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Cloning, Characterization, and Effects of Overexpression of the *Escherichia coli rnd* Gene Encoding RNase D

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RNase D is a 3′-exoribonuclease whose in vitro specificity has suggested that it is involved in the processing of tRNA precursors. Its in vivo role has remained unclear, however, because mutant cells devoid of the enzyme display no defect in growth or tRNA processing. To learn more about the structure and function of RNase D, we cloned the *Escherichia coli rnd* gene, which is thought to code for this enzyme. The *rnd* gene was isolated from a cosmid library based on elevated RNase D activity and was subcloned as a 1.4-kilobase-pair fragment in pUC18. Maxicell analysis of the cloned fragment revealed that a single protein of ~40 kilodaltons, which is the size of RNase D, was synthesized. The *rnd* gene is present as a single copy on the *E. coli* chromosome and is totally absent in a deletion mutant. Cells that harbored the cloned *rnd* gene displayed RNase D activity that was elevated as much as 20-fold over that of the wild type. As growth of the culture progressed, however, RNase D specific activity declined dramatically, together with a similar decrease in plasmid copy number. In contrast, no decrease in copy number was observed with an inactive *rnd* gene. Placement of the *rnd* gene downstream from the *lac* promoter led to inducible RNase D overexpression and concomitantly slowed cell growth. These findings support the idea that *rnd* is the structural gene for RNase D and indicate that elevated RNase D activity is deleterious to *E. coli.*

In recent years a number of new RNases have been identified in *Escherichia coli* (8). One of these, RNase D, is a 3′-exoribonuclease that displays a high degree of specificity for certain tRNA-related molecules (6, 11). tRNAs that lack the 3′-terminal CCA sequence or those that contain several nucleotide residues following the CCA sequence are both active substrates for the enzyme, whereas intact tRNA CCA is hydrolyzed relatively poorly (6, 11). Thus, in the case of tRNAs with extra 3′ residues, the additional nucleotides are removed in a random fashion, generating tRNA molecules with functional CCA termini (5). This activity of RNase D has led to the suggestion that the enzyme might participate in the 3′ processing of *E. coli* tRNA precursors (5), all of which appear to contain an encoded CCA sequence followed by additional residues (7, 24).

Attempts to confirm this suggestion by the use of mutants that are deficient in RNase D (*rnd* mutants) have not been successful. Neither a strain with a temperature-sensitive RNase D (22) nor one with a deletion in the *rnd* gene (2) shows any growth impairment and, in the latter case, any defect in tRNA biosynthesis. We have also shown (23) that an *E. coli* strain that is deficient in three exoribonucleases, RNase D, RNase II, and RNase BN, likewise, displays no defect in growth or tRNA processing. Thus, it is not yet clear what the role of RNase D might be in vivo or which exoribonuclease is involved in the 3′ processing of tRNA precursors.

To learn more about the structure and function of RNase D, we cloned the *rnd* gene, which is thought to code for RNase D (2, 22), from a cosmid library. In this report we describe the cloning and characterization of the *rnd* region of the *E. coli* chromosome, the overexpression of RNase D, and results of our initial studies on the effect of elevated RNase D activity on cells. Our results indicate that *rnd* is the structural gene for RNase D and that elevation of RNase D activity is deleterious to *E. coli*.

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MATERIALS AND METHODS

Bacterial strains and plasmids. Wild-type *E. coli* K-12 strains CA265 and its multiple RNase-deficient derivative 20-12E/18-11 (RNase I$^-$, II$^-$, D$^-$, BN, T$^-$) have been described previously (23). Strain MC1061 (3) and strain UT481 [Δ(*lac-pro*) *hsdS* (r$^-$ m$^-$) *lacI*^{q} *lacZ*], which was obtained from Gordon Carmichael, were used for the growth of phage and plasmids. Strain CSR603 was used to prepare maxicells (17). Plasmids pHC79 (12), pBR328 (18), pUC13 (20), pUC18 (21), and the runaway plasmid pOU61 (13) have been described previously. Plasmid pMLB1107, which contains a *lacI*^{q} gene and a *lac* promoter, was constructed by M. L. Berman and given to us by Asis Das.

Culture conditions. Cells were routinely grown at 37°C in YT medium or on YT plates (15). The minimal medium used was M9 (15). Glucose, when present, was included at 1%, and glycerol was included at 0.2%. Antibiotics were added at the following concentrations: ampicillin, 50 μg/ml; tetracycline, 12.5 μg/ml; chloramphenicol, 35 μg/ml. Growth in liquid culture was followed by determination of the A_{550}.

Chemicals and enzymes. Amino acids, dithiothreitol, phenylmethylsulfonyl fluoride, D-cycloserine, sodium dodecyl sulfate (SDS), and RNase A were purchased from Sigma Chemical Co. (St. Louis, Mo.). [α-^{32}P]CTP and [^{3}H]poly(A) were obtained from Amersham Corp. (Arlington Heights, Ill.); and $^{32}P_i$, [^{14}C]ATP, and [^{35}S]methionine were obtained from New England Nuclear Corp. (Boston, Mass.). Restriction endonucleases, T4 DNA ligase, and BAL 31 nuclease were products of New England BioLabs, Inc. (Beverly, Mass.). DNA polymerase I (Klenow fragment) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.), and DNase I and snake venom phosphodiesterase were obtained from Cooper Biomedical. Hexadeoxyribonucleotide primers for probe labeling were obtained from Pharmacia Fine Chemicals (Piscataway, N.J.). Ultrogel AcA44 and AcA54 were purchased from LKB Instruments, Inc. (Rockville, Md.).

RNase substrates and assays. The RNase substrates,

[32P]diesterase-treated tRNA (dtRNA), tRNA-CCA-[14C]C-C, tRNA-C-C-[14C]A, and [32P]rRNA were prepared as described previously (23). Assays for RNase D, RNase II, RNase T, RNase R, and RNase I were carried out according to our standard conditions for these enzymes (23).

Preparation of extracts. Cells and transformants were grown until the A_{550} reached ~1 or as indicated in the figure legends. Cells were centrifuged, suspended in a 1/5th or 1/10th volume of 20 mM glycine–NaOH (pH 8.9), and ruptured by sonication with two 15-s pulses. Cell debris was removed by centrifugation, and the supernatant fraction was used for assay.

Preparation and screening of cosmid library. The cosmid library, a gift from P. de Boer, was prepared from a partial *Sau*3A digest of DNA (fragment size, 45 to 50 kilobases [kb]) from strain ED51 (F'126 *pro trpA his metE*), ligated into pHC79, and packaged in vitro. Infection of strain 20-12E/18-11 was carried out under standard conditions and gave 443 Amp^r transformants. For rapid screening of the library, extracts were prepared as described above from an equal mixture of five transformants and assayed against each of the RNase substrates to find a group with elevated RNase D activity. Each of the transformants from a group that showed elevated activity was then grown and assayed individually. Potential positive *rnd* clones were examined in more detail (see below).

Recombinant DNA techniques. Standard recombinant DNA techniques described by Maniatis et al. (14) were used for the isolation of plasmid DNA, restriction endonuclease and BAL 31 exonuclease digestions, agarose gel electrophoresis, filling in of DNA and DNA ligation reactions, transformations, and hybridizations. Genomic DNA from strains CA265 and 20-12E/18-11 was prepared by the method described by Nakamura et al. (16). Radioactive labeling of DNA probes was done with [α-32P]CTP to a specific activity of 10^8 cpm/μg of DNA by the procedure described by Feinberg and Vogelstein (10). Maxicell preparation and labeling and analysis of labeled proteins by SDS-polyacrylamide gel electrophoresis (PAGE) were as described previously (17).

Determination of plasmid copy number. Total DNA was extracted by an SDS lysis procedure (1) and subjected to electrophoresis in a 0.9% agarose gel. DNA was transferred to nitrocellulose (19) and hybridized to 32P-labeled pUC18. The hybridized bands were localized by autoradiography, cut from the filter, and quantitated by liquid scintillation counting. The suitability of the procedure was confirmed by the use of increasing amounts of standard pUC18 DNA.

Construction of a mutant *rnd* gene. Plasmid pDB14, which contained a 1.4-kb *rnd* fragment in pUC18, was cleaved with *Bgl*II within the *rnd* gene (see Fig. 1). The four-nucleotide overlapping ends were filled in with DNA polymerase I and closed with T4 DNA ligase. The mutated plasmid pBF5, which contained a four-nucleotide GATC addition in the *rnd* gene, was resistant to digestion by *Bgl*II, and UT481 cells transformed with this plasmid did not show elevated RNase D activity (see Fig. 5B).

RESULTS

Cloning of the *rnd* gene. Since no selection procedure was available for isolation of the *rnd* gene, we screened a cosmid library containing relatively large fragments of the *E. coli* genome for transformants that displayed elevated levels of RNase D or other RNases. To facilitate screening, an *E. coli* strain, 20-12E/18-11, that was deficient in multiple RNase activities was used as the recipient for infection with the cosmids, and transformants were combined in groups of five for RNase assays. The low background levels of RNases in the recipient strain ensured that even small increases in activity levels would be detected. Four hundred transformants were screened in this fashion, and several strains with elevated activity for various RNases were identified. One strain, 236, that displayed elevated activity against [32P]dtRNA, the RNase D substrate, but not against other RNase substrates was chosen for further study.

To confirm that the elevated activity in transformant 236 was RNase D, a supernatant fraction was prepared by high-speed centrifugation and applied to a column of either Ultrogel AcA44 or AcA54. A single peak of activity against [32P]dtRNA was found in each case that corresponded to a molecular mass of ~40 kilodaltons (kDa), which is the known size of RNase D (6). Furthermore, the substrate specificity of this activity was identical to that of purified RNase D (Table 1). Based on these findings, we tentatively concluded that cosmid 236 contains the *rnd* gene.

Subcloning and restriction analysis of the *rnd* region. Purified cosmid 236 DNA was digested with restriction endonuclease *Eco*RI, and fragments were ligated into plasmid pBR328. Assays of the cells that were transformed with this mixture demonstrated that elevated RNase D activity is associated with a 5.7-kb DNA fragment. The 5.7-kb fragment from this plasmid, pYR32, was excised and subsequently ligated into pUC18 to generate plasmid pYU19, which also displayed elevated RNase D activity on transformation of strain UT481. The 5.7-kb DNA fragment was subjected to restriction cleavage with a variety of enzymes, and the deduced restriction map is shown in Fig. 1.

Localization of the *rnd* gene. Localization of the *rnd* gene was carried out with BAL 31 exonuclease and restriction endonuclease digestion of the 5.7-kb fragment. Each of the shortened fragments was cloned into pUC18 and analyzed for its coding potential by using maxicells and for its ability to overexpress RNase D activity. Maxicell analysis indicated that the original 5.7-kb fragment coded for three proteins of 41, 18, and 69 kDa (Fig. 2). The last two proteins were also found when the 3.5-kb *Bgl*II-*Eco*RI fragment was subcloned (Fig. 3), suggesting that they were localized to the right of the *Bgl*II site (Fig. 3).

More detailed localization of the three genes on the 5.7-kb fragment was ascertained by BAL 31 deletion analysis. Removal of nucleotides from the right side of the fragment led to the progressive loss of first the 18-kDa and then the 69-kDa proteins (Fig. 3). A 2.7-kb fragment from the left-hand side of the insert retained its coding capacity for the 41-kDa protein (Fig. 2) and for elevated RNase D activity. Removal of an additional 300 nucleotides from the right side

TABLE 1. Substrate specificity of the partially purified RNase activity encoded by cosmid 236

Substrate	Nucleotide (nmol/10 min [%]) released by:	
	RNase D	Fraction 46[a]
[32P]dtRNA	5.8 (100)	6.7 (100)
[32P]tRNA	1.8 (31)	2.5 (37)
[3H]poly(A)	0.2 (3.4)	0.2 (3.0)
[32P]rRNA	0.1 (1.7)	0.2 (3.0)

[a] Fraction 46, which was obtained from an Ultrogel AcA54 column, was concentrated 10-fold by ultrafiltration and assayed against the indicated substrates as described in the text, and results were compared with those of similar assays done with purified RNase D.

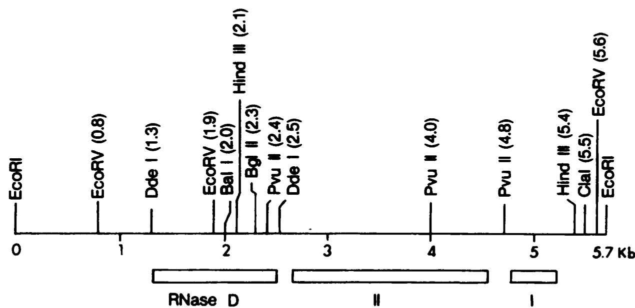

FIG. 1. Restriction map of the *rnd* region of the *E. coli* chromosome. The 5.7-kb *Eco*RI subclone of cosmid 236 was digested with a variety of restriction endonucleases, and the resulting cleavage map was derived from these data. The approximate positions of the genes for RNase D and unknown proteins I and II, which were obtained from the data given in Fig. 3, are indicated. The numbers in parentheses indicate the position of each restriction site, in kilobases, relative to the left-hand side of the fragment.

of the 2.7-kb fragment, however, led to the loss of both elevated RNase D activity and the 41-kDa protein. Further BAL 31 exonuclease trimming from the left-hand *Eco*RI site afforded precise localization of the *rnd* gene to a 1.4-kb fragment. Removal of ∼100 additional nucleotides from the left side to give a 1.3-kb fragment led to the loss of RNase D activity; but a slightly larger protein of 44 kDa was synthesized in the maxicells, presumably because of the removal of the normal translation termination site. Removal of an additional ∼100 nucleotides to generate a 1.2-kb fragment led to the synthesis of a smaller protein of 34 kDa. These data strongly suggest that *rnd* is the structural gene for RNase D, and that the initiation site for RNase D synthesis is in the region between protein II and *rnd*. In fact, results of sequence analysis of this region have revealed a possible promoter, a Shine-Delgarno sequence, and the beginning of an open reading frame (unpublished data).

Number of copies of the *rnd* gene in wild-type and in a deletion mutant of *E. coli*. DNA from wild-type strain CA265 was individually digested with several restriction enzymes (*Pst*I, *Cla*I, *Bam*HI, *Eco*RI) and hybridized to a labeled probe made from the 1.4-kb fragment that encompasses the complete *rnd* gene. Only a single band in each digestion hybridized, indicating that the *rnd* gene is present as a single copy on the *E. coli* chromosome (Fig. 4). When the same experiment was carried out with DNA from the putative *rnd* deletion strain 20-12E/18-11, no hybridization occurred, confirming that the complete *rnd* gene was deleted. Further analysis of the extent of the deletion by use of labeled probes from other parts of the 5.7-kb fragment indicated that the deletion extended at least from the left-hand *Eco*RI site to the *Pvu*II site at 4.8 kb. Some DNA between 4.8 kb and the right-hand end of the 5.7-kb fragment, however, was present since a probe from this region did hybridize (data not shown). The extent of the deletion past the left-hand side of the 5.7-kb fragment is not known.

RNase D overexpression. Results of our initial studies of RNase D overexpression when a variety of vectors was used revealed that the specific activity of RNase D changes substantially during growth of the transformed cells. Detailed examination of this point with the 1.4-kb fragment in the high-copy-number plasmid pUC18 is shown in Fig. 5A. RNase D elevation early in growth was as much as 20-fold compared with that of control cells containing pUC18 without an insert, and its level declined as much as 5-fold during the course of logarithmic growth. The basal level of RNase D in strain UT481 remained unchanged during cell proliferation, indicating that RNase D activity is not normally affected by the phase of growth. It should be noted that although the specific activity of RNase D declined during growth, the total RNase D activity in the culture increased, although not as fast as cell mass, suggesting that the enzyme does not undergo an inactivation process but, rather, is diluted out during cell growth. Similar decreases in RNase D specific activity were also observed with the original 5.7-kb *Eco*RI fragment that was cloned into pBR328 or pUC18, with the 2.7-kb fragment (Fig. 3) that was cloned into pUC18, and with the 1.4-kb fragment that was cloned into pBR328. Likewise, induction of the runaway plasmid pOU61 (13), which contained the 1.4-kb fragment, led to a 25-fold elevation of RNase D activity within 2 h of a shift to 42°C; this was followed by a rapid decline in activity.

Effect of elevated RNase D on plasmid copy number. To ascertain the reason for the decreasing specific activity of RNase D during the growth of transformed cells, we examined the copy number of pUC18 in cells that harbored this plasmid with or without an *rnd* insert. From the data in Fig. 5A it can be determined that whereas the copy number of pUC18 remained relatively constant throughout the log phase, when the 1.4-kb *rnd* fragment was present, the initial plasmid copy number was only 40% of the control value; and it decreased during growth to a level that was only 5 to 10% of that in the absence of the insert. The overall decreases during growth in the RNase D specific activity and plasmid copy number paralleled each other very closely, suggesting that the decreasing activity may be a consequence of the decreasing copy number.

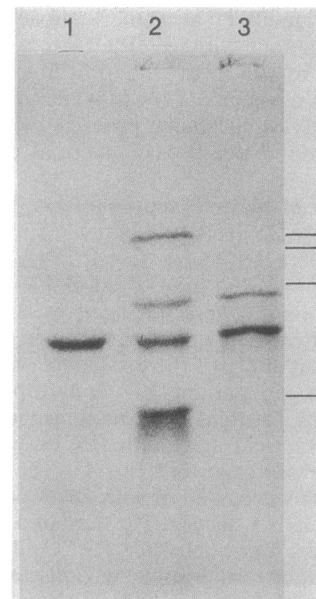

FIG. 2. Maxicell analysis of plasmid-encoded proteins. Maxicell strain CSR603 cells were transformed with pUC18 (lane 1); pYU19, which contained the 5.7-kb subclone of the *rnd* region (lane 2); or pDB27, which contained the 2.7-kb BAL 31 fragment noted in Fig. 3 (lane 3). Cells were labeled with [^{35}S]methionine, and extracts were run on SDS-PAGE, as described by Sancar and Rupp (17). The migration position of the following molecular mass standards, from top to bottom, are shown on the right: bovine serum albumin, 68 kDa; catalase, 59 kDa; ovalbumin, 44 kDa; trypsin inhibitor, 21.5 kDa. The common band at 32 kDa in all lanes was the β-lactamase that is encoded by the vector.

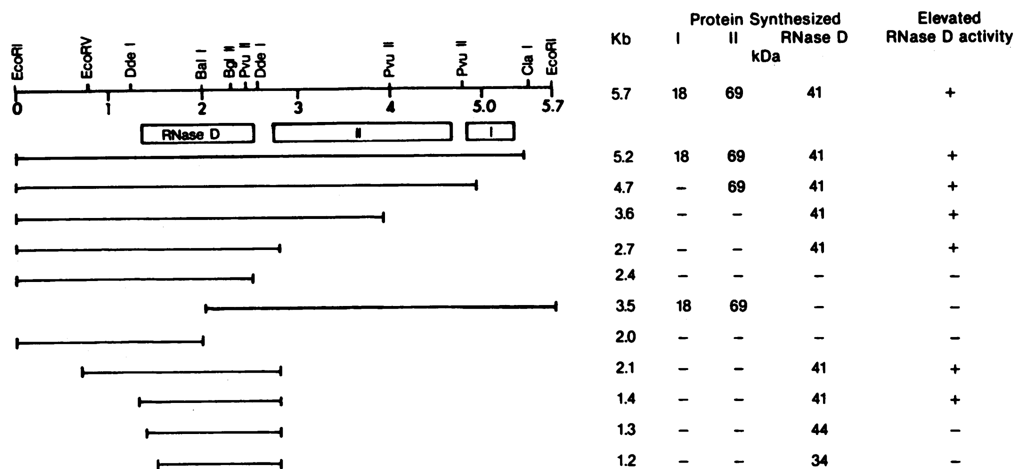

FIG. 3. Localization of the *rnd* gene on the 5.7-kb fragment. Various fragments with deletions from each end of the 5.7-kb *Eco*RI fragment were generated by restriction endonuclease or BAL 31 treatment, and were tested for their ability to elevate RNase D activity and to code for the synthesis of proteins in maxicells. The diagram at the left shows the approximate length of the fragments compared with that of the original 5.7-kb fragment. The adjacent columns indicate the length of the fragment based on agarose gel electrophoresis, the proteins that were synthesized in the maxicells and their sizes on SDS-PAGE, and whether elevated RNase D activity was observed. The approximate positions of the genes for RNase D and proteins I and II, which were based on the results of this analysis, are shown. All fragments, except those of 2.4, 3.5, and 2.0 kb, were generated by BAL 31 nuclease.

To determine whether it was *rnd* DNA or elevated RNase D activity that affected the plasmid copy number, a mutant *rnd* gene that contained a four-nucleotide insert at the *Bgl*II site was constructed in pUC18; and the mutant plasmid was used to transform strain UT481. RNase D activity in this strain was not increased above the basal level, and likewise, the copy number of the mutant plasmid remained constant throughout growth and at the same level as pUC18 (Fig. 5B). These findings indicate that it is elevated RNase D activity that adversely affects the copy number of the plasmid.

Effect of elevated RNase D on cell growth. The experiment for which the results are shown in Fig. 5A is unsatisfactory for accurately assessing effects on cell growth because the decreasing plasmid copy number and RNase D activity during growth may reflect dilution by faster-growing cells with lower plasmid copy numbers and less RNase D activities. In fact, in a number of experiments, the initial growth of the transformants that contained a cloned *rnd* gene was slower than that in subsequent experiments with the same cells, suggesting that the original phenotype was lost. Likewise, the earliest measurement of plasmid copy number was already decreased 60% compared with that of plasmids without an *rnd* insert (Fig. 5A).

To circumvent these potential problems, a *Dde*I fragment that contained the *rnd* gene (see Fig. 1) but that lacked the *rnd* promoter was cloned adjacent to the inducible *lac* promoter of plasmid pMLB1107, and the effect of RNase D induction on cell growth was examined. This plasmid contained a *lacI*^{q} gene, so that the consequent high level of repressor served to keep the *rnd* gene almost fully repressed prior to induction. The absence of the *rnd* promoter was confirmed by the finding that only one of the two orientations of the *Dde*I fragment led to elevated and inducible RNase D activities, whereas the 1.4-kb fragment (Fig. 3) led to elevated RNase D activities in either orientation (data not shown).

In the absence of isopropyl-β-D-thiogalactopyranoside, RNase D specific activity was only slightly elevated above the control levels that were found without an *rnd* insert (Fig. 6A). The addition of isopropyl-β-D-thiogalactopyranoside led to a rapid increase in RNase D specific activity and a decreased growth rate (Fig. 6B). With time, however, as observed previously (Fig. 5), RNase D specific activity fell, and this was accompanied by an increase in the growth rate,

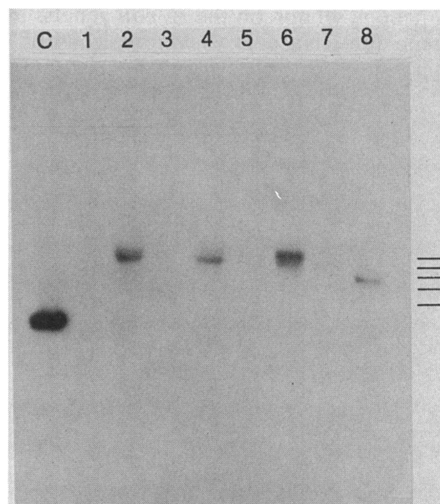

FIG. 4. Hybridization analysis of the *rnd* gene in the wild type and in a deletion mutant. Chromosomal DNA from wild-type strain CA265 and its *rnd* deletion derivative 20-12E/18-11 were each digested with the restriction endonucleases *Pst*I (lanes 1 and 2), *Cla*I (lanes 3 and 4), *Bam*HI (lanes 5 and 6), and *Eco*RI (lanes 7 and 8) and were run on a 0.8% agarose gel. DNA was transferred to nitrocellulose by the method described by Southern (19) and hybridized to a ^{32}P-labeled, 1.4-kb *rnd* fragment (Fig. 3). Hybridization was carried out at 68°C for ~15 h in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.01 M EDTA–5× Denhardt solution–0.5% SDS–100 μg of denatured salmon sperm DNA per ml. DNA was obtained from strain 20-12E/18-11 (lanes 1, 3, 5, and 7), CA265 (lanes 2, 4, 6, and 8), and a self-hybridization control (lane C). Size standards, in kilobases, shown on the right were as follows, from top to bottom: 23.1, 9.4, 6.6, 4.4, and 2.3 kb.

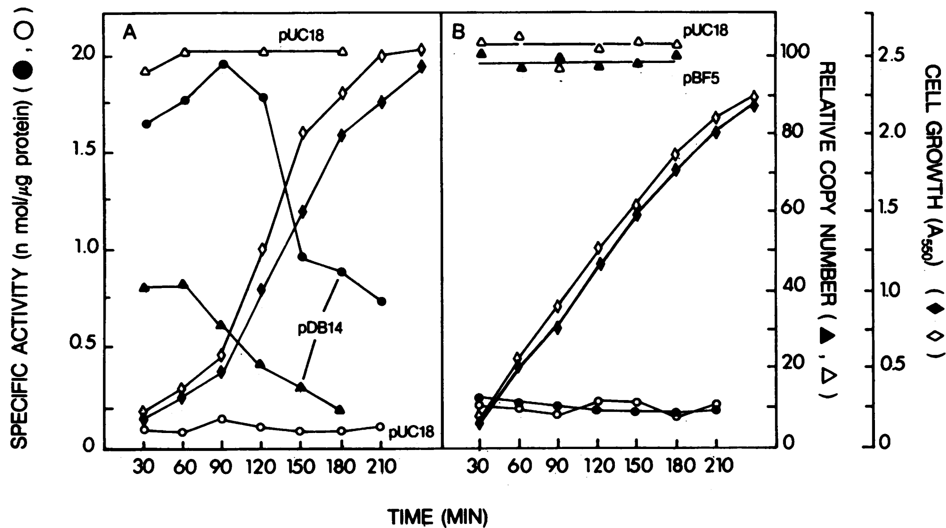

FIG. 5. RNase D activity and copy number throughout the growth of cells harboring pUC18 and containing wild-type or mutant *rnd* genes. Overnight cultures were diluted into YT-ampicillin medium and grown at 37°C until the beginning of the stationary phase. Portions were removed at various times for measurement of the A_{550}, RNase D activity, protein, and copy number, as described in the text. (A) UT481 cells with pUC18 or pDB14 that contained the 1.4-kb wild-type *rnd* insert. (B) UT481 cells with pUC18 or pBF5 that contained a mutant *rnd* gene (see text). The relative copy number of the plasmid was normalized to the same A_{550} of the culture at each time point.

which reached that of the control cells. These data demonstrate that elevated RNase D affects cell growth, but that a rapid alteration of phenotype ensues.

DISCUSSION

Results of previous genetic studies (2, 22) have suggested that the *rnd* gene at 40 min on the *E. coli* genetic map is the structural gene for RNase D. Several pieces of evidence described here support this assignment. (i) A cloned DNA fragment of 1.4 kb that contained the *rnd* gene led to the synthesis of a 40,000-Da protein, which is the known size of RNase D (5), in maxicells, and to elevated RNase D activity in transformed cells; (ii) changes in *rnd* DNA that altered the size of the protein or abolished its synthesis also abolished elevated RNase D activity; (iii) a deletion mutant that was devoid of RNase D activity lacked *rnd* DNA; and (iv) a four-nucleotide insert in the *rnd* gene abolished RNase D activity. While these data indicate that it is likely that *rnd*

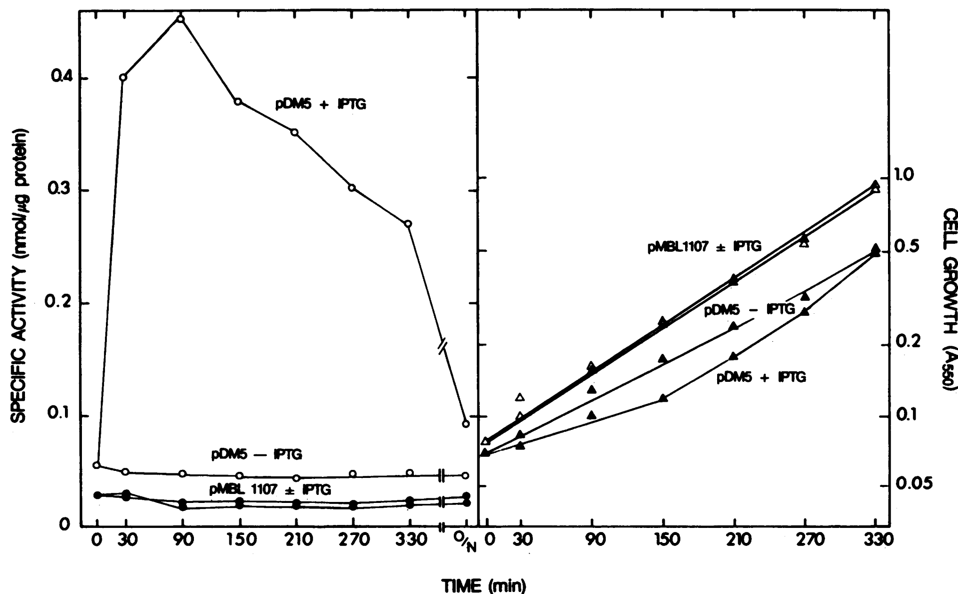

FIG. 6. Effect of RNase D induction on cell growth. UT481 cells, which were transformed with plasmid pMLB1107, which contained (pDM5) or lacked the *DdeI* insert, were grown overnight in M9 medium-glucose-ampicillin. The cells were diluted 1:200 and grown for an additional 2 h in the same medium. Cells were then spun down, washed once with M9 medium-glycerol, and suspended in the initial volume of M9 medium-glycerol-ampicillin. The cultures were divided into two portions, and to one of each portion isopropyl-β-D-thiogalactopyranoside (IPTG) was added to 0.5 mM at time zero. Cultures were then grown and analyzed as described in the legend to Fig. 5. (A) RNase D specific activity; (B) A_{550} of cultures.

encodes RNase D, sequencing of the *rnd* gene and comparison with the sequence of the purified protein will be required to conclusively prove this point; these studies are under way.

The maxicell experiments also showed that *rnd* is in close proximity to genes for two other proteins of 18 and 69 kDa. The identity of these proteins is unknown, however, and it appears that *rnd* is transcribed from its own promoter independently of these genes. Preliminary sequencing data have confirmed that a possible promoter sequence is present between the gene for protein II and *rnd* that could support *rnd* transcription. By use of the *rnd* promoter and the multicopy plasmid pUC18 or the runaway plasmid pOU61, we have been able to overexpress RNase D to a maximum of 20- to 30-fold. These systems should facilitate purification of RNase D for additional structural and mechanistic studies.

The data presented here also indicate that elevated levels of RNase D activity are deleterious to *E. coli*. It has not been possible to maintain cells with more than a fewfold elevation of the RNase. Rather, there is a tendency for the culture to lower the levels of RNase D, concomitant with a decrease in plasmid copy number. We suspect that this represents selection for faster-growing cells that contain fewer plasmids, since slow-growing transformants rapidly lost their phenotype and induction of RNase D overexpression led to slower growth. However, we have not eliminated the possibility that elevated RNase D levels might also have a direct effect on the RNAs that regulate plasmid maintenance or that some other type of regulation also occurs.

The deleterious nature of elevated RNase D activity differs from that seen with two other cloned RNases, RNase II and polynucleotide phosphorylase (4, 9), and is therefore somewhat surprising since these two enzymes show much less specificity in vitro. These findings may indicate that excess RNase D is accessible to some essential RNA in vivo, whereas the other nucleases are not. Nevertheless, it raises the possibility of identifying an in vivo substrate for RNase D, which could help in elucidating the physiological role of this enzyme.

ACKNOWLEDGMENTS

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This is paper 49 in the series "Reactions at the 3′ Terminus of tRNA." The previous paper in this series is listed in reference 24.

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