ISOLATION, GENETIC MAPPING AND SOME CHARACTERIZATION OF A MUTATION IN *ESCHERICHIA COLI* THAT AFFECTS THE PROCESSING OF RIBONUCLEIC ACID

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ABSTRACT

Temperature-sensitive mutants were isolated from an *rnc* (RNase III-) strain of *Escherichia coli*, and their rRNA metabolism was analyzed on 3% polyacrylamide gels. One of these mutants was unable to produce 23S and 5S rRNAs at the nonpermissive temperature. When an *rnc*+ allele was introduced to this strain, it remained temperature sensitive. At the nonpermissive temperature, this strain could then produce 23S rRNA but was unable to make normal levels of 5S rRNA. In matings and transduction experiments, the defect in rRNA metabolism and temperature sensitivity behaved as a syndrome caused by a single point mutation, which was mapped at min 23.5 on the *E. coli* chromosome. This mutation probably affects an enzyme, ribonuclease E (RNase E), which introduces a cut in the nascent rRNA transcript between the 23S and the 5S rRNA cistrons. The mutation *rne* is recessive with respect to temperature sensitivity and the pattern of rRNA. Revertants able to grow at 43° and with normal metabolism of rRNA were isolated; genetic analysis showed that they do not contain the original *rne* mutation, suggesting that they were true revertants. By combining the *rne* mutation with an *rnc* mutation, double *rnc rne* strains were synthesized, which behaved very similarly to the original *rnc* strain from which the *rne* mutation was isolated. Such strains have RNA metabolism that is similar to that of *rnc* strains at permissive temperatures, but at the nonpermissive temperature they fail to synthesize p23, m23 and 5S rRNAs. Thus, the experiments reported here, together with previous studies, suggest the existence of a new processing ribonuclease activity in *Escherichia coli*, which is called ribonuclease E.

STUDIES with strains of *Escherichia coli* lacking the enzyme RNase III (GEGENHEIMER and APIRION 1975; APIRION, NEIL and WATSON 1976; GEGENHEIMER, WATSON and APIRION 1976, 1977) suggested that maturation of rRNA is a complex process of events involving a fairly large number of enzymatic cleavages, two of which are carried out by RNase III. These cleavages can be classified as to primary or secondary processing events; primary processing events occur during transcription, while the secondary processing events occur after transcription (GEGENHEIMER, WATSON and APIRION 1977; APIRION and LASSAR 1978).

In *rnc* (RNase III⁻) strains, two of the primary maturation cuts, which remove p23 and p16 from the growing transcript, do not occur, and the maturation of rRNA is carried out by the remaining primary and secondary processing enzymes (GEGENHEIMER, WATSON and APIRION 1977; APIRION and LASSAR 1978). Consequently, one observes, in addition to the customary stable 23S and 16S ribosomal RNAs, a number of transitory RNA species referred to as 30S, 25S, 18S and 17S. The final mature rRNAs in *rnc* and *rnc*⁺ strains seem to be identical (GEGENHEIMER, WATSON and APIRION 1977). In *rnc* strains, the different types of rRNA molecules are formed under all conditions in which the strains grow and are all caused by a single *rnc-105* mutation, since true revertants, which regain normal levels of RNase III, synthesize only the customary 23S, 17S and 16S rRNA molecules (APIRION, NEIL and WATSON 1976).

It was evident that RNase III is not involved in the maturation of 5S rRNA since in *rnc* cells the metabolism of 5S rRNA was normal (GEGENHEIMER, WATSON and APIRION 1977). Therefore, it was proposed that a cut is introduced between the 23S and the 5S rRNA cistrons by a putative enzyme that was named RNase "E" (see Figure 12 in GEGENHEIMER, WATSON and APIRION 1977). These findings and considerations suggested that in *rnc* cells the remaining primary cuts would be indispensible. Therefore, I set out to isolate temperature-sensitive mutants from RNase III⁻ strains and to examine their rRNA metabolism. I shall report here on the genetics of one temperature-sensitive mutant in which a cleavage between the 23S and the 5S cistrons does not seem to occur at elevated temperatures.

MATERIALS AND METHODS

All procedures used are published and will be referred to at the appropriate places. Strains used are described in Table 1.

RESULTS

Isolation of ts⁻ mutants defective in rRNA metabolism from an rnc *strain*: Strains carrying the *rnc-105* mutation can grow at almost all temperatures at which *rnc*⁺ strains can grow, albeit at a slower rate (APIRION and WATSON 1974, 1975). They grow well at 43° in minimal medium or enriched medium, and therefore we isolated temperature-sensitive mutants that fail to grow at 43°. In order to isolate ts⁻ mutants, two *rnc* strains (N2097 and N2311) were treated with N-methyl-N-nitro-N′-nitrosoguanidine (40 μg/ml at 30° for 40 min) in broth at pH 6.5 and the treated cells were plated on broth agar medium (APIRION 1966) at 30° to give rise to about 200 colonies per Petri dish (85 mm diameter). After three days, the colonies were replica plated to the same medium and incubated at 43°. Colonies that failed to grow at 43° were picked, purified (twice) and retested. Only the mutants that did not acquire any mutations to auxotrophy were kept for further analysis.

Screening of mutants for rRNA metabolism: Cultures were grown in 0.3 ml of a low phosphate rich medium (MEYHACK, MEYHACK and APIRION 1973) at

TABLE 1

Bacterial strains used

Strain	Genotype	Source/reference
N2077	F⁻ *thi-1 argH1 nadB4 lacY1 malA1 xyl-7 ara-13 mtl-2 rpsL9 tonA2? λ⁻ supE44? rnc-105*	APIRION and WATSON (1975)
N2097	As N2077, but *lac+ mal+*	APIRION *et al.* (1976)
N2311	As N2097, but *rpsE2311*	Sp* from N2097
N3071	As N2311, but *rne3071*	NG from N2311
N3403	As N3071, but *nad+ rnc+*	D10→N3071†
N3421	Hfr POl *thi-1 rel-1 lacZ43 rne3071*	N3403→3050–U6
N3422	Hfr POl *thi-1 rel-1 lacZ43 rne+*	N3403→3050–U6
N3423	As X7014 (see below) but *pyr+ rne3071*	N3421→X7014
N3520	F⁻ *nadB4 lacY1 malA1 xyl-7 mtl-2 rpsL9 rnc-105 rne-3071*	N3421 × N2077
D10	F⁻ *metB1 rna-10*	GESTELAND (1966)
LA2–89	F⁻ *fabD1 thi-1 gltA ara 14 lac Y1 galK2 xyl-5 mtl-1 tfr-5 tsx-57 rpsL20* (λ)	DAVID SILBERT
LA2–130	As LA2–89, but *fabD2*	DAVID SILBERT
3050–U6	Hfr POl *thi-1 pyrC46 rel-1 lacZ43*	J. BECKWITH‡
X7014	F⁻ *pyrC46 purB51 thi-1 lacZ43* or *13 ma A1 xyl-7 mtl-2 rpsL125*	J. BECKWITH via D. SILBERT
KLF26/181	F′ episome F126 $\xrightarrow{gal\ \ rnc}$ chromosomal markers *pyrD rec A1 mtl xyl his mal trp thi gal rpsL*	K. BROOKS LOW‡

* Sp=spontaneous, NG=nitrosoguanidine.
† Denotes a transduction; arrow leads from donor to recipient.
‡ Strains supplied by B. J. BACHMANN, Yale University, Coli Genetic Stock Center.

30°. At an absorbancy of about 0.4 (at 560 nm), the cultures were transferred to 43° for one hour, and then to each culture 18 μCi of $^{32}P_i$ were added. Labeling was terminated 40 min later by addition of ethanol and diethylpyrocarbonate (to 80% and 1%, respectively). The cultures were centrifuged and the pellets opened in a hot sodium dodecyl containing buffer, as described by GEGENHEIMER, WATSON and APIRION (1977). Samples containing about 100,000 CPM were applied in 5 to 20 μl volumes to thin slab 3% polyacrylamide gels. Samples from 20 strains were applied to each gel and electrophoresis was carried out, and the gels were dried and autoradiographed (GEGENHEIMER, WATSON and APIRION 1977).

In this manner, 380 strains were screened and the analysis of one of these, designated N3071, is described here.

Genetic analysis of strain N3071: While the parental strain of N3071, strain N2311, synthesized 23S, 16S, 30S, 25S, "p23", 18S and 5S rRNAs at all temperatures, strain N3071 synthesized all these molecules at permissive temperatures such as 30° or 37°, but failed to produce either "p23", 23S or 5S rRNA at 43°, (APIRION and LASSAR 1978). In order to assess the precise contribution of the new mutation(s) in strain N3071 to abnormal rRNA metabolism, we introduced an *rnc+* allele into the strain.

The *rnc* gene is co-transduced with the *nadB* gene (APIRION and WATSON 1975). Since strain N3071 is *nadB*, it was infected with P1 bacteriophage (LENNETTE and APIRION 1971) grown in strain D10 (*nad*$^+$ *rnc*$^+$), and selection was carried out for Nad$^+$ transductants at 37°. All the Nad$^+$ transductants (40 tested) remained ts$^-$ since they still failed to grow at 43°. The rRNA metabolism of ten transductants was analysed on a 3% polyacrylamide gel as explained above. Six of the ten transductants became *rnc*$^+$. This was evident since they did not produce 30S, 25S and 18S rRNAs. The other four transductants retained the *rnc-105* allele. All the abnormal features of rRNA metabolism observed in RNase III$^-$ strains are caused by a single point mutation, *rnc-105* (APIRION and WATSON 1975; APIRION, NEIL and WATSON 1976). The strains that retained the *rnc* mutation grew somewhat more slowly than the strains that did not.

RNA metabolism in the *rnc*$^+$ transductants at the permissive and nonpermissive temperatures was analysed on 3% and 5%/12% tandem polyacrylamide gels (GEGENHEIMER, WATSON and APIRION 1977; LEE, BAILEY and APIRION 1978). In a 3% gel, large RNAs can be observed, while in the other gel small RNAs such as tRNA and 5S rRNA can be analysed. At the permissive temperature, RNA metabolism of large and small molecules was normal, while at the nonpermissive temperature (43°) large rRNAs were synthesized as in regular strains, but the small RNAs were not normal (see Figures 1, 2 and 3). At the nonpermissive temperature a number of new species of RNA appear (see Figure 1 arrows) and 5S rRNA did not accumulate to any appreciable extent. Since these six strains all behaved in a similar fashion, one of them was designated N3403, and the analysis was continued with this strain. To ascertain whether or not the temperature sensitivity and the new pattern of RNA metabolism were caused by the same mutation, ts$^+$ revertants for growth at 43° were isolated, and analysed. Their RNA metabolism was normal (see below), suggesting that these two characteristics are indeed caused by the same mutation.

In order to localize the ts mutation on the chromosome, strain N3403, which is SpcR (spectinomycin resistant), and Arg$^-$ (*argH*) were crossed (LENNETTE and APIRION 1971) to ten different Hfr's with different points of origin and which are sensitive to spectinomycin and are Arg$^+$. About 60 SprR Arg$^+$ recombinants were analysed for their ability to grow at 43°. Crosses that yielded ts$^+$ recombinants suggested that the ts mutation is between the point of origin of the Hfr strain and *argH*. These experiments suggested that the *ts* mutation is located between minutes 21 and 28 of the *E. coli* map (BACHMANN, LOW and TAYLOR 1976).

Many genes in this region were tested for co-transduction with the ts mutation in strain N3403. Co-transduction was found with *pyrC* and *purB*, about 50% and 10%, respectively. In one type of experiment, strain N3403 was the donor and strain 3050-U6 (*pyrC*) was the recipient, and selection was carried out at 37° for Pyr$^+$. From 60 Pyr$^+$ transductants, 29 also became ts$^-$, thus indicating 48% co-transduction between these two genes. Forty of these 60 transductants were analysed on 5%/12% tandem polyacrylamide gels for their RNA metabolism. All the ts$^-$ transductants behaved like the parental donor strain

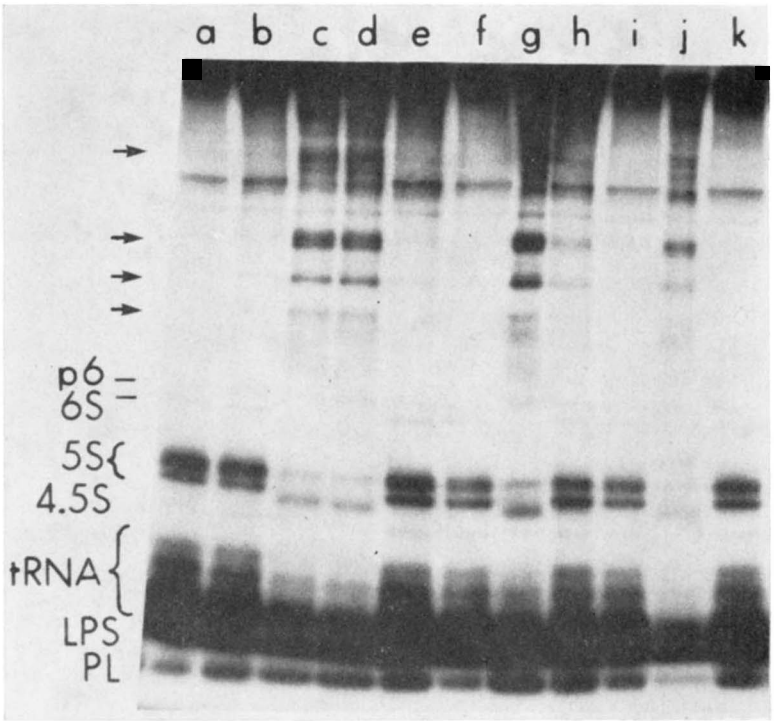


FIGURE 1.—Analysis of recombinants from a transduction involving an *rne* strain as a donor and an *rne*+ strain as the recipient. Strains containing an *rne* mutation are temperature sensitive, and when shifted to a nonpermissive temperature such as 43°, they fail to mature a variety of RNA molecules, including 5S rRNA, 6S RNA and some tRNAs. Instead, one observes the accumulation of larger precursor molecules (arrows on the left). The pattern of the RNA synthesized in *rne*+ strains can be seen in lanes a, b, e, f, h, i, and k, while the pattern of RNA synthesized in *rne* strains at the restrictive temperature can be seen in lanes c, d, g, and j. Since the *rne* mutation is very closely linked to the *pyrC* locus, a transduction was carried out in which an *rne pyr*+ (N3403) strain was the donor and an *rne*+ *pyrC* (3050-U6) was the recipient. Selection was carried out for the ability to grow without uracil. A large proportion (48%) of the *Pyr*+ transductants became ts− like the *rne* parental strain. RNA synthesis was analyzed in a sample of *pyr*+ transductants, some of which were ts+ and some ts−, as well as in the parental, donor, and recipient strains. All of the strains were grown in a low phosphate medium at 30°, transferred to 43° and 40 min after the shift, the cells were labelled with $^{32}p_i$ for 30 min. The cells were processed and their content was analyzed on 5%/10% tandem polyacryylamide slab gel (LEE, BAILEY and APIRION 1978). The gel was dried and autoradiographed, and a picture of the autoradiograph is shown here. The RNA of the parental donor *rne* strain is in lane c and the parental recipient *rne*+ strain is in lane b. The ts+ transductants are in lanes a, e, f, h, i, and k, and the ts− transductants are in lanes d, g, and j. It can be seen that all the ts+ recombinants behave like the ts+ parental strain (*rne*+), while all the ts− recombinants behave like the ts− parental strain (*rne*). Most of the 5% part of the gel, which contains the large nucleic acids, is not shown in this picture.

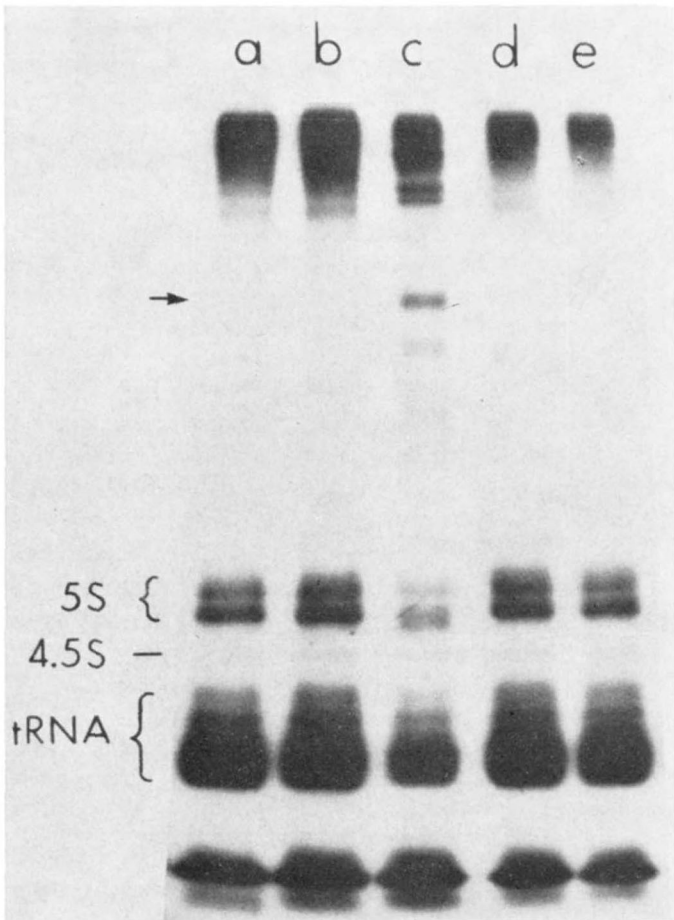


FIGURE 2.—ts+ revertants. Strain N3421 *rne* (lane c) and four ts+ revertants (lanes a, b, d, e) were grown at 30°, transferred to 43°, labeled with $^{32}p_i$ and analyzed as described in the legend to Figure 1.

N3403, while all the ts+ transductants behaved like the parental recipient strain (3050-U6). At the permissive temperature, RNA metabolism was normal in all strains, while at 43° RNA metabolism was abnormal in the ts- transductants. The result of the analysis of some of the transductants is shown in Figure 1. It can be seen that when these ts- strains are labeled at 43°, they accumulate some larger molecules, while they fail to accumulate 5S rRNA and normal levels of tRNA. One of these larger molecules (the top one indicated by the second arrow on the left in Figure 1) contains 5S rRNA sequences (B. GHORA and D. APIRION, unpublished).

One of the ts- transductants was designated N3421, and one of the ts+ transductants was designated N3422; the analysis continued with these strains. To explain the observations with strains such as N3421, we assume that such strains

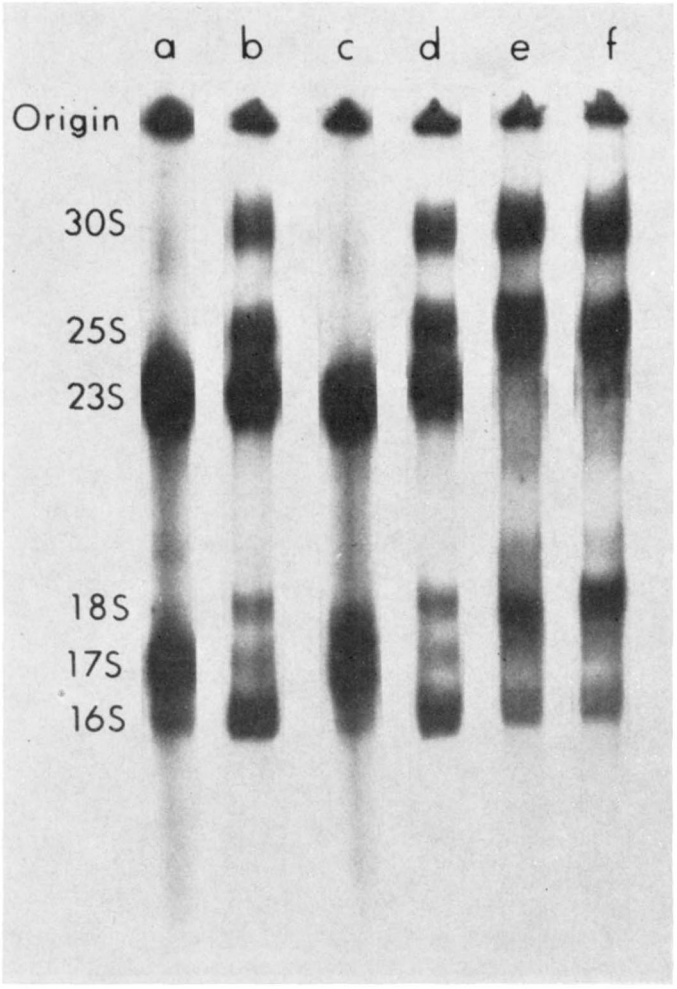
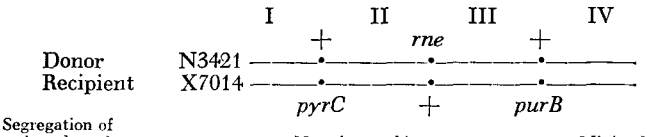


FIGURE 3.—Recombinants from a cross *rne*×*rnc*. The parental strains N3421 (*rne*) and N2077 (*rnc*) and some of their recombinants were labeled as described in Figure 1 and analyzed on a 3% gel. a, N3421 *rne*; b, N2077 *rnc*; c, a wild-type recombinant; d, an *rnc* recombinant; e, f, *rnc rne* recombinants.

contain a mutation which affects rRNA processing, and that at the nonpermissive temperature they fail to cut the nascent rRNA chain between the 23S and the 5S cistrons. Such a cut was previously predicted on the basis of studies with (RNase III⁻) *rnc* mutants, and the enzyme responsible for this cut was designated RNase E (GEGENHEIMER, WATSON and APIRION 1977). Therefore the ts mutation in strain N3421 is designated *rne-3071*, and it is assumed that such strains contain a temperature-sensitive RNase E. (Preliminary experiments by BASANTA GHORA support such an assumption. (For the nomenclature of RNase genes, see APIRION and WATSON 1975.)

TABLE 2

Mapping of rne-3071 *with relation to* pyrC *and* purB



Segregation of unselected markers		No. of recombinants in the class	Minimal no. of necessary crossovers
Rne	*Ade*		
+	−	34	I and II
−	−	11	I and III
−	+	7	I and IV
+	+	0	I, II, III and IV

Selection was carried out for *Pyr*+, by plating P1-infected recipient cells in minimal medium plates containing adenine at 30°.

In order to assess the orientation of the *rne* gene with respect to *pyrC* and *purB*, three point transduction experiments were carried out involving mutations in the genes *rne*, *pyrC* and *purB* (Table 2). It can be seen from the results in Table 2 that the *rne* gene is located between *pyrC* and *purB*, closer to *pyrC*. In a cross similar to that shown in Table 2, when selection was carried out for Pur+ Pyr+, all 84 transductants tested were ts−, again indicating that *rne* is located between *pyrC* and *purB*. Since *fabD* maps in this region of the chromosome (SEMPLE and SILBERT 1975), linkage of *fabD* to *rne* was tested. In this case both parental strains are ts−, but the *rne-3071* strains can grow at 40°, while *fabD* strains cannot. For this reason N3421 (*rne*) was used as donor, and LA2–89 or LA2–130 (both are *fabD*) were used as recipients. Selection was carried out for transductants able to grow at 40° on rich medium. The results from both experiments were similar; therefore, the data from both experiments were pooled. From a total of 52 ts+ transductants tested, 25 were ts+ even at 45°, indicating that they do not carry either the *rne* or the *fabD* alleles, and indicating a 52% co-transduction of these two genes. Since the co-transduction frequency of *rne* and *purB* is about 10% and of *pyrC* and *fabD* is at most 13% (SEMPLE and SILBERT 1975), I deduced that the order of the genes studied here is *pyrC rne fabD purB*. Thus, the *rne* gene was located at about 23.5 minutes of the *E. coli* map (BACHMANN, LOW and TAYLOR 1976).

The mutation rne-3071 *is recessive*: In order to determine whether the *rne-3071* mutation is recessive or dominant, an F′ (F126) which covers the *rne* gene was transferred to the *rne-3071* strain N3423. This strain is also *purB*, which is co-transduced with *rne* (see above). In order to transfer this F′, strain KLF26/181 was mated to strain N3423 (LENNETTE and APIRION 1971), and selection was carried out on minimal medium at 37° for Ade+ merodiploids; strain KLF26/181 requires L-histidine and therefore could not grow on minimal medium. Ten Ade+ merodiploids were purified and analysed; all grew well at 43° and had a normal pattern of RNA synthesis at that temperature. To ascertain if these strains

are merozygotes and not recombinants, they were tested for the ability to donate the F′ 126 plasmid. In crosses they could donate the *purB*$^+$ *rne*$^+$ and *pyrC*$^+$ genes to F$^-$ strains at high frequency, as compared to other genes that are not carried on the plasmid.

Analysis of revertants: From strain N3421, ts$^+$ revertants with the ability to grow at 43° were isolated. In five independent experiments, the frequency of revertants was between 6 and 8.8×10^{-9}, indicating a frequency commensurate with *rne-3071* being a single point mutation. RNA metabolism at 43° was analysed in some of these revertants, (Figure 2). In all the revertants tested, RNA metabolism was normal. To determine if these are true revertants, seven of them were further studied; they included at least one revertant from each of the five experiments. Strain 3050-U6, which is *pyrC*, was infected with P1 phage prepared from these seven strains. In each case Pyr$^+$ recombinants were selected for at 37°. From each cross 60 Pyr$^+$ transductants were analysed, and in no case was a ts$^-$ recombinant found, indicating that none of these seven ts$^+$ revertants harbors a ts mutation. I therefore conclude that these seven strains are true revertants.

Building rnc rne *double-mutant strains*: To verify our hypothesis that the original strain N3071, which was isolated by mutagenesis from strain N2311 (*rnc-105*), contains two mutations that affect RNA processing, *rnc-105* and *rne-3071*, and that all the abnormalities in RNA metabolism observed in this strain were caused by these two mutations, *rnc rne* double-mutant strains were constructed. For this experiment a prolonged mating was set up between strain N3421, which is an Hfr (PO1) *rne-3071* and which should be able to mobilize the *rne* gene, and an F$^-$ strain N2077 (*rnc-105*). Selection was carried out for StrR Arg$^+$ recombinants. Thirty such recombinants were purified, and their RNA metabolism was analysed on gels, at 37° and 43°. The results observed with some of them at 43° are shown in Figure 3 (on a 3% gel). Of the 30 recombinants analyzed, 15 had the wild-type phenotype, ten had the *rnc* phenotype, while five had the *rnc rne* phenotype, as indicated by failure to produce p23 or 23S rRNA at 43°. The recombinant class, which numbered 15 strains, contained *rne* parental types and wild-type recombinants; on a 3% gel they are not easy to distinguish from one another. This experiment indicates that the original N3071 strain does contain only two mutations that affect its RNA metabolism, *rnc* and *rne*. One of the double-mutant strains was kept and designated N3520 (Table 1).

DISCUSSION

The results presented here describe the isolation of a mutant that defines a new gene affecting RNA processing. The simplest interpretation of the results described here is that the *rne* gene codes for a processing enzyme, RNase E, and that the *rne-3071* allele renders this protein thermolabile. Other more complex possibilities are not excluded, however; for instance the mutation could affect an RNA modifying enzyme, the activity of which is obligatory for the RNase E action to take place. While the experiments performed here cannot distinguish

between these two possibilities or point to another avenue, they clearly establish the existence of a new gene that affects RNA processing events not affected by other genes governing RNA processing, such as *rnc* or *rnp*. The results suggest rather strongly that an enzyme is affected, since the cells carrying the *rne-3071* mutation are thermolabile, the appearance of the abnormal RNA metabolism is confined to the nonpermissive temperature and the mutation is recessive.

Two other genes that govern RNA processing (cleaving) enzymes have been identified in *E. coli* K12; one is *rnp*, which affects RNase P (SCHEDL and PRIMAKOFF 1973), and the other is *rnc*, which affects RNase III (DUNN and STUDIER 1973; APIRION, NEIL and WATSON 1976; GEGENHEIMER, WATSON and APIRION 1977). Each of the three genes defined by the mutations (*rnc, rne, rnp*) affects a different set of reactions since the patterns of RNA metabolism observed in each of these mutants are very different. The *rne* mutation affects production of 5S rRNA, and the putative enzyme RNase E seems to introduce a cut in the nascent rRNA chain somewhere between the 23S and the 5S rRNA cistrons, exactly as predicted from previous experiments (GEGENHEIMER, WATSON and APIRION 1977; APIRION and LASSAR 1978).

Thus far, RNase III has been shown to affect mainly rRNA metabolism (DUNN and STUDIER 1973; GEGENHEIMER, WATSON and APIRION 1977), while RNase P affects mainly tRNA metabolism (SCHEDL and PRIMAKOFF 1973; ALTMAN 1975; SEIDMAN and McLAIN 1975). RNase E, however, seems to affect the metabolism of rRNA, tRNA and other RNA molecules. For instance, in Figures 1 and 2 it can be seen that while normal levels of 5S do not accumulate at the nonpermissive temperature (most of the RNA accumulating at the 5S region is not 5S rRNA, B. GHORA and D. APIRION, unpublished observations), a number of larger molecules accumulate but not all of them contain 5S rRNA sequences (B. GHORA and D. APIRION, unpublished). It can be seen clearly in Figures 1 and 2 that the amount of tRNA that accumulates in *rne* mutants at the nonpermissive temperature is rather diminished, and some of the larger molecules are most probably tRNA precursors (different size tRNA precursors accumulate in *rnp* strains). There is a third class of small stable RNA molecules, 4.5S 6S and 10S (LEE, BAILEY and APIRION 1978), which are neither tRNA nor 5S rRNA, and the maturation of two of these molecules is also affected in *rne* mutants at the nonpermissive temperature. While the maturation of 4.5S rRNA (see Figures 1 and 2) does not seem to be affected, the maturation of 6S and 10S is. (The maturation of 4.5S RNA is affected by RNase P according to BOTHWELL, GARBER and ALTMAN 1976.)

The amount of label analysed in Figures 1 and 2 is not sufficient to permit a clear observation of the 6S RNA species, but a careful examination of these gels shows that while both p6 and m6 RNAs can be observed in *rne*$^+$ strains, only p6 is observed in *rne* strains. For clear observation of the 10S region, less concentrated gels have to be used.

Since a number of RNA molecules are affected by the *rne* mutation, it is not obvious which one's absence causes the temperature sensitivity of the strain. It

is interesting that while the original strain, N3071, into which the *rne-3071* mutation was introduced and the *rne rnc* strains synthesized from the single mutants are very similar, there is at least one difference between them. Strain N3071 fails to mature 16S rRNA at the elevated temperature (see APIRION and LASSAR 1978, Figure 1), while the synthesized *rnc rne* strains do accumulate 16S rRNA, albeit at a reduced rate (Figure 3, lanes e and f). This difference is most likely due to another mutation that is responsible for some of the temperature sensitivity of strain N3071. When strain N3403 was crossed to the different Hfrs (see RESULTS), it became apparent that besides the *rne* mutation there was another mutation unlinked to *rne-3071* that did not affect the major features of RNA metabolism, but that caused some temperature sensitivity. Since the *rne* mutation was transferred from the original genetic background to a completely different genetic background in transduction experiments (Figure 1), and since revertants from such strains were analyzed (Figure 2), I feel confident that all the effects on RNA metabolism and temperature sensitivity observed in strains such as N3421 are caused by the *rne-3071* mutation.

While primary processing of rRNA in *E. coli*, *i.e.*, processing occurring during transcription, is not affected by protein synthesis, secondary processing is, *e.g.*, formation of m16 from p16 (PACE 1973; GEGENHEIMER, WATSON and APIRION 1977). Therefore, changes observed in the pattern of primary processing cuts usually means that an enzyme involved in primary processing is affected, while changes observed in secondary processing, in particularly a failure to mature 16S rRNA, do not necessarily indicate that an enzyme involved in secondary processing is affected. In the case of formation of 16S from 17S, many factors that influence protein synthesis can affect this step. It is most unlikely, therefore, that the mutation which affects formation of 16S in strain N3071 is directly related to processing of rRNA. Indeed, the level of protein synthesis is rather negligible in strain N3071 after it is shifted to 43° for 40 min (less than 2% of the 30° level), while the level of protein synthesis in the *rnc rne* strains synthesized from the two single parental strains is appreciable, about 50% of the normal rate at 30°.

SEMPLE and SILBERT (1975) encountered certain anomalies in their transduction experiments using strain X7014. We encountered similar difficulties in obtaining *pur*$^+$ transductants, using strain N3421 as donor and X7014 or its derivatives as recipients, which suggests that there might be some chromosomal nonhomology between the two strains in this region. Yet, all the data obtained, together with former studies on co-transduction frequencies in this region (SEMPLE and SILBERT 1975), are in agreement with the suggested order *pyrC rne fabD purB*. In the absence of any evidence to the contrary, this should be the preferred order. At present a number of genes that affect ribonucleases, processive and degradative, have been mapped, but in no case has a cluster of such genes been found (BACHMAN, LOW and TAYLOR 1976).

It has been reported that nitrosoguanidine tends to induce mutations in clusters (CERDÁ-OLMEDO, HANAWALT and GUEROLA 1968). We have now analyzed a

fair number of nitrosoguanidine-induced mutations, and while it is clear that nitrosoguanine can induce multiple mutations, in no case were these mutations clustered. For instance in the analyses of strain N4752, *sts-4752* (LENNETTE and APIRION 1971) and N4721, *rimH 4721* (JOHNSON, WATSON and APIRION 1976), and in the case studied here, *rne-3071* (N3071), it is clear from the genetic analysis that the strains do not contain many mutations and that the particular mutations of interest are not co-transduced (by P1) with any other new mutation. Even in strain AB301–105, which was isolated in another laboratory (KINDLER, KEIL and HOFSCHNEIDER 1973) and analyzed in mine (APIRION and WATSON 1974, 1975), we found a large number of mutations (at least eight) only one of which was co-transduced (by P1) with the *rnc-105* mutation. It is possible that our handling of nitrosoguanidine mutagenesis, *i.e.*, lower concentration of the drug, shorter time and higher pH, reduced multiple clustered mutations.

Continued studies with the new RNA processing mutant described here, as well as with other processing mutants, should help us understand RNA processing in the cell.

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