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Demonstration of Separate Genetic Loci Encoding Distinct Membrane-Bound Respiratory NADH Dehydrogenases in *Escherichia coli*

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The nature of the *Escherichia coli* membrane-bound NADH dehydrogenases and their role in the generation of the proton motive force has been controversial. One *E. coli* NADH:ubiquinone oxidoreductase has previously been purified to homogeneity, and its corresponding gene (*ndh*) has been isolated. However, two biochemically distinct *E. coli* NADH:ubiquinone oxidoreductase activities have been identified by others (K. Matsushita, T. Ohnishi, and H. R. Kaback, Biochemistry 26:7732–7737, 1987). An insertional mutation in the *ndh* gene has been introduced into the *E. coli* chromosome, and the resulting strain maintains membrane-bound NADH dehydrogenase activity, demonstrating that a second genetically distinct NADH dehydrogenase must be present. By standard genetic mapping techniques, the map position of a second locus (*nuo*) involved in the oxidation of NADH has been determined. The enzyme encoded by this locus probably translocates protons across the inner membrane, contributing to the proton motive force.

The aerobic respiratory chain of *Escherichia coli* contains several dehydrogenases which catalyze the oxidation of various substrates. These dehydrogenases, including succinate dehydrogenase, D-lactate dehydrogenase, NADH dehydrogenase, and pyruvate oxidase, donate electrons to ubiquinone, which transfers the electrons to either of the two terminal oxidase complexes (1). The function of this electron transport chain is to generate a proton motive force across the membrane. This force, in turn, is responsible for a number of energy-dependent processes, such as ATP synthesis and active transport.

The nature of the *E. coli* membrane-bound NADH dehydrogenases and their role in the generation of the proton motive force have been controversial. Young and Wallace isolated mutants deficient in membrane-bound NADH dehydrogenase activity by the inability of such mutants to utilize mannitol as the sole carbon source (28). These mutants were believed to contain single-locus mutations which were mapped to min 22 of the *E. coli* linkage map (2, 28). Subsequently, a gene encoding an *E. coli* NADH dehydrogenase (*ndh*) was cloned and sequenced (26, 27). This membrane-associated NADH dehydrogenase was then purified from a strain harboring a recombinant plasmid (10). The purified enzyme (NDH) consists of a single polypeptide with a molecular mass of 47 kDa which contains flavin adenine dinucleotide but no iron (10, 11). It is highly active with ubiquinone-1 (Q1) as an electron acceptor (11). The data presented by Young and colleagues suggest that there are no iron-sulfur centers and no energy-coupling site associated with this NADH dehydrogenase.

However, studies with inside-out membrane vesicles from *E. coli* strongly suggested that NADH oxidation is coupled to the generation of a proton motive force (14). In addition, Owen and Kaback (18) demonstrated that *E. coli* inner membranes contain at least two immunologically distinct NADH dehydrogenases. One of these (antigen 15) contains nonheme iron and can be distinguished from the other (antigen 19/27) by the ability of antigen 15 to react with reduced nicotinamide hypoxanthine dinucleotide (deamino-NADH).

Recently, Matsushita et al. (15) demonstrated that *E. coli* membranes contain two biochemically distinct NADH dehydrogenase activities. One of these activities (NADH dhI) utilizes both deamino-NADH and NADH as substrates, and its turnover generates an electrochemical gradient in the presence of cyanide and Q1. The other activity (NADH dhII) utilizes NADH exclusively, and electron flow from NADH to Q1 catalyzed by NADH dhII does not generate an electrochemical gradient. It was proposed (15) that the NADH dhII activity results from the enzyme NDH, which is the *ndh* gene product.

The data presented here confirm the data of Matsushita et al. (15) by demonstrating that the two *E. coli* NADH dehydrogenase activities correspond to genetically distinct loci. The cloned NADH dehydrogenase gene (*ndh*) has been interrupted with a gene cartridge conferring kanamycin resistance. This *ndh*::Km^r construction was then introduced into the *E. coli* chromosome by genomic replacement techniques which rely on homologous recombination (17, 25). The *ndh*::Km^r construction has been confirmed by bacteriophage P1 cotransduction and by Southern blotting. The resulting *ndh*::Km^r strain retains membrane-bound NADH dehydrogenase activity and shows no obvious growth defects. The NADH dehydrogenase activity in this *ndh*::Km^r strain uses the substrates deamino-NADH and NADH with approximately equal efficiency.

It has also been demonstrated that the mutants defective in NADH dehydrogenase activity (26, 28) are not single-locus mutations but in fact contain mutations in two genetically distinct loci which encode two biochemically distinct NADH:ubiquinone oxidoreductases (*ndh* encoding NADH dhII and a new locus, *nuo*, encoding NADH dhI). With this information, standard genetic techniques have been used to locate the genetic map position of the second mutation in the strain lacking NADH dehydrogenase activity. The second mutation is cotransduced by bacteriophage P1 with a marker located at min 49 on the *E. coli* genetic map. This locus is

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TABLE 1. Selected *E. coli* K-12 bacterial strains and plasmids used in this study

Strain or plasmid	Relevant properties	Source or derivation
Strains		
CAG18484	*zej-223*::Tn*10*	20
CY288	*zce*::Tn*10 fabB*(Ts)*fabF*	J. Cronan
GR19N	*cyd*	8
GR70N		9
HB101	F$^-$ *hdsS20 recA13*	Laboratory collection
IY12	*ndh nuo* Man$^-$	26[a]
JC7623	*recB21 recC sbcB15*	25
MW01	HB101(pMW01)	This work
MW02	HB101(pMW02B)	This work
MW03	JC7623 *ndh*::Km^r	Transform with pMW02B
MWC003	GR19N *zej-223*::Tn*10 nuo* Man$^+$	P1(TN49-102) × GR19N
MWC007	GR19N *zej-223*::Tn*10* Man$^+$	P1(TN49-102) × GR19N
MWC008	GR19N *zej-223*::Tn*10 nuo ndh*::Km^r Man$^-$	P1(NKS02) × MWC003
MWC169	JC7623 *ndh*::Cm^r	Transform with pMC12
MWC190	GR70N *zej-223*::Tn*10 nuo* Man$^+$	P1(TN49-102) × GR70N
MWC215	GR70N *ndh*::Cm^r Man$^+$	P1(MWC169) × GR70N
MWC232	GR70N *ndh*::Cm^r *zej-223*::Tn*10 nuo* Man$^-$	P1(MWC169) × MWC190
MWC233	GR19N *ndh*::Km^r Man$^+$	P1(NKS02) × GR19N
NKR01	*ndh*::Km^r *zce*::Tn*10* Man$^+$	P1(MW03) × CY288
NKS02	*ndh*::Km^r Tc^s Man$^+$	P1(MW03) × CY288
TN49-1	IY12 *zej-223*::Tn*10 ndh* Man$^+$	P1(CAG18484) × IY12
TN49-102	IY12 *zej-223*::Tn*10 ndh nuo* Man$^-$	P1(CAG18484) × IY12
Plasmids		
pBR322	Ap^r Tc^r	4
pHP45ΩCm	Ap^r Cm^r	7
pIY10	*ndh*$^+$ Tc^r	26
pMW01	*ndh*$^+$ Ap^r	This work
pMW02B	*ndh*::Km^r Ap^r	This work
pMC12	*ndh*::Cm^r Ap^r	This work
pUC4-K	Km^r Ap^r	3

[a] The addition of the mutation in *nuo* to the genotype of strain IY12 is based on data presented in this work.

likely to be the site of the gene encoding NADH dhI. The proposed name for this new locus is *nuo* (NADH:ubiquinone oxidoreductase).

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. The Hfr strains (20, 22) are listed in Fig. 1, and the strains from the Tn*10* mapping set (20) are listed in Table 2. For routine manipulations and cell growth for enzymatic assays, a rich medium was used (10 g of tryptone, 5 g of yeast extract, 5 g of NaCl per liter). For minimal media, M63 salts were used (19). Antibiotics were used in the following concentrations (in micrograms per milliliter): tetracycline, 12.5; kanamycin, 50; ampicillin, 50; streptomycin, 25; chloramphenicol, 25. Markers were moved between strains by generalized transduction with bacteriophage P1 as previously described (19). Hfr matings were performed in liquid culture as previously described (16).

Materials. NADH, deamino-NADH, and antibiotics were purchased from Sigma Chemical Company. T4 DNA ligase and restriction endonucleases were from Bethesda Research Laboratories, Inc., and from International Biotechnologies, Inc. Biotinylated DNA probes for Southern blotting were constructed with the Photobiotin Labeling System from Bethesda Research Laboratories, and the Southern blots were visualized with the BluGene Nonradioactive Nucleic Acid Detection System from Bethesda Research Laboratories. Filters used in Southern blotting were from Millipore. GeneClean was purchased from Bio 101 Inc., La Jolla, Calif. BCA Protein Assay Reagent was purchased from Pierce Chemical Company, Rockford, Ill. Q1 was prepared according to a procedure provided by Chang-An Yu (29). All other chemicals were reagent grade from commercial sources.

General recombinant DNA techniques and Southern blotting. Methods for large-scale isolation of plasmid DNA, minipreparation of plasmid DNA, restriction enzyme digestions, ligase reactions, transformations, and isolation of DNA from acrylamide gels have been described elsewhere (13). DNA fragments were isolated from agarose gels with GeneClean according to the manufacturer's instructions. Chromosomal DNA was extracted from *E. coli* as previously described (19). Southern blotting has been previously described (13). DNA probes were photobiotin labeled as recommended by the manufacturer. Prehybridization and hybridization conditions and the method of detection were according to the manufacturer's instructions.

Membrane preparations and enzyme assays. For routine screening of new strains, aerobically grown cells were harvested, washed once with 50 mM potassium phosphate (pH 7.5), and resuspended in 1/10 volume of buffer per original culture volume. The cells were then disrupted on ice by sonication, and cell debris was removed by low-speed centrifugation. This cell extract was used directly to assay NADH and deamino-NADH oxidase activity. This method of assaying whole-cell extracts was found to be a reliable predictor of the results obtained with assays of membrane preparations. Membranes were prepared as described previously with a French pressure cell at high shear forces (15).

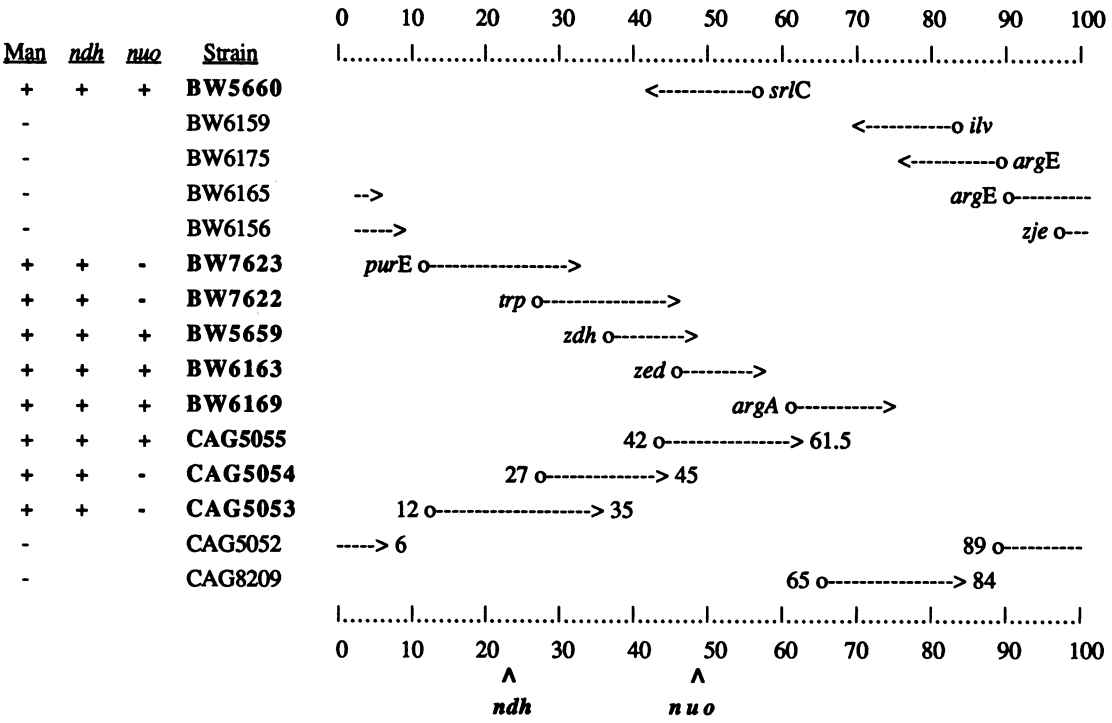

FIG. 1. Hfr matings. The points of origin and the directions of the Hfr transfer are depicted by the arrowheads. The circles indicate the positions of the Tn*10* insertions used for selection of exconjugants. The phenotypes of the exconjugants resulting from the mating of the Hfr strain with strain IY12 are designated either Man$^+$ or Man$^-$ for ability or inability, respectively, to grow with mannitol as the sole carbon source. The deduced presence or absence of a functional *ndh* or *nuo* gene is indicated by + or −, respectively. The deduced map positions of *ndh* and *nuo* are shown in relationship to their genetic map positions.

Oxidoreductase activities were measured spectrophotometrically as previously described (15).

Protein assays. Protein concentrations were determined by the Pierce BCA Protein Assay according to the manufacturer's instructions with bovine serum albumin as a standard.

RESULTS

Construction of Km^r and Cm^r insertion mutants. Plasmid pMW01 was constructed to create a unique *Eco*RV restriction site within the *ndh* gene (Fig. 2). Mutagenesis of the *E. coli ndh* gene was accomplished (Fig. 2) by linearizing plasmid pMW01 with *Eco*RV and inserting the 1.3-kb *Hin*cII Km^r cartridge from plasmid pUC4-K. Restriction endonuclease mapping of one of the resulting plasmids confirmed that the direction of transcription of the Km^r gene was as indicated in Fig. 2 (data not shown), leaving 1.2 and 0.8 kb of *E. coli* DNA flanking the Km^r gene. *E. coli* chromosomal mutants were selected on kanamycin plates following transformation of strain JC7623. The rare event of a double crossover which replaced the chromosomal *ndh* gene with the plasmid *ndh*::Km^r construction was identified by screening Km^r transformants for Ap^s. Initial screening of the Km^r colonies yielded 100% Ap^r colonies. One of the colonies was grown in Luria broth overnight without antibiotic selection, and colonies were selected on kanamycin plates. Screening of the resultant colonies yielded only 6% (3 of 50) Ap^r colonies. One of the Ap^s colonies (designated strain MW03) was chosen for further characterization. A similar technique was used to introduce the Cm^r cartridge from plasmid pHP45ΩCm (7) into pMW01. The resultant plasmid (pMC12) was used to introduce *ndh*::Cm^r into the *E. coli* chromosome, to make strain MWC169.

Strain CY288 contains a Tn*10* insertion at minute 23, which cotransduces with *ndh* at a frequency of 28% (data not shown). In these experiments, transductants were selected for Tc^r and then screened for the ability to grow on mannitol and for deamino-NADH oxidase activity. This result is consistent with the map position of *ndh* as identified by Young and Wallace (28), as well as with the physical map position identified by Calhoun et al. (5). The chromosomal *ndh*::Km^r construction was transferred from strain MW03 into strain CY288 by bacteriophage P1 transduction. Transductants (NKR01 and NKS02) were selected for Km^r and then screened for Tc^r. The cotransduction frequency between Km^r (*ndh*::Km^r) and Tc^s (with *zce*::Tn*10* replaced) is 25%. The location of the *ndh*::Cm^r chromosomal insert was also confirmed by P1 transduction into strain CY288. Transductants were selected for Cm^r and screened for Tc^r. The cotransduction frequency between the Cm^r (*ndh*::Cm^r) and the Tc^s (replacing *zce*::Tn*10* of strain CY288) is 50%.

Southern blot analysis. Southern blot analysis of NKR01 and NKS02 genomic DNAs demonstrated that the chromosomal *ndh* gene had been interrupted by the Km^r gene by double-reciprocal recombination. Figure 3 shows the expected change in the size of the *Eco*RI-*Sal*I restriction fragment which contains the *ndh* gene (lanes 1 to 4). These experiments also documented colinearity of the interrupted *ndh* gene with the Km^r gene on the 3.3-kb *Eco*RI-*Sal*I restriction endonuclease fragment in genomic DNA digests derived from strains NKR01 and NKS02 (lanes 5 to 8).

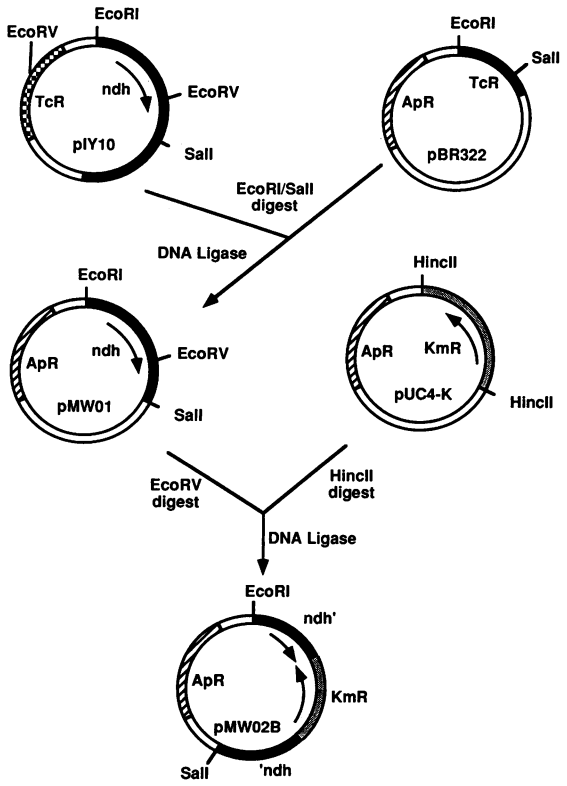

FIG. 2. Construction of plasmids pMW01 and pMW02B for chromosomal mutation of the *E. coli ndh* gene. Plasmid pMW01 was constructed to create a unique *Eco*RV blunt-end restriction site in *ndh* for insertion of the 1.3-kb blunt-end *Hin*cII Km^r cartridge from plasmid pUC4-K (3). A similar strategy was used to construct plasmid pMC12, which contains the Cm^r cartridge from pHP45ΩCm (7).

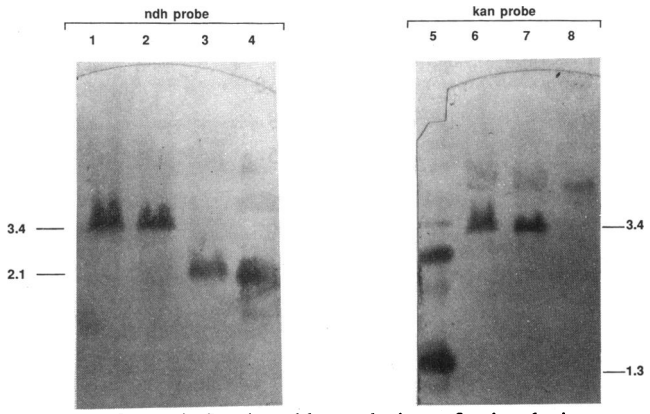

FIG. 3. Genomic Southern blot analysis confirming the interruption of *ndh* in strains NKR01 and NKS02. Shown are the results obtained when restricted genomic DNAs from wild-type strain CY288 and mutant (*ndh*::Km^r) strains NKR01 and NKS02 were hybridized with the DNA probes indicated. Genomic DNA samples (NKR01, NKS02, and CY288) digested with *Eco*RI and *Sal*I are in lanes 1 to 3. Lane 4 contains plasmid pIY10 (*ndh*$^+$) digested with *Eco*RI and *Sal*I. Lane 5 contains plasmid pUC4-K (Km^r) digested with *Eco*RI. Genomic DNA samples (NKR01, NKS02, and CY288) digested with *Eco*RI and *Sal*I are in lanes 6 to 8. The DNA probes used in this analysis were (i) the *ndh* probe (the 2.05-kb *Eco*RI-*Sal*I restriction endonuclease fragment from plasmid pIY10), and (ii) the kan probe (a 1.3-kb *Eco*RI restriction endonuclease fragment generated from plasmid pUC4-K). The numbers next to the blots indicate the sizes (in kilobases) of restriction endonuclease fragments predicted to be homologous to the individual DNA probes, which were comparable to the sizes of λ *Hin*dIII DNA size standards.

Enzyme assays of the *ndh*::Km^r and *ndh*::Cm^r strains. The results of the assays of NADH and deamino-NADH membrane-bound oxidase activities, dichlorophenol indophenol (DCIP) reductase activities, and Q1 reductase activities of several wild-type and mutant strains are shown in Fig. 4. These assays demonstrate that absence of the *ndh* gene product does not disrupt the ability of the cell to oxidize NADH.

As has been previously noted (15), the deamino-NADH dehydrogenase activity is labile, and its level varies among different preparations of the same strain. For example, in the wild-type strain GR19N, the deamino-NADH oxidase level was found to vary between 20 and 80% of the NADH oxidase level. The activity levels shown in Fig. 4 are representative. The discrepancy between the oxidase (Fig. 4B) and the Q1 reductase (Fig. 4C) activities was replicated with different preparations. From comparison of the activities of the isogenic strains, the discrepancy is apparently the result of the lower activity of NADH dhI with Q1 as the electron acceptor than with the endogenous ubiquinone electron acceptor.

Genetic mapping of the second mutation in strain IY12. To determine the map location of the second mutation in strain IY12, an overlapping set of Hfr strains (20, 22) was used to locate the region of interest. Each Hfr was mated with strain IY12, which cannot grow on mannitol as the sole carbon source (MAN$^-$). Matings that produced colonies which could grow on mannitol minimal medium (MAN$^+$) identified Hfr strains that could transfer a functional NADH dehydrogenase gene into strain IY12. The results of the Hfr mapping are shown in Fig. 1. As expected, the Hfr matings define two regions of the chromosome which can restore growth on mannitol as the sole carbon source. One of these is the region around min 22 of the *E. coli* genetic map. The other region is located between min 45 and 51. Activity assays of sonicated cells of the MAN$^+$ exconjugants revealed that Hfrs transferring min 22 have NADH oxidase but not deamino-NADH oxidase activity, while Hfrs transferring min 45 to 51 have both activities.

Eight strains containing Tn*10* markers between min 45 and 51 were selected from the set constructed by Singer et al. (20). These strains were used as donors for bacteriophage P1 transduction into strain IY12, and transductants were selected for the resistance to tetracycline which is conferred by Tn*10*. Resultant colonies were then screened for their abilities to grow on mannitol minimal medium. The results of the P1 transductions are shown in Table 2. The Tn*10* marker in strain CAG18484 (*zej-223*::Tn*10*, min 49.50) cotransduced at high frequency with the phenotype of growth on mannitol. A lower frequency of cotransduction was found with the Tn*10* marker in strain CAG12178 (*zei-723*::Tn*10*, min 48.50) suggesting that the mutation is located near minute 49. Two transductants from donor CAG18484 were selected for further characterization. Both strains contain *zei-223*::Tn*10*, but strain TN49-1 grows on mannitol minimal medium, while strain TN49-102 does not. On rich medium, TN49-1 grows at the same rate as the wild-type P1 donor CAG18484. Strain TN49-102 exhibits slow growth and lower final cell density, characteristics which it shares with the MAN$^-$ parent strain IY12. Assays of sonicated cells from strain TN49-1 showed

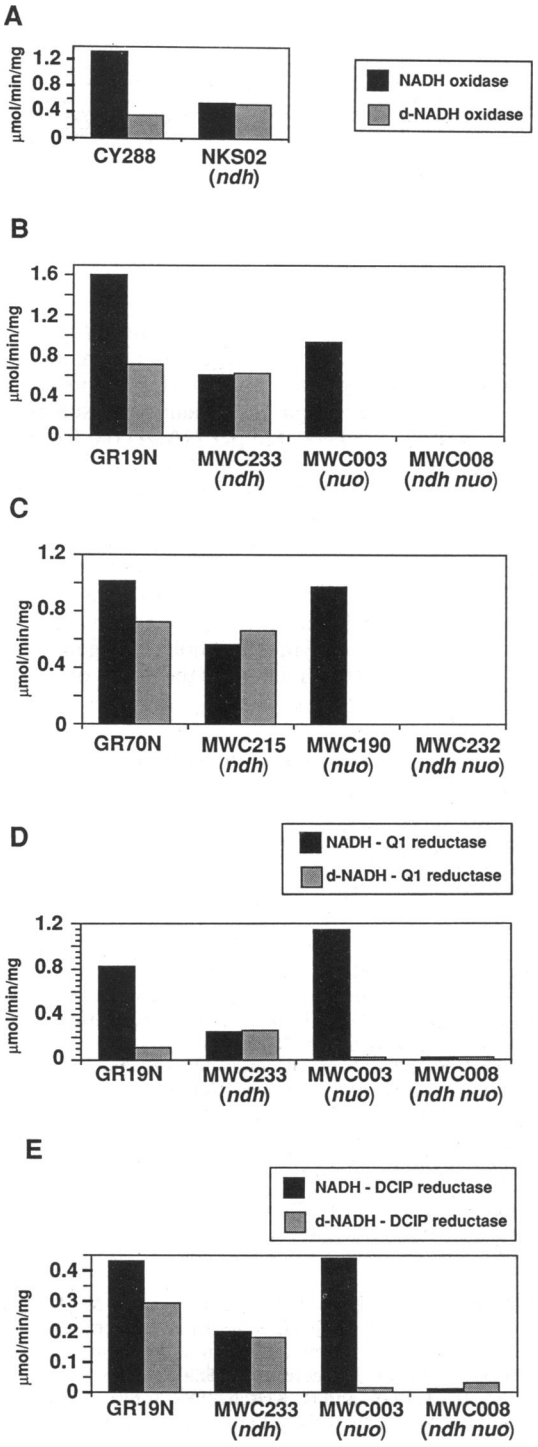

FIG. 4. Oxidase, Q1 reductase, and DCIP reductase activities of wild-type and mutant strains with NADH or deamino-NADH (d-NADH) as substrates. Methods of measurement are outlined in Materials and Methods. (A) NADH and deamino-NADH oxidase activities of the isogenic strains CY288 and NKS02 (*ndh*::Km^r). (B) NADH and deamino-NADH oxidase activities of the isogenic strains GR19N, MWC233 (*ndh*::Km^r), MWC003 (*nuo*), and MWC008 (*ndh*::Km^r *nuo*). All strains in this set lack the cytochrome *bd* oxidase (*cyd*). (C) NADH and deamino-NADH oxidase activities of the isogenic strains GR70N, MWC215 (*ndh*::Cm^r), MWC190 (*nuo*), and MWC232 (*ndh*::Cm^r *nuo*). (D) NADH:Q1 and deamino-NADH:Q1 reductase activities of the isogenic strains GR19N, MWC233 (*ndh*::Km^r), MWC003 (*nuo*), and MWC008 (*ndh*::Km^r *nuo*). All strains in this set lack the cytochrome *bd* oxidase (*cyd*). (E) NADH:DCIP and deamino-NADH:DCIP reductase activities of the isogenic strains GR19N, MWC233 (*ndh*::Km^r), MWC003 (*nuo*), and MWC008 (*ndh*::Km^r *nuo*). All strains in this set lack the cytochrome *bd* oxidase (*cyd*).

TABLE 2. Bacteriophage P1 cotransduction frequencies of Tn*10* markers with restoration of growth on mannitol minimal medium to strain IY12

Strain name	Tn*10*	Min	Cotransduction frequency (%)
CAG12179	*mgl-500*	45.75	0
CAG12098	*zeg-722*	46.50	0
CAG12177	*zeh-298*	47.75	0
CAG12178	*zei-723*	48.50	16
CAG18484	*zej-223*	49.50	69
CAG18483	*fadL771*	50.50	0
CAG18467	*zfb-1*	51.00	0
CAG18468	*nupC-510*	51.75	0

that this strain contains membrane-bound NADH dehydrogenase which can utilize NADH and deamino-NADH with equal efficiency. Strain TN49-102 cannot oxidize either substrate.

The mutation (*nuo*) from strain TN49-102 was transferred by P1 transduction into strain GR19N by selecting for the genetically linked Tn*10*. Several transductants were selected, and activity assays were performed. Resultant colonies grew equally well but were of two types: those which retained wild-type NADH and deamino-NADH oxidase activities (e.g., strain MWC007) and those which showed NADH oxidase activity but lacked deamino-NADH oxidase activity (e.g., strain MWC003). Strains which lack deamino-NADH oxidase activity are strains in which the mutation affecting the dehydrogenase cotransduced with the Tn*10* marker.

The *ndh*::Km^r mutation was combined with the mutation at min 49 (*nuo*) from strain IY12 by transducing strain MWC003 with a P1 lysate from strain NKS02. The resulting Km^r Tc^r transductants (e.g., strain MWC008) do not grow on mannitol minimal medium and grow poorly on rich medium. Membranes from these strains are unable to oxidize NADH or deamino-NADH (Fig. 4). These characteristics are identical to those of IY12, the original NADH dehydrogenase-defective strain (26, 28). These results show that mutations at two sites, one in *ndh* and one near min 49 (*nuo*), are sufficient to remove the NADH oxidase activity from *E. coli* membranes.

To confirm that these results were independent of strain background, particularly of the mutation in *cyd*, the gene encoding the *bd*-type terminal oxidase, analogous strains (GR70N, MWC190, MWC215, and MWC232) were constructed in a different wild-type strain background with the *ndh*::Cm^r mutation. Strain MWC169 was the P1 donor for *ndh*::Cm^r and strain TN49-102 was the donor for *zej-223*::Tn*10* and *nuo*. The results from activity assays of these strains were consistent with those measured for the *cyd* strains (Fig. 4C).

DISCUSSION

The data presented in this work demonstrate that the two biochemically distinct NADH: ubiquinone oxidoreductase

activities present in aerobically grown *E. coli* arise from distinct proteins encoded by more than one genetic locus. The results of this study suggest that the NADH dhII activity is the product of the *ndh* gene, as previously proposed (15). Additionally, the NADH dhI activity described by Matsushita et al. (15) is associated with the *nuo* locus.

The absence of either enzyme produces no obvious growth defects. The growth rates of the mutant strains in rich medium are indistinguishable from the growth rate of the wild-type parent. However, simultaneous mutations in both enzymes are deleterious to cell growth. Such strains form smaller colonies than does the wild-type strain, and their doubling times are longer than that of the wild-type parent.

Strains containing either one or both of the NADH dehydrogenases grow well on mannitol minimal medium, as expected of strains that contain NADH:ubiquinone oxidoreductase activity. Strains containing mutations in both NADH dehydrogenases are unable to grow with mannitol as the sole carbon source because of the deficiency in NADH oxidase activity. The work presented here demonstrates that the mutant strain reported by Young et al. (26, 28) contains two mutations which affect the NADH dehydrogenase activity. This double mutation was necessitated by the selection used to identify mutants in NADH dehydrogenase (28), since the data presented in the current work demonstrate that either enzyme alone is sufficient to support growth with mannitol as the sole carbon source.

This work confirms that the *ndh* gene is located at approximately min 22 of the *E. coli* linkage map, as previously identified (28) and supported by other work from this laboratory (5). In addition, a second genetic locus (*nuo*) encoding a membrane-bound NADH dehydrogenase has been located at min 49 of the *E. coli* chromosome. Weidner et al. have recently reported the cloning and partial DNA sequence of an *E. coli* gene which bears a high similarity to subunits of the mitochrondrial NADH dehydrogenase (complex I) (23). With the *E. coli* lambda bank of Kohara et al. (12), it has been shown that this gene is located between min 49.2 and 49.6 of the *E. coli* chromosome (24). This suggests that the mutation identified by the current work (*nuo*) is located within the structural genes encoding this enzyme.

The role of the two NADH dehydrogenases is unclear. The major functional difference between the two activities reported by Matsushita et al. (15) is the correlation of a coupling site with the NADH dhI activity but not with the NADH dhII activity. This suggests that electron flow through NADH dhII (the *nuo* gene product) will conserve more energy per NADH than will flow through NADH dhII (the *ndh* gene product). Therefore, *E. coli* may be able to regulate the energy recovered from NADH oxidation by controlling the relative levels of the two NADH dehydrogenases. This idea is supported by studies of the regulation of the *ndh* gene which show that transcription of the *ndh* gene decreases as the cells approach the late-exponential-growth phase (21). The regulation of *nuo* awaits further investigation.

The branched NADH dehydrogenase segment of the respiratory chain is reminiscent of the branched terminal oxidase segment of the aerobic respiratory chain (Fig. 5). The oxidase composition of *E. coli* membranes changes with variations in oxygen tension. The *bo*-type oxidase complex is expressed when oxygen tension in the medium is high, while the *bd*-type oxidase complex is expressed under low oxygen tension. In addition, the *bo*-type oxidase has been shown to function as a proton pump, creating a larger proton

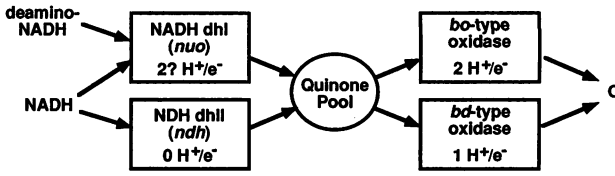

FIG. 5. Diagram of the *E. coli* respiratory chain depicting the branched electron pathways and measured or proposed H^+/e^- ratios (6).

motive force ($H^+/e^- = 2$) than does the *bd*-type oxidase complex ($H^+/e^- = 1$). Simultaneous alterations in the levels of NADH dehydrogenases and of the terminal oxidases would allow the cell to tune the oxidation of substrates to a wide range of H^+ translocated per NADH oxidized. Studies of the relative bioenergetic efficiencies of these enzymes in vivo are consistent with in vitro results (6).

Identification of an NADH dehydrogenase in *E. coli* which is genetically distinct from *ndh* provides the impetus for many future experiments. This second NADH dehydrogenase is of particular interest because of its similarities to its counterpart in mitochondria (23). The relative ease of manipulation of *E. coli* enzymes by molecular genetics makes NADH dhI an attractive model for the study of its mammalian counterpart.

ACKNOWLEDGMENTS

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