Cloning and Sequencing of the Genes from Salmonella typhimurium Encoding a New Bacterial Ribonucleotide Reductase

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A plasmid library of Salmonella typhimurium was used to complement a temperature-sensitive nrdA mutant of Escherichia coli. Complementation was obtained with two different classes of plasmids, one carrying the E. coli nrdAB-like genes and the second containing an operon encoding a new bacterial ribonucleotide reductase. Plasmids harboring these new reductase genes also enable obligately anaerobic nrdB::Mud1 E. coli mutants to grow in the presence of oxygen. This operon consists of two open reading frames, which have been designated nrdE (2,145 bp) and nrdF (969 bp). The deduced amino acid sequences of the nrdE and nrdF products include the catalytically important residues conserved in ribonucleotide reductase enzymes of class I and show 25 and 28% overall identity with the R1 and R2 proteins, respectively, of the aerobic ribonucleoside diphosphate reductase of E. coli. The 3' end of the sequenced 4.9-kb fragment corresponds to the upstream region of the previously published proU operon of both S. typhimurium and E. coli, indicating that the nrdEF genes are at 57 min on the chromosomal maps of these two bacterial species. Analysis of the nrdEF and proU sequences demonstrates that transcription of the nrdEF genes is in the clockwise direction on the S. typhimurium and E. coli maps.

In both eukaryotic and prokaryotic cells, the biosynthesis of deoxyribonucleotides (dNTPs) from the corresponding ribonucleotides is catalyzed by ribonucleotide reductases (RR), with the exception of dTTP, whose synthesis is derived from dCTP and dUDP. RR plays a crucial role in the balanced supply of DNA precursors. Imbalances of the dNTPs have been associated with different genetic effects, besides modifying the sensitivity of cells to DNA-damaging agents (26). Three classes of RR have been described so far (for a review, see reference 35). Class I enzymes are aerobic and exist in higher organisms and in some prokaryotes. Escherichia coli ribonucleoside diphosphate reductase (RDP reductase) is the best known of this group. The *nrdA* and *nrdB* genes encode the α and β polypeptide chains, which form the R1 (α_2) and R2 (β_2) subunits of the RDP reductase. The E. coli RDP enzyme contains a stable radical located at Tyr-122 as an essential component for catalysis (27). Class II enzymes, present in many prokaryotes, employ adenosyl cobalamin as a radical generator (3). Finally, the only enzyme of class III identified is the anaerobic RTP reductase of E. coli, encoded by the nrdD gene (39). This enzyme requires S-adenosylmethionine, which probably is a glycine radical generator (for a review, see reference 36).

The *nrdAB* genes of *E. coli* have been proposed as genes of vital importance because for many years only conditionallethal *nrdAB* mutants were found (34, 40). Some years ago, it was shown that null *nrdB* mutants, obtained as a consequence of a Mud1 insertion, are viable in the absence of oxygen (21), since under these conditions, an anaerobic RTP reductase is active (13). In this respect, *E. coli* cells growing under either fermentative or nitrate-respiring conditions have a lower basal level of *nrdAB* gene transcription (6). Several regions involved in either positive or negative control of the *nrdAB* genes of *E. coli* have been detected upstream of the coding region (41, 42). The activity of the *E. coli* RDP reductase is increased when DNA replication is inhibited (12, 16). Furthermore, DNA damage enhances the transcription of *nrdAB* genes (18). This increase is related to the SOS response (18).

We were interested in cloning the *nrdAB* genes of *Salmo-nella typhimurium* to determine if these genes have the same control regions as those of *E. coli*. Nevertheless, during these experiments we isolated not only the *nrdAB* genes but also an operon encoding a new RR. The DNA sequences and locations of these new RR-encoding genes and their predicted amino acid sequences are reported in this paper.

The bacterial strains and plasmids used and their relevant characteristics are shown in Table 1. E. coli and S. typhimurium strains were grown at either 30, 37, or 42°C in Luria-Bertani medium, Terrific Broth, or AB minimal medium containing 0.2% glucose and 0.4% Casamino Acids (38). Anaerobic growth was in either plates or filled flasks for liquid cultures, both being incubated in anaerobic jars with the GasPak system (Becton Dickinson). Heterologous triparental matings were done as described previously (4). Complementation of temperature sensitivity was determined by either plating samples of a liquid culture or streaking single colonies on minimal medium plates at both 30 and 42°C. Abolition by the chromosomal insert of the nrdB1 mutation, which in the KK450 strain decreases R2 activity (15), was tested by hydroxyurea resistance (34). This characteristic was determined by plating equal portions of appropriately diluted exponential-phase cultures of the KK450 strain on minimal agar plates containing hydroxyurea at 1 mg/ml. After 3 days of incubation, the presence or absence of growth in these plates was analyzed. Restriction enzyme digestions, subcloning procedures, plasmid extractions, gel electrophoresis, DNA labelling with digoxigenin, and hybridization procedures were carried out by standard methods (38). E. coli DH5 α F' cells were transformed either by electroporation (9) or by using frozen competent cells (20). Prior to DNA sequencing, a set of exonuclease III-mediated nested deletions of the isolated S. typhimurium insert was created by using the Erase-a-Base system (Promega Corporation). The DNA sequence of each one of these clones was determined by the dideoxy method with fluorescent primers and the Auto-

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Strain or plasmid	Description	Source (reference)
E. coli K-12 strains		
DH5aF'	recA1 endA1 hsdR17 supE44 thi-1 gyrA96 relA1 Δ(lacZYA-argF)U169 deoR	Laboratory stock
KK450	nrdA(Ts) nrdB1 thyA thr leu thi deo tonA lacY supE44 gyrA	B. M. Sjöberg (34)
H1491	MC4100 aroB nrdB::Mud1	K. Hantke (21)
UA4851	KK450 Riff	This study
Plasmids		-
F'143		B. Bachmann
pPS2	Tc ^r NrdAB ⁺	B. M. Sjöberg (30)
pRK404	Tc ^r Mob ⁺	G. Ditta (8)
pRK2013	Km ^r Tra ⁺ Mob ⁺	G. Ditta (8)
pBluescript $SK(+)$	Amp ^r	Laboratory stock
pUA326	pRK404 containing a 4.9-kb fragment carrying the <i>nrdEF</i> genes	This study
pUA335	pBSK containing a 4.9-kb XmaI-PstI fragment carrying the nrdEF genes	This study
pUA338	pBSK containing a 4-kb fragment by 3' exonuclease III deletion from pUA335	This study
pUA341	pBSK containing a 3.45-kb NotI-AccI fragment from pUA338, under P _{lac} control	This study
pUA343	Like pUA341 but with the 3.45-kb NotI-AccI fragment in inverted orientation	This study
pUA344	pBSK containing a 3.2-kb fragment by <i>ClaI-ClaI</i> deletion from pUA341	This study
pUA345	pBSK containing a 3.2-kb fragment by SacI-SacI deletion from pUA341	This study
pUA395	pBSK containing a 3.6-kb fragment by 3' exonuclease III deletion from pUA335	This study
pUA396	pBSK containing a 4.7-kb fragment by 5' exonuclease III deletion from pUA335	This study

TABLE 1. Bacterial strains and plasmids

matic Laser Fluorescent DNA Sequencer (Pharmacia). The entire nucleotide sequence was determined with both DNA strands. Computer analysis was carried out with the University of Wisconsin Genetics Computer Group package (version 7.2). Sequences from several RR were obtained from the Swiss-Prot data bank.

Isolation of genes encoding a new RR. A library of S. typhimurium LT2 chromosomal DNA was constructed by cloning size-fractioned, partially digested Sau3AI restriction fragments with an average size of 5 to 8 kb into the BamHI site of the broad-host-range plasmid pRK404 (8) and then was electrotransformed into E. coli DH5aF'. To isolate nrdAB genes from S. typhimurium, the library was transferred by triparental mating into the temperature-sensitive nrdA(Ts)mutant of E. coli (strain UA4851), plating exconjugants onto rifampin-, tetracycline-, and thymidine-supplemented Luria-Bertani agar plates at 42°C. Several temperature-resistant clones were observed after 2 days of incubation. Plasmid DNA of each of these exconjungants was extracted and retransformed into the KK450 strain to confirm the temperatureresistant phenotype. Restriction and Southern analysis (with a 5.5-kb EcoRI-PstI fragment of the pPS2 plasmid containing the nrdAB genes of E. coli as a probe [34]) of plasmid DNA isolated from these retransformed clones indicated the presence of two kinds of clones. One kind hybridized with the nrdAB genes from E. coli (Fig. 1A, lane 3), whereas the second did not (Fig. 1A, lane 4). Only two types of RR genes have been described for enterobacteria, i.e., the nrdAB and nrdD genes from E. coli, encoding the aerobic and anaerobic enzymes, respectively. Since the anaerobic RR does not complement nrdAB mutants under aerobic conditions, these other clones would contain the genes for a new aerobic RR. For this reason, we decided to concentrate on the smallest chromosomal insert (about 4.9 kb) of this bacterium which did not hybridize with the nrdAB genes from E. coli. The plasmid carrying this insert was designated pUA326.

The hydroxyurea hypersensitivity of the *nrdB1* mutant strain KK450 was also abolished by the chromosomal fragment contained in the pUA326 plasmid (Fig. 2). The O_2 sensitivity of the *nrdB*::Mud1 mutant of *E. coli* H1491 (21) was also suppressed by pUA326. Thus, the efficiency of plating of H1491

(pUA326) cells in the presence of oxygen was 0.75. Furthermore, Southern blot hybridization of total DNA from *E. coli* and *S. typhimurium* digested with *Eco*RI-*Pst*I and probed with a 2.8-kb *Cla*I-*Hind*III fragment of the 4.9-kb insert corroborated that the cloned fragment is present in the *S. typhimurium* chromosome as well as in the *E. coli* chromosome (Fig. 1B), although the species showed different banding patterns: the chromosomal DNA of *E. coli* exhibited a band at 11 kb (Fig.



FIG. 1. Southern hybridization of chromosomal DNA from *E. coli* (lanes 1), *S. typhimurium* (lanes 2), a pRK404 plasmid derivative containing the *nrdAB*-like genes from *S. typhimurium* (lanes 3), and plasmid pUA326 carrying the *nrdEF* genes from *S. typhimurium* (lanes 4), all doubly digested with *Eco*RI and *PstI*. DNA was hybridized with either the 5.5-kb *Eco*RI-*PstI* fragment of the pPS2 plasmid carrying the *nrdAB* genes from *E. coli* (A) or the 2.7-kb *ClaI-HindIII* internal fragment of the *nrdEF* genes from *S. typhimurium* contained in the pUA326 plasmid (B). Lane 5 of panel B is a *HindIII* digest of digoxigenin-labelled lambda DNA as a molecular weight marker. Numbers on the right are sizes in kilobases.

		Complementation		
Acci EcoRi Acci Hincii BstXi Ndei Hindii Ndei	Plasmid	nrdA(ts)	nrdB1	
nrdE nrdF proV	pUA326	0.67	0.3	
	pUA335	0.93	0.32	
	pUA396	2 .1x10 ⁻⁶	<1x10 ⁻⁷	
	pUA338	0.70	0.38	
C	pUA395	2x10 ⁻⁵	<5.5x10 ⁻⁸	
Acci	pUA343	1.5x10 ⁻⁶	4.5x10 ⁻⁷	
P _{lac}	pUA341	0.10	0.015	
P _{lac} soci	pUA345	0.13	0.015	
Plac	pUA344	0.11	0.020	
1.14	pBSK	<4.1x10 ⁻⁹	1.25x10 ⁻⁸	
<u>⊢1 KD</u>	F'143	1	ND	

FIG. 2. Physical map and complementation analysis of the inserts contained in plasmid pUA326 and their derivatives, obtained by either subcloning or nested deletions of the original 4.9-kb fragment of the *S. typhimurium* chromosome abolishing temperature sensitivity of the KK450 mutant of *E. coli*. P_{lac} denotes plasmids at which the *lac* promoter has been inserted immediately upstream of the chromosomal fragment. The complementation of the *nrdA*(Ts) and *nrdB1* mutations by hybrid plasmids was derived from the efficiency of plating either on minimal medium at 42°C or in the presence of hydroxyurea at 1 mg/ml, respectively. P_{lac} -mediated complementation was carried out in the absence of isopropyl- β -D-thiogalactopyranoside (IPTG). ND, not determined.

1B, lane 1), while the DNA of S. typhimurium exhibited two bands whose sizes were 0.9 and 13 kb (Fig. 1B, lane 2). Furthermore, in both E. coli and S. typhimurium, the banding pattern of the chromosomal DNA obtained with the nrdAB probe was different from the one exhibited when the nrdEF probe was used (Fig. 1).

Taking advantage of the XmaI and PstI sites of the polylinker of the pRK404, which were flanking our insert, we subcloned it in the pBluescriptSK(+) vector, giving rise to plasmid pUA335. Starting with this plasmid, a combination of subcloning and deletion analyses was performed, showing that practically all of the 4.9-kb original insert was required to support the growth of the KK450 strain at $42^{\circ}C$ (Fig. 2).

Nucleotide and amino acid sequences of the new RR genes. The nucleotide sequence of the 4.9-kb fragment of plasmid pUA335 is presented in Fig. 3. Several open reading frames can be identified in the insert, although the deduced products of only the two largest have some identity with other RR enzymes. The sequence between positions 836 and 2980 (consisting of 2,145 bp) is suggested to correspond to one subunit (designated *nrdE*) of the new reductase (Fig. 3). This protein has been obtained in pure form and shown to have the sequence ATTTPERVMQXTMD at its N-terminal end (24).

This is in complete agreement with the amino acid sequence deduced from the 42 bp starting at position 839. We suggest that translation starts with Met at the UUG triplet (positions 836 to 838), with eventual loss of this Met. In fact, it is known that the UUG codon also specifies initiation in bacteria, although at a low frequency (7). The loss of the N-terminal methionine has also been reported to occur for the R1 and R2 subunits of the aerobic RR of E. coli (5). The second open reading frame, called nrdF, is 960 bp long (Fig. 3, positions 2991 through 3950). The predicted molecular weights of the deduced proteins of the nrdE and nrdF genes were 80,519 and 36,281, respectively. The nrdE and nrdF open reading frames were separated by a small noncoding region of 10 bp. Putative ribosomal binding sites were located 16 bp upstream of the initiation codon for nrdE and 12 bp upstream of that for nrdF. No canonical TATA box preceded by a - 35 promoter consensus sequence was detected upstream of the nrdE gene. However, the subcloning and deletion analysis shown in Fig. 2 (plasmids pUA396, pUA341, and pUA343) indicated that the 836 bp upstream of the nrdE open reading frame contains the promoter from which the transcription of these genes occurs. Downstream from the stop codon of the nrdF gene (TAA), a putative transcriptional terminator was detected as a perfect

1	GATCGGGCGTTCACGCCGCCATCCGGCAAAAATTAGCCATGCCTCCCCTACCCCGCGGCG	60	2461	GCAACCGAAAACAGCGAAAGTCAGGGCGCTATTTGCCCGCAGCGGCATTACGCTGCCCAC	2520
61		120	542	O P K T A K V R A L P A R S G I T L P T	561
121		180	2521		2580
181		240	562		581
241	GITTCHCTCLACTCLACCACACAAAAAAAAAAAAAAAAAAAA	300	2581	TRODEDOTODEDORADITAL ANTIONTICADOTTODODOSODOS ANTIONIONIONI	2640
301		360	582	TOAVPPTGSTSYTNHATSST	601
361	TTACCGGTGGTGGTGGCGGCGGCGTTTGGGCTGCTCCGCCCGGGCATGATTAAC	420	2641	TCATCCGATTGTGGCCAAAATTGAGATTCGCAAAGAGGCCAAAACCGGGCGTGTGTATTA	2700
421	CGTCTGC ACCCCGAC ACGCCGCCGCGCGCGCGCGCGCGCGCGC	480	602	H P T V A K T R T R K R G K T G R V Y Y	621
481	GCTCTGAAAATACGCACCGCTTTATGCAGCGTCTGGGGCCTGCCT	540	2701	CCCCCCCCCTTTATGACCAATGAAAACCTGGACATGTATCAGGATGCTTACGATATCGG	2760
541	TC & ATGACCCCC & ATTC ACCTACACCC & ACCTACATCTCCCCCCCCCC	600	622	PAPFMTNENTOMYODAYDIG	641
601	CCGCGCGCATGCCCGTGCCGCGCGCGCGCGCGCGCGCGCG	660	2761	TCCGGAAAAAATTATTGATACCTATGCCGAGGCCACGCCCCCCGACGATCAAGGGCTGTC	2820
661	a case of the second	720	642	PRKTTDTYARATERVDOGTS	661
721	Gegergergergergergergeraat also a steregegerererergergeraereering	780	2821	GCTCACCCTGTTTTTTCCCCCGATACCGCCACGACGCCGCGATATCAACAACGCCGCAGATCTA	2880
781	AGCTCATGGGCACACAGCGCACATCGATAATGTCCGAAAAGGAGTAAATGAATTTTCGC	840	662	LTLFFPDTATTRDINKAOIY	681
/01	RBS RBS A	1	2881	TGCCTGGCGAAAAGGTATTAAGTCCCTGTATTACATCCGGCGCTTCGGCGAGTGGCGCGAGA	2940
841	AACAACTACCCCGGAGCGCGTAATGCAGGAAACCATGGATTACCACGCCCTGAACGCGAT	900	682	AWRKGIKSLYYIRLROLALB	701
2	TTTPERVMOETMDYHALNAM	21	2941	AGGTACTGAAATTGAAGGCTGCGTATCCTGCGCGCTATAAGGAAAGCCATATGAAATTAT	3000
901	GCTGAATCTTTACGATAAAGCAGGCCATATTCAGTTCGACAAGGACCAGCAGGCGATCGA	960	702	GTELEGCVSCAL*RBS MKLS	4
22	LNLYDKAGHIOFDKDOOAID	41	3001	CTCGTATTAGCGCCATCAACTGGAACAAGATCCAGGACGACAAAGATCTGGAGGTATGGA	3060
961	CGCCTTCTTTGCCACCCACGTCCGCCCGCATTCCGTGACGTTTGCCAGCCA	1020	5	RISAINWN KIODD KDL BVWN	24
42	AFFATHVRPHSVTFASOHER	61	3061	ACCGGCTGACCAGTAACTTCTGGCTGCCGGAAAAAGTGCCGTTATCGAATGATATTCCGG	3120
1021	TCTGGGGACGCTGGTTCGGGAAGGGTATTACGATGACGCCGTCCTCGCGCGTTACGACCG	1080	25	R L T S N F W L P E K V P L S N D I P A	44
62	LGTLVREGYYDDAVLARYDR	81	3121	CCTGGCAGACGCTGAGCGCCGCCGAACAGCAGCTCACCATTCGCGTGTTTACGGGACTTA	3180
1081	CGCCTTCGTCCTTCGCCTGTTCGAGCACGCCCATGCCAGCGGCTTTCGCTTCCAGACGTT	1140	45	WOTLSAAEOOLTIRVFTGLT	64
82	AFVLRLFEHAHASGFRFOTF	101	3181	CGCTGCTCGACACTATCCAGAACATCGCAGGCGCGCCGTCGTTAATGGCAGATGCCATCA	3240
1141	TCTTGGCGCCTGGAAGTTCTATACCAGTTACACGCTGAAAACCTTCGACGGCAAACGTTA	1200	65	L L D T I O N I A G A P S L M A D A I T	84
102	LGAWKFYTSYTLKTFDGKRY	121	3241	CGCCGCATGAAGAGGCAGTGCTGTCGAACATCAGCTTTATGGAAGCGGTACACGCCCGCT	3300
1201	TCTGGAACACTTTGAAGATCGGGTGACAATGGTGGCGTTGACGCTGGCGCAGGGTGACGA	1260	85	PHEEAVLSNISFMEAVHARS	104
122	L B H F B D R V T M V A L T L A Q G D B	141	3301	CTTACAGTTCTATTTTCTCCACGCTGTGCCAGACGAAAGAGGTTGATGCCGCCTACGCCT	3360
1261	AACGCTGGCCACCCAACTGACCGATGAAATGCTTTCTGGTCGCTTTCAGCCCGCTACCCC	1320	105	Y S S I F S T L C Q T K E V D A A Y A W	124
142	T L A T Q L T D B M L S G R F Q P A T P	161	3361	GGAGCGAAGAAAACCCACCGCTTCAGCGTAAGGCGCAGATTATTTTAGCTCATTACGTCA	3420
1321	GACTTTTTTAAATTGCGGCAAACAGCAGCGTGGGGGAACTGGTCTCCTGCTTCCTGCTCCG	1380	125	S E E N P P L Q R K À Q I I L A H Y V S	144
162	T F L N C G K Q Q R G E L V S C F L L R	181	3421	GCGATGAACCGCTAAAGAAAAAGATTGCCAGCGTCTTTTTAGAGTCTTTTCTGTTCTATT	3480
1381	TATCGAAGACAACATGGAGTCGATCGGGCGGGGGGGGAATTCGGCGCTGCAACTCTCCAA	1440	145	DEPLKKKIASVFLESFLFYS	164
182	I E D N M E S I G R A V N S A L Q L S K	201	3481	CCGGCTTCTGGTTGCCGATGTATTTCTCCAGCCGCGGTAAGCTCACGAACACTGCCGACC	3540
1441	ACGCGGCGGCGCGCCCCCTTTTTACTCTCCAATCTGCGCGAGCGGGCGCGCCCCAATCAA	1500	165	G F W L P M Y F S S R G K L T N T A D L	184
202	R G G G V A F L L S N L R E A G A P I K	221	3541	TGATTCGTTTAATCATTCGCGATGAAGCGGTTCACGGTTATTATTATTGGCTATAAGTATC	3600
1501	ACGCATTGAGAATCAGTCTTCCGGCGTGATCCCCGGTGATGAAAATGCTGGAAGACGCGTT	1560	185	I R L I I R D B A V H G Y Y I G Y K Y Q	204
222		241	3601	AGATAGCGCTACAAAAACTATCGGCAATCGAGCGTGAAGAGTTAAAGCTTTTCGCGCTGG	3660
1201	TTEGTATGCCAACCTAGCGCGCGCGCGCGGGCGGGCGGGGGGGG	1620	205		224
1621		201	3001	ATTTGTTGATGGAACTGTACGACAACGGAAATCCGCTACACAGAAGCGTTATATGCGGAAA	3/20
262		201	2721		3790
1691		1740	245		264
2021		201	2791		3840
1741		1800	265		284
302	N & O M & T. F.S. P.Y. D.T. O.R.R.Y.G.K.P.F.	321	3841	TTOTA STTERATED SOCTET TOTTE A STANDARD A STAND SOTAL A STANDARD SOCTET TO STANDARD SOCTET TO STANDARD SOCTET STANDARD SOCTET TO STANDARD SOCTE TO STANDARD SOCTET TO STANDARD SOCTE	3900
1801	TGGCGATATCGCCATTAGCGAACGGTACGATGAATTAATT	1860	285	A L S P N A D R N H D F F S G S G S S Y	304
322	G D I A I S B R Y D B L I A D P H V R K	341	3901	ATGTGATGGGGAAAACAGTCGAAACCGAAGACGAAGACTGGAATTTTTAACCTTACGGGC	3960
1861	AACCTATATTAACGCCCGTGACTTTTTTCAAACACTGGCGGAGATTCAGTTCGAATCCGG	1920	305	VMGKTVETEDEDWNF*	320
342	TYINARDFFQTLAEIQFESG	361	3961	ATGGGAAATAACGTTACATTTCCCATGCCTTTATTTCAAGCAATAGGGAGTCAAATCGCG	4020
1921	GTATCCCTACATCATGTTTGAAGATACGGTAAACCGCGCGAATCCCATTGCTGGTCGCAT	1980			
362	Y P Y I M F E D T V N R A N P I A G R I	381	4021	CAAATATTACAACATGTCCTACACTCAATACGAGTGACATTATTCACCTGGATTCCCCCCA	4080
1981	TAATATGAGCAACCTGTGCTCAGAAAATTTTACAGGTCAATAGCGCTTCCCGTTACGACGA	2040	4081	ATTCAGGTGGATTTTTGCTGGTTGTTCCAAAAAATATCTCTTCCTCCCCATTCGCGTTCA	4140
382	N M S N L C S E I L Q V N S A S R Y D D	401	4141	GCCCTTATATCATGGGAAATCACAGCCGATAGCACCTCGCAATATTCATGCCAGAAGCAA	4200
2041	TAACCTTGACTATACCCACATCGGGCATGACATCTCCTGCAATCTCGGCTCGCTGAATAT	2100	4201	attc aggg ttgtctcagattctgagtatgttagggtagaaaaaggtaactatttctatca	4260
402	N L D Y T H I G H D I S C N L G S L N I	421	4261	ggtaacatatcgacataagtaaataac <u>agga</u> atcattctattgc <u>atg</u> gcaattaaattag	4320
2101	CGCTCACGTCATGGATTCACCGGACATTGGCCGTACCGTAGAAACCGCTATTCGCGGCCT	2160	4321	AAGTGAAGAATCTGTATAAAATATTTGGAGAGCATCCGCAGCGTGCCTTCAAATATATTG	4380
422	A H V M D S P D I G R T V B T A I R G L	441	4381	AAAAGGGACTATCGAAAGAGCAAATACTGGAAAAAACGGGGGCTATCGCTTGGCGTTAAAG	4440
2161	GACGGCGGTGTCGGACATGAGCCATATACGCAGCGTGCCCTCAATAGCCGCCGGTAATGC	2220	4441	ACGCCAGTCTGGCCATTGAAGAAGGCGAGATATTTGTCATCATGGGATTATCCGGCTCGG	4500
442	TAVSDMSHIRSVPSIAAGNA	461	4501	GTAAATCCACAATGGTACGCCTTCTCAATCGCCTGATTGAACCCACCC	4560
2221	CGCCTCTCATGCCATCGGTCTGGGCCAGATGAATCTGCATGGCTATCTGGCGAGGGAAGG	2280	4561	TGATTGACGGCGTTGATATTGCCAAAATATCAGACGCTGAGCTTCGCGAGGTGCGCAGGA	4620
462	A 5 H A 1 G L G Q M N L H G Y L A R B G	481	4621	AAAAGATTGCGATGGTCTTCCAGTCATTTGCGCTCATGCCGCATATGACCGTGCTGGATA	4680
2281	TATTGCCTACGGTTGGAGGCGTTGGATTTCACCAATCTCTATTTTACACCATTAC	2340	4681	ATAUGGCATTCGGTATGGAATTAGCGGGCATCGCGGCGCAAGAGCGTCGCGAAAAAGCGC	4740
482		501	4741	TGGACGCCTTGCGTCAGGTGGGGGCTTGAGAATTACGCTCACGCCTACCCCGGATGAACTTT	4800
2341	W H & V H T C M D I & D T D C T T T C	24UU 521	4001	UCGGTGGGATGCGTCAGCGTGTTGGGCTTGCCCGCGCGCGC	4000
2401		2460	4001	TATTAATGGATGAAGCGTTTTTCCGCCCTCGATCC	4094
522	F A O S R Y A S G D Y F T O Y T. O D D W	541			
		~			

FIG. 3. Nucleotide and deduced amino acid sequences of the nrdEF genes of S. typhimurium LT2. The putative ribosome binding site (RBS) sequences are underlined. A 12-bp palindrome which may serve as a transcription terminator is indicated by inverted arrows. The ribosome binding site and the first coding triplet of the first gene (proV) of the proU operon of S. typhimurium (33) are boldface and underlined. The TTG triplet proposed as the translation start of the nrdE subunit is boxed.

inverted repeat of 12 nucleotides separated by 8 nucleotides (positions 3958 to 3989), followed by a run of AT base pairs.

The sequence of the 3' end of our 4.9-kb fragment corresponds to the 5' regions of the *proU* operons of *S. typhimurium* and *E. coli*, indicating that in the chromosomes of both species, the *nrdEF* genes are upstream of the *proU* operon and, consequently, are located at 57 min on the chromosomal maps of these two bacteria (Fig. 3). In agreement with this, plasmid F'143 (which contains the region around 57 min of the *E. coli* chromosome) abolishes the temperature sensitivity of the KK450 mutant (Fig. 2). From previously known data about the *proU* operon (19, 33), it is possible to conclude that the transcription of *nrdEF* genes is in the clockwise direction on the *S. typhimurium* and *E. coli* maps.

Comparison of the *nrdEF* predicted protein sequences with those of other aerobic RR of class I shows a limited identity (Table 2). Thus, the *nrdE* and *nrdF* products have, respectively, 25 and 28% overall amino acid sequence identities with the R1 and R2 subunits of the aerobic RR of *E. coli*. Amino acid sequence alignments of the class I RR from different species have shown that they are homologous within three groups: the

TABLE 2. Amino acid identity of the large and small subunits of
the class I RR from different species with products of the nrdEF
genes, respectively, of S. typhimurium ^a

	% identity of RR subunit with:					
Species	nrdE product	nrdF product				
E. coli	25.0	27.7				
Bacteriophage T4	23.3	22.1				
Human	27.5	24.3				
Mouse	27.6	24.3				
S. cerevisiae	17.8	22.3				
Vaccinia virus	25.8	19.2				
HSV-1	24.7	20.9				
HSV-2	27	21.2				
Varicella-zoster virus	26.2	26.8				
EBV	23.2	22.3				
Rir3-Yeast	24.5					

^a The sequences were aligned by using the Gap program of the University of Wisconsin Genetics Computer Group package. HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; EBV, Epstein-Barr virus; Rir3-Yeast, product of the *RNR3* gene of *S. cerevisiae*, encoding a duplicated R1 subunit.

	-									
1161/4	1	COCADEETK	NUDDATECCO							82
HSVI Varicella	NKEPALMLET		RVPPRIFGSP			LULUVPPVPP	NAYMPYYLKE	YVIKLVNGFK	PLVSRSARLY	RILGVLVHL.
Mouse			KTTSRTOKIC		AOTTMKVTOG		ESTIPVELME	I DTI AAFTA		ATLAA
Vaccinia	MEVIKR	NGYKENVMED	KTTSRIGKEC	YGI NTDHTDP	TKTAMKVIQG	TYNGVTTV	E	I DTI AAFTA		ATLAA
E. coli	MNONLLVTKR	DGSTERINLD	KIHRVLDWAA	EGLH NVSI	SOVELRSHIO	FYDGIKTS	D	THETTIKAAA	DITSRDAPDY	
NrdE								ATTTP	ERVMOETMDY	HALNAMENLY
Consensus				1					Y	1
	83									164
HSV1	.RIRTREASF	EEWLRSKEVA	LDFGLTERLR	EH.EAQLVIL	AQALDHYDCL	IHSTPHTLVE	RGLQ.SA	LKYE.EFYLK	RFGGHYMESV	FQMYTRIAGF
Varicella	.RINLRSVSM	ETWLTSP	.ILCLTPRVR	QAIEGRRDEI	RRAILEPF	LKDQYPALAT	LGLQ.SA	LKYE.DFYLT	KLEEGKLESL	CQFFLRLAAT
Mouse	. KIAVSNLHK	EIK	KVFS	DVMEDLYNYI	NPHNGRHSPM	VASSTLDIVM	ANKORLN.SA	IIYDRDFSYN	YFGFKTLERS	YLLKINGK
	- KIAVSNLHK		KLFS		NPKNGKHSP1	15511MD1VN	KYKUKLN.SV	11YERDESYN	YEGEKILEKS	YLLKINNK
NrdE				ASOUEDI CTI	VECVVDDAV	LADVODACVI		TDHDKDM1FS		TLVUNKVIGE
Consensus	DKAGHIQFDK	DQQAIDAFFA	INVKPNSVIF	ASQUEREGIE	VREGITODAV	LAKTUKAFVL	KLFENANASG	FKFQI	FLGAWKFTIS	TILKIFUGKK
consensus	•						3-			
	165									259
HSV1	LACRATRGMR	HIALG	REGSWWEMFK	FFFHRLYDHQ	IVPSTPAMLN	LGT.RNYYTS	SCYLVNPOA.	.TTNKATLRA	ITSNVSAILA	RNGGIGLCVO
Varicella	VTT.EIVNLP	KIATLIPGIN	DGYTWTDVCR	VFFTALACQK	IVPATPVMMF	LGR. ETGATA	SCYLMDPES.	. ITVGRAVRA	ITGDVGTVLQ	SRGGVGISLQ
Mouse	VAERPOHMLM	RVSVGI	.HKEDIDAAI	ETYNLLSEKW	FTHAPPTLFN	AGT.NRPQLS	SICIFILISMIKD.	.DSIE.GIYD	TLKQCALISK	SAGGIGVAVS
Vaccinia	IVERPQHMLM	RVAVGI	.HQWDIDSAI	ETYNLLSEKW	FTHASPTLFN	AGT.SRHQMS	SCIFLLNMID.	.DSIE.GIYD	TLKRCALISK	MAGGIGLSIS
E. coli	IYESAQFLYI	LVAACLFSNY	PRETRLQYVK	RFYDAVSTFK	ISLPTPIMSG	VRT.PTRQFS	SCVLIE.CG.	.DSLD.SINA	TSSAIVKYVS	QRAGIGINAG
NrdE	YLEHFEDRVT	MVALTL	.AQGDETLAT	QLTDEMLSGR	FQPATPTFLN	CGKQQRGELV	SOFLLRIEDN	MESIGRAVNS	ALQLSK	RGGGVAFLLS
Consensus		a			P	-g	sq-L			gG-g
	260									254
UCV1	200	DECOCTACINA						ODCONTECAL		351
Varicella		DIENOTICI		MATNEDCE D	PTGACVILEP	WHIVDLOTVLA				
Mouse	CTRATESYTA			PVVDOCCNKP	DCAEATVIED	WHIDLOIVLA	IKUMLYKDET			LOILKUADAV
Vaccinia	NTRASGSYTS	GTNGTSNGTT	PMI RVYNNTA	RYTDOGGNKR	PGVMATYLEP	WHSDTMAFLD	I KKNTGNEE			KDDG
E. coli	RIRALGSPIR	GGEAFHTGCT	PEYKHEOTAV	KSCSOGG, VR	GGAATI FYPM	WHI EVESILIV	I KNNRGVEG.	NRVRHMDYGV	OTNKI MYTRI	IKGE
NrdE	NLREAGAPIK	RIENOSSGVI	PVMKMLEDAF	SYANOLGA.R	OGAGAVYLHA	HHPDILRFLD	TKRENA . DEK	IRIKTLSLGV	VIPDITFR	LAKENA
Consensus		g	p	R	y	wH-dL-	-ke-	-R	pdlr-	
					-					
	352								-	449
HSV1	TWTLFDRDTS	MSLADFH	GEEFEKLYQH	LEVMG.FG.E	QIPIQELAYG	IVRSAATTGS	PFVMFKDAVN	RHYIYDTQGA	AIAGSNUCTE	IVHP
Varicella	QWTLFDNRAD	I.LRTLH	GEAFTSTYLR	LEREG.LGVS	SVPIQDIAFT	IIRSAAVTGS	PFLMFKDACN	RNYHMNTQGN	AITGSNUCTE	IVQK
Mouse	DWSLMCPNEC	PGLDEVW	GEEFEKLYES	YEKQGRVRKV	. VKAQQLWYA	IIESQTETGT	PYMLYKDSCN	RKSN.QQNLG	TIKCSNUCTE	IV
Vaccinia	EWSLMCPDEC	PGLDNVW	GDEFERLYTL	YERERRYKSI	.IKARVVWKA	IIESQIETGT	PFILYKDACN	KKSN.QQNLG	TIKCSNUTE	II
E. COLL	DITLESPSON	PGLYDAFFAD	QEEFERLYIK	YEKDDSIRKQ	RVKAVELFSL	MMQERASTGR	1Y1QNVDHCN	THSPEDPALA	PVRQSNLICLE	IALPIKPL
Nrde	QMALESPYDI	QKKYGKPFGD	IAISEK. YDE	LIADPHVKKI	YINAKDEEQI	LAEIQFESGY	PYIMFEDIVN	KANPIAG	RINMSNLCSE	ILQVNSASRY
Consensus	[1	rei	-e			pN		-1SNUG-E	T
	450									539
HSV1		รรดงไฟเ ดรง	NI ARCVSRO.	. TEDEGRI RD	AVOACVI MVN	TMTDSTLO, P	TPOCTRGNDN		I HTACI KI GI	DIESAFFODI
Varicella	ADAH	OHGVONLASI	NLTTCLSKGP	VSFNLNDLOL	TARTTVIFLN	GVLAAGNF.P	CKKSCKGVKN	NRSLGIGIOG	LHTTCLRLGF	DLTSOPARRL
Mouse	EYTSKD	EVAVONLASL	ALNMYVTPEH	.TYDFEKLAE	VTKVIVRNLN	KIIDINYY.P	IPEAHLSNKR	HRPIGIGVOG	LADAFILMRY	PFESPEAOLL
Vaccinia	QYADAN	EVAVONLASV	ALNMFVIDGR	FDFLKLKD	VVKVIVRNLN	KIIDINYY.P	IPEAEISNKR	HRPIGIGVQG	LADAFILLNY	PFDSLEAQDL
E. coli	NDVNDENG	EIAUCTLSAF	NLGA	.INNLDELEE	LAILAVRALD	ALLDYQDY.P	IPAAKRGAMG	RRTLGIGVIN	FAYYLANDGK	RYSDGSANNL
NrdE	DDNLDYTHIG	HDISONLGSL	NIAHVM	DSPDIGR	TVETAIRGLT	AVSDMSHIRS	VPSIAAGNAA	SHAIGLGQMN	LHGYLAREGI	AYGSPEALDF
Consensus		{QnL-s-	-1	l	v1-	dp	-p	-rGiG	1	sal
	F 40									
UCV1		CANNERNALC	VOCADOCNU				ABDOVECE			625
Varicelle		SAME I SNALC	KTCCLADERC	FRESHTRAGE	I HODCECT	•••••	T CVIDID	WCTI DDD	TCAVCI VNCO	
Mouse	NKOIEETTYV	CALEASCELA	KEVC DVET	VECSDASKCT		•••••		WKDIKEK	TAKYCTONSI	I TADMDTAST
Vaccinia	NKKIFFTIYY	GALEASCELA	FKEG PYDT	YVGSYASNGT		•••••	VVPSDI WN		TRTYGIRNSI	
E. coli	THKTFEAIOY	YLLKASNELA	KEOG. ACPW	FNETTYAKGI	LPIDTYKKDL	DT	TANEPLHYD.	WEALRES	TKTHGI RNST	L SAL MPSETS
NrdE	TNLYFYTITW	HAVHTSMRLA	RERG.KTFAG	FAOSRYASGD	YFTOYLODDW	OPKTAKVRAL	FARSGITLPT	REMWLKLRDD	VMRYGIYNON	LOAVPPTGSI
Consensus	e	S1-	G	G-				₩L	GNS-	A-mPt
	626									688
HSV1	AQISDVSEGF	APLFTNLFSK	VTRDGETLRP	NTLLLKELER	TFS.GKRLLE	VMDSLDAKQW	SVAQALPCLE	PTHPLRRFKT	AFDY.DQKLL	IDLCADRAPY
Varicella	AQVTECSEGF	SPIYNNESK	VTTSGELLRP	NLDLMDELRD	MYSCEEKRLE	VINILEKNOW	SVIRSFGCLS	NSHPLLKYKT	AFEY.EQEDL	VDMCAERAPF
Mouse	AQILGNNESI	EPYISNIYTR	KVLSGEFQIV	NPHLLKDLTE	KGLWNKE	MKNQIIACNG	SIQSIPEIPD	DLKQLYKT	VWEIS.QKTV	LKMAAERGAF
ναςςιηια	AQILGNNESV	EPTISNITR	KVLSGEFQVV	NPHLLRVLTE	KKLWNDE	IKNKIMADGG	SIQN. INLPE	DIKKV. YKT	IWELP.QKTI	LAUVETHE
E. COLL NedE	SUISNAINGI	HDTVAVTET	ASKUGILKUV		•••••	•••••	E		AVDICOEV T	LULVGIMUK
Consensus	-01	-P		FAFFMIN				CNLUMIQU	AIDIGPEK.I	IUITALAIKH
consensus	4.		9					y		u
	689									761
HSV1	VDHSQSMTLY	VTEKADGT	LPASTLVRLL	VHAYKRGLKT	GMYYCKVRKA	TNSGVFGGDD	NIVOMSCAL.			
Varicella	IDQSQSMTLF	IEERPDGT	IPASKIMNLL	IRAYKAGLKT	GMYYCKIRKA	TNSGLFAGGE	.LTIOTSIONL.			• • • • • • • • • • • • • • • • • • • •
Mouse	IDQSQSLNIH	IAEPNYG	KLTSMH	FYGWKQGLKT	GMYYLRTRPA	ANPIQFTLNK	EKLKOKEKAL	KEEEEKERNT	AAMVCSLENR	EECLMCGS
Vaccinia	IDQSQSMNIH	IADPSYS	KLTSMH	FYGWSLGLKT	GMYYLRTKPA	SAPIQFTLDK	DKIK	• • • • • • • • • • •	PPVVCDS	EIGTSQSG
t. coli	IDQSISANTN	YDPSRFPSGK	VPMQQLLKDL	LTAYKFGVKT	.LYYQNTRDG	AEDAQ		• • • • • • • • • • •	DDLVPSIQD.	DOGLESCARCKI
Nrde	VDQGLSLTLF	FPDT	ATTRDINKAQ	IYAWRKGIK.	SLYYIRLRQL	ALEGTE	TEODARDAL.	•••••	• • • • • • • • • • • •	•••••
consensus	-vas-5			G-Kt	1 1 r					

FIG. 4. Amino acid sequence alignment of the large subunit of RR from herpes simplex virus type 1 (HSV1), varicella-zoster virus, mouse, vaccinia virus, and *E. coli* and the *ndE* product of *S. typhimurium*. The N-terminal domain of the herpes simplex virus type 1 sequence, which is unrelated to all other sequences shown here, has been omitted. The numbering refers to the *E. coli* sequence. A residue is given in the consensus sequence if it is present in at least all but one of the sequences and is in capital letters if it is completely conserved. The five specific cysteine residues representing the essential thiols of the R1 protein (11) are boxed.

	1									97
HSV1	PAI TAI TOOS		KCPDPERY . E	YTSOCPDTNH	. I RSI STI NR		DEEDVSKI SE	GELSEYRELE	AFISAADDIV	TENI GG. I SG
Varicella		M	DOKDCSHE, F	YRPECPDINN	I RAL STSNR	ESDETTED	DYOYI DCI TE	DEL TEYRETE	TEL SAADDLV	NVNI GS. I TO
Mouse	DSAELESKAP	TNPSVEDEPL	LRENPRREVV	FPIEYHDIWO	.MYKKAEASE	WTAEEVDLSK	DIOHWEALKP	DERHFISHVL	AFFAASDGIV	NENLVERFSO
Vaccinia		MEPI	LAPNPNRFVI	FPIOYYDIWN	.MYKKAEASF	WITVEEVDISK	DINDWNKLTP	DEKYFIKHVL	AFFAASDGIV	NENLAERFCT
E. coli	AYTTFSOTKN	DOLKEPMFFG	OPVNVARY	.DOOKYDIFE	KLIEKOLSFF	WRPEEVDVSR	DRIDYOALPE	HEKHIFISNL	KYOTLUDSIO	GRSPNVALLP
NdrF		MKLS	RISAINWNKI	ODDKDLEVWN	RLTSNF	WLPEKVPLSN	DIPAWOTLSA	AEQOLTIRVF	TGLTLUDTIO	NIAGAPSLMA
Consensus				di		M	dL	-E	d	
							L)		L L	
	98	_								193
HSV1	LFEQKDILHY	YVEQECTEVV	HSRVMNIIQL	VLFHNNDQAR	REYVAGTINH	PAIRAKVDWL	EA			.RVRE.CASV
Varicella	LFSQKDIHHY	YIEQECTEVV	HARVYSQIQL	MLFRGDESLR	VQYVNVTINN	PSIQQKVQWL	EE			.KVRD.NPSV
Mouse	EVQVTEARCF	YGFQIAMENI	HSEMYSLL.I	DTYIKDPKER	EYLFNAIETM	PCVKKKADWA	LR`			.WIGDKEATY
Vaccinia	EVQITEARCF	YGFQMAIENI	HSEMYSLL.I	DTYVKDSNEK	NYLFNAIETM	PCVKKKADWA	QK			.WIHD.SAGY
E. coli	LISIPELETW	VETWAFSETI	HSRSYTHIIR	NIVNDPSV	VFDDIVTN	EQIQKRAEGI	SSYYDELIEM	TSYWHLLGEG	THTVNGKTVT	VSLRELKKKL
NdrF	DAITPHEEAV	LSNISFMEAV	HARSYSSIFS	TLCQTKEV	DAAYAWSEEN	PPLQRKAQII	LAHY			VSDEPLKKKI
Consensus			HM			pk				
	194	<u>п. п. п</u> .								291
HSV1	PEKFILMILI	EGIFFAASFA	AIAYLRTNNL	LRVTCQSNDL	ISRDEAVHTT	ASCYIY	NNYL	GGHAKPPPDR	VYGLFRQAVE	IEIGFIRSQA
Varicella	AEKYILMILI	EGIHEVSSHA	AIAYLRNNGL	FVVTCQFNDL	ISRDEATHTS	ASCCIY	NNYV	PEKPAITR	IHQLFSEAVE	IECAFLKSHA
Mouse	GERVVAFAAV	FOTHEROHA	SIFWLKKRGL	MPGLIFSNEL	ISRDEGUHCD	FACLMF	KHLV	HKPAEQR	VREIIINAVR	IEQEFLIEAL
ναςςιηια	GERLIAFAAV	EGIHHSGSHA	SIFWLKKRGL	MPGLTFSNEL	ISRDEGUHCD	FACLMF	KHLL	HPPSEET	VRSIITDAVS	IEQEFLTAAL
E. COLL	YECEMSVNAL	EATHHYVSHA	CSFAFAEREL	MEGNAKIIRL	LIARDEALH	LIGIQHMENE	LRSGADDPEM	AETAEECKQE	CYDLFVQAAQ	QEKDWADYLF
Nart	ASVFL	ESFLHYSOH	LPMYFSSRGK	LINIADLIRL	INRDEAVINGY	YIGYKYQIAL	QKLSAIER	EELKLF	ALDLLMELYD	NEIRYTEALY
Consensus		F-1-R-allo	l	L					a	-t
	202								276	
1101/4						CTDVITN	FFF CD CT CD	CANAMO	5/0	
	PIDSHILSPA	ALAALENTVK	FSAUKLLGLI	NUDELENTED	PUASFPLSLM		FFECKSISTA	GAVVNUL	••••	
Varicella		NUTLAKOVIE	FSAUKLESAL	CENETERVEN	PUSUFPLAFM		FFERHSISTA	GIVINUL		
Nouse		NCEMMATTE		GENELEKVEN		ENISLEGKIN	FFERRYCEMO			
	POCS MTC		VTTNTPMOAV	CLUI DEOTRE	NDTDWTNTWI		EVENEN	VCOTDSEVDT		
	AETC WVND	WKAELC	TT THE MUL	CVEALEDDEM	ADVNDATI AA		EESCOCOM			
Consonsus	ALIG W/ND	*MILC		GILALIFFEM	ADVIIFATLAA	LJFNADENND	ffo M	muk	LD.EDWINF.	
consensus		y	u							

FIG. 5. Amino acid sequence alignment of the small subunit of RR from herpes simplex virus type 1 (HSV1), varicella-zoster virus, mouse, vaccinia virus, and *E. coli* and the *nrdF* product of *S. typhimurium*. The N-terminal domains of the mouse and herpes simplex virus type 1 sequences, which are unrelated to all other sequences shown here, have been omitted. The numbering refers to the *E. coli* sequence. A residue is given in the consensus sequence if it is present in at least all but one of the sequences and is in capital letters if it is completely conserved. Conserved amino acid residues involved in the iron ligands, the hydrogen-bonding network, or the R1-binding surface, as well as the tyrosyl radical and its environment (31), are boxed.

eukaryotic proteins, the herpesvirus-type virus proteins, and the *E. coli* and bacteriophage T4 proteins (31). Among these three groups there is a low level of homology, i.e., between 20 and 30% identity. It is worth noting that the homology of the *nrdEF* products with all of these RR is in this range of identity (Table 2), suggesting that NrdEF proteins may belong to a fourth group.

Several consensus residues of aerobic RR of class I are conserved in both the *nrdE* and *nrdF* deduced products, including the five specific cysteine residues representing the essential thiols (35) of the R1 protein, Cys-225, Cys-439, Cys-462, Cys-754, and Cys-759 (Fig. 4). Likewise, the iron ligands (Asp-84, Glu-115, His-118, Glu-204, Glu-238, and His-241), the tyrosyl radical and its environment (Tyr-122, Phe-208, Phe-212, and Ile-234), the hydrogen-bonding network (Asp-237 and Trp-48), and the R1-binding surface (Asp-58, Arg-236, and Tyr-356) of the R2 protein (14) are also preserved in the *nrdF* protein (Fig. 5). These homologies suggest that this new RR belongs to class I.

The results presented in this paper clearly show that in both *S. typhimurium* and *E. coli* there are two sets of genes encoding the function of aerobic synthesis of dNTPs. Other examples of duplication of the same function in which the homologous genes map to distinct loci in *E. coli* include *argI* and *argF* (2, 43) and *tufA* and *tufB* (1, 44). The *arg* genes have diverged to the point that the two enzymes are biochemically distinguishable (28, 29) despite the fact that their amino acid sequences differ by not more than 5% (28). The *tuf* genes are nearly identical, and the only difference between the 339-residue structural

proteins is a single substitution at the C terminus (17). Since the gene products of the *tuf* loci are functionally indistinguishable (30), it has been suggested that the duplication provided an emergency backup when demand for the translational elongation factor EF-Tu was high (1). In this respect, and because of the great importance of the RR to any organism, it seems reasonable that more than one *nrd* system is available in bacterial cells to guarantee the production of dNTPs required for DNA replication.

In Saccharomyces cerevisiae the R1 gene of RR is duplicated (for a review, see reference 10). This organism contains the genes for the large subunit (RNR1) and the small subunit (RNR2) but in addition a third gene (RNR3). RNR1 and RNR3 proteins are 80% identical in the portions of their genes that have been sequenced (10). RNR1 and RNR2 mutations are lethal, whereas RNR3 mutants have no obvious growth defects and are not sensitive to hydroxyurea. Also, similarly to the case with the nrdEF genes in nrdAB mutants of E. coli, an RNR1 mutant cannot be complemented by the RNR3 chromosome copy, but the RNR3 gene can abolish the deficiency of RNR1 mutants when introduced in a high-copy-number vector (10). On the other hand, NrdAB and NrdEF proteins are very divergent despite the fact that both seem to be fully functional. In fact, preliminary analysis showed that extracts of E. coli nrdA(Ts) cells containing a multicopy plasmid carrying nrdEF genes have, at the restrictive temperature, RR activity (25). In this respect, it is also worth noting that the principal functional residues of RR enzymes of class I are conserved in the NrdEF proteins. Complementation data for several deleted plasmids (Fig. 2) also indicate that both *nrdEF* genes must be present to suppress the *nrdAB*-defective phenotype, indicating that some interaction must exist between NrdE and NrdF proteins to form an active RR. Furthermore, R2 and NrdE proteins cannot form a functional complex in vivo, since plasmid pUA395 lacking the 3' end of the *nrdF* gene was unable to abolish a *nrdA*(Ts) phenotype (Fig. 2).

This work raises a very intriguing question: why does the nrdA(Ts) KK450 mutant of E. coli die at 42°C if it has another gene for aerobic RR? The answer to this question is not known, but the following alternatives appear to be excluded by our results: (i) an additional mutation in the nrdEF genes of this strain, (ii) the *nrdEF* genes being repressed genes belonging to either a cryptic prophage or a plasmid integrated in the bacterial chromosome, and (iii) expression of the nrdEF genes requiring a high gene dosage. The first explanation must be discarded since the temperature sensitivity of the nrdA(Ts) mutation when present in other genetic backgrounds is also abolished by cloned *nrdEF* genes. The second possibility does not account for the fact that the nrdEF genes are present in the same locus of both E. coli and S. typhimurium, since in this case the hypothetical prophage or plasmid would already be present in the ancient ancestor of these two bacterial species, which are believed to have diverged between 120 and 160 million years ago (32). Moreover, there are characteristics that help to identify genes acquired by a bacterial species from a foreign source such as a phage or a plasmid. One of these characteristics is the G+C content, and another is the codon usage (37). The G+C content (52.9%) as well as the codon usage of the *nrdEF* genes are within the range of values typical for S. typhimurium genes. Our results with plasmid F'143 (Fig. 2) and the low-copy-number plasmid pHSG575 carrying these genes (data not shown) lead us also to discard the third hypothesis.

Finally, the remaining possibility is that the structure of the region of the chromosome containing nrdEF genes has a negative effect, as with the expression of other genes in *E. coli*, such as *bgl* and *tonB*, which is influenced by supercoiling (22). Likewise, in the *proU* operon (which is downstream of the *nrdEF* genes), the only *trans*-acting mutations isolated so far affect DNA supercoiling (23). Further work is in progress to better characterize the new aerobic RR as well as the regulation of its expression.

Nucleotide sequence accession number. The nucleotide sequence in Fig. 3 has been assigned accession number X73226 in the EMBL data library.

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