demonstrating intragenic complementation at this temperature, and that functionally active *nadC*-encoded QAPRTase is a multimeric enzyme.

DISCUSSION

A fine structure genetic map of the *nadC* gene of *S. typhimurium* was constructed using P22-mediated

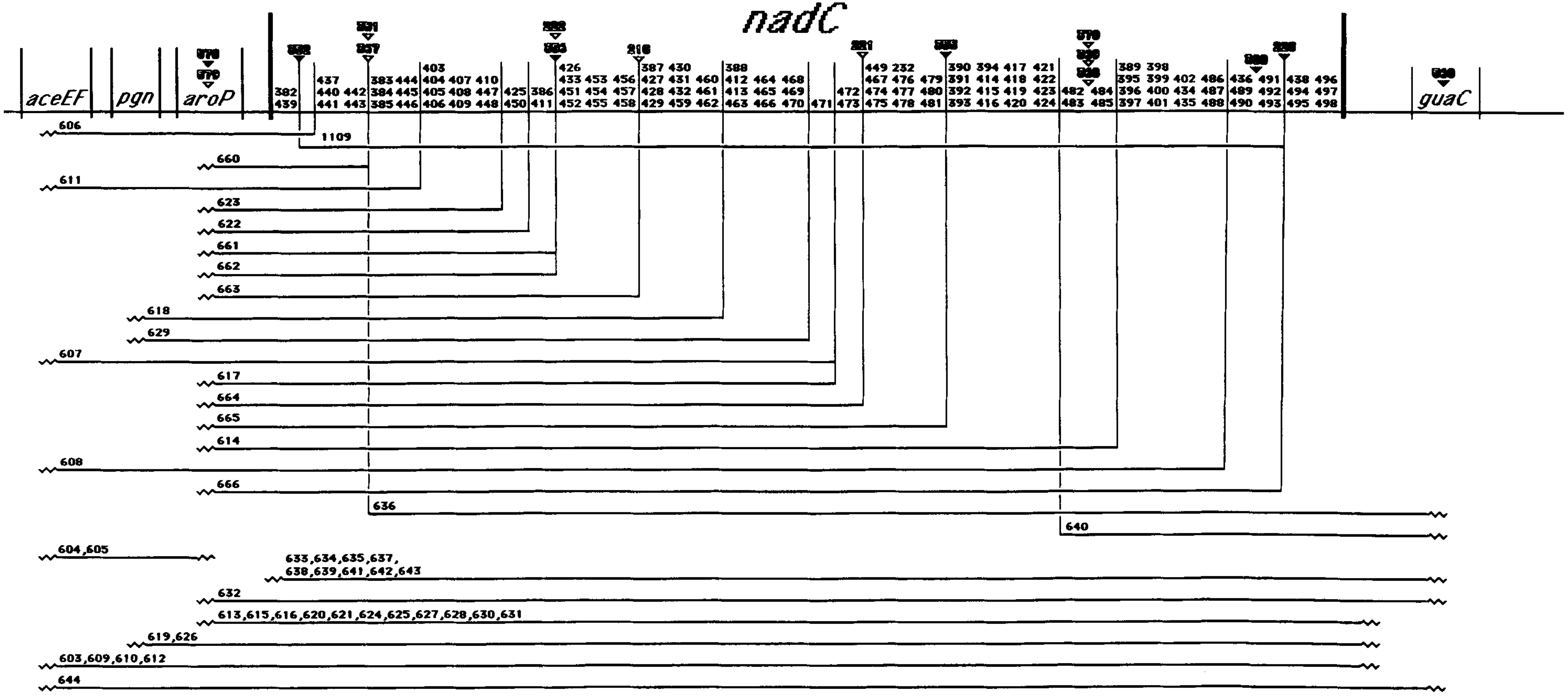

FIGURE 5.——Fine structure map of the *nadC* gene of *S. typhimurium*. For deletions, the extent of the chromosome included within the duplication is indicated by the horizontal line. Point mutations are presented above the top horizontal line. Insertions are indicated by a triangle: Mud insertions inserted in Lac$^+$ and Lac$^-$ orientations are indicated by closed and open triangles, respectively, the Tn*10*dTc insertions (*nadC380* and *guaC568*) are indicated by checkered triangles.

TABLE 2

Characterization of nadC conditional auxotrophs

	Suppressor[a]							
nadC allele	*supC*	*supD*	*supE*	*supI*	*supJ*	*supK*	*supU*	*sumA*
383, 384, 385, 389	+	+	+		+			
390, 391, 393, 394	+	+	+		+			
395, 396, 397, 398	+	+	+		+			
399, 400, 401, 402	+	+	+		+			
449	+	+	+		+			
386	+				+			
387, 427			+		+			
388, 392		+	+		+			
403, 404, 406, 414	+			+				
405, 407, 408, 409	+							
410, 411, 412, 413	+							
467	+							
415, 417, 418, 412						+	+	
416, 419, 420, 422						+		
423, 424						+		
425, 426, 428, 429								+
430, 431, 432, 434								+
435, 436, 473								+
437, 438 = temperature sensitive								
439, 440, 441, 442, 443 = cold sensitive								

[a] + = Growth on minimal medium without pyridine source.

transductional recombination tests between *nadC* point mutants generated by hydroxylamine mutagenesis and *nadC* deletion mutants. The deletion mutants were generated by three different protocols. One protocol involved selection for spontaneous *aroP* mutants. It was fortuitous that the *aroP* gene is linked to *nadC,* since loss of *aroP* can be selected for directly by selecting for resistance to azaserine and there are no essential genes between *aroP* and *nadC*. Spontaneous deletions could also be selected which entered the *nadC* gene from the opposite direction. This was done by selecting for tetracycline sensitivity in a *guaC*::Tn*10*dTc insertion mutant background. Since no essential genes lie between *nadC* and *guaC, nadC* deletions were obtained among the spontaneous Tc^s mutants. An advantage of using the Tn*10*dTc element is that deletions are due to imprecise excision generated by the flanking sequences independent of transposase since the Tn*10*dTc element does not carry the Tn*10* transposase gene. Tc^s deletions obtained in a Tn*10* background occur at a much higher frequency than deletions obtained in a Tn*10*dTc background. In addition, Tn*10* transposase-generated deletions show recurring hotspots. Thus, the Tn*10*dTc element has the advantage of yielding a wider distribution of deletion endpoints than does Tn*10*. We observed that the Tc^s selection works best at 42°. A third technique used in obtaining deletions with endpoints in the *nadC* gene was through recombination between MudA insertions in the *aroP* and *nadC* genes. This technique has the advantage that the MudA element inserts essentially at random and every insertion can be used to generate a new deletion endpoint. The deletion endpoints are predefined by the location of the original MudA insertions, and one can generate a deletion to a particular MudA insertion from either end of the gene provided there is no essential gene between parental MudA insertions. One can even recombine different *nadC*::MudA insertions to generate internal *nadC* deletions. This technique could be very useful in generating deletion maps of genes for which no other selections for deletions are available.

TABLE 3

Transcription orientation of the *aroP* gene

Donor(s)	No. of Ap^r transductants	Parental insertion recombinants	No. of deletion recombinants (Aza^r, Nad$^-$)	No. of duplication recombinants (Aza^r, Nad$^-$)
aroP578	275	272[b] (Aza^r)	0	0
aroP579	267	265 (Aza^r)	0	0
nadC220	270	268 (Nad$^-$)	0	0
nadC222	173	172 (Nad$^-$)	0	0
aroP578 × *nadC220*	926	551 (375 = *aroP*) (286 = *nadC*)	147	118
aroP578 × *nadC222*	458	454 (293 = *aroP*) (161 = *nadC*)	0	4[a]
aroP579 × *nadC220*	487	485 (298 = *aroP*) (187 = *nadC*)	0	2
aroP579 × *nadC222*	663	467 (318 = *aroP*) (149 = *nadC*)	109	87

The recipient in all crosses was LT2. The donors were TT10705 (*aroP578*::MudA, Lac$^+$), TT10706 (*aroP579*::MudA, Lac$^-$), TT8786 (*nadC220*::MudA, Lac$^+$), TT8788 (*nadC222*::MudA).

[a] MudA phage transposes under zygotic induction conditions at a low (<1%) background frequency (HUGHES and ROTH 1984).

TABLE 4

Transcription orientation of the *nadC* gene

Donor(s)[a]	No. of Ap^r transductarts	No. of theonine auxotrophs	No. of niacin auxotrophs	No. of prototrophs
thr-458::MudA (B)	~500	~500	0	0
thr-469::MudA (A)	~1000	~1000	0	3[b]
nadC220::MudA	~500	0	~500	2
nadC222::MudA	~1000	0	~1000	2
thr-458 (B) × *nadC220*	453	157	195	101
thr-458 (B) × *nadC222*	436	195	238	3
thr-469 (A) × *nadC220*	443	266	175	2
thr-469 (A) × *nadC222*	598	214	221	162

The recipient in all crosses was LT2. The donors were TT8370 (*thr-458*::MudA, Lac$^-$), TT8371 (*thr-469*::MudA, Lac$^+$), TT8786 (*nadC220*::MudA, Lac$^+$), TT8788 (*nadC222*::MudA, Lac$^-$).

[a] The designation (A) or (B) signifies the orientation of the Mud insertion on the chromosome (HUGHES and ROTH 1985).

[b] MudA phage transposes under zygotic induction conditions at a low (1%) background frequency (HUGHES and ROTH 1984).

TABLE 5

***nadC* complementation and dominance studies**

	nadC436	*nadC437*	*nadC444*	*nadC445*	*nadC447*	*nadC493*	*nadC494*	*nadC495*	*nadC*$^+$
nadC436	−	−	−	−	−	−	−	−	+
nadC437	−	−	−	−	−	−	−	−	+
nadC444	−	−	−	−	−	−	−	−	+
nadC445	−	−	−	−	−	−	−	−	+
nadC447	−	−	−	−	−	−	−	−	+
nadC493	−	−	−	−	−	−	+	−	+
nadC494	−	−	−	−	−	+	−	−	+
nadC495	−	−	−	−	−	−	−	−	+

[a] +, growth on minimal medium without an exogenous pyridine source at 37° in the presence of 15 μg/ml ampicillin.

TABLE 6

Intragenic recombination of *nadC* merodiploids

	nadC436	*nadC437*	*nadC444*	*nadC445*	*nadC447*	*nadC493*	*nadC494*	*nadC495*
nadC436	−	++	++	++	++	+	+	+
nadC437	++	−	+	+	+	++	++	++
nadC444	++	+	−	+	+	+	++	++
nadC445	++	+	+	−	+	++	++	++
nadC447	++	+	+	+	−	++	++	++
nadC493	+	++	++	++	++	−	+[a]	+
nadC494	+	++	++	++	++	+[a]	−	+
nadC495	+	++	++	++	++	+	+	−

Merodiploids were plated on minimal medium lacking an exogenous pyridine source in the absence of ampicillin selection. A + symbol represents between 100 and 800 colonies per 10^9 cells plated; a ++ symbol represents >2,000 colonies per 10^9 cells plated.

[a] These recombination tests were performed at 42°.

Another advantage of obtaining MudA insertions in *nadC* and *aroP* was that they could be used to determine transcriptional orientation of these operons in simple transduction experiments. Both *nadC* and *aroP* are transcribed counterclockwise on the *S. typhimurium* chromosome in opposite orientation to DNA replication. It has been observed that most operons are transcribed in the same direction as chromosomal replication, possibly to avoid collisions between active DNA and RNA polymerases (BREWER 1988, 1990). The *nadC* gene is not highly transcribed (HOLLEY and FOSTER 1982) nor probably is *aroP*; so their transcriptional orientation is not likely to have a significant effect on chromosomal replication.

The distribution of the different classes of *nadC* mutants isolated is somewhat skewed on the deletion map. Of five cold-sensitive (CS) and two temperature-sensitive (TS) alleles, one TS allele mapped to the last deletion interval while the other TS and 5 CS alleles mapped to the first two deletion intervals. Since we did not actively seek conditional alleles, it is not clear if this result has significance. These results may reflect the multimeric structure of the functional enzyme. Of the suppressible alleles, 22 amber mutants mapped to 7 deletion intervals, 13 ochre mutants mapped to 4 deletion intervals, 11 *sumA*-suppressed alleles mapped to 5 deletion intervals while all 10 UGA alleles mapped to a single deletion interval, interval 14. This

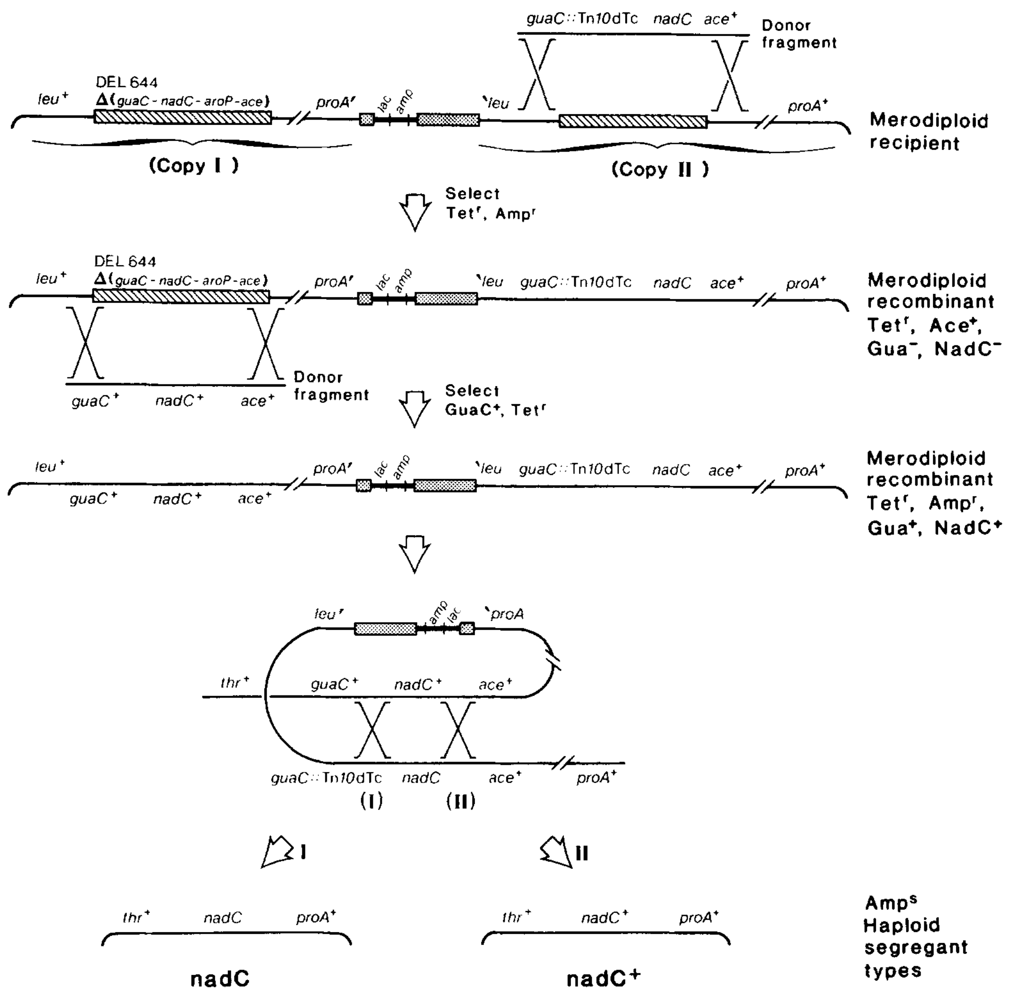

FIGURE 6.—Merodiploid construction for *nadC* complementation and dominance studies. A strain duplicated for a *nadC* deletion mutant was constructed (see MATERIALS AND METHODS). One *nadC* allele was introduced via P22 transduction by selecting for a closely linked Tn*10*dTc insertion in *guaC*. The second *nadC* allele was also introduced via P22 transduction selecting for *guaC*$^+$ transductants which retained the *guaC*::Tn*10*dTc insertion in the other duplicated *nadC* region. The duplication was maintained by selecting for ampicillin resistance of the MudA insertion at the join point of the duplication. The duplications were allowed to segregate and the two parental haploid allele phenotypes were identified to ensure that *nadC* merodiploids had been obtained.

result is unlikely to be due to a single hot spot for hydroxylamine mutagenesis which results in a particular UGA mutant because some of the UGA alleles were suppressed by both UGA suppressors tested while the others were suppressed by only one of the two UGA suppressors tested. Curiously, this deletion interval had more point mutants than any other interval. Of the 16 *nadC* mutant alleles mapping to deletion interval 14 all were nonsense alleles; 10 were UGA, 5 were amber and 1 was ochre.

The most unexpected result was obtained in the complementation experiments. Eight point mutants were chosen for these studies. Four of the alleles were chosen from the carboxy-terminal portion of *nadC* and four alleles were chosen from the amino-terminal portion of *nadC*. Two alleles, *nadC493* and *nadC494*, both from the carboxy-terminal end were found to complement at 37°, but only poorly at 42°. The following observations support intragenic complementation: (1) *nadC436* which is from the same deletion interval as *nadC493* would not complement *nadC494*; (2) *nadC495* which is from the same deletion interval as *nadC494* would not complement *nadC493*; (3) complementation of *nadC493* and *nadC494* is not due to additive leakiness since neither would complement *nadC437* which is a TS allele used in the complementation experiments nor would cells become NadC$^+$ when diploid for the individual alleles; and (4) complementation is poor at 42°, suggesting thermal instability of the active which one would not expect from intergenic complementation.

The construction of the *nadC* deletion map and determination of transcription orientation provides the groundwork for the comprehensive characterization of quinolinic acid phosphoribosyl transferase. Of interest is the differences between the *nadC* enzyme, QAPRTase, and the *pncB* enzyme, NAPRTase. Both enzymes are specific for their given substrates, yet, quinolinate and nicotinate are similar substrates. Mutants have been isolated which alter the substrate specificity of the *nadC* enzyme, allowing it to act on nicotinic acid (K. T. HUGHES, unpublished results).

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