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Location on the *Salmonella typhimurium* Chromosome of the Gene Encoding Nucleoside Diphosphokinase (*ndk*)

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The gene encoding nucleosidediphosphate kinase (*ndk*) was located at 55 units on the *Salmonella typhimurium* chromosome. The *ndk* locus was 83% cotransducible with *hisS* and 2% cotransducible with *glyA* in phage P22-mediated crosses. A nucleosidediphosphate kinase mutant that produced only 10% of the wild-type enzyme activity (*ndk-1*) grew normally and produced a heat-labile enzyme.

Nucleosidediphosphate kinase (ATP:nucleosidediphosphate phosphotransferase [EC 2.7.4.6]; hereafter abbreviated Ndk) catalyzes the last reaction in the synthesis of nucleoside triphosphates. By a ping-pong mechanism (2, 3), the γ-phosphate from a nucleoside 5′-triphosphate is transferred to the enzyme, generating a high-energy intermediate capable of phosphorylating nucleoside 5′-diphosphates. Any of 11 ribo- and deoxyribonucleoside triphosphates can donate a phosphoryl group to the enzyme, and any of 11 diphosphates can accept it from the enzyme. Thus, Ndk can catalyze at least 121 distinct reactions. In *Salmonella typhimurium*, it is the only enzyme that catalyzes these reactions (4; C. L. Ginther, Ph.D. thesis, University of California, Davis, 1973).

A mutant strain of *S. typhimurium* (JL2097 [Table 1]) that produces low levels of Ndk has been previously described (4). Assuming that Ndk is an essential enzyme and that Ndk function is essential to the toxicity of certain base analogs, a cold-sensitive 8-azaguanine-resistant mutant was sought: after mutagenesis with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and counter-selection with penicillin at 20°C, clones resistant to 8-azaguanine were selected. One of them produced a low level of Ndk activity.

To aid mapping, the following procedure was used to attempt to insert a Tn*10* element near the lesion causing low levels of Ndk to be synthesized. A Gal$^+$ derivative of strain JL2097 (JL2848) was transduced to grow at 20°C, using a lysate prepared by growing phage P22 on a population of *S. typhimurium* LT2 clones containing Tn*10* elements inserted at random sites on the chromosome (7–9). Four Tet^r transductants were examined and found to continue to produce low levels of Ndk activity and to be resistant to 8-azaguanine. Thus, at least two mutations were introduced into strain JL2097, one that conferred cold sensitivity and one that decreased expression of Ndk and probably conferred resistance to 8-azaguanine (*ndk*-1). The Tn*10* element inserted near the cold sensitivity lesion was located at 61 units on the *S. typhimurium* linkage map; it cotransduces at a frequency of 8% with *argA* in phage P22-mediated crosses.

8-Azaguanine resistance scores proved to be an unreliable indication of the *ndk-1* lesion; therefore, a sensitive radiochemical technique suitable for estimating the Ndk activity of a single colony was developed (S. B. Rodriguez, Ph.D. thesis, University of California at Davis, 1982). This microassay enabled colonies of *ndk-1*-carrying and wild-type strains to be distinguished clearly. To determine the approximate location of *ndk-1* on the linkage map, an Hfr

TABLE 1. Bacterial strains

Strain	Genotype/phenotype	Origin or reference
JL2021	*glyA1*	
JL2097	*galE1121 ndk-1 csg-1*	4
JL2848	*ndk-1 csg-1*	JL2097 × LT2 phage
JL3387	*ndk-1* Hfrk5 *hisF8539*::Tn*10*	—[a]
JL3408	*hisG200 pro-621 tryA541 gal*	—
JL3559	*metC argA69 cysA* Nal^r *serA1395*::Tn*10*	—
JL3569	*glyA1 gal*	—
JL3571	*hisS6334*	B. Ames[b] (TA2484)
JL3573	*glyA418*::Tn*10*	—
JL3574	*ndk-1*	—
JL3652	*glyA1 zfg-801*::Tn*10*	5

[a] —, S. B. Rodriguez, Ph.D. thesis, University of California, Davis, 1982.
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TABLE 2. Phage P22- and P1-mediated transductional crosses between *ndk* and nearby genes

Recipient	Phage	Donor	Selected marker	Unselected marker	% Cotransduction (no. of Ndk⁻ transductants/total)
JL3408	P1	JL2097	Tyr⁺	Ndk⁻	0 (0/112)
JL3569	P1	JL2097	Gly⁺	Ndk⁻	24 (28/117)
JL3571	P22	JL2848	His⁺	Ndk⁻	83 (40/48)

strain carrying *ndk-1* (JL3387) was constructed. In crosses between this strain and a variety of auxotrophic strains, the frequency of coinheritance of *ndk-1* and 10 auxotrophic markers was determined by the microassay. The relatively high frequency of coinheritance of *ndk-1* and *glyA* (70%) suggested that *ndk-1* lay near *glyA*. Confirmation of this was obtained from the results of an interrupted mating between strain JL3387 and an appropriate multiply marked recipient (JL3559). Mating was interrupted at 2-min intervals by spreading the mixture on nalidixic acid-containing plates selective for the three auxotrophic markers. The coinheritance of *ndk-1* and one of the markers (*cysA*) began to increase 22 min after mating was initiated, again locating *ndk-1* near *glyA* on the linkage map.

More precise location was accomplished by phage P22- and P1-mediated transductional crosses (Table 2). The *ndk-1* marker and *glyA* (JL3569) cotransduced at a frequency of 24% in a P1-mediated cross and with *hisS6334* (JL3571) at 83% in a P22-mediated cross. A three-factor P22-mediated cross indicated that *zfg*-801::Tn*10* (5) lay between *ndk* and *glyA* (Table 3, footnote *a*). The position of *ndk* on the *S. typhimurium* chromosome is shown in Fig. 1.

The transductional linkage between *ndk-1* and *glyA* enabled transfer of *ndk-1* to a *glyA*-carrying strain (JL3573, which grew at wild-type rates in glycine-supplemented media). The resulting prototrophic *ndk-1*-carrying strain (JL3574) grew at approximately 90% of the wild-type rate but produced only 10% of the wild-type levels of Ndk (Table 4).

To determine whether *ndk* is a regulatory or a structural gene for Ndk (which is composed of a single class of subunits [3]), the heat labilities of the activities produced by a strain carrying *ndk-1* and a strain carrying the wild-type allele were compared. At 37°C, Ndk activities in crude extracts of wild-type (LT2) and mutant (JL3574) cells decayed with half-lives of 7.7 and 1.8 min, respectively. The marked heat lability of the mutant activity suggests that *ndk-1* lies within the structural gene encoding Ndk. Further support of this contention comes from observations that regulation of the residual activity produced by the mutant was unchanged by the mutation. Ndk activity decreased as growth rate of the culture increased, and quite low levels were

TABLE 3. Three-factor phage P22-mediated cross (donor, JL2848; recipient, JL3652) establishing the location of *ndk* relative to *glyA* and *zfg801*::Tn*10*[a]

Recombinant class	No. of transductants
GlyA⁺ Tet^s Ndk⁻	2
GlyA⁺ Tet^r Ndk⁻	0
GlyA⁺ Tet^s Ndk⁺	10
GlyA⁺ Tet^r Ndk⁺	90

[a] Indicated gene order (dotted lines indicate crossover pattern yielding rare recombinant class):

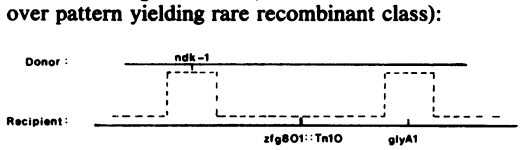

TABLE 4. Growth rates (k) and Ndk activities of JL3574 (*ndk-1*) and LT2 (wild type) grown anaerobically and aerobically on rich and minimal media at 37°C

Growth condition[a]	LT2		JL3574	
	k (h^{-1})[b]	Ndk sp act[c]	k (h^{-1})	Ndk sp act
Aerobic growth[c]				
Rich medium[d]	1.48	4.8	1.26	0.7
Minimal medium[e]	0.67	38.7	0.61	4.0
Anaerobic growth				
Rich medium[d]	0.66	3.2	0.51	0.3
Minimal medium[e]	0.55	20.4	0.48	0.1

[a] Aerobic cultures (25 ml) were grown in 250-ml cotton-plugged Erlenmeyer flasks shaken at 250 rpm on a rotary shaker. Anaerobic cultures were grown in Klett tubes completely filled with culture and closed with a rubber stopper. Rich medium, Modified Luria broth (S. B. Rodriguez, Ph.D. thesis, University of California, Davis, 1982) with 1.25% glucose. Minimal media, Vogel-Bonner basal salts media (11) with 0.4% glucose.

[b] Calculated from: $k = 2.303\ [(\log X - \log X_0)/(t - t_0)]$, where X and X_0 are dry weights at times t and t_0, respectively.

[c] Micromoles of CDP utilized per minute per milligram of protein (S. B. Rodriguez, Ph.D. thesis).

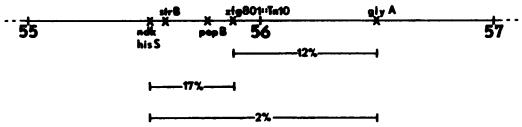

FIG. 1. Genetic map of the *ndk* region of the *S. typhimurium* chromosome. Percentages refer to cotransduction in a phage P22-mediated cross (Table 3). Numbers indicate units.

produced during anaerobic growth (Table 4). Saeki et al. (10) suggested that the low levels of Ndk produced by anaerobic cultures reflect the fact that when rates of gycolysis are high, pyruvate kinase replaces Ndk in synthesizing most of the nucleoside triphosphates required by the cells. Saeki et al. showed that the low level of nucleoside diphosphate specificity of *Escherichia coli* pyruvate kinase is consistent with such a proposal.

That strain JL3574 grew at a near-normal rate in spite of producing low levels of Ndk probably reflects the fact that this enzyme is so highly active in enteric bacteria. Its activity is about 10-fold higher than that of the nucleoside monophosphokinases (1, 6). The intracellular concentrations of nucleoside di- and triphosphates are probably maintained at thermodynamic equilibrium by Ndk.

LITERATURE CITED

1. **Beck, C. F., J. Neuhard, E. Thomassen, J. L. Ingraham, and E. Klecker.** 1974. *Salmonella typhimurium* mutants defective in cytidine monosphosphate kinase (*cmk*). J. Bacteriol. **120**:1370–1379.
2. **Garces, E., and W. W. Cleland.** 1969. Kinetic studies of yeast nucleoside diphosphate kinase. Biochemistry **8**:633–640.
3. **Ginther, C. L., and J. L. Ingraham.** 1974. Nucleoside diphosphokinase of *Salmonella typhimurium*. J. Biol. Chem. **249**:3406–3411.
4. **Ginther, C. L., and J. L. Ingraham.** 1974. Cold-sensitive mutants of *Salmonella typhimurium* defective in nucleoside diphosphokinase. J. Bacteriol. **118**:1020–1026.
5. **Green, L., and C. G. Miller.** 1980. Genetic mapping of the *Salmonella typhimurium pepB* locus. J. Bacteriol. **143**:1524–1526.
6. **Ingraham, J. L., and J. Neuhard.** 1972. Cold-sensitive mutants of *Salmonella typhimurium* defective in uridine monophosphate kinase (*pyrH*). J. Biol. Chem. **247**:6259–6265.
7. **Kleckner, N., R. K. Chan, B.-K. Tye, and D. Botstein.** 1975. Mutagenesis by insertion of a drug resistant element carrying an inverted repetition. J. Mol. Biol. **97**:561–575.
8. **Kleckner, N., J. Roth, and D. Botstein.** 1977. Genetic engineering *in vivo* using translocatable drug-resistance elements. J. Mol. Biol. **116**:125–159.
9. **Kleckner, N., D. A. Steele, K. Reichardt, and D. Botstein.** 1979. Specificity of insertion by the translocatable tetracycline-resistant element Tn10. Genetics **92**:1023–1040.
10. **Saeki, T., M. Hori, and H. Umezawa.** 1974. Pyruvate kinase of *Escherichia coli*. Its role in supplying nucleoside triphosphates under anaerobic conditions. J. Biochem. **76**:631–637.
11. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *E. coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.