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Mapping of *nrdA* and *nrdB* in *Escherichia coli* K-12

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The structural genes coding for the B1 and B2 subunits of the enzyme ribonucleoside diphosphate reductase, *nrdA* (formerly designated *dnaF*) and *nrdB*, respectively, have been mapped in *Escherichia coli*. They are located at approximately 48 min. The gene order in this region of the *E. coli* chromosome was found to be *purF glpT nrdB nrdA nalA cdd dcd his*.

In *Escherichia coli*, the enzyme ribonucleoside diphosphate reductase (RDP reductase) (E.C. 1.17.4.1) is responsible for the conversion of ribonucleotides to deoxyribonucleotides. It thus catalyzes the first reaction in a pathway specific for the synthesis of deoxyribonucleic acid precursors (4). RDP reductase contains two subunits designated B1 and B2. Each of the subunits is composed of two polypeptide chains (4). A mutant containing a structurally altered B1 subunit (8) and a mutant containing a structurally altered B2 subunit (7, 9) have been characterized. The mutations present in these strains have been designated *nrdA* and *nrdB*, respectively. The mutated B1 subunit was shown to be extremely thermolabile in vitro (8), and the mutated B2 subunit was found to have a greatly decreased activity, which could be partially restored by the addition of high concentrations of sodium acetate (7). The *nrdA* mutation prevents the growth of strains harboring it at temperatures above 40°C even in enriched media. The *nrdB* mutant was isolated from a specially constructed strain as a deoxyuridine auxotroph (7), but the mutation was found to be more easily recognizable by the increased sensitivity to hydroxyurea that it conferred to strains harboring it. We have used these properties to map the *nrdA* and *nrdB* genes. In the course of these studies we also located the approximate map position of two additional deoxyribonucleotide-metabolizing genes, *cdd* and *dcd*, the genes coding for deoxycytidine (cytidine) deaminase and deoxycytidine triphosphate deaminase, respectively.

MATERIALS AND METHODS

Materials. [^{3}H]cytidine diphosphate was obtained from Schwarz/Mann. Hydroxyurea was a gift from E. R. Squibb and Sons. Purified thioredoxin and thioredoxin reductase obtained from *E. coli* B were available in our laboratory. Davis minimal medium (6) and L broth (15) were used.

Bacterial strains. All of the bacterial strains used in this study were derivatives of *E. coli* K-12 and are described in Table 1.

Selection and testing of phenotypes. The following conditions were used to detect the phenotypes caused by the indicated mutations:

nrdA (formerly *dnaF* [20]): Failure of cells to grow at 40°C on either enriched or minimal plates supplemented with 20 μg of thymidine per ml.

nrdB: Inhibition of growth by hydroxyurea (0.25, 0.50, or 1.0 mg/ml) in minimal media in the presence of 20 μg of thymidine per ml. Sensitivity of both *nrdB* and *nrdB*$^+$ is increased at a lower temperature (30°C) and in thymine auxotrophs.

nalA: Resistance to 40 μg of nalidixic acid per ml.

cdd in *thyA*$^+$ strains: Inhibition of growth by 5-fluorodeoxycytidine (0.2 μg/ml).

cdd in *thyA* strains: Growth with 5-methyl deoxycytidine as thymine source.

cdd in *pyr* strains: Growth with deoxycytidine (20 μg/ml) as pyrimidine source.

cdd in *thyA pyr* strains: Growth with 5-methyl deoxycytidine and deoxycytidine as thymine and pyrimidine sources, respectively.

glpT: Inability to utilize 0.4% α-glycerol phosphate as a carbon source. Isolation of *glpT* mutants was accomplished by planting a culture on minimal plates containing 0.4% glycerol as a carbon source and 0.2 mg of phosphonomycin per ml. Resistant colonies were purified and screened for those unable to use 0.4% α-glycerol phosphate as a carbon source (13).

dcd (formerly *pax A* [16]): Sensitivity to 100 μg of 5-bromodeoxyuridine per ml at 42°C.

Conjugation. Matings between many different Hfr strains and a single female were carried out as follows. A total of 25 Hfr strains were grown overnight in 0.1 ml of L broth in separate compartments of an autoclavable nylon microculture container (E.L.E.S.A., 20129 Milan, Italy). The cultures were diluted 10-fold and grown for 2 h. A 0.3-ml sample of each Hfr was transferred to a new block and combined with 0.3 ml of the exponentially growing female. At various times samples from each mating

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TABLE 1. *E. coli* strains used

Strain	Sex	Genotype of chromosome	Source of derivation/reference
KL16	Hfr		
AB2572	Hfr		
CGSC4280 (KLF29/JC1553)	F'129	*argG metB leu his recA mtl xyl malA gal lacY* or *lacZ str*	Barbara Bachman
LD195	F⁻	*his argG metB leu lacY* or *lacZ malA xyl mtl gal str tpp cdd dcd nrdB* (P1)	7, 9
E101	F⁻	*thr leu thi thyA dra drm pup tonA lacY supE nrdA*	J. Gross (20)
K-43	F⁻	*thi tonB trp his met cys str gal lac tonA tsx* (λ)	G. Bertani
KK391	F⁻	*thi met cys str gal lac tonA tsx nrdB cdd*	See text
KK395	F⁻	*thr leu thi thyA dra drm pup tonA lacY supE nrdA nalA*	From E101
KK408	F⁻	*thi met cys str gal lac tonA tsx nrdB cdd thyA*	From KK391
KK419	F⁻	*met drm thyA nalA glpT*	Constructed in our lab
KK420	F⁻	*thi met cys str gal lac tonA tsx nrdB cdd thyA glpT*	From KK395
KK424	F⁻	*thr leu thi thyA dra drm pup tonA lacY supE nrdA nalA glpT*	From KK395
KK342	F'129	*his metB leu lacY* or *lacZ nalA str xyl dcd cdd tpp nrdB* (P1)	See text
KK343	F'129	*his metB leu lacY* or *lacZ nalA str xyl dcd cdd tpp nrdB* (P1)	See text
HD1038	F⁻	*his metB leu argG lacY* or *lacZ malA str xyl mtl dcd gal*	17
JC411	F⁻	*his metB leu argG lacY* or *lacZ malA str xyl gal mtl*	17
LD181	F⁻	*his metB leu lacY* or *lacZ malA gal str xyl mtl dcd cdd tpp*	7
CGSC4249 (KLF3/JC1153)	F'103	*argG metB his trp leu mtl xyl malA gal lacY* or *lacZ str*	Barbara Bachman
LD188	F⁻	*his metB his trp leu lacY malA str xyl mtl dcd cdd tpp pyrE thyA*	6

were transferred to selective agar plates by inverted nails held by a Plexiglas template.

Interrupted and long-term matings were performed as described by Curtiss et al. (5).

P1 transduction. P1 transductions were conducted essentially as described by Lennox (12) except that phage P1 *vir-1* was used. Map locations are given according to the 100-min map (1).

Enzyme assays. RDP reductase assays were conducted as previously described (4).

Protein estimation. Protein concentrations in crude extracts were estimated from the absorbance at 280 and 260 nm (1-cm light path) by the following equation: protein concentration (milligrams per milliliter) = $1.55\,A_{280} - 0.76\,A_{260}$ (19).

RESULTS AND DISCUSSION

Mapping of *nrdB* by conjugation. A series of Hfr strains was crossed to the *nrdB* mutant LD195, and after 30 min samples were spotted on plates containing streptomycin (500 μg/ml) and hydroxyurea (1 mg/ml). Hfr KL16, which transfers counterclockwise with an origin at approximately 60.5 min, gave numerous recombinants, whereas Hfr AB2572, which also transfers counterclockwise but with an origin of transfer of approximately 45.5 min, gave no recombinants. This indicates that *nrdB* lies between 45.5 and 60.5 min. An interrupted mating utilizing strains KL16 and LD195 was carried out, selecting for either hydroxyurea and streptomycin resistance or histidine prototrophy and streptomycin resistance. The results indicated that hydroxyurea resistance is transferred 2.7 to 5.0 min before *his*, and thus *nrdB* is located between 47 and 49 min. To confirm the orientation of *nrdB* with respect to *his*, recombinants from noninterrupted matings were analyzed for unselected markers. Since the recipient contains *cdd* (mutation in the gene coding for cytidine [deoxycytidine] deaminase) which had previously been located clockwise from *his* (O. Karlstrom, unpublished data, and in *Salmonella* [2]), *cdd*⁺ was analyzed as well. Table 2 indicates that the selection of *nrd*⁺ resulted in a low frequency of inheritance of unselected markers. Selection of *his*⁺ was accompanied by a much higher frequency of *cdd*⁺ and *nrd*⁺. Furthermore, *cdd*⁺ and *nrd*⁺ usually appeared together. This would indicate a gene order of *nrdB cdd his*. In this experiment, unselected markers were underrepresented due to the restriction exerted by phage P1, which is present as a prophage in LD195.

Before the *nrdB* mutation could be transferred via P1 transduction, it was necessary to transfer it to a nonlysogenic strain. F'129, which includes the region of the *E. coli* chromosome from 44 to 50 min including the *nrd*⁺ genes, from strain CGSC428 was transferred to strain LD195 by selecting for *his*⁺ to obtain strain KK342. An *nrdB cdd* homozygote KK343 was obtained from strain KK342 by selecting for resistance to 0.1 μg of 5-fluorodeoxycytidine per ml. Since difficulties were experienced in transferring the *nrdB* mutation to the chromosome of another strain via episome transfer and homozygotization, strain KK343 was subjected to ultraviolet light and crossed to strain K-43 in an Hfr-type mating selecting for *trp*⁺ recombinants. The *nrdB cdd* strain, KK391, was identified among the recombinants. KK391 is F⁻.

P1 transductions utilizing strain KK391 and its derivatives indicated that *nrdB* was closely linked to *nalA* (not shown). This observation led to our characterization of the *nrdA* mutant (8). P1 transductions were then used to determine the gene order of *nrdB* and *nrdA* and surrounding markers.

Mapping of *glpT*, *nrdA*, *nrdB*, and *nalA* by P1 transduction. Transduction 1 (Table 3) shows that *nrdA* is closely linked to both *nalA* and *glpT* and has a cotransduction frequency with either of approximately 80%. In the four-factor transduction with *nrdB* in the donor and *nrdA glpT* and *nalA* in the recipient, selection for *glpT*$^+$ indicates that *glpT* has a cotransduction frequency with either *nrdA* or *nrdB* of approximately 85%, but a cotransduction frequency with *nalA* of only 65% (transduction 2, Table 3). Thus, the gene order is *glpT* (*nrdA*,*B*) *nalA*. Furthermore, since only 6 out of 171 transductants exhibited a recombination between *nrdA* and *nrdB*, these genes must be closely linked (96% cotransduction frequency).

To determine the order of *nrdA* and *nrdB* relative to outside markers, 267 *nrdA*$^+$ *nrdB*$^+$ transductants were selected in the above transduction. These recombinants can only arise when there is a crossover between *nrdA* and *nrdB*, so that the *nrdB*$^+$ allele of the recipient is retained in the recombinant. This crossover decreases the frequency of cotransduction of the marker that is on the same side as *nrdB*. With this selection, the donor allele of *glpT* was recovered in only 5% of the transductants, whereas *glpT* and *nrdA* were 80 to 85% cotransductable when *nrdB*$^+$ was not selected. This experiment indicates a gene order of *glpT nrdB nrdA nalA*.

As an independent test of the order between *nrdA* and *nrdB* with respect to outside markers, transduction 3 (Table 3) was conducted. When the donor was *nrdA nalA* and the recipient was *nrdB glpT*, selection for *nrdA*$^+$ *nrdB*$^+$ yielded recombinants in which the *glpT nalA* class occurred most frequently. With the gene order derived from transduction 2, this class of recombinants would occur with only a double crossover. If the gene order was *glpT nrdA nrdB nalA*, a quadruple crossover would be required. Thus, the gene order is *glpT nrdB nrdA nalA*.

By using Wu's formula (21), the map distance can be estimated to be 0.1 min for *glpT-nrdA*,*B* and 0.3 min for *glpT-nalA*.

Enzyme assays. To verify that the phenotypes used corresponded to the assumed genotype of the strain, we assayed various types of recombinants obtained in transduction 2 (Table 3) to correlate their enzymatic defect to their phenotype. Table 4 shows that the recombinants sensitive to 0.5 mg of hydroxyurea per ml were defective in subunit B2 of RDP reductase, whereas recombinants that failed to grow at 40°C were defective in the B1 subunit. A defect in both subunits was observed in strain KK442, which is sensitive to hydroxyurea and also fails to grow at 40°C, indicating that the expression of one *nrd* mutation does not affect the expression of the phenotype caused by a mutation in the other *nrd* gene.

Mapping of the *cdd* and *dcd* genes. The preliminary crosses indicated that the *cdd* mutation maps between *nrdB* and *his*. Of the transductants to *glpT*$^+$ in transduction 2 of Table 3, 46 were also checked for *cdd*. Since the donor was *cdd* and the recipient *cdd*$^+$ *thyA*, any *cdd* transductant should lose the ability to use 5-methyl deoxycytidine as a thymine source. No *cdd* cotransductants were found. If one assumes that cotransduction frequency depends only on the distance between markers, one can calculate a minimum distance between the markers applying Wu's formula (21). Using a "confidence limit" of 95%, we obtain the minimum distance of 1.2 min between *glpT* and *cdd*.

We tried to detect linkage between *cdd* and *nalA* using strain KK395, the nearest progenitor of strain KK424, as a donor in P1 transduction and LD188 (*cdd nalA*$^+$) as a recipient. Among 248 *cdd*$^+$ transductants selected on plates with 5-methyl deoxycytidine and deoxycytidine as thymine and uracil sources, respectively, none was resistant to nalidixic acid. Using a "confidence limit" of 95% and Wu's formula (21), we obtain a minimum distance between *nalA* and *cdd* of 1.5 min.

Another mutation affecting nucleotide metabolism, *dcd* (defect in the gene coding for deoxycytidine triphosphate deaminase), was of particular interest since it was used in the original isolation of the *nrdB* mutation (9). The observation that the *dcd* mutation potentiates the toxic effect of 5-bromodeoxyuridine, partic-

TABLE 2. *Unselected markers in mating of KL16 × LD195*

Selection	Unselected markers			No. of colonies
	nrdB	*cdd*	*his*	
His$^+$	−	−		60
	−	+		5
	+	−		2
	+	+		24
Nrd$^+$		−	−	93
		−	+	2
		+	−	2
		+	+	3

TABLE 3. *P1 transductions of nrdA and nrdB genes*

Transduction	Donor	Recipient	Selection	No. of transductants	Unselected markers	No. of transductants
1	KK419 (*glpT nalA*)	E101 (*nrdA*)	NrdA$^+$	59	*glpT nalA*	42
					glpT$^+$ *nalA*	6
					glpT nalA$^+$	4
					glpT$^+$ *nalA*$^+$	7
2	KK391 (*nrdB*)	KK424 (*glpT nrdA*) (*nalA*)	GlpT$^+$	173	*nrdB nrdA nalA*	1
					nrdB nrdA nalA$^+$	0
					nrdB nrdA$^+$ *nalA*	37
					nrdB nrdA$^+$ *nalA*$^+$	107
					nrdB$^+$ *nrdA nalA*	19
					nrdB$^+$ *nrdA nalA*$^+$	2
					nrdB$^+$ *nrdA*$^+$ *nalA*	3
					nrdB$^+$ *nrdA*$^+$ *nalA*$^+$	2
			NrdB$^+$ NrdA$^+$		*glpT*	258
					nalA	139
3	KK395 (*nrdA nalA*)	KK420 (*glpT nrdB*)	NrdB$^+$ NrdA$^+$	39	*glpT*$^+$ *nalA*$^+$	22
					glpT nalA$^+$	13
					glpT$^+$ *nalA*	1
					glpT nalA	3

TABLE 4. *Activity of RDP reductase subunits in various recombinants of P1 transduction from KK391 to KK424*

Recombinant strain	Phenotype		B1 activity[c]	B2 activity[c]	B1 activity/B2 activity
	HU[a]	40C[b]			
KK449	r	+	7.2	12.7	0.6
KK448	r	+	6.7	11.6	0.6
KK447	s	+	15.0	0.8	19.0
KK446	s	+	14.7	1.1	13.0
KK445	r	−	0.3	11.3	0.03
KK444	r	−	0.5	19.6	0.03
KK443	r	−	0.8	13.5	0.06
KK442	s	−	0.3	1.0	0.3

[a] Sensitivity (s) or resistance (r) to 0.5 mg of hydroxyurea per ml.
[b] Growth at 40°C.
[c] Nanomoles of cytidine diphosphate reduced per 10 min per milligram of protein.

ularly at high temperature (J. A. Fuchs, unpublished data), enabled us to map the *dcd* mutation. In conjugations between Hfr KL16 and strain HD1038, which is streptomycin resistant, and *dcd* and *his*, 5-bromodeoxyuridine-resistant Str^r and His$^+$ Str^r recombinants were selected. Of 22 5-bromodeoxyuridine-resistant recombinants 14 were also His$^+$, and of 36 His$^+$ recombinants 30 were 5-bromodeoxyuridine resistant. These results show that the two markers are linked and suggest that *dcd* is located clockwise from *his*.

Of 91 His$^+$ P1 transductants of strains HD1038 using a *dcd*$^+$ donor, none was 5-bromodeoxyuridine resistant. The minimum distance between *dcd* and *his* is 1.3 min at "95% confidence," again with the previous reservations.

Two F′ factors were used to establish the order between *cdd* and *dcd*. The F′129 is an unusually large F′, including both *his* and *dsdA*; F′103 includes *his* to *metG* (14). F′ derivatives were prepared from strain LD181, which is *cdd dcd his*, using strains CGSC4280 and CGSC4249 as donors and selecting for His$^+$ Arg$^+$ and His$^+$ Arg$^+$ Trp$^+$, respectively.

The F′129 derivatives of strain LD181 were 5-fluorodeoxycytidine sensitive and 5-bromodeoxyuridine resistant, indicating that the wild-type alleles of both *cdd* and *dcd* are carried on this F′ and that, as expected, both mutant phe-

notypes are recessive. Twenty recombinants were shown to be male by an MS2 phage test and to revert to the mutant phenotypes upon curing by acridine orange treatment. The F'103 derivatives of strain LD181 were also 5-bromodeoxyuridine resistant, but were still as 5-fluorodeoxycytidine resistant as LD181. We conclude that F'103 covers *dcd* but not *cdd*. Assuming that our F'103 does not contain a deletion, this finding establishes the order *cdd dcd his*.

The chromosome deletions caused by P2-mediated eduction include *his* and end near or in *mglABC* (18). Neuhard and Thomassen have recently shown that an eductant QE1 lacks deoxycytidine triphosphate deaminase (16). In agreement with this result, we find that QE1 and the two other eductants that we have tested, QE5 and QE982 (18), are sensitive to 5-bromodeoxyuridine. These eductants are not resistant to 5-fluorodeoxycytidine. The deletions thus seem to remove *dcd* but not *cdd*, confirming the order derived from F' mapping. If, indeed, the deletions end in *mglABC* and if *metG* (gene coding for methionyl transfer ribonucleic acid synthetase [10]) is an essential gene, we can conclude that *metG* is clockwise from *mglABC*. This establishes the order *cdd metG mglABC dcd his*.

Many of the markers discussed in this section have been mapped by P1 transductions in *Salmonella typhimurium* (2). In *Salmonella*, linkage between *glpT* and *cdd* has been claimed (Vinopal, quoted in reference 2). Our negative results cannot exclude a low frequency of cotransduction between *cdd* and *glpT* or *nalA* in *E. coli*. The data from *Salmonella* include *udk* (the gene coding for uridine kinase), which has been mapped close to *dcd* in *Salmonella* (16). The map order found in *Salmonella* is *cdd metG udk his* (3), in complete agreement with our results for *E. coli*.

It has previously been reported that *nrdA* can be transduced with *purF*, *nrdA* mapping counterclockwise from *purF* (20). Also by cotransduction with *purF*, *glpT* has been placed clockwise from *nalA* (11). In conjugation with the present results these data give the map order *purF glpT nrdB nrdA nalA cdd metG mglABC dcd his*.

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