Genetic Characterization of Pyridine Nucleotide Uptake Mutants of *Salmonella typhimurium*

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Two classes of pyridine nucleotide uptake mutants isolated previously in a strain of *Salmonella typhimurium* defective in both *de novo* NAD biosynthesis (*nad*) and pyridine nucleotide recycling (*pncA*) were analysed in terms of their genetic relationship to each other and their roles in the transport of nicotinamide mononucleotide as a precursor to NAD. The first class of uptake mutants, *pnuA* (99 units), failed to grow on nicotinamide mononucleotide (NMN) as a precursor for NAD. The second class, *pnuB*, grew on lower than normal levels of NMN and suppressed *pnuA* mutations. A third class of uptake mutant, *pnuC*, isolated in a *nadB pncA pnuB* background, also failed to grow on NMN. Transport studies and enzyme analyses confirmed these strains as defective in NMN uptake. A fourth locus, designated *pnuD*, was found to diminish NMN utilization in a *nad pncA*⁺ background. Tn10 insertions near *pnuA*, *pnuC* and *pnuD* were isolated and utilized in mapping studies. *pnuA* was found to map between *thr* and *serB* near *trpR*. The *pnuC* locus was cotransducible with *nadA* at 17 units while *pnuD* mapped at approximately 60 units. The biochemical and genetic data suggest that the *pnuA* and *pnuC* gene products cooperate in the utilization of NMN under normal conditions. A *pnuB* mutant, however, does not require the *pnuA* gene product for NMN uptake but does rely on the *pnuC* product. Fusion studies indicate that *pnuC* is regulated by internal NAD concentrations.

INTRODUCTION

The maintenance of a proper oxidation–reduction balance is of the utmost importance to living cells. NAD and NADP are two major compounds involved with cellular oxidation–reduction reactions. Consequently, the synthesis, recycling and regulation of these coenzymes is of great interest.

NAD may be recycled in *Salmonella typhimurium* via two pathways, known as pyridine nucleotide cycles (PNC) (Foster & Moat, 1980). PNC IV recycles NAD through a series of four reactions while PNC VI requires six enzyme steps to accomplish this task (Fig. 1). All available evidence indicates that PNC IV is used primarily for the intracellular recycling of NAD while the major role of PNC VI is in the utilization of preformed pyridine derivatives present in the extracellular environment (Foster & Baskowsky-Foster, 1980).

Cells with an intact PNC VI can efficiently utilize exogenously supplied nicotinamide mononucleotide (NMN), nicotinamide (NA_m) and nicotinic acid (NA) at 10^{−5} M. However, a cell containing a mutation in *pncA*, which eliminates all NA_m deamidase activity, cannot utilize NA_m and requires an NMN concentration of 10^{−4} M or more. NMN can apparently traverse the membrane intact via a system dependent upon the *pnuA* locus, which maps at 99 units (Foster *et al.*, 1979*a, b*; Liu *et al.*, 1982). The efficiency of *pnuA*-dependent NMN transport

Abbreviations: PNC, pyridine nucleotide cycles; NMN, nicotinamide mononucleotide; NA_m, nicotinamide; NA, nicotinic acid; NA_mR, nicotinamide ribonucleoside.

Table 1. *Strains of Salmonella typhimurium LT-2 used*

Strain	Genotype	Source or reference*
SF24	<i>serB54</i>	K. Sanderson
SF27 (TT459)	<i>pyrC691::Tn10</i>	J. Roth
SF67	<i>nadA540::Tn10</i>	J. Roth
SF71 (TT6734)	<i>nadA56 pncA15 zjj-1004::Tn10 trpA49</i> (Tn10 near <i>pnuA</i> *)	J. Roth
SF77	<i>nadB51 pncA15 pnuB1 trpA49</i>	Liu <i>et al.</i> (1982)
SF78 (TR5654)	<i>thrA9 rpsL</i>	J. Roth
SF81 (TR5657)	<i>purE rpsL</i>	J. Roth
SF82 (TR5658)	<i>pyrC rpsL</i>	J. Roth
SF83 (TR5659)	<i>purB rpsL</i>	J. Roth
SF84 (TR5660)	<i>pyrF rpsL</i>	J. Roth
SF102 (SGSC183)	<i>trpR520 thrC59</i>	K. Sanderson
SF103 (SGSC194)	<i>trpR520 serB80</i>	K. Sanderson
SF110	<i>pyrF146 pde-1 aphA nap gal</i>	Uerkvitz & Beck (1981)
JF22	<i>nadB31 purC7 proA46 ilv-405 rha-461 rpsL</i>	
JF63	<i>nadB51 pncA15 trpA49</i>	Foster <i>et al.</i> (1979a)
JF66	<i>nadA56 pncA15 trpA49</i>	Foster <i>et al.</i> (1979a)
JF76	<i>nadA56 pncA15 pnuA11 trpA49</i>	Kinney <i>et al.</i> (1979)
JF85	<i>nadB31 pnuD2 purC7 proA46 ilv-405 rha-461 rpsL</i>	
JF181	<i>nadB31 purC7 proA46 ilv-405 zga-21::Tn10</i>	
JF184	<i>nadA56 pncA15 pnuA11 trpA49 zjj-1004::Tn10</i>	
JF204	<i>nadB51 zcf-1::Tn10</i> (Tn10 near <i>pncA</i> *) <i>trpA49</i>	
JF211	<i>nadA::Tn10 galE hutR49 ΔpncX503</i>	
JF339	<i>galE ΔnadA ΔpncA hutR49</i>	
JF349	<i>nadB51 pncA15 pnuB1 pnuC1 trpA49</i>	
JF350	<i>nadB51 pncA15 pnuB1 pnuC2 trpA49</i>	
JF436	<i>galE pyrC rpsL</i>	
JF439	<i>nadB51 nadA::Tn10 pnuB1 pnuC1 trpA49</i>	
JF454	<i>nadB51 pncA15 pnuB1 pnuC3 trpA49</i>	
JF455	<i>nadB51 pncA15 pnuB1 pnuC4 trpA49</i>	
JF456	<i>nadB51 pncA15 pnuB1 pnuC5 trpA49</i>	
JF457	<i>nadB51 pncA15 pnuB1 pnuC6 trpA49</i>	
JF536	<i>trpA49 nadA56 pncA15 ΔpnuA1</i>	
JF572	<i>trpA49 nadB51 pncA15 thrA9 zjj-1004::Tn10</i>	
JF573	<i>trpA49 nadB51 pncA15 pnuB1 thrA9 zjj-1004::Tn10</i>	
JF666	<i>nadB51 pncA15 pnuB1 pyrC691::Tn10</i>	
JF680	<i>ΔnadB pncA::Tn10 pyrF146 pde-1 aphA nap gal</i>	
JF681	<i>nadB51 pncA15 trpA49 pyrC691::Tn10</i>	
JF682	<i>nadA56 pncA15 trpA49 pnuA11 pyrC691::Tn10</i>	
JF731	<i>pnuC8::Mud1-8 nadB541::Tn10 supD10</i>	
JF756	<i>nadB51 pncA15 trpA49 pnuB1 pnuC2 rpsL</i>	
JF806	<i>ΔnadB nadA540::Tn10 pnuC8::Mud1-8</i>	
JF808	<i>thrA9 pnuC8::Mud1-8 rpsL</i>	
JF858	<i>nadB51 pncA15 trpA49 pnuB1 pnuC8::Mud1-8</i>	
JF869	<i>pnuC8::Mud1-8 nadR1 pyrD95 zff-28::Tn10</i>	
JF870	<i>nadB51 pncA15 trpA49 pnuC8::Mud1-8</i>	
JF871	<i>ΔnadB pnuC8::Mud1-8 cya::Tn10</i>	
JF873	<i>nadA56 pncA15 pnuA11 trpA49 pnuC8::Mud1-8</i>	
MU27	<i>ΔnadB pncA::Tn10 serA13 rfa-3058</i> (Hfr 117' cw)	
MU28	<i>ΔnadB pncA::Tn10 serA13 pur-268</i> (Hfr 123' ccw)	
MU30	<i>zga-21::Tn10 serA13 pur-268</i> (Tn10 near <i>pnuD</i> *) (Hfr 123' ccw)	

* Strains for which no source is given were derived in the work described in this paper. K. Sanderson, Department of Biology, University of Calgary, Calgary, Alberta, Canada T2N 1N4; J. Roth, Department of Biology, University of Utah, Salt Lake City, Utah 84112, USA.

Extracts were prepared and NMN deamidase or NMN glycohydrolase activities determined as described earlier (Foster, 1981). Specific activities are expressed as nmol h⁻¹ (mg protein)⁻¹. Protein was determined by the Lowry method.

The various *pnu* mutants described in this paper were tested for their ability to transport [¹⁴C]NMN and [¹⁴C]NA as described earlier (Kinney *et al.*, 1979).

RESULTS AND DISCUSSION

pnuB suppresses the expression of pnuA mutations

To determine whether the PnuB⁻ phenotype, i.e. growth of a *nadA pncA pnuB* mutant on 10⁻⁵ M-NMN, could be partially or totally reversed by the introduction of *pnuA*, a strain containing a Tn10 insertion located near *pnuA* was constructed. HT transducing phage propagated on strain SF71 (Tn10 near *pnuA*⁺) was used to transduce strain JF76 (*nadA pncA pnuA*) to tetracycline resistance (Tc^R). The Tc^R colonies were scored on minimal medium supplemented with NA or 10⁻⁴ M-NMN. Clones that grew on NA but not on NMN should possess the genotype *nadA pncA pnuA zjj-1004::Tn10* and thus contain Tn10 near *pnuA*. One such strain (JF184) was cured of HT phage and used to prepare another HT lysate. This lysate was crossed with the *pnuB* mutant (SF77) and its parent (JF63), selecting for Tc^R. The Tc^R transductants were then scored for the PnuA and PnuB phenotypes. The results (Table 2) indicate that when *pnuA* was transduced into a *nadB pncA* strain (JF63) the PnuA⁻ phenotype was observed. The PnuA⁻ phenotype, however, was not observed in the *nadB pncA pnuB* background. Demonstration that *pnuA* was successfully transferred to the *pnuB* strain (SF77) was accomplished by growing phage on 10 of the Tc^R transductants and subsequently transferring the cryptic *pnuA* locus to strain JF63. Six of these lysates yielded Tc^R *pnuA* progeny. Thus, the *pnuB* mutation suppresses *pnuA* mutations. While *pnuB* seems to map near *pnuA*, *pnuA* and *pnuB* are nevertheless separate loci.

The *pnuB* suppression of the PnuA⁻ phenotype was not the result of an unusual subunit interaction between the *pnuA* and *pnuB* gene products. This was shown by constructing a Tn10-directed *pnuA* deletion and cotransducing this deletion into JF572 and JF573, *thrA* derivatives of JF63 (*nadB pncA*) and SF77 (*nadB pncA pnuB*) respectively. Transductants which were *thrA*⁺ were scored for their ability to utilize NMN. As expected, the *pnuA* deletion phenotype was observed in the JF572 cotransductants (NMN⁻) but not in the JF573 cotransductants (NMN⁺). Three-point crosses were performed to determine more accurately the position of *zjj-1004::Tn10* and thus *pnuA* and *pnuB*. The results (Fig. 2) show that the Tn10 insertion mapped between the *serB* and *trpR* loci.

Isolation of pnuC mutants

To define the relationship between *pnuA* and *pnuB* more precisely an attempt was made to isolate *pnuB* reversion mutants or mutants altered in a potential regulatory locus affecting *pnuA* and/or *pnuB*. SF77 was mutagenized and enriched using penicillin selection to select for mutants which would not grow on 10⁻⁴ M-NMN but which could still utilize NA. From approximately 2700 colonies, six were found which were unable to grow on 10⁻⁴ M-NMN. Two of these were picked for further study. To show that the defect did not alter NAD metabolism at a point following NMN transport, NMN deamidase activity was measured. NMN deamidase activity was normal in both *pnuC* strains [~ 60 nmol h⁻¹ (mg protein)⁻¹] indicating they were not NMN deamidase mutants but were probably defective in NMN transport (we show below that they were not defective in NMN glycohydrolase). Accordingly, we designated these strains as pyridine nucleotide uptake mutants, *pnuC*. The biochemical reactions subsequent to NMN deamidase in PNC IV were considered normal since a defect in any one of them would preclude growth on NA as well as on NMN.

Table 2. *Transduction of pnuA and pnuB with zjj-1004::Tn10*

Donor	Recipient	Selected marker	Unselected marker	Number scored	Percentage cotransduction
SF71	JF76 (<i>nadA pncA pnuA</i>)	Tc ^R	<i>pnuA</i> ⁺	99	71
JF184	JF63 (<i>nadB pncA</i>)	Tc ^R	<i>pnuA</i>	155	67
JF184	SF77 (<i>nadB pncA pnuB</i>)	Tc ^R	<i>pnuA</i>	111	<1
SF71	SF77 (<i>nadB pncA pnuB</i>)	Tc ^R	<i>pnuB</i> ⁺	169	11.5

Fig. 2. Location of *pnuA* in the *serB thrA* region of the *S. typhimurium* linkage map. The numbers below the lower bars are the cotransduction frequencies.

Table 3. Transport of [^{14}C]NMN and [^{14}C]NA by *pnu* mutants

Cells ($2 \times 10^8 \text{ ml}^{-1}$) were suspended in a minimal medium supplemented with $0.1 \mu\text{Ci}$ [^{14}C]NA or [^{14}C]NMN ml^{-1} ($10 \mu\text{Ci} \mu\text{mol}^{-1}$; $37 \text{ kBq} \mu\text{mol}^{-1}$). Samples (2.0 ml) were taken at 60 min, washed twice rapidly with 5.0 ml cold E buffer using a Millipore syringe apparatus and the filter suspended and counted directly in 10 ml scintillation fluid.

Strain	Pertinent genotype	nmol bound per sample	
		[^{14}C]NMN	[^{14}C]NA
JF66	<i>pncA pnuA⁺ pnuB⁺ pnuC⁺</i>	0.45	0.83
JF76	<i>pncA pnuA11 pnuB⁺ pnuC⁺</i>	0.05	0.94
SF77	<i>pncA pnuA⁺ pnuB1 pnuC⁺</i>	0.79	0.75
JF349	<i>pncA pnuA⁺ pnuB1 pnuC1</i>	0.04	0.71
JF350	<i>pncA pnuA⁺ pnuB1 pnuC2</i>	0.07	0.86
JF454	<i>pncA pnuA⁺ pnuB1 pnuC3</i>	0.04	0.81
JF455	<i>pncA pnuA⁺ pnuB1 pnuC4</i>	0.04	0.99
JF456	<i>pncA pnuA⁺ pnuB1 pnuC5</i>	0.06	0.72
JF457	<i>pncA pnuA⁺ pnuB1 pnuC6</i>	0.05	1.01
JF870	<i>pncA pnuA⁺ pnuB⁺ pnuC8</i>	0.05	0.91

pnuC mutants are defective in the transport of NMN

Table 3 presents the results of several transport experiments. The *pnuA* mutant (JF76) was defective in NMN transport while the *pnuB* mutant (SF77) was able to transport NMN more readily than its parent, as expected. *pnuC* mutations prevent the transport of NMN in a *pnuB* background (e.g. JF349) as well as in a *pnuB⁺* background (JF870). A model suggested by these results is that the *pnuB* mutation results in a membrane alteration which facilitates NMN uptake as suggested previously (Liu *et al.*, 1982) but which is independent of the *pnuA⁺* system. The *pnuC* gene product, however, must interact with the *pnuA* gene product under normal conditions to permit NMN utilization. The *pnuB* mutation would result in a gene product that, while not requiring the *pnuA* gene product, still depends upon the presence of the *pnuC* gene product for NMN uptake.

pnuC is distinct from *pnuA* and *pnuB*

Since two other *pnu* loci have been identified (*pnuA* and *pnuB*) it was necessary to determine if *pnuC* was linked to these loci. The *pnuC* locus was shown to be different from *pnuA* by crossing SF71 (Tn10 near *pnuA⁺*) with JF349, selecting for Tc^R and looking for repair of the *pnuC* mutation. There was no repair of the *pnuC* locus, indicating that *pnuA* and *pnuC* are separate loci (data not shown).

Table 4. *Transduction of *pnuC* with *nadA* and *galE**

Phage P22 was grown on JF211 and this was then crossed with JF349. Initial selection was on LB medium (Miller, 1972) containing tetracycline (selection for Tc^R) or minimal E medium supplemented with 10⁻⁴ M-NMN (selection for *pnuC*⁺).

Selected marker	Unselected marker	Number scored	Percentage cotransduction
Tc ^R (<i>nadA</i>)	<i>pnuC</i> ⁺	174	83.9
<i>pnuC</i> ⁺	<i>galE</i>	174	<0.5
Tc ^R (<i>nadA</i>)	<i>galE</i>	174	6.9

Table 5. *Effect of nicotinic acid concentration on the expression of a *pnuC*-lacZ fusion in various genetic backgrounds*

Strain	Genotype	β -Galactosidase specific activity (Miller units)*		Derepression (fold)
		10 ⁻⁴ M-NA	10 ⁻⁶ M-NA	
JF731	<i>nadB::Tn10 pnuC8::Mud1-8</i>	29.6	604.5	20.4
JF806	Δ <i>nadB nadA::Tn10 pnuC8::Mud1-8</i>	36.4	520.1	14.3
JF808	<i>pnuC8::Mud1-8 nad</i> ⁺	36.9	64.7	1.8
JF858	<i>nadB pncA pnuB pnuC8::Mud1-8</i>	29.0	690.7	23.8
JF870	<i>nadB pncA pnuC8::Mud1-8</i>	23.8	529.2	22.2
JF869	<i>nadR1 pnuC8::Mud1-8</i>	842.1	909.3	1.1
JF873	<i>nadA pncA pnuA pnuC8::Mud1-8</i>	24.0	995.3	41.5

* Miller, 1972. Means of three independent experiments.

The *pnuC* mutation was also shown to be distinct from the *pnuB* locus in that *pnuB* strains repaired for the *pnuC* mutation were once again able to grow on 10⁻⁵ M-NMN. Thus, the *pnuB* phenotype remained even though *pnuC* was repaired. Consequently, *pnuA*, *pnuB* and *pnuC* are all distinct genetic loci which affect the utilization of NMN.

*Repair of *pncA* in a *pnuC* mutant*

To determine if *pnuC* codes for a transport system totally distinct from the proposed PNC VI NMN glycohydrolase-dependent system (Foster *et al.*, 1979a), the *pncA*⁺ allele was introduced into JF349 by transduction. If *pnuC* is distinct from the NMN glycohydrolase system, repair of *pncA* should enable the strain to grow on NMN. Alternatively, if *pnuC* codes for a common transport system then the resultant strain should still be unable to grow on NMN. JF349 was transduced with P22 phage grown on JF204 and 20 transductants showing the *pncA*⁺ phenotype (growth on NAm) were tested for their ability to grow on 10⁻⁴ M-NMN. All 20 showed a renewed ability to grow on 10⁻⁴ M-NMN, indicating that *pnuC* is distinct from NMN glycohydrolase-dependent utilization of NMN.

*Mapping of the *pnuC* locus*

The relative location of the *pnuC* locus on the *S. typhimurium* linkage map was determined by time of entry experiments. Hfr strains MU27 and MU28 were crossed with JF756 with selection for *pnuC*⁺ or Tc^R. The results indicated a general map position for *pnuC* between 17 and 22 units. Further cotransductional mapping studies with various Tn10s distributed throughout this region revealed that *pnuC* was cotransducible with *nadA::Tn10*. Cotransduction frequencies varied from 83 to 90% depending upon the *pnuC* mutant used. Transductional analyses presented in Table 4 indicate the gene order *pnuC*-*nadA*-*gal*.

*Expression of *pnuC*-lacZ gene fusions*

A *pnuC::Mud1-8 Lac*⁺ fusion strain (JF731) was isolated by standard Mud(Aplac) phage gene fusion techniques. Measurement of β -galactosidase activity indicated that *pnuC* could be regulated by varying the amount of NA supplied to the *nadB pnuC::Mud1-8* strain (Table 5).

Fig. 3. Kinetics of *pnuC* derepression. An overnight culture of JF731 was washed twice to remove NA and used to inoculate minimal medium supplemented with either 10^{-4} M-NA (circles) or 10^{-6} M-NA (squares). Growth (filled symbols) and β -galactosidase activity (in Miller units; Miller, 1972) (open symbols) were measured periodically.

Kinetics of *pnuC-lacZ* derepression during NA starvation are presented in Fig. 3. Derepression occurred midway through starvation. This indicates that *pnuC* is regulated by fluctuations in intracellular NAD levels as are *nadA* and *nadB* (unpublished observations).

The *pnuC::Mud* insertion, when transferred to a *nad⁺* background, did not result in a *Nad⁻* phenotype. Neither did a *nadA::Tn10* insertion affect *pnuC-lacZ* expression (JF806, Table 5). Thus, *nadA* and *pnuC* represent two distinct and separate loci.

Regulation of *pnuC* by *nadR*

A mutation which results in the constitutive expression of *nadA* and *nadB* was recently isolated in a gene designated *nadR* (unpublished observations). To determine whether *nadR* also affects *pnuC* regulation, *pnuC8::Mud1-8* was transferred to the *nadR* strain (JF869) and β -galactosidase activity monitored during growth in high and low concentrations of NA. The results (Table 5) reveal a high constitutive expression of *pnuC* in the *nadR* background indicating a role for *nadR* in the regulation of *pnuC*.

The effect of *pnuB* or *pnuA* mutations on *pnuC-lacZ* expression was examined. While *pnuB* did not affect *pnuC-lacZ* expression (JF858), the *pnuA* mutation tested (JF873) allowed greater derepression of *pnuC* indicating that *pnuA* as well as *nadR* may act as a negative effector of *pnuC* (Table 5).

Isolation and mapping of a *pnuD* mutant affected in PNC VI-dependent NMN transport

To reveal potential genetic determinants involved in the PNC VI-dependent transport of NMN, a search was made for mutations in a *nad pncA⁺* strain (JF22) which would prevent growth on 10^{-5} M-NMN. Again, EMS mutagenesis was used with penicillin enrichment. One of the mutants isolated (JF85) grew well on 10^{-5} M-NAm and 10^{-4} M-NMN but failed to grow on 10^{-5} M-NMN, in contrast to the parent, JF22, which grew well on 10^{-5} M-NMN. Extracts of the *pnuD* mutant were compared with those of JF22 to determine the relative activities of NMN glycohydrolase and NMN deamidase. Both enzymes appeared normal in JF85 (data not shown).

Tn10 insertions were isolated near the *pnuD* locus by selecting for *pnuD⁺* (NMN⁺) Tc^R strains following the transduction of JF85 with a P22HT lysate propagated on a pool of *Tn10* insertion mutants. The *Tn10* insertion isolated (JF181) was transferred to the Hfr strains SA722 and SA464 and the resultant *Tn10* derivations used to transfer Tc^R in interrupted matings to suitable auxotrophic mutants. The time of entry experiments indicate a map position for *pnuD* of 56–60 units on the *S. typhimurium* linkage map.

Table 6. *Growth characteristics of *pnuC* in various genetic backgrounds*

Strain	Pertinent genotype	Growth on minimal media plus:					
		10^{-5} M-NA	10^{-5} M-NA _m	10^{-4} M-NMN	10^{-5} M-NMN	10^{-4} M-NA _m R*	10^{-4} M-NAD
JF235	<i>nadA</i>	+	+	+	+	+	+
JF63	<i>nadB pncA</i>	+	—	+	—	+	+
JF76	<i>nadA pncA pnuA</i>	+	—	—	—	+	—
SF77	<i>nadB pncA pnuB</i>	+	—	+	+	+	+
JF470	<i>nadA pncA pnuA pnuB</i>	+	—	+	+	+	ND
JF349	<i>nadB pncA pnuB pnuC</i>	+	—	—	—	+	—
JF870	<i>nadA pncA pnuC</i>	+	—	—	—	+	ND
JF439	<i>nadB pnuB pnuC</i>	+	+	+	+	+	ND

+, Growth; —, no growth; ND, not determined.

* Growth of strain JF76 on NA_mR was less than that of the other strains tested.

Table 7. *Effect of *pnu* and phosphatase mutations on the utilization of NMN and UMP*

NA and NA_m were used at a concentration of 10^{-5} M, and uracil at 2.5×10^{-4} M.

Strain	Pertinent genotype	Growth on minimal medium plus:						
		Ura	Ura + NA	Ura + NA _m	Ura + 10^{-4} M- NMN	Ura + 10^{-5} M- NMN	10^{-3} M- UMP + NA	10^{-4} M- UMP + NA
SF27	<i>pyrC</i>	+	ND	ND	ND	ND	+	—
JF681	<i>nadB pncA pnuA⁺ pyrC</i>	—	+	—	+	—	+	—
JF682	<i>nadB pncA pnuA pyrC</i>	—	+	—	—	—	+	—
JF666	<i>nadB pncA pnuB pyrC</i>	—	+	—	+	+	+	—
JF680	<i>nadB pncA pyrF aphA nap pde</i>	—	+	—	+	—	—	—

+, Growth; —, no growth; ND, not determined.

*Genetic relationships between *pnu* loci*

Further insight into the relationships between *pnuC* and the other *pnu* loci was afforded by introducing *pnuC* by transduction into strains with various genetic backgrounds. These were then tested on minimal media supplemented with various components of the PNC. Although *pnuC* was identified in a *pnuB* mutant the *pnuC* phenotype does not require a *pnuB* background to prevent growth on NMN (Table 6). This can be seen by comparing the growth patterns of JF349 (*pnuB pnuC*) with JF870 (*pnuB⁺ pnuC*), neither of which grow on 10^{-4} M-NMN. This information complements the transport studies presented above, as does the observation that the *pnuB* mutation suppresses the expression of *pnuA* mutations. This is seen by comparing JF76 (*pnuA*), which did not grow on NMN supplemented medium, to JF470 (*pnuA pnuB*), which grew well on this medium.

*The *pnu* system is not associated with nonspecific phosphatases*

The fact that *phoP*, a gene involved in nonspecific acid phosphatase regulation, is also found in this region led to the idea that *pnuC* may be the *phoP* locus (Kier *et al.*, 1979). Both purine and pyrimidine nucleotides are acted upon by nonspecific acid phosphatases to yield their respective nucleosides which may then enter the cell. Since NMN is structurally similar to pyrimidine nucleotides it was reasonable to suppose that NMN might be utilized through the same reaction. However, the lack of *pnuC* linkage to *purB* contradicts this since *phoP* is 96% linked to *purB* by P22 phage transduction (Kier *et al.*, 1979).

To determine more directly the possible involvement of *pnu* loci with the transport of other nucleotides, *nadB* and *pncA* were introduced into SF110, a *pde aphA nap* mutant lacking nonspecific acid phosphatase I (*nap*), nonspecific acid phosphatase II (*aphA*) and 2':3'-cyclic-nucleotide 2'-phosphodiesterase (*pde*). *S. typhimurium* strains lacking these phosphatases fail to degrade nucleoside monophosphates such as UMP and thus cannot utilize nucleotides to satisfy auxotrophic requirements (Uerkvitz & Beck, 1981). If the *pnuA*⁺ *pnuB*⁺ system for NMN utilization is related to these nucleotide phosphatases, then the *nadB pncA pde aphA nap* strain (JF680) should be unable to grow on 10⁻⁴ M-NMN. This strain, however, did grow normally on this concentration of NMN, indicating that *pnuA*⁺ *pnuB*⁺ dependent transport is unrelated to systems used to degrade and transport UMP (Table 7). Further evidence for the specificity of NMN utilization is provided by JF682, a *pnuA pyrC* mutant, which grew normally on 10⁻³ M-UMP but failed to grow on NMN. The data in Table 7 also reveal that the *pnuB pyrC* strain, JF666, while it could utilize lower concentrations of NMN did not grow any better on lower UMP levels. Thus, NMN is not utilized by systems involved in the transport of other structurally similar nucleotides.

In summary, the genetic and biochemical data suggest that under normal conditions the *pnuA*⁺ and *pnuC*⁺ gene products form a system for NMN uptake. The *pnuB*⁺ gene product plays no role in this system. Selective pressure for a more efficient transport system, however, appears to result in an alteration of *pnuB* allowing it to interact either directly or indirectly with the *pnuC*⁺ gene product to transport NMN more efficiently. In this system *pnuA*⁺ plays no role. None of the above loci appeared to function in the utilization of NMN via the PNC VI/*pncA* route, but a fourth locus, *pnuD*, was found to be involved in this system.

It is interesting that both *pnuA* and *pnuC* mutants are capable of growing on NAmR. This suggests that the *pnu* system is not involved with NAmR uptake and that a separate uptake system is required for this nucleoside. This is supported by the recent finding of a NAmR salvage pathway in *S. typhimurium* (Liu *et al.*, 1982).

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