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The *udhA* Gene of *Escherichia coli* Encodes a Soluble Pyridine Nucleotide Transhydrogenase

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The *udhA* gene of *Escherichia coli* was cloned and expressed in *E. coli* and found to encode an enzyme with soluble pyridine nucleotide transhydrogenase activity. The N-terminal end of the enzyme contains the fingerprint motif of a dinucleotide binding domain, not present in published *E. coli* genome sequences due to a sequencing error. *E. coli* is hereby the first organism reported to possess both a soluble and a membrane-bound pyridine nucleotide transhydrogenase.

Pyridine nucleotide transhydrogenases catalyze the reversible transfer of reducing equivalents between NAD and NADP pools according to the following equation:



Based on the stereospecificity of the transfer, two groups of transhydrogenases have been defined. The AB transhydrogenases specifically transfer the 4A hydrogen of the nicotinamide ring of NADH to NADP⁺ and the 4B hydrogen from NADPH to NAD⁺, while the BB transhydrogenases transfer the 4B hydrogen from both NADPH and NADH (16). The AB transhydrogenases are found in mitochondria and in some bacteria, such as *Escherichia coli* (6). They are integral membrane proteins bound to the inner mitochondrial membrane or the bacterial membrane where they couple the transfer of hydride from NADH to NADP⁺ with proton import (12). The genes encoding these enzymes have been cloned from numerous sources (1, 4, 27), facilitating the study of these membrane-bound transhydrogenases, and their physiological role is assumed to be the generation of NADPH, which can be used for reductive biosynthetic reactions.

The BB transhydrogenases are structurally unrelated to the AB transhydrogenases and have been found in *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Azotobacter vinelandii* (17). They are soluble flavoproteins containing flavin adenine dinucleotide (FAD) and are remarkable for their formation of large polymers. The enzyme from *P. aeruginosa* has a minimal active form of approximately 1.6 × 10⁶ Da (23), probably composed of four stacked rings of seven or eight monomers (23), and upon isolation filaments exceeding 500 nm in length were observed (13). The *P. fluorescens* and *A. vinelandii* enzymes also form polymers (7, 21); however, the structure of the *A. vinelandii* polymers appears to be different from that of the *P. aeruginosa* enzyme (22). These soluble transhydrogenases (STH) also display interesting kinetic behavior, with NADPH and 2'-AMP strongly activating the enzyme and NADP⁺ inhibiting its activity (24). Ca²⁺ is found to increase the activation and decrease the inhibition effect. The inhibition of STH by NADP⁺ suggests that its physiological role is the

conversion of NADPH, generated by peripheral catabolic pathways, to NADH, which can enter the respiratory chain for energy generation (20).

The first soluble pyridine nucleotide transhydrogenase gene (*sth*) cloned was from *P. fluorescens* and revealed that the enzyme was related to the family of flavoprotein disulfide oxidoreductases (7). This family of enzymes includes the well-characterized dihydrolipoamide dehydrogenase, glutathione reductase, and mercuric reductase. These enzymes are active as homodimers and possess a characteristic redox-active disulfide bond. The subunits of these enzymes consist of an N-terminal FAD binding domain, a central NAD(P) binding domain, and a C-terminal dimerization domain (26). However, one of the cysteines involved in the redox-active disulfide bond characteristic of this family of enzymes was lacking in the *P. fluorescens sth* sequence. The *sth* sequence was found to be most similar to an *E. coli* gene of unknown function (*udhA*), showing 60% sequence identity and 77% similarity (7).

In order to investigate the identity of *udhA* from *E. coli*, and because no transhydrogenase activity could be detected in extracts of *E. coli* grown on rich medium under standard conditions, we cloned and overexpressed this gene in order to study its product.

Cloning and sequence analysis of *udhA* from *E. coli*. Based on the published *E. coli* genome sequence (GenBank no. U00006), oligonucleotides were designed in order to amplify by PCR the *udhA* gene from *E. coli* JM109 cells. The primers 5'-AGGGATCCAATAAAAACGTCAGGGC-3' and 5'-CCATCGATGGGGTTGTTTATCTGC-3' (with restriction sites underlined), annealing at positions approximately 150 bp upstream and downstream, respectively, of the potential structural gene, were used. PCR was performed with 30 s of denaturing (94°C), 30 s of annealing (55°C), and 90 s of polymerization (72°C) for 30 cycles, with an additional 90 s of denaturing prior to the first cycle (polymerase added after the 90 s) and 3 min of polymerization after the last cycle. The 1.6-kb PCR product was digested with *Bam*HI and *Cla*I and cloned into the multiple cloning site of pBluescript SK(+) (no. 52325; Stratagene). The resulting construct was designated pUDHA1 and the insert was sequenced in both orientations. The *udhA* sequence and the deduced amino acid sequence are given in Fig. 1. An open reading frame encoding a protein of 466 amino acids (including the initiating Met) was identified, with *E. coli*-like -35 and -10 promoter sequences and a Shine-Dalgarno sequence upstream of the initiating ATG (Fig. 1). The molecular mass of the protein excluding the initiating methionine was determined by the Genetics Computer Group PEPTIDESORT program

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<i>Bam</i> HI			
GGATCCAATA	AAACGTCAGG	GCAAAAGTAA	GAACAGACA AAGCAAAGGC -104
CGCTCAGGAT	ATAGCCAGAT	AAATGACGGG	GATCAATTGG CTTACCCGGG -54
ATAAAATGTT	ACCAATTCGT	TGCTTTTATG	TATAAGAACA GGTAAGCCCT -4
ACCATGCCAC	ATTCCTACGA	TTACGATGCC	ATAGTAATAG GTTCCGGCCC +47
M P H S Y D	Y D A	I V I	G S G P
CGGCGCGAA	GGCGCTGCAA	TGGGCTGGT	TAAGCAAGGT GTGCGCGTCG +97
G G E G A A	M G L V	K Q G	V R V
CAGTTATCGA	GCCTTATCAA	AATGTTGGC	GCGGTTGCAC CCACTGGGGC +147
A V I E R Y Q	N V G	G G C T	H W G
ACCATCCCGT	CGAAAGCTCT	CGTCCAGCC	GTCAGCCGCA TTATAGAATT +197
T I P S K A L	R H A	V S R	I I E F
CAATCAAAC	CCACTTTACA	GCGACCATTC	CGACTGCTC CGCTCTTCTT +247
N Q N P L Y	S D H S	R L L	R S S
TTGCCGATAT	CCTTAACCAT	GCCGATAACG	TGATTAAATCA ACAAACGCGC +297
F A D I L N H A	A D N V	I N Q Q T R	
ATGCGTCAGG	GATTTTACGA	ACGTAATCAC	TGTGAAATAT TGCAGGAAA +347
M R Q G F Y E	R N H	C E I	L Q G N
CGCTCGCTTT	GTTGACGAGC	ATACGTTGGC	GCTGGATTGC CCGGACGGCA +397
A R F V D E	H T L A	L D C	P D G
GCGTTGAAAC	ACTAACCGCT	GAAAATTTG	TTATTGCCCTG CCGCTCTCGT +447
S V E T L T A	E K F	V I A C	G S R
CCATATCATC	CAACAGATGT	TGATTTACC	CATCCACGCA TTTACGACAG +497
P Y H P T D V	D F T	H P R	I Y D S
GCACTCAATT	CTCAGCATGC	ACCAGCAACC	GCGCCATGTA CTTATCTATG +547
D S I L S M	H H E P	R H V	L I Y
GTGCTGGAGT	GATCGGCTGT	GAATATGCGT	CGATCTTCCG CCGTATGGAT +597
G A G V I G C	E Y A	S I F R	G M D
GTAAAGTGG	ATCTGATCAA	CACCCGCGAT	CGCCTGTTGG CATTTCTCGA +647
V K V D L I N	T R D	R L L	A F L D
TCAAGAGATG	TCAGATTCTC	TCTCCTATCA	CTTCTGGAAC AGTGGCGTAG +697
Q E M S D S	L S Y H	F W N	S G V
TGATTCTGTC	CAACGAAGAG	TACGAGAAGA	TCGAAGGCTG TGACGATGGT +747
V I R H N E E	Y E K	I E G C	D D G
GTGATCATGC	ATCTGAAAGTC	GGGTAAAAAA	CTGAAAGCTG ACTGCCTGCT +797
V I M H L K S	G K K	L K A	D C L L
CTATGCCAAC	GGTCGCACCG	GTAATACCGA	TTCGCTGGCG TTACAGAACA +847
Y A N G R T	G N T D	S L A	L Q N
TTGGGCTAGA	AACTGACAGC	CGCGGACAGC	TGAAGGTCAA CAGCATGTAT +897
I G L E T D S	R G Q	L K V N	S M Y
CAGACCGCAC	AGCCACACGT	TTACGCGGTG	GGCGACGTGA TTGGTATCC +947
Q T A Q P H V	Y A V	G D V	I G Y P
GAGCTGGCG	TCGGCGGCCCT	ATGACCAGGG	GCGCATTGGC GCGCAGGGCG +997
S L A S A A	Y D Q G	R I A	A Q A
TGGTAAAGG	CGAAGCCACC	GCACATCTGA	TTGAAGATAT CCCTACCGGT +1047
L V K G E A T	A H L	I E D I	P T G
ATTTACACCA	TCCCGGAAAT	CAGCTCTGTG	GGCAAAACCG AACAGCAGCT +1097
I Y T I P E I	S S V	G K T	E Q Q L
GACCGCAATG	AAAGTGCCAT	ATGAAGTGGG	CCGCGCCAGC TTAAACATC +1147
T A M K V P	Y E V G	R A Q	F K H
TGGCACRGA	ACAAATCGTC	GGCATGAACG	TGGGCAGCT GAAAATTTG +1197
L A R A Q I V	G M N V	G T L	K I L
TTCCATCGGG	AAACAAAAGA	GATTCTGGGT	ATTCAGTCTG TTGGCGAGCG +1247
F H R E T K E	I L G	I H C	F G E R
CGCTGCCGAA	ATTATTCATA	TCGGTCAGGC	GATTATGGAA CAGAAAAGTG +1297
A A E I I H	I G Q A	I M E	Q K G
GCGCAACAC	TATTGAGTAC	TTCGTCAACA	CCACCTTTAA CTACCCGACG +1347
G G N T I E Y	F V N	T T F N	Y P T
ATGGCGGAAG	CCTATCGGGT	AGTCGCGTTA	AACGGTTTAA ACCGCGCTGT +1397
M A E A Y R V	A A L N G L	N R L F	
TTAAAACCTT	ATCGAAATGG	CCATCCATTC	TTGCGGGAT GGCCTCTGCC +1447
*			
AGCTGCTCAT	AGCGGCTCGC	CAGCGGTGAG	CCAGGACGAT AAACCAGGCC +1497
AATAGTGGCG	CGTGGTCCG	GCTTAATGCA	CGGCAGATAA ACAACCCCAT +1547
CGAT +1551			
Clal			

FIG. 1. Sequence of *udhA* and deduced amino acid sequence of STH. Nucleotides are numbered, with 1 being the A of the initiating ATG. Restriction sites indicate the insertion sites in pBluescript SK(+). Putative promoter (-35 and -10) and terminator regions are indicated. *rbs*, ribosome binding site.

(8) to be 51,457 Da. The sequence differed from previously published genomic sequences (GenBank no. U00006, X66026, and AE000470) in having a 1-base deletion (a C) after nucleotide 40 (Fig. 1). This extra base in previously published sequences had given rise to a deduced protein sequence (P27306) which started at the methionine 22 amino acids downstream of the methionine presented in this study to be the start of the protein (see below). The N-terminal part of the protein missing in previously deduced sequences contains the amino acid sequence Gly-X-Gly-X-X-Gly/Ala, which is characteristic for a dinucleotide binding domain (Rossman fold). Homology with the disulfide pyridine nucleotide oxidoreductases (see Fig. 4) suggests that this is the FAD binding domain. The protein was found to be 59% identical and 77% similar to *P. fluorescens* STH.

Expression of recombinant STH in *E. coli*. Crude extracts were prepared from JM109/pUDHA1, grown to saturation in SOB medium (Difco) at 37°C, and assessed for STH activity. STH activity was assayed by monitoring the reduction of thio-nicotinamide adenine dinucleotide (tNAD⁺) at 400 nm in a reaction mixture consisting of 0.1 mM NADPH and 0.1 mM tNAD⁺ (Sigma Chemical Co.) in 50 mM Tris-HCl buffer (pH 7.0) at 30°C. The molar extinction coefficient of tNADH at 400 nm was taken as 11,300 liters mol⁻¹ cm⁻¹ (5). One unit of enzyme activity was defined as the amount of activity reducing 1 μmol of tNAD⁺ per min under these conditions. Protein

concentration was measured by using a reagent from Pierce (Rockford, Ill.). Control extracts of JM109 grown under the same conditions did not have measurable STH activity (lowest detectable activity, approximately 0.001 U/mg) while extracts of the recombinant cells, JM109/pUDHA1, possessed about 2.9 U of STH activity per mg. This result implies that the *udhA* gene of *E. coli* encodes an enzyme with STH activity, and its putative protein was therefore designated STH.

Purification and characterization of recombinant *E. coli* STH. The recombinant STH was purified to apparent homogeneity in a single affinity chromatography step by using adenosine-2',5'-diphosphate agarose. The protocol used for the purification of *P. fluorescens* STH was applied except that the 0.7 M NaCl wash step was replaced by 0.5 M NaCl (7). A total of 1,600 U was loaded onto a column with a 6-ml packed volume in the presence of 5 mM CaCl₂. The most active eluted fractions, totalling 9.6 ml, were pooled and dialyzed against 50 mM Tris-HCl buffer (pH 7.0) with 5 mM dithiothreitol (DTT) in order to remove salts and reduce the pH. The product contained 1,150 U of STH at a specific activity of 85 U/mg. The specific activity of the purified *E. coli* STH is about 3.5-fold lower than that of the *P. fluorescens* STH under standard assay conditions (7). An expression level of about 3.5% of total soluble protein was estimated. The protein appeared to be homogeneous, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2), and the sub-

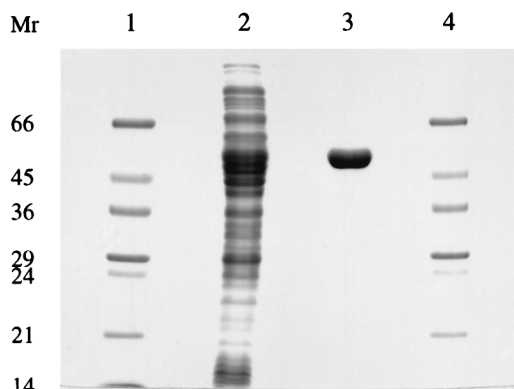


FIG. 2. SDS-PAGE showing purification of STH from *E. coli* JM109/pUDHA1. Lanes 1 and 4, M_r markers; lane 2, crude extract (20 μ g); lane 3, purified STH (5 μ g). Numbers on the left are in thousands.

unit M_r determined from SDS-PAGE correlates well with the calculated size and with that reported for *P. fluorescens*, *P. aeruginosa*, and *A. vinelandii* STHs (7, 17, 23). The N-terminal sequence of the recombinant enzyme was determined by automated Edman degradation and was found to be P-H-S-Y-D-Y-D-A-I-V-I-G-S-G-P-G-G-E-(R/G)-A-A-M-G-L-V-K. This is consistent with the deduced amino acid sequence excluding the initiating methionine and confirmed the translation start of the *udhA* gene and the presence of a Rossman fold fingerprint motif at the N terminus.

The UV-visible absorption spectrum of the purified STH (Fig. 3) has a peak at 444 nm with a shoulder at 472 nm, and it is characteristic of an oxidized flavoprotein (15). When the enzyme was boiled and the denatured protein was removed by centrifugation, the supernatant retained the yellow coloration and showed the visible absorption spectrum of a free flavin. The flavin liberated in this way was subjected to thin-layer chromatography together with the flavin standards riboflavin, flavin mononucleotide, and FAD. The flavin liberated from STH comigrated with FAD and not with riboflavin or flavin mononucleotide in two different thin-layer chromatography

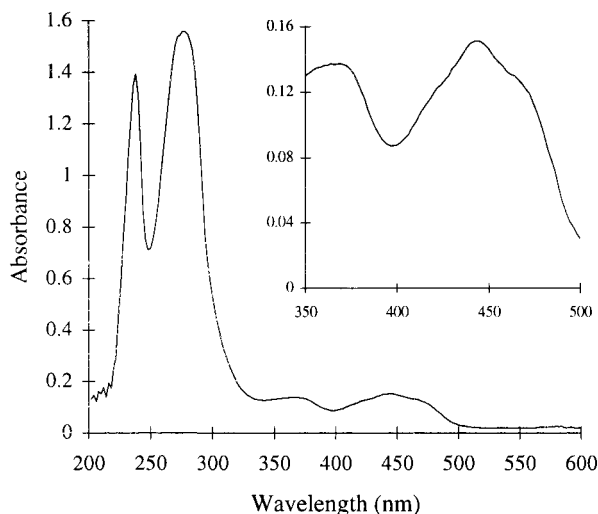


FIG. 3. UV-visible spectrum of STH. The spectrum of purified *E. coli* STH (1.0 mg/ml) in 50 mM Tris-HCl buffer (pH 7.0), with 5 mM DTT and 10% glycerol, was measured against a blank consisting of the same buffer. The inset shows an enlargement of the region characteristic of an oxidized flavoprotein.

systems (system I, 3% [wt/vol] $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ in distilled H_2O [d H_2O]; system II, *n*-butanol-d H_2O -acetic acid-methanol [14:14:1:6]). This suggests that *E. coli* STH contains the noncovalently bound prosthetic group FAD. This is also consistent with earlier reports of STH from *P. aeruginosa* and *A. vinelandii* (17). Assuming the extinction coefficient of FAD given by Siegel (18), the absorbance spectrum suggests a flavin content of 0.76 mol of FAD per mol of STH subunit.

In order to determine whether the *E. coli* STH was capable of forming large polymers, as found for the *Pseudomonas* (7, 13) and *Azotobacter* (22) STHs, samples were adsorbed to glow-discharged carbon Formar films from a 1.0-mg/ml solution, negatively stained with 1% (wt/vol) uranyl acetate, and examined by transmission electron microscopy with a Philips CM100 electron microscope operated at 80 kV. These electron microscopy studies showed that the *E. coli* enzyme differs from the other STHs in that it does not form filaments under these conditions (data not shown).

In order to get an estimate of the native size of the *E. coli* STH, gel filtration on Superose 6 10/30 (Pharmacia) was performed. The column was equilibrated with 50 mM Tris-HCl (pH 7.0) containing 100 mM NaCl and 5 mM DTT, and the STH was dialyzed against the same buffer prior to application. The column was calibrated with appropriate standards (thyroglobulin, ferritin, catalase, aldolase, albumin, and ovalbumin [from Pharmacia Biotech]; 200 μ l of 1- to 5-mg/ml solutions), and STH (200 μ l at a concentration of 1.0 mg/ml) was loaded, all at a flow rate of 0.2 ml/min. STH was eluted as a broad peak with an average molecular mass (\pm standard error) of 386 ± 31 kDa and a shoulder at the monomer size, 51.6 ± 3.5 kDa (data not shown). This shows that *E. coli* STH does form multimeric structures; however, the enzyme is different from the *Pseudomonas* and *Azotobacter* STHs as it is devoid of large polymers. The average molecular weight suggests that the *E. coli* STH is present in a form consisting of seven or eight monomers. As the *A. vinelandii* STH is thought to have a minimal active form consisting of eight subunits (22), this could suggest that these two STHs have a similar subunit arrangement.

Sequence comparisons. The deduced amino acid sequence of the *E. coli* STH was compared to other sequences in protein databases by using the BLAST 2 service of the National Center for Biotechnology Information (NCBI) accessed at <http://www.bio.cam.ac.uk> (Fig. 4). Various dihydrolipoamide dehydrogenases showed up to 27% identity and 45% similarity to the *E. coli* STH. The purified *E. coli* STH did not show significant activity with lipoamide (<0.1 U/mg) in an assay system consisting of 0.2 mM lipoamide and 0.2 mM NADH or NADPH in 50 mM Tris-HCl (pH 7.0) at 30°C. Under the same conditions, dihydrolipoamide dehydrogenase from porcine heart (Sigma) displayed very high activity with NADH (35 U/mg) but no significant activity with NADPH (<0.3 U/mg). Dihydrolipoamide dehydrogenase did not display significant STH activity (<0.1 U/mg). Lack of dihydrolipoamide dehydrogenase activity is consistent with earlier reports on STH (5, 7).

In contrast to the flavoprotein disulfide oxidoreductases (7), both the *E. coli* and *P. fluorescens* STHs have a threonine at the position of one of the redox-active cysteines characteristic of this family (amino acids 50 and 49, respectively). The cysteine residue missing in the STH sequences is directly involved in the electron transfer between the nonnicotinamide substrate and the FAD in dihydrolipoamide dehydrogenase, glutathione reductase, and mercuric reductase. The conservation of the threonine residue at this position in STH suggests that this residue might be of importance in the STH reaction. The

sponse to hydrogen peroxide (2, 3). The expression of *oxyR* is maximal in the exponential phase of aerobic growth, when cells are most likely to encounter oxidative stress (9). It has been demonstrated that it is the OxyR protein itself which is sensitive to hydrogen peroxide, activating the protein through the reversible formation of a disulfide bond (3, 28). The oxidized OxyR binds to promoter elements of the genes it regulates, resulting in the onset of their expression, and the protein also represses its own synthesis (3, 19). Hoek and Rydström proposed that the mitochondrial transhydrogenase is particularly important during oxidative stress (11), and we can in this respect speculate whether the STH and the membrane-bound transhydrogenase of *E. coli* have complementing functions in the cell. A study of the wild-type expression of *udhA* in *E. coli* under various conditions will assist in understanding the physiological role of this enzyme.

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REFERENCES

1. Arklad, E. L., C. Betsholtz, and J. Rydström. 1996. The cDNA sequence of proton-pumping nicotinamide nucleotide transhydrogenase from man and mouse. *Biochim. Biophys. Acta* **1273**:203–205.
2. Christman, M. F., R. W. Morgan, F. S. Jacobsen, and B. N. Ames. 1985. Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. *Cell* **41**:753–762.
3. Christman, M. F., G. Storz, and B. N. Ames. 1989. OxyR, a positive regulator of hydrogen peroxide-inducible genes in *Escherichia coli* and *Salmonella typhimurium*, is homologous to a family of bacterial regulatory proteins. *Proc. Natl. Acad. Sci. USA* **86**:3484–3488.
4. Clarke, D. M., T. W. Loo, S. Gillam, and P. D. Bragg. 1986. Nucleotide sequence of the *pntA* and *pntB* genes encoding the pyridine nucleotide transhydrogenase of *Escherichia coli*. *Eur. J. Biochem.* **158**:647–653.
5. Cohen, P. T., and N. O. Kaplan. 1970. Purification and properties of the pyridine nucleotide transhydrogenase from *Pseudomonas aeruginosa*. *J. Biol. Chem.* **245**:2825–2836.
6. Fisher, R. R., and S. R. Earle. 1982. Membrane-bound pyridine dinucleotide transhydrogenases, p. 279–324. *In* J. Everse, B. Anderson, and K. S. You (ed.), *The pyridine nucleotide coenzymes*. Academic Press Inc., New York, N.Y.
7. French, C. E., B. Boonstra, K. A. J. Bufton, and N. C. Bruce. 1997. Cloning, sequence, and properties of the soluble pyridine nucleotide transhydrogenase of *Pseudomonas fluorescens*. *J. Bacteriol.* **179**:2761–2765.
8. Genetics Computer Group. 1997. Program manual for the Wisconsin package. Version 9.1. September 1997. Genetics Computer Group, Madison, Wis.
9. Gonzalez-Flecha, B., and B. Demple. 1997. Transcriptional regulation of the *Escherichia coli oxyR* gene as a function of cell growth. *J. Bacteriol.* **179**:6181–6186.
10. Gustafsson, C., and S. R. Warne. 1992. Physical map of the *oxyR-trmA* region (minute 89.3) of the *Escherichia coli* chromosome. *J. Bacteriol.* **174**:7878–7879.
11. Hoek, J. B., and J. Rydström. 1988. Physiological roles of nicotinamide nucleotide transhydrogenase. *Biochem. J.* **254**:1–10.
12. Jackson, J. B., N. P. J. Cotton, R. Williams, T. Bizouarn, M. N. Hutton, L. A. Sazanov, and C. M. Thomas. 1993. Proton-translocating transhydrogenase in bacteria. *Biochem. Soc. Trans.* **21**:1010–1013.
13. Louie, D. D., N. O. Kaplan, and J. D. McLean. 1972. Allosteric effect of 2'-adenylic acid on the *Pseudomonas* pyridine nucleotide transhydrogenase. *J. Mol. Biol.* **70**:651–664.
14. McKie, J. H., and K. T. Douglas. 1991. Evidence for gene duplication forming similar binding folds for NAD(P)H and FAD in pyridine nucleotide-dependent flavoenzymes. *FEBS Lett.* **279**:5–8.
15. Palfey, B. A., and V. Massey. 1998. Flavin-dependent enzymes, p. 81–153. *In* M. Sinnott (ed.), *Radical reactions and oxidation/reduction*, vol. III. Academic Press Limited, London, United Kingdom.
16. Rydström, J., J. B. Hoek, and L. Ernster. 1976. Nicotinamide nucleotide transhydrogenases, p. 51–89. *In* P. D. Boyer (ed.), *The enzymes*, 3rd ed., vol. 13. Academic Press, New York, N.Y.
17. Rydström, J., B. Persson, and E. Carlenor. 1987. Transhydrogenases linked to pyridine nucleotides, p. 433–461. *In* D. Dolphin, O. Aramovic, and R. Poulson (ed.), *Pyridine nucleotide coenzymes: chemical, biochemical and medical aspects*, part B. John Wiley & Sons, New York, N.Y.
18. Siegel, L. M. 1978. Quantitative determination of noncovalently bound flavins: types and methods of analysis. *Methods Enzymol.* **53**:419–429.
19. Toledano, M. B., I. Kullik, F. Trinh, P. T. Baird, T. D. Schneider, and G. Storz. 1994. Redox-dependent shift of OxyR-DNA contacts along an extended DNA-binding site: a mechanism for differential promoter selection. *Cell* **78**:897–909.
20. Voordouw, G., S. M. van der Vies, and A. P. N. Themmen. 1983. Why are two different types of pyridine nucleotide transhydrogenase found in living organisms? *Eur. J. Biochem.* **131**:527–533.
21. Voordouw, G., C. Veeger, J. F. L. van Breemen, and E. F. J. van Bruggen. 1979. Structure of pyridine nucleotide transhydrogenase from *Azotobacter vinelandii*. *Eur. J. Biochem.* **98**:447–454.
22. Voordouw, G., S. M. van der Vies, J. K. Eweg, C. Veeger, J. F. van Breemen, and E. F. van Bruggen. 1980. Pyridine nucleotide transhydrogenase from *Azotobacter vinelandii*: improved purification, physical properties and subunit arrangement in purified polymers. *Eur. J. Biochem.* **111**:347–355.
23. Wermuth, B., and N. O. Kaplan. 1976. Pyridine nucleotide transhydrogenase from *Pseudomonas aeruginosa*: purification by affinity chromatography and physicochemical properties. *Arch. Biochem. Biophys.* **176**:136–143.
24. Widmer, F., and N. O. Kaplan. 1977. *Pseudomonas aeruginosa* transhydrogenase: affinity of substrates for the regulatory site and possible hysteretic behavior. *Biochem. Biophys. Res. Commun.* **76**:1287–1292.
25. Wierenga, R. K., M. C. H. Demeyer, and W. G. J. Hol. 1985. Interaction of pyrophosphate moieties with alpha-helices in dinucleotide binding-proteins. *Biochemistry* **24**:1346–1357.
26. Williams, C. H. 1992. Lipoamide dehydrogenase, glutathione reductase, thioredoxin reductase, and mercuric ion reductase—a family of flavoenzyme transhydrogenases, p. 121–211. *In* F. Müller (ed.), *Chemistry and biochemistry of flavoenzymes*, vol. III. CRC Press Inc., Boca Raton, Fla.
27. Williams, R., N. P. J. Cotton, C. M. Thomas, and J. B. Jackson. 1994. Cloning and sequencing of the genes for the proton-translocating nicotinamide nucleotide transhydrogenase from *Rhodospirillum rubrum* and the implications for the domain-structure of the enzyme. *Microbiology—UK* **140**:1595–1604.
28. Zheng, M., F. Aslund, and G. Storz. 1998. Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science* **279**:1718–1721.