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Characterization of the mRNA Coding for Ribonucleoside Diphosphate Reductase in *Escherichia coli*

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Total *Escherichia coli* RNA was separated by electrophoresis on methyl mercury agarose gels, transferred to diazobenzyloxymethyl-paper, and hybridized to various DNA probes containing different segments of the *nrd* genes to determine the organization of these genes. A 3.2-kilobase polycistronic mRNA transcript which hybridizes to both the *nrdA* and *nrdB* genes indicated that the *nrdA* and *nrdB* genes are organized in an operon. The polycistronic transcript contained the *nrdA* gene at the 5′ end and the *nrdB* gene at the 3′ end. The size of the polycistronic mRNA was sufficient to code for the 80,000-molecular-weight B1 protein and the 40,000-molecular-weight B2 protein. The results also indicated that the *nrdA* and *nrdB* genes are the only genes in *E. coli* that code for ribonucleoside diphosphate reductase. Two smaller RNA species that hybridized to *nrd* DNA were observed and probably overlap with the 3.2-kilobase *nrd* mRNA.

Ribonucleoside diphosphate (RDP) reductase is the enzyme responsible for conversion of ribonucleotides to deoxyribonucleotides and thus catalyzes the first reaction in the pathway unique to DNA synthesis in *Escherichia coli* (26). This enzyme is composed of two subunits, B1 and B2 (4), encoded by the *nrdA* and *nrdB* genes, respectively (11). The B2 subunit is composed of two identical 40,000-molecular-weight polypeptides (25). The B1 subunit is composed of two nonidentical 80,000-molecular-weight polypeptides which have different amino-terminal amino acids but identical carboxy-terminal amino acids (25). Since the B1 subunit contains two identical substrate binding sites (27) and two pairs of identical allosteric binding sites (5), it would appear that the two polypeptides are very similar. There are several possible explanations for the differences observed between the B1 polypeptides. The B1 subunit may be composed of two homologous polypeptides with the amino-terminal portion of one polypeptide proteolytically removed, or alternatively, the two polypeptides could be synthesized from one structural gene with two different translational start signals. It is also possible that there are two genes coding for B1, and a mutation has not been identified for one of these genes.

The synthesis of RDP reductase is regulated at the level of transcription (13). Inhibition of DNA synthesis by thymine deprivation, chemical inhibitors, or shifting of temperature-sensitive mutants defective in DNA synthesis to nonpermissive temperatures all lead to increased synthesis of RDP reductase (8, 9, 13). Furthermore, the synthesis of the two subunits has been shown to be coordinately controlled (10).

A *PstI* restriction fragment containing the genes for RDP reductase has been cloned into pBR322 (22). *E. coli* cells containing this plasmid have amplified levels of RDP reductase activity, suggesting that this plasmid contains all the genetic information necessary to code for RDP reductase (22). The amplification of RDP reductase is independent of the orientation of the *PstI* restriction fragment in pBR322, indicating that amplification of the enzyme is due to transcription from the *nrd* promoter and not a plasmid promoter (22). Further subcloning of the *nrd* region localized the *nrdB* gene to a *Bam*HI restriction fragment (22). This subclone can complement a *nrdB* mutation but does not have amplified levels of the *nrdB* gene product, suggesting that the promoter for *nrdB* has been removed by subcloning and that expression occurs via a plasmid promoter (22).

In this study, total *E. coli* RNA was isolated and separated by electrophoresis on methyl mercury agarose gels. The RNA was then transferred to diazobenzyloxymethyl-paper and hybridized to various nick-translated DNA fragments containing different segments of the *nrdA* or *nrdB* gene. The results show that the genes for RDP reductase are transcribed as a 3.2-kilobase (kb) polycistronic mRNA with *nrdA* at its 5′ end and *nrdB* at the 3′ end. The size of

this polycistronic mRNA is of sufficient size to code for only one 80,000-molecular-weight B1 polypeptide and one 40,000-molecular-weight B2 polypeptide. This suggests that RDP reductase is encoded by only two genes, *nrdA* and *nrdB*, and that the difference seen in the two B1 polypeptides is not due to two genes coding for B1. Two smaller *nrd* RNA species were also observed, which probably overlap the 3.2-kb *nrd* mRNA.

MATERIALS AND METHODS

Materials. [α-^{32}P]dATP was purchased from Amersham Corp. Nitrobenzyloxymethyl paper and nitrocellulose filters (BA85) were obtained from Schleicher and Schuell Inc. DNase I was obtained from Millipore Corp. Restriction endonuclease enzymes, T4 DNA ligase, DNA polymerase I, and the Klenow fragment of DNA polymerase I were obtained from Bethesda Research Laboratories. All other chemicals were obtained from Sigma Chemical Co. The M13mp8 and M13mp9 subclones of the *pst*I-*Eco*RI fragment containing the *nrdAB* genes were a gift of John Carlson (this laboratory).

Bacterial strains and plasmids. *E. coli* strains C600 (*hsdR hsdM*$^+$) and KK535 (*thr leu thi deo tonA lacY supE44 recA gyrA nrdA nrdB*) were obtained from B.-M. Sjoberg (22) and were used for the propagation and maintenance of the plasmids in this study. *E. coli* CR34 (*thr leu thi thyA deoC deoB lacY tonA rpsL*) was used to isolate total *E. coli* RNA (13). *E. coli* JM103 [Δ(*lac-pro*) *thi rpsL supE endA sbcB15* F'*traD36 proAB lacI*^{q} Δ*lacZ* M151] was used for propagation of M13 subclones (16). *E. coli* CSH26 [*ara* Δ(*lac-proAB*) *thi*] harboring plasmid pHM235 was used to prepare primers (16). The recombinant *nrd* plasmid pPS2 was constructed and obtained from Platz and Sjoberg (22).

Growth of bacteria. *E. coli* CR34 cells were grown in supplemented Davis-Mingioli minimal medium as previously described (8). Cultures were grown exponentially for several generations to a density of 1×10^8 to 2×10^8 cells per ml before beginning an experiment. To remove thymine, a culture was rapidly chilled in an ice bath, and the cells were collected by centrifugation, washed, and then suspended in 37°C minimal medium lacking thymine. Cells were grown another 2 h in medium lacking thymine to induce *nrd* mRNA synthesis before RNA was isolated. To isolate RNA for determination of a possible endonucleolytic cleavage of the *nrd* mRNA, a thymine-starved culture of *E. coli* CR34 grown at 37°C was made permeable to rifampin by EDTA treatment (13). After EDTA treatment, the culture was treated with rifampin (80 μg/ml), and RNA was isolated as discussed below.

Isolation of RNA. RNA was isolated from *E. coli* by the diethyl pyrocarbonate method of Summers (24) except that one phenol:chloroform (1:1) extraction was performed before the RNA was precipitated with ethanol.

Isolation of plasmid DNA and single-stranded M13 DNA. Plasmid DNA was isolated by the method of Guerry et al. (12), followed by treatment with RNase A (20 μg/ml) for 1 h, two extractions with chloroform:isoamyl alcohol (24:1), and then precipitation with 1/10 volume of 3.0 M sodium acetate and 2.5 volumes of 95% ethanol. The DNA was further fractionated by resuspending it in 6 ml of TES buffer (30 mM Tris-hydrochloride, 50 mM NaCl, 5 mM disodium EDTA, pH 8.0), adding 7 g of CsCl and 1 ml of ethidium bromide (4 mg/ml), and centrifuging at 35,000 rpm in a Beckman type 40 rotor for 48 to 72 h. The plasmid band was removed with a syringe. Ethidium bromide was removed by extracting the DNA twice with an equal volume of isopropyl alcohol saturated with CsCl. The DNA was then dialyzed overnight at 4°C against 0.01 M Tris-hydrochloride–0.001 M EDTA, pH 7.9.

Single-stranded M13 DNA was isolated by the method of Heidecker et al. (14), except that the dialysis step was omitted.

Isolation of DNA restriction fragments. DNA restriction fragments were isolated after electrophoresis on agarose gels by method 2 of Yang et al. (29).

Nick translation of DNA fragments and plasmid DNA. Nick translations were performed by the method of Rigby et al. (23), except that the reactions were carried out in 0.05 M Tris-hydrochloride–0.005 M MgCl–0.01 M β-mercaptoethanol, pH 7.8, instead of 0.05 M potassium phosphate–0.005 M MgCl, pH 7.4.

Single-stranded DNA hybridization probes. Strand-specific hybridization probes were made from the subclones of the *Pst*I-*Eco*RI fragment into M13mp8 and M13mp9 (21) by the method of Hu and Messing (16) with the following modifications. The 21-base pair primer was separated by electrophoresis through an 8% polyacrylamide gel (38:2) in 89 mM Tris–89 mM borate–2.5 mM disodium EDTA, pH 8.2, for 30 min. The gel was then stained with ethidium bromide, and the band containing the 21-base pair fragment was excised. The DNA was eluted by soaking the gel particles overnight in 500 mM ammonium acetate–10 mM magnesium acetate–1 mM EDTA–0.1% sodium dodecyl sulfate at 37°C (20). Gel particles were removed from the DNA solution by centrifugation through a cotton plug. The DNA was then precipitated by the addition of 1/10 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 95% ethanol. The precipitate was then washed in 70% ethanol, dried, and resuspended in 10 mM Tris-hydrochloride–1 mM EDTA, pH 7.9.

Templates were radiolabeled by hybridizing approximately 20 ng of heat-denatured primer to about 0.5 mg of template in 50 mM Tris-hydrochloride–5 mM MgCl–10 mM β-mercaptoethanol in a final volume of 0.01 ml at 65°C for 15 min and then adding 3 nmol each of dGTP, dCTP, and TTP, 2.5 μmol of Tris-hydrochloride, pH 7.8, 0.25 μmol of MgCl, 0.5 μmol of β-mercaptoethanol, 200 μCi of [α-^{32}P]dATP (10 mCi/ml, 3,000 Ci/mmol), and 7 U of the Klenow fragment of DNA polymerase I to the hybridization mixture in a final volume of 0.05 ml. The reaction was monitored by trichloroacetic acid precipitation of 1 μl of the reaction mixture. When the reaction was completed, the mixture was fractionated on a G50 column to separate the labeled DNA from the unincorporated nucleotides.

Northern transfers. Total *E. coli* RNA (2 μg per lane) was separated by electrophoresis on 1% agarose gels containing 10 mM methyl mercury according to the procedure of Lehrach et al. (17). RNA samples were prepared by adding 0.5 volumes of 10% Ficoll–0.004% bromophenol blue and methyl mercury at a final concentration of 20 mM. Transfer of the RNA to

diazobenzyloxymethyl paper was performed according to the procedure of Alwine et al. (2), except that 0.2 M sodium acetate buffer, pH 4.0, was used instead of 50 mM sodium borate, pH 8.0. The hybridizations were carried out at 42°C for 36 to 48 h (2). Autoradiography with preflashed Kodak XAR-5 X-ray film and Cronex Lightning Plus (Du Pont Co.) intensifying screens was performed for various lengths of time at −70°C.

Subcloning of restriction fragments into pBR322. Plasmid pPH108 was constructed by isolating the 5.0-kb *Eco*RI-*Pst*I restriction fragment from pPS2, which contains the *nrd* genes, and ligating it to the large *Eco*RI-*Pst*I restriction fragment of pBR322. Plasmid pPH5 was constructed by isolating the 1.7-kb *Eco*RI-*Bam*HI restriction fragment from pPS2 and ligating it to the large *Eco*RI-*Bam*HI restriction fragment of pBR322. The ligation reactions were performed according to manufacturer specifications by using approximately equal molar amounts of vector fragment and insert fragment. *E. coli* C600 and *E. coli* KK535 were transformed by the method of Cohen et al. (6).

Quick plasmid preparation. The rapid alkaline isolation procedure of Birnboim and Doly (3) was used to obtain plasmid DNA for restriction analysis of the pBR322 subclones of the *nrd* genes. Replicative intermediates of the M13mp8 and M13mp9 subclones were isolated by the method of Holmes and Quigley (15) and characterized by restriction enzyme analysis.

DNA-DNA hybridization. One microgram of plasmid DNA (pBR322, pPH108, and pPH5) was loaded onto nitrocellulose filters (BA85) and hybridized at 42°C for 48 h according to the procedure of Denhardt (7).

Endonuclease digestion. Restriction digestions of DNAs were done according to the specifications of the manufacturers.

DNA electrophoresis. Agarose slab gels (0.7%) were run in 89 mM Tris–89 mM borate–2.5 mM disodium EDTA, pH 8.2. DNA bands were visualized by staining with ethidium bromide (0.4 μg/ml).

RESULTS

The hybridization probes used in this study were constructed from plasmid pPS2, which carries the *E. coli nrd* genes on a *Pst*I fragment cloned into the *Pst*I site of pBR322 (22). The restriction map generated in the present study (Fig. 1) agrees with a previously published map of the insert in this plasmid (22). Our restriction map also agrees with a restriction map of the corresponding segment of the *E. coli* chromosome generated by Yamada et al. while working on the *ftsB* gene, which maps adjacent to the *nrdB* gene (28).

Plasmid pPH108 was constructed by subcloning the 5.0-kb *Eco*RI-*Pst*I fragment from pPS2 into *Eco*RI-*Pst*I-restricted pBR322. This plasmid complements both the *nrdA* and *nrdB* genes when transformed into *E. coli* KK535. Plasmid pPH5 was constructed by subcloning the 1.7-kb *Eco*RI-*Bam*HI fragment into *Eco*RI-*Bam*HI-restricted pBR322. This plasmid did not complement either the *nrdA* or *nrdB* mutation in *E. coli* KK535. A *Bam*HI fragment cloned by Platz and Sjoberg which contains the 3.0-kb *Bam*HI-*Pst*I

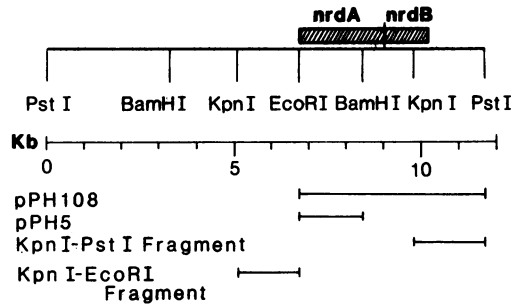

FIG. 1. Restriction endonuclease map of the *nrd* region of the *E. coli* chromosome. The bars indicate plasmids and restriction fragments used as hybridization probes in this study.

region (Fig. 1) complements only the *nrdB* gene (22). Therefore, the 1.7-kb *Eco*RI-*Bam*HI fragment did not appear to contain any of the *nrdB* gene and was used as a *nrdA*-specific hybridization probe. The 1.9-kb *Kpn*I-*Pst*I fragment isolated from pPH108 was used as a *nrdB*-specific hybridization probe based on the following information. Preliminary DNA sequencing results (J. Carlson, personal communication) indicated that the *nrdA* gene sequence begins very near the *Eco*RI restriction site, and approximately 2.2-kb would be required to code for the 80,000-molecular-weight *nrdA* gene product. The *Kpn*I restriction site is 3.05 kb from the *Eco*RI restriction site, and therefore, the 1.9-kb *Kpn*I-*Pst*I fragment does not contain any of the *nrdA* gene but should contain some of the *nrdB* gene since at least 3.2 kb would be required to code for *nrdA* and *nrdB*. The 1.6-kb *Kpn*I-*Eco*RI fragment was isolated from pPS2 and used as a hybridization probe to determine whether *nrd* RNAs were complementary to DNA in this region.

The direction of transcription of *nrd* RNA transcripts was determined by using the 5.0-kb *Eco*RI-*Pst*I fragment of pPH108 subcloned in the double-stranded replicative form of the single-stranded M13 bacteriophage vectors M13mp8 and M13mp9 (21). The M13mp8 and M13mp9 are constructed such that when the 5.0-kb *Eco*RI-PstI restriction fragment is cloned into these vectors, the resultant single-stranded form of the recombinant phage contains complementary strands of the inserted DNA. The *Eco*RI-*Pst*I fragment cloned into M13mp8 will have the *Eco*RI restriction site on the 5′ end of the cloned fragment and the *Pst*I restriction site on the 3′ end. Alternatively, the fragment cloned into M13mp9 will have the *Eco*RI restriction site on the 3′ end and the *Pst*I restriction site on the 5′ end. Since RNA is transcribed from 5′ to 3′, the single-stranded probe that hybridizes to *nrd* RNA will have a 3′ to 5′ orientation.

Total *E. coli* RNA was separated by electro-

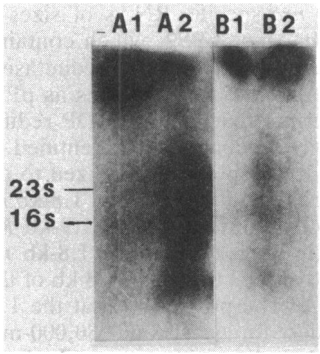

FIG. 2. Direction of transcription of the *nrd* genes. The A lanes were hybridized to a recombinant M13mp9 phage containing an *Eco*RI-*Pst*I insert. The B lanes were hybridized to a recombinant M13mp8 phage containing an *Eco*RI-*Pst*I insert. Lane 1 is RNA isolated from an exponentially growing culture and lane 2 is RNA isolated from a thymine-starved culture.

phoresis on methyl mercury agarose gels (17), transferred to diazobenzyloxymethyl paper, and hybridized (2) to the M13 probes labeled by extension of a primer 3′ to the inserted DNA by the method of Hu and Messing (16) (Fig. 2). The results show that the M13mp9 probe, with the *Eco*RI site at the 3′ end of the *Pst*I site at the 5′ end of the *nrd* DNA, hybridizes to the *nrd* RNAs. This demonstrates that all *nrd* RNA species are transcribed from the same DNA strand and that they are transcribed from left to right on the restriction map (Fig. 1). The reason for the faint hybridization seen with the probe cloned in the opposite orientation is not known. The hybridization seen at the top of the gel is presumably due to chromosomal DNA that is present in the RNA samples.

To further characterize the *nrd* RNA transcripts, the various *nrd*-specific probes (Fig. 1) were nick translated and hybridized to total *E. coli* RNA that had been separated by electrophoresis on a methyl mercury agarose gel and transferred to diazobenzyloxymethyl paper (Fig. 3). Using the 23S and 16S rRNAs as size markers, three RNA species were found to hybridize to pPH108: one with a size of 3.2 kb, one of 1.8 kb, and a third minor RNA species of 0.7 kb. The same size *nrd* RNA species also hybridized to pPS2 (data not shown). Comparison of Fig. 3 lanes A and B shows the difference between noninduced and induced levels of *nrd* mRNA. The 3.2- and 1.8-kb *nrd* RNAs are seen in noninduced cells, and although the smallest *nrd* RNA is not seen in this autoradiogram, it has been observed in other experiments (not shown). This indicates that the smaller RNAs are present in exponentially growing cells and are not artifacts of thymine starvation. The *nrdA*-specific hybridization probe, pPH5, also hybridized to all three *nrd* RNA species (Fig. 3, lane D) but the *nrdB*-specific hybridization probe, the 1.9-kb *Kpn*I-*Pst*I fragment, hybridized to only the two larger *nrd* RNAs (Fig. 4, lanes E and F). Even after very long exposures of the autoradiogram, hybridization of the 1.9-kb *Kpn*I-*Pst*I fragment of the 0.7-kb *nrd* RNA species could not be detected. The 1.6-kb *Kpn*I-*Eco*RI fragment did not hybridize to any RNA species (Fig. 3, lane G), indicating that the *nrd* RNAs must start very near or to the right of the *Eco*RI restriction site (Fig. 1). These results indicate that the 3.2-kb *nrd* RNA is a polycistronic mRNA which codes for both the *nrdA* and *nrdB* gene products. This is consistent with the amount of DNA necessary to code for an 80,000-molecular-weight polypeptide and a 40,000-molecular-weight polypeptide.

There is a possibility that the 1.8-kb *nrd* RNA could code for a second 80,000-molecular-weight B1 polypeptide and be transcribed from a gene located in the *Kpn*I-*Pst*I fragment. If such a gene exists in the *Kpn*I-*Pst*I fragment, this fragment should be homologous to the *nrdA* gene since the *nrdA*-specific hybridization probe, pPH5, also hybridizes to the 1.8-kb *nrd* RNA. To examine this possibility, a DNA-DNA hybridization experiment was conducted to determine whether the *Kpn*I-*Pst*I fragment would hybridize to plasmid pPH5 (Fig. 4). The results show that the *Kpn*I-*Pst*I fragment does not hybridize to the *Eco*RI-*Bam*HI fragment of pPH5. As positive and negative controls, pPH108 and pBR322, respectively, were also hybridized to the *Kpn*I-*Pst*I fragment. Therefore, a second gene for the B1 subunit of RDP reductase does not exist in the *Kpn*I-*Pst*I region.

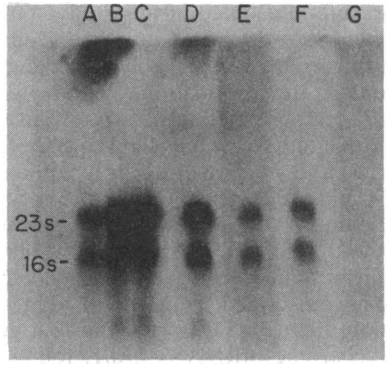

FIG. 3. Hybridization of various *nrd* DNA probes to total *E. coli* RNA. Lanes A, B, and C were hybridized to pPH108. Lane A, RNA isolated from an exponentially growing culture; Lanes B and C, RNA isolated from two separate thymine-starved cultures; lanes D, E, F, and G, RNA samples isolated from a thymine-starved culture and hybridized to pPH5, the *Kpn*I-*Pst*I fragment, the *Kpn*I-*Pst*I fragment purified by electrophoresis through two agarose gels, and the *Kpn*I-*Eco*RI fragment, respectively.

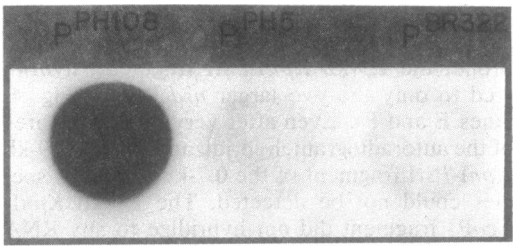

FIG. 4. Dot hybridization of pBR322, pPH5, and pPH108 to the *Kpn*I-*Pst*I restriction fragment.

To investigate whether the reason for multiple *nrd* RNA species is due to breakdown of the 3.2-kb *nrd* RNA, we added rifampin to cells induced for RDP reductase, and total *E. coli* RNA was isolated at various times after rifampin addition. The RNA was fractionated by electrophoresis on methyl mercury agarose gels, transferred to diazobenzyloxymethyl paper, and then hybridized to the *Kpn*I-*Pst*I fragment (Fig. 5). If the 1.8-kb *nrd* RNA is derived from the 3.2-kb *nrd* mRNA, one would expect the 3.2-kb *nrd* mRNA to disappear more rapidly than the 1.8-kb *nrd* RNA species after inhibition of RNA synthesis. When the RNA is hybridized to the *Kpn*I-*Pst*I fragment, the intensity of the 3.2-kb *nrd* RNA species decreases more rapidly than the 1.8-kb *nrd* RNA (Fig. 5). The reason that the 6- and 8-min time points do not decrease any further is not clear, but it may be due to incomplete inhibition of RNA synthesis by rifampin. Therefore, these results suggest, but do not prove, that the 1.8-kb *nrd* RNA species may be a degradation or processing product of the 3.2-kb *nrd* RNA.

DISCUSSION

The results presented in this study show that the *nrdA* and *nrdB* genes are organized in an operon that codes for one polycistronic mRNA that contains the information for both the *nrdA* and *nrdB* gene products. The size of this 3.2-kb polycistronic mRNA is sufficient to code for one B1 polypeptide and one B2 polypeptide. The results explain the coordinate control of the B1 and B2 subunits of RDP reductase (10). Furthermore, the hybridization experiment which used the single-stranded probes showed that the *nrd* mRNA is transcribed from left to right on the restriction map (Fig. 1). Thus, the first gene of the polycistronic mRNA is *nrdA*, followed by *nrdB*, and therefore, the promoter for both *nrd* genes should be located to the left of the *nrdA* gene. This conclusion is consistent with the observation of Platz and Sjoberg that the *nrdB* subclone does not have amplified levels of the B2 subunit (22). It also explains the observation of Yamada et al. (28) that the region close to the *Eco*RI site affects *nrdB* activity.

RNA-DNA hybridization experiments identified three *nrd*-specific RNAs of sizes 3.2, 1.8, and 0.7 kb. Since pPS2, which contains all the genetic information for RDP reductase, hybridizes the same *nrd* RNA species as pPH108, all the transcripts that contain RDP reductase sequences have probably been identified. Since all three *nrd* RNA species hybridized to the *nrdA*-specific probe and the 3.2- and 1.8-kb *nrd* RNA species both hybridize to the 1.9-kb *Kpn*I-*Pst*I region, this suggests that the 1.8-kb *nrd* RNA probably overlaps the distal 1.8 kb of the 3.2-kb *nrd* mRNA. The possibility that the 1.8-kb *nrd* RNA codes for a second 80,000-molecular-weight B1 polypeptide transcribed from the *Kpn*I-*Pst*I region was eliminated because the *Kpn*I-*Pst*I restriction fragment did not hybridize to pPH5, which also hybridizes to the 1.8-kb *nrd* RNA. The possibility that the 1.8-kb *nrd* RNA species codes for a functional *nrdA* gene from some other location on the *E. coli* chromosome is unlikely because it contains sequences from both the *nrdA* gene and the *Kpn*I-*Pst*I region and therefore does not have sufficient coding capacity for an *nrdA* gene product. The possibility that the 1.8-kb *nrd* RNA is transcribed from an *nrdB* gene from some other location on the *E. coli* genome is also unlikely since the B2 protein appears to be composed of identical polypeptides (25) and a single mutation eliminates the *nrdB* gene product (11). Since all the *nrd* RNA species have probably been identified, these results indicate that RDP reductase is encoded by only two genes, *nrdA* and *nrdB*, located within the 5.0-kb *Eco*RI-*Pst*I fragment. Therefore, the two polypeptides of B1 must be encoded by the same *nrdA* gene. The differences in the B1 polypeptides may be due to post-transla-

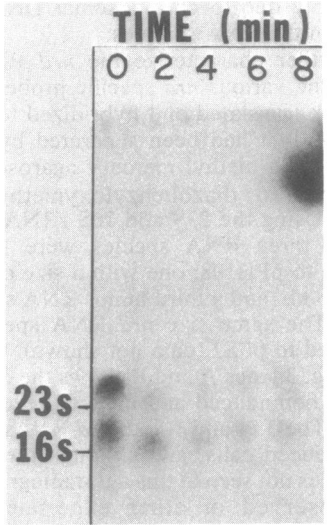

FIG. 5. *nrd* mRNA decay. Total *E. coli* RNA was isolated from a thymine-starved culture 0, 2, 4, 6, and 8 min after addition of rifampin and hybridized to the *Kpn*I-*Pst*I restriction fragment.

tional modification, two different promoters for the *nrd* operon, or they may be an artifact of the protein isolation procedure.

The smaller *nrd* RNA species could be degradation products caused by a specific endonucleolytic cleavage or transcription termination products or even products of internal promoters. One possibility is that the 0.7-kb *nrd* RNA is a transcription termination product and the 1.8-kb *nrd* RNA is a product of an internal promoter that terminates at the same site that the 3.2-kb *nrd* mRNA does. If there is an internal promoter it would also have to be regulated the same way as the main *nrd* promoter since both the 3.2- and 1.8-kb *nrd* RNAs increase by similar amounts during thymine starvation. A specific endonucleolytic cleavage seems more likely since the rifampin experiment showed that the 3.2-kb *nrd* mRNA decays faster than the 1.8-kb *nrd* RNA, suggesting that the 1.8-kb *nrd* RNA may be an endonucleolytic cleavage product of the 3.2-kb *nrd* mRNA. The fate of the other part of the 3.2-kb *nrd* mRNA is not known. It may have a very short half-life or, alternatively, the 0.7-kb *nrd* RNA may be part of the 5′ end of the endonucleolytic cleavage. Specific endonucleolytic cleavage as a model for mRNA decay has been proposed for several operons, including the *lac* operon (18), the *trp* operon (19), and the *gal* operon (1). The possibility that the 1.8-kb *nrd* RNA is transcribed from an internal promoter and has a longer half-life than the 3.2-kb *nrd* mRNA or that the 1.8-kb *nrd* RNA is a product of exonucleolytic degradation and is stabilized against further degradation cannot be eliminated by the results presented in this study.

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