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Mapping of the *nadB* Locus Adjacent to a Previously Undescribed Purine Locus in *Escherichia coli* K-12

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It is proposed that all mutants blocked in the de novo pathway of nicotinamide adenine dinucleotide biosynthesis be designated *nad* rather than *nic*. It is further suggested that mutants blocked in the pyridine nucleotide cycle be designated *pnc*. The *nadB* locus and a previously unidentified *pur* locus are cotransducible. These two loci have been mapped near minute 49 on the standard genetic map of *Escherichia coli*. The order of genes in that region is *purC-guaB-purG-glyA-pur-nadB-tyrA-pheA*.

In *Escherichia coli* nicotinamide adenine dinucleotide (NAD) is synthesized from nicotinic acid (12) by the Preiss-Handler pathway (18) previously elucidated in higher organisms. Nicotinic acid is formed from nicotinamide by deamidation (10). Sundaram (22) has demonstrated that *E. coli* can form NAD from nicotinamide only via nicotinic acid. However, nicotinic acid is not on the de novo pathway of NAD biosynthesis, which proceeds via quinolinate in *E. coli* (1; Fig. 1) as in higher organisms (7). In *E. coli*, quinolinate is synthesized from aspartate and a three-carbon precursor (17), whereas in *Neurospora* and mammals it is formed from tryptophan (7). The de novo pathway of NAD biosynthesis in *E. coli* is shown with solid arrows in Fig. 1. The reactions shown with dashed arrows constitute a salvage pathway for the reutilization of degraded NAD (7).

Mutants of *E. coli* blocked in the synthesis of NAD have been designated previously as *nicA* (24), *nicB* (24), and *nicC* (8), since nicotinic acid supports the growth of these mutants. However, only those mutants blocked in NAD biosynthesis before nicotinate mononucleotide can use nicotinic acid for growth. We therefore propose that mutants blocked in any step in the de novo pathway (solid arrows) be designated as *nad* to reflect more accurately the biochemistry of this pathway. We further propose that mutants blocked in any step of the salvage pathway (dashed arrows) be designated *pnc* for the pyridine nucleotide cycle (7).

The *nadB* locus, which controls the catalysis of a step leading to quinolinic acid (Fig. 1), is positioned on the Taylor and Trotter genetic map of *E. coli* (8) between *supN* and *ctr* at about 45 min. Entry time determinations carried out in this laboratory yielded results consistent with this general map position. However, Wang, Morse, and Morse (25) concluded that the *nadB* gene was mismapped when it could not be cotransduced with *ctr*.

The present study reports the mapping of the *nadB* locus between *glyA* and *tyrA*, adjacent to a previously undescribed locus involved in purine biosynthesis.

MATERIALS AND METHODS

Bacterial strains. The properties of the *E. coli* K-12 derivatives used in this study are described in Table 1.

Media. The minimal medium utilized was that of Davis and Mingioli (5), supplemented with 0.005 g of thiamine per liter. Minimal agar contained 15 g of agar per liter. Where required, the minimal medium was supplemented with 0.02 mg of the appropriate amino acid(s) per ml, 0.005 mg of nicotinic acid per ml, and 0.2 mg of guanine or adenine, or both, per ml.

L-broth and L-broth agar (14) and Penassay Broth (Difco Antibiotic Medium no. 3) were used as complex media. Eluting fluid contained 8 g of sodium chloride and 3 g of Difco Peptone per liter of water.

Transduction procedure. A temperate strain of bacteriophage P1 was used in all transductions. All lysates were recycled on the same host at least once before use.

Donor phage stocks were produced in soft L-broth agar seeded with the donor bacterium and about 10^6 P1 phage particles, and overlayed on hard L-broth agar plates. After 6 hr of incubation at 37 C, the phage was eluted from the soft agar. Debris was removed by centrifugation, and the suspension was stored over chloroform at 5 C.

In a typical transduction experiment, a stationary

phase culture of the recipient bacterium in Penassay Broth was diluted 1/50 into L-broth and incubated at 37 C for 3 hr on a New Brunswick model VS rotary shaker. Approximately 30 min before the recipient bacterium was ready to use, 0.005 ml of 0.5 M $CaCl_2$ was added to a sterile test tube along with 0.3 ml of the donor phage suspension. The mixture was incubated in a water bath at 37 C. The recipient culture (0.7 ml) was then added to the preincubated phage suspension, and the mixture was incubated at 37 C for another 30 min, after which the cells were plated at 10^{-1} and 10^{-2} dilutions onto hard minimal medium supplemented with appropriate growth factors when necessary. These plates were incubated at 37 C for 48 hr, and then the transductant colonies were replicated to the proper hard minimal medium to check for the presence of unselected markers.

All genetic nomenclature is according to Demerec et al. (6).

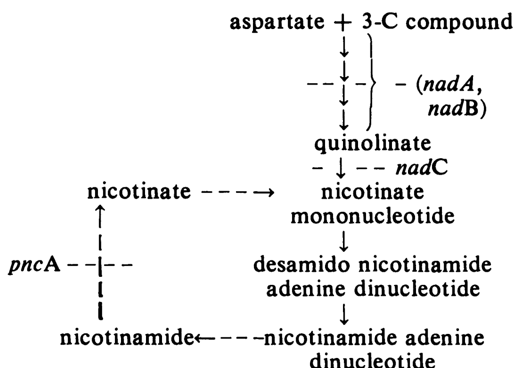

FIG. 1. *Pyridine nucleotide cycle in E. coli and the location of known mutations.*

RESULTS

Preliminary mating experiments yielded entry times of 21 min for *nadB* and 31 min for *his*. Thus *nadB* was positioned 10 min from *his* at approximately minute 49 on the Taylor and Trotter map (24).

UTH 4664 contained a purine mutation which was cotransducible with *nadB;* it was thought to be *purF* because of the published proximity of the two genes. Thus a transduction was performed in which UTH 4035, which contained a known *purF* mutation, was used as a donor and UTH 4664 (*nadB*, *pur*) as the recipient. Transductants were selected on minimal agar supplemented with adenine and arginine; it was found

TABLE 1. *Properties of Escherichia coli K-12 derivatives*

Stock no.[a]	Mutations present	Source
UTH 620	*bio, pheA*	H. P. Treffers
UTH 4067	*his, tyrA, trp, purC, guaA*	P. G. DeHaan (H-725)
UTH 4069	*his, tyrA, trp, purC, guaB, thi*	P. G. DeHaan (H-724)
UTH 4083	*F′his⁺/nadB42, his, ileu*	T. S. Matney
UTH 4105	*his, trp, purG, thi*	P. G. DeHaan (H-888)
UTH 4133	*glyA, thi*	A. L. Taylor (AT-2457)
UTH 4144	*nadB11*	E. A. Adelberg
UTH 4146	*nadB20, his, thr, leu*	F. Jacob
UTH 4148	*nadB36*	T. S. Matney
UTH 4451	*nadB35, met*	R. Lavalle (P_4X)
UTH 4462	*nadB29*	ATCC 9723b
UTH 4464	*nadB30*	Sundaram (W-3899)
UTH 4469	*nadB33, met*	J. Imasande (E-4)
UTH 4471	*nadB6*	J. Imasande (E-36)
UTH 4617	*nadB40*	T. S. Matney
UTH 4660	*nadB1*	J. Imasande (E-2)
UTH 4661	*nadB2, met, pur*	J. Imasande (E-3)
UTH 4662	*nadB3*	E. A. Adelberg
UTH 4663	*nadB4*	J. Imasande (E-25)
UTH 4664	*nadB5, pur, argH*	A. L. Taylor (PA 3306)
UTH 4668	*nadB7, met*	E. A. Adelberg
UTH 4669	*nadB8*	E. A. Adelberg
UTH 4671	*nadB10*	E. A. Adelberg
UTH 4673	*nadC13*	J. Imasande (E-126)
UTH 4674	*nadB15, met*	R. Lavalle (P_4X SB16)
UTH 4675	*nadB16, met*	R. Lavalle (P_4X SB41)
UTH 4676	*nadB17, met*	R. Lavalle (P_4X SB84)
UTH 4677	*nadB21, thr, leu*	A. L. Taylor (AT 1124)
UTH 4680	*nadB24, arg, trp*	R. Lavalle (PA2-35-5)

[a] The University of Texas/Houston Stock Collection.

that 62% of these no longer required adenine for growth. Thus, the *pur* marker in strain UTH 4664 was shown to be closely linked to *nadB* and not to be a *purF* mutation.

In addition to *purF*, there are two other purine genes, *purC* and *purG*, which map within possible cotransduction range of *nadB*. Transductions were carried out between a *purC* mutant (UTH 4069) and the recipient UTH 4664 and between a *purG* mutant (UTH 4105) and UTH 4664 in which the number of Arg^+ recombinants and the number of Pur^+ recombinants were scored separately (Table 2). The ratio of Pur^+/Arg^+ in each transduction was nearly identical. Thus, it was concluded that the purine mutation in UTH 4664 was neither a *purC* nor a *purG* mutation.

The new *pur* mutation was found to be cotransduced with a number of genes in the 48- to 50-min portion of the chromosomal map (Table 3, part A). On the basis of these data and the known proximity of *tyrA* and *pheA* (24), the *pur* mutation in UTH 4664 is shown to lie between *glyA* and *tyrA*, and much closer to *glyA*.

TABLE 2. *Transductional crosses between pur mutants*

Donor	Recipient	Selected marker	No. of recombinants	Ratio of recombinants (*pur/arg*)
UTH 4069	UTH 4664	*pur*+ *arg*+	330 585	.564
UTH 4105	UTH 4664	*pur*+ *arg*+	292 505	.578

Since it was known that *nadB* is closely linked to the purine mutation in UTH 4664, we attempted to cotransduce *nadB* with various markers in that portion of the chromosomal map. The *nadB* gene is not only cotransducible with the newly mapped purine locus, but also with *guaB*, *tyrA*, *pheA*, and *glyA* (Table 3, part B). The order of the known genes in this area was verified by further cotransductional experiments (Table 3, part C). When all of these data are taken into consideration, the apparent gene order is (*guaB-purG*)-*glyA-pur-nadB-tyrA-pheA* (Fig. 2). This gene order for *guaB* and *purG* is consistent with the order previously established in *E. coli* by Stouthamer et al. (21) and is apparently reversed in *Salmonella* (19). Further evidence that the new purine locus in UTH 4664 is not *purG* is provided by the large difference in linkage values between *glyA-purG* and *glyA-pur*. There appears to be 0.9-min distance between these two purine genes. Also, *purG* is not cotransducible with *pheA* at a detectable level, whereas the mutation in UTH 4664 is cotransduced at a 3% frequency.

The position of the *nadB* locus relative to *glyA*, *pur*, and *tyrA* was confirmed by three-factor analyses. The possible combinations of the unselected markers in these analyses are listed in Table 4. The lowest frequency in the first cross is

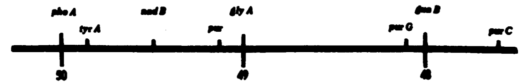

FIG. 2. *Portion of the linkage map of E. coli taken from Taylor and Trotter (24).*

TABLE 3. *Linkage between various genes in the 48 to 50-min region of the Escherichia coli chromosomal map*

Part	Donor	Recipient	Selected marker	Unselected marker	Cotransduction frequency	Distance between markers (min)[a]
A	UTH 4067	UTH 4664	*pur*+	*tyrA*	.081 (13/160)	1.14
	UTH 620	UTH 4664	*pur*+	*pheA*	.031 (5/160)	1.38
	UTH 4664	UTH 4133	*glyA*+	*pur*	.717 (114/159)	0.20
B	UTH 4673	UTH 4664	*nadB*+	*pur*+	.565 (174/308)	0.35
	UTH 4069	UTH 4664	*nadB*+	*guaB*	.027 (8/300)	1.40
	UTH 4069	UTH 4664	*nadB*+	*tyrA*	.201 (23/114)	0.83
	UTH 620	UTH 4664	*nadB*+	*pheA*	.165 (53/322)	0.90
	UTH 4664	UTH 4133	*glyA*+	*nadB*	.170 (27/159)	0.90
C	UTH 4105	UTH 4133	*glyA*+	*purG*	.088 (15/171)	1.11
	UTH 620	UTH 4105	*purG*+	*pheA*	.000 (0.142)	>2.00
	UTH 620	UTH 4069	*guaB*+	*pheA*	.000 (0.494)	>2.00
	UTH 4069	UTH 4133	*glyA*+	*guaB*	.074 (11/149)	1.16
	UTH 4069	UTH 4133	*glyA*+	*tyrA*	.033 (5/149)	1.36

[a] Calculated from the equation: cotransduction frequency $= (1 - x/2)^3$ where x is the distance between the selected and unselected genes (26).

TABLE 4. *Three-factor analyses of the genes located in the nadB region of the chromosomal map of Escherichia coli*

Transduction	Genotype			Total no. of each genotype
	Selected marker	Unselected markers		
UTH 4067 × UTH 4664	pur^+	$tyrA^+$	$nadB^+$	74
	pur^+	*tyrA*	$nadB^+$	15
	pur^+	$tyrA^+$	*nadB*	313
	pur^+	*tyrA*	*nadB*	0
UTH 4133 × UTH 4664	$nadB^+$	$glyA^+$	pur^+	101
	$nadB^+$	$glyA^+$	*pur*	144
	$nadB^+$	*glyA*	pur^+	76
	$nadB^+$	*glyA*	*pur*	1

observed in the transductant having the genotype pur^+-*tyrA-nadB*. Since the donor carried pur^+ *tyrA*, and the recipient carried *nadB*, it demonstrates that the *nadB* locus lies between *pur* and *tyrA*. In the second cross, the donor carried *glyA* and $nadB^+$ and the recipient carried *pur*. In this one case the recombinant genotype $nadB^+$-*glyA-pur* is the lowest frequency indicating that *pur* must be between *glyA* and *nadB*. These results confirm the gene order depicted in Fig. 2.

Thirty-five *nad* mutants in our stock collection were surveyed for linkage to *tyrA;* 22 of these exhibited linkage to *tyrA*, i.e., they were *nadB* mutants. Of the remaining *nad* mutants, seven exhibited linkage to *gal* (*nadA*) and six were linked to *leu* (*nadC*). No *nad* mutant tested gave linkage to the genes in the 45-min area of the chromosomal map of *E. coli*, including *glpT*, *ctr*, *dsdA*, *purF*, and *aroC*.

DISCUSSION

Of the 12 enzymes involved in purine biosynthesis, mutations affecting eight of these enzymes have been mapped in *E. coli* (2, 4, 13, 16, 20, 21, 23). The mutant purine locus in strain UTH 4664 appears to involve a ninth gene. Two additional genes, *purI* and *purJ*, have been mapped in *Salmonella* (19, 9). The *purI* locus maps very near *guaB* and may be analogous to the purine locus which we have mapped. If this is the case, then it should be designated *purI*. However, if this purine locus is one which has not previously been described in either *E. coli* or *Salmonella*, the proper designation is *purK*. Therefore, we have refrained from attaching a letter designation to this new purine locus until the biochemical nature of the block in purine biosynthesis has been determined. This investigation is presently in progress.

The length of the *E. coli* chromosome is the equivalent of 90 min of transfer time (24). Since the transducing fragment has a weight of 6×10^7 daltons (11) and the *E. coli* chromosome has a weight of 2.8×10^9 daltons (3), the length of the transducing fragment can be calculated to be slightly less than 2 min of transfer time. Our data place *nadB* approximately 4 min from the published position and 3.5 min from *ctr*. This is outside of the P1-cotransducing range and explains the lack of cotransduction, reported by Wang and co-workers (25), between *ctr* and *nadB*.

The distances between the various genes in the 48- to 50-min segment of the chromosomal map which were estimated from the theoretical relationship between cotransduction frequency and map distance do not, in all cases, correspond to those published (24), although we do confirm the order of the genes. There may be a number of reasons for this. The equation is only a means of estimating these distances and may not hold at the extreme limits of cotransduction frequencies. There also appears to be an inherent variability in transductional experiments. This has been amply discussed by McFall (15). She points out the lack of reciprocity in transductional experiments and the possible influence of a deletion on the transductional frequency.

The cotransductional frequencies between the 22 *nadB* mutants and *tyrA* varied between 0.106 and 0.369 (Table 5). This variation could be due to the inherent variability in transductional experiments or to sampling error. On the other hand, there may be more than one gene at or near the *nadB* locus, and the observed differences in cotransduction frequencies may be real. Since 60% of the *nad* mutations studied are located at the *nadB* locus and the remaining 40% are distributed about equally between the *nadA* and the *nadC* loci, this is a distinct possibility. Complementation analysis of the *nadB* locus with stable F′ merodiploids is expected to help clarify this question.

The *nadA* locus has been carefully mapped by

TABLE 5. *Linkage of tyrA (unselected marker) with nadB (selected marker) in various mutants of Escherichia coli with UTH 4067 as donor*

Donor	Recipient	Cotransduction frequency	Distance between markers (min)[a]
UTH 4067	UTH 4660	.232 (37/160)	0.78
UTH 4067	UTH 4661	.194 (31/160)	0.84
UTH 4067	UTH 4662	.294 (47/160)	0.67
UTH 4067	UTH 4663	.225 (36/100)	0.79
UTH 4067	UTH 4471	.319 (51/160)	0.63
UTH 4067	UTH 4668	.131 (21/160)	0.98
UTH 4067	UTH 4669	.288 (46/160)	0.68
UTH 4067	UTH 4671	.331 (53/160)	0.62
UTH 4067	UTH 4144	.263 (42/160)	0.71
UTH 4067	UTH 4674	.125 (20/160)	1.00
UTH 4067	UTH 4675	.150 (24/160)	0.94
UTH 4067	UTH 4676	.256 (41/160)	0.73
UTH 4067	UTH 4146	.344 (55/160)	0.60
UTH 4067	UTH 4677	.288 (46/160)	0.68
UTH 4067	UTH 4680	.369 (59/160)	0.56
UTH 4067	UTH 4462	.300 (48/160)	0.66
UTH 4067	UTH 4464	.169 (27/160)	0.90
UTH 4067	UTH 4469	.213 (34/160)	0.81
UTH 4067	UTH 4451	.106 (17/160)	1.06
UTH 4067	UTH 4148	.344 (55/160)	0.60
UTH 4067	UTH 4617	.219 (35/160)	0.80
UTH 4067	UTH 4083	.219 (35/160)	0.80

[a] Calculated according to Wu (26).

Taylor and Trotter (24) adjacent to *gal* and we have confirmed this map location (*unpublished data*). Both *nadA* and *nadB* control biosynthetic steps before formation of quinolinate, since mutants possessing mutations at these loci can use quinolinate in place of nicotinic acid for growth and have a functional quinolinate phosphoribosyl transferase (1). The *nadC* locus controls the synthesis of this enzyme. Genetic and biochemical studies involving *nadC* mutants will be described in a subsequent paper.

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