GENETIC MAPPING AND SOME CHARACTERIZATION OF THE *rnpA49* MUTATION OF *ESCHERICHIA COLI* THAT AFFECTS THE RNA-PROCESSING ENZYME RIBONUCLEASE P

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ABSTRACT

A mutant defective in the enzyme RNase P was isolated by P. SCHEDL and P. PRIMAKOFF (1973). The mutation *rnpA49* found in this strain, which confers temperature sensitivity on carrier strains, was mapped by conjugation and transduction experiments and located around minute 82 of the *E. coli* map, with the suggested order *rnpA bglB phoS rbsP ilv*. As expected, the *rnpA49* mutation is recessive. Even though this mutation is conditional, it is manifested at temperatures at which the carrier strains can grow.

IT has become apparent in the past few years that rRNA and tRNA molecules in the bacterial cell are not synthesized as the mature species, but are processed from larger transcripts (PACE 1973; ALTMAN 1975; APIRION *et al.* 1980; see also papers in DUNN 1974; and ABELSON, SCHIMELL and SÖLL 1979). At present, three enzymes involved in the primary endonucleolytic processing of rRNA in *E. coli* have been identified. These are: RNase III (GINSBURG and STEITZ 1975; APIRION, NEIL and WATSON 1976a,b; GEGENHEIMER, WATSON and APIRION 1977), RNase E. (APIRION 1978; APIRION and LASSAR 1978; GHORA and APIRION 1978) and RNase P (GEGENHEIMER and APIRION 1978). Mutants have been isolated and characterized in these three enzymes (KINDLER, KEIL and HOFSCHNEIDER 1973; SCHEDL and PRIMAKOFF 1973; APIRION and WATSON 1975; APIRION 1978). However, while the mutations affecting RNase III (*rnc*) and *RNase E* (*rne*) have been mapped, the mutation *rnpA49*, which was isolated by SCHEDL and PRIMAKOFF (1973), has not. In order to gain insight into the contributions of these three enzymes to the processing of RNA, we set out to construct *E. coli* strains that carry these three mutations in all possible combinations (GEGENHEIMER and APIRION 1978; APIRION *et al.* 1980). In order to do so, it was useful to map the *rnpA49* mutation. The experiments presented here describe the mapping of this mutation and its further characterization.

MATERIALS AND METHODS

All procedures used are published and will be referred to at the appropriate places. All strains used are *E. coli* K12 derivatives, and are described in Table 1. Colonies were tested for the

TABLE 1

Strains used

Strain	Characteristics	Source
PP47	Hfr PO1 *thi-1 relA1 bglB11 crp-1*	Perlman and Pasten* Hfr Hayes derivative transfers *uxuA,B pil* early
A49	F⁻ *rnpA49 argA52 trpA36 glyA34 rpsL lacZ*	SCHEDL and PRIMAKOFF (1973)
PP113	F⁻ *argA52 trpA36 glyA34 rpsL lacZ*	SCHEDL and PRIMAKOFF (1973)
N141	F⁻ *thi-1 ilv-158 argE3 his-4 proA2 lacY1 galK2 mtl-1 xyl-5 tsx 29* λ⁻ *supE44 mal*	This laboratory
N1130	F⁻ *trp-37 phoS3 rbsP1 lacI22,Z13 xyl-13 rpsL106 tna-4* ?	LENNETTE and APIRION (1971)
N1211	F⁻ *trp-37 phoS3 rbsP1 bg1B11 lacI22,Z13 xyl-13 rpsL106 tna-4* ?	PP47 → N1130†
N2020	Like A49, but resistant to valine (Val^R)	This laboratory
N2027	F⁻ *rnpA49 bglB11 argA52 trpA36 glyA34 rpsL lacZ* Val^R	PP47 → N2020
N2029	F⁻ Ts⁺ revertant fromN2020, *rnp*+	This paper
N2035	F⁻ Ts⁺ revertant from N2020, *rnpA49 sup-2035*	This paper
N2039	F⁻ *mal thi-1 ilv-158 argE3 his-4 proA2 lacY1 galK2 mtl-1 bglB11 rnpA49 xyl-5 tsx-29* λ⁻ *supE44*	N2027 → N141

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

ability to utilize ribose, as well as other sugars, by using either minimal medium that contained a particular sugar as the only carbon source or MacConkey agar medium (BBL), which contains 1% w/v of the desired sugar. Colonies were tested for the *phoS* mutation by replicating colonies to a low phosphate peptone medium (10 g peptone, 5 g glucose, 18 g agar per liter) and staining them for alkaline phosphatase by naphtol phosphoric acid and fast violet B salt, as described by APIRION (1966). Colonies carrying the *phoS* mutation stain dark red, while *phoS*+ colonies remain unstained. Strains that are *bgl* can be selected for, since they can utilize salicin (0.2%) as the only carbon source, whereas *bgl*+ strains cannot.

All transductions were mediated by bacteriophage P1 (LENNETTE and APIRION 1971). For media used, see APIRION (1966) and LENNETTE and APIRION (1971). (I found that in replication to rich medium at 43° or 45°, strains carrying the *rnpA49* mutation keep growing sufficiently to obscure the scoring. Therefore, replicated plates incubated at the elevated temperature were routinely re-replicated and further incubated. This led to unambiguous scoring; see also Table 3.)

I followed the notations suggested by APIRION and WATSON (1975), which were adopted by BACHMANN, LOW and TAYLOR (1976). Therefore, a mutation that affects the enzyme RNase P is designated *rnp*. Since there might be at least two genes that affect this enzyme (OZEKI *et al.* 1974), the gene defined by the mutation described here is designated *rnpA*.

RESULTS

Mapping the rnpA49 *mutation by conjugation and P1 mediated transduction:* SCHEDL and PRIMAKOFF (1973) isolated strain A49 (*rnpA49*) from strain PP113 and showed that strain A49 is Ts⁻ (temperature sensitive) and is defective in the enzyme RNase P. They suggested, from analysis of revertants, that both effects are caused by a single mutation.

In order to map the *rnpA49* mutation, strain N2020 (*rnpA49*), which is F^-, was crossed to a number of Hfr strains (Low 1973; JOHNSON, WATSON and APIRION 1976). The results of these experiments indicated that the *rnpA49* mutation (if the Ts^- and the Rnase P^-, Rnp^- phenotypes are pleiotropic effects of the same mutation) is located between the points of origin PO46 and PO18, which corresponds roughly to the area between minutes 81 and 87 on the *E. coli* map (BACHMANN, LOW and TAYLOR 1976).

In order to pinpoint the location of *rnpA49* with more precision, a series of transduction experiments were performed using strain N2020 as the recipient and strains carrying mutations in this region of the chromosome as donors. Co-transduction was detected with the *bglB*, *rbsP*, *phoS* and *ilv* genes. The highest co-transduction, about 50%, was between *rnpA* and *bglB*; the lowest, about 3%, was with an *ilv* gene. These genes are all located between 82 and 83.5 min on the current *E. coli* map. From these experiments, a number of transductants, some Ts^+ and some Ts^-, were tested on gels to ascertain their pattern of RNA synthesis (see Figures 1 and 2). In such experiments, cultures are grown at 30° or 37° in a low phosphate medium and transferred to 43°; 40 minutes after the shift, $^{32}P_i$ is added to the culture and, 30 minutes later, the growth of the cells is terminated, cells are concentrated and opened in a sodium dodecyl sulfate containing buffer, and their content of RNA molecules is displayed in polyacrylamide gels (GEGENHEIMER, WATSON and APIRION 1977; LEE, BAILEY and APIRION 1978). In such experiments, the *rnpA49* phenotype is very distinct and is characterized by the appearance of prominent new RNA molecules that are not seen in rnp^+ strains (Figures 1 and 2). During the past two years, we tested about 80 Ts^+ and 50 Ts^- transductants, where the donors were rnp^+ and *rnpA49* strains, and invariably Ts^- recombinants showed the Rnp^- phenotype while the Ts^+ transductants showed the Rnp^+ phenotype. This indicates that the Ts^- and Rnp^- phenotypes are caused by a single point mutation, or by two extremely closely linked mutations.

In order to map the *rnpA49* mutation more precisely, strain N1211 (Table 1), which carries three co-transducible markers in this region, *bglB11*, *phoS3* and *rbsP1*, was crossed to strain N2020 in a transduction experiment. (Strain N1211 was derived by transferring the *bglB* marker from strain PP47 to strain N1130, which is *phoS* and *rbsP1*; see Table 1). One experiment, where strain N1211 (rnp^+) was the donor and strain N2020 (*rnp*) was the recipient, is summarized in Table 2. The data from this experiment, as well as from a number of other experiments, suggest that the order of the genes tested in this region is *rnpA-bglB-phoS-rbsP-ilv*. In all these experiments, the co-transduction frequency between *rnpA49* and *bglB* was always the highest. Therefore, according to the recent linkage map of *E. coli* (BACHMANN, LOW and TAYLOR 1976), this places the *rnpA* gene between min 82.0 and 82.5. According to our data, the order of the genes in this region is *bglB-phoS-rbsP* rather than *phoS-bglB-rbsP* as shown in the *E. coli* map. (Personal communication from ANDREW WRIGHT and JEF-

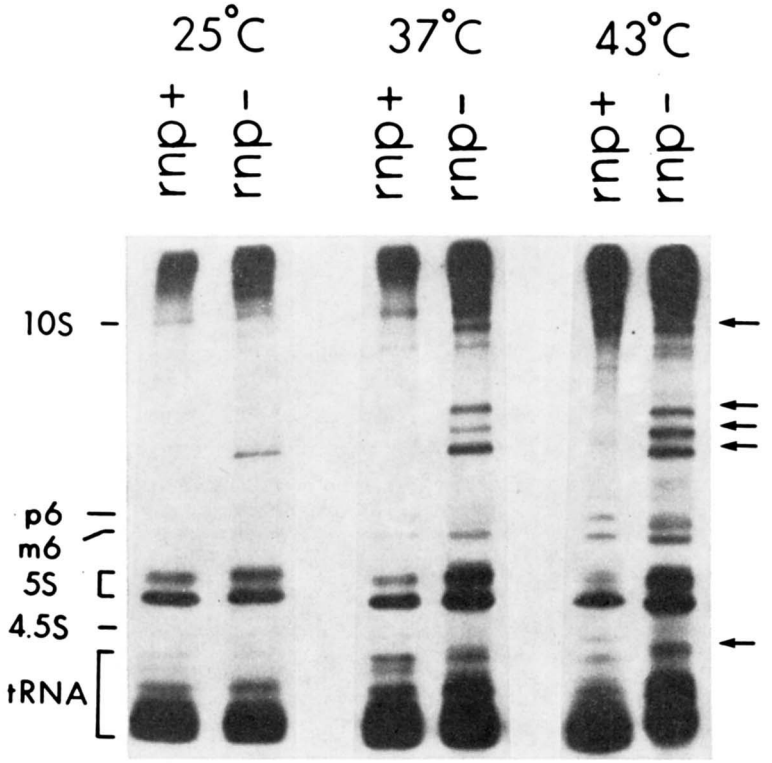


FIGURE 1.—Patterns of RNA synthesized in a *rnp*+ and a *rnp* strain at various temperatures. Cultures were grown in a low phosphate medium (GEGENHEIMER, WATSON and APIRION 1977) at 30° and at A_{560} of about 0.5 transferred to the various temperatures. Forty minutes later, $^{32}P_i$ was added to the cultures (100–200 μC/ml) and, after 30 minutes, the cultures were processed and analyzed on 5%/10% thin (1.5 mm) polyacrylamide slab gels (for further details see LEE, BAILEY and APIRION 1978). A photograph of an autoradiograph is shown. The 5% portion of the gel was removed prior to the autoradiography in order to obtain a clearer view of the top part of the 10% gel. The arrows on the right indicate RNA molecules unique to *rnpA49* strains. The *rnp*+ strain is PP113, and the *rnp* is N2020. All lanes were from the same gel.

FREY FELTON, Tuft University, of some new mapping data is in agreement with the order suggested here.)

A convenient way to transfer the *rnpA49* mutation is by co-transduction with the *bglB* marker. (Strain N2039 was constructed in this way; see Table 1.) Phenotypically, the strains into which the *rnpA49* mutation was transferred behaved exactly like the original strain A49 or N2020; their patterns of RNA synthesis were identical.

Characterization of strain carrying the rnpA49 *mutation:* Our previous experiments (GEGENHEIMER and APIRION 1978) suggested that RNase P is inactivated, at least to some extent, in *rnpA49* strains, even at permissive temperatures. Therefore, we tested colony formation of some of the strains used, at vari-

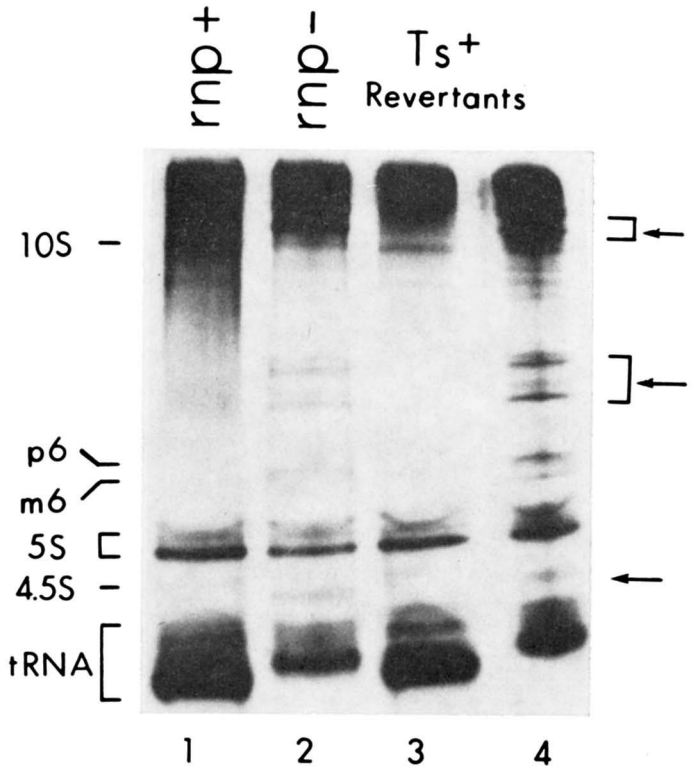
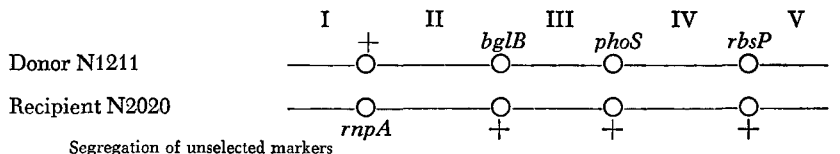


FIGURE 2.—Patterns of RNA synthesized in various strains at 43°. Cultures were grown at 30°, shifted to 43° and, 40 minutes after the shift, were labeled for 30 minutes with $^{32}P_i$ (for further details see legend to Figure 1). The arrows on the right indicate RNA molecules unique to *rnpA49* strains. 1,PP113, 2,N2020; 3,N2029; 4,N2035. Notice the difference in patterns between Figure 1 and Figure 2, which were caused by minor differences in the experimental procedure and, in particular, in the distance that the various molecules migrated in the gel. All lanes were from the same gel.

ous temperatures. As seen in Table 3, while the *rnp*+ strain PP113 and N141 can form colonies in rich medium at all temperatures up to 45°, strains N2020 and N2039, which carry the *rnpA49* mutation, cannot. Both form colonies at 30°, 37° and 40°, but at the higher temperatures their growth rate is slower than that of *rnp*+ strains, as judged from the size of the colonies formed. (This method of comparing growth of strains in the same medium at different temperatures was chosen, rather than the customary measuring of doubling time, since this is a much more sensitive method. Here, growth is measured after a relatively long period of time, two days, without interfering with the culture, as is the case when growth curves are measured.) These results suggested that the RNase P is somewhat inactivated in a physiologically meaningful way, even at temperatures where growth is still feasible. As could be expected, the severity of the decrease in growth rate depends on the genetic background of the strain. While both *rnp*+ strains into which the *rnpA49* mutation was introduced can grow at

TABLE 2

Linkage of rnpA49 *to* bglB phoS *and* rbsP*



Segregation of unselected markers			No.	Minimal necessary crossing overs
bgl	*phoS*	*rbsP*		
+	+	+	60	I and II
—	—	+	28	I and IV
—	—	—	9	I and V
—	+	+	6	I and III
—	+	—	2	I, III, IV and V
+	+	—	2	I, II, IV and V
+	—	+	0	I, II, III and IV
+	—	—	0	I, II, III, and V

* Selection was carried out for Rnp+ transductants, by plating P1-infected recipient cells in rich medium plates at 45°. Suggested order and percentage of co-transduction:

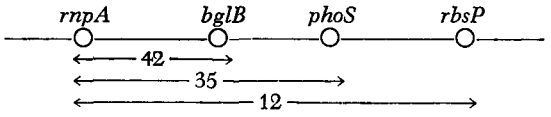


TABLE 3

Plating efficiency and size of colonies

Strain		Temperature 30°	37°	40°	43°	45°
PP113	No.	312	285	303	321	292
(*rnp*+)	Size*	2.4	2.7	3.2	2.9	3.0
N2020	No.	248	212	201	253	$<10^{-6}$†
(*rnpA49*)	Size	2.5	1.9	0.3	0.1	—
N2029	No.	212	236	218	197	224
(*rnp*+)	Size	2.6	2.8	3.2	3.0	2.2
N2035	No.	210	196	187	194	51×10^{-5}†
(*rnpA49 sup*)	Size	1.8	2.6	2.7	1.9	0.4
N141	No.	192	211	187	165	219
(*rnp*+)	Size	2.9	3.1	3.7	1.8	0.7
N2039	No.	192	217	183	< 10	$<10^{-6}$†
(*rnpA49*)	Size	2.2	2.0	0.4	—	—

* Diameter in mm.

† Calculated from plating less diluted samples.

Cultures were grown at 30° in liquid rich medium, diluted, and the same volumes from the same dilution were plated on rich medium plates. The Petri dishes were incubated at the various temperatures for two days; then they were counted and measured. The size shown is the average diameter in mm of about five to ten colonies.

45°, when the *rnpA49* mutation was introduced into them, strain N2020 still grew, albeit extremely slowly, at 43°, while strain N2039 failed to grow at 43°. Further support for the notion that the *rnpA49* mutation can be manifested at temperatures at which the strain can grow can be observed in Figure 1. In this experiment, RNA synthesis in an *rnp*$^+$ strain (PP113) and in an *rnp* strain (N2020) was analyzed at 25°, 37° and 43°. It can be seen that some of the RNA molecules that clearly characterized the *rnp* mutation at 43° appear at 25° to a limited extent and at 37° to a larger extent.

Revertants from rnpA49 *strains:* To find out whether the *rnpA49* is a point mutation, revertants able to grow at higher temperatures were isolated from *rnp* strains. To avoid the isolation of possible siblings, this was carried out by replicating large colonies grown at 37° on rich medium into the same medium, and the Petri dishes were incubated at 45°. A number of patches of good growth appeared. These were purified and retested. Two types of Ts$^+$ revertants appeared; one class grew faster than the other at the higher temperatures (Table 3). The faster-growing class behaved, in all respects, like the original strain (PP113); as can be seen in Figure 2, the pattern of RNA synthesis in the Ts$^+$ revertant strain N2029 became normal and seems to have regained a normal RNase P. In this class of revertants, probably a true reversion event occurred. This was further supported by the fact that, in transduction experiments, we failed to recover the *rnpA49* allele from such strains. The other class grew slower (Table 3, strain N2035) and, as can be seen in Figure 2, it retained the *rnpA49* mutation.

The rnpA49 *mutation is recessive*: To find out whether the *rnpA49* mutation is recessive or dominant, an F′ (F111), which covers the proper region of the *E. coli* chromosome (Low 1972), was transferred (Low 1972) to strain N2039 (*ilv argE3 rnpA49*). Ilv$^+$ Arg$^+$ recombinants were isolated, purified and retested. Many of these strains (15 of 25) also became Ts$^+$. Strain N2039 is particularly suitable for such an analysis since it contains two markers, *argE3* and *ilv158*, in the vicinity of *rnpA49* and many other markers (see Table 1) scattered all over the *E. coli* chromosome. These 15 colonies retained all the other markers of the parental strain N2039, thus indicating that they are likely to contain the F′111 factor rather than being recombinants that could have resulted from the mobilization of the donor chromosome by the F′. This was further indicated by the fact that some of the strains isolated could easily donate the *ilv*$^+$ and *arg*$^+$ genes, but not others. RNA metabolism was analyzed in some of the Ts$^+$ and Ts$^-$ colonies isolated from the cross; all the Ts$^+$ colonies showed an Rnp$^+$ phenotype, while the Ts$^-$ colonies showed an Rnp$^-$ phenotype. It is concluded, therefore, that the *rnpA49* mutation is recessive.

DISCUSSION

The studies described here confirm that *rnpA49* is a recessive point mutation and show that it maps at about minute 82 of the *E. coli* chromosome. The experiments also suggest that it is a missense mutation since the enzyme in a modified form must exist in growing cells (see Figure 1).

OZEKI *et al.* (1974) isolated a number of mutants defective in the enzyme RNase P. These mutants seem to fall into two groups. They suggested from studying the phenotype of RNase P⁻ strains after a series of F′ was transferred to them that one of these two groups is located between minutes 78 and 83 on the *E. coli* map (BACHMANN, LOW and TAYLOR 1976). It is important to note that, while it is possible that the *rnpA49* mutation is allelic with the *rnp* mutation *ts-241* described by OZEKI *et al.* (1974) (which maps between 78 and 83 min), this cannot be firmly stated, since precise mapping for the latter mutations is unavailable, and complementation tests between these mutant strains were not carried out.

An interesting feature of the *rnpA49* mutation is its manifestation at almost all the temperatures where strains carrying the mutation grow (Figure 1). The defects in RNA processing at the permissive temperatures seem to be physiologically significant since the growth rates of *rnpA49* strains are affected (see Table 3). Since RNase P is involved in the processing of a large number of RNA molecules (SCHEDL and PRIMAKOFF 1973; OZEKI *et al.* 1974; ALTMAN 1975), the slower maturation of only one of these molecules could be responsible for the physiological effects.

It is interesting to compare the *rnpA49* mutation to the *rnc-105* and the *rne-3071* mutations, since all of them affect RNA processing enzymes. The *rnc-105* mutation abolishes completely the enzyme RNase III (APIRION, NEIL and WATSON 1976a,b), and cells can survive without it. On the other hand, the *rne-3071* mutation is manifested only at the nonpermissive temperature (APIRION 1978; APIRION and LASSAR 1978). These unique characterizations of each of these mutations helped us build up strains that carry all the possible combinations of these mutations (APIRION *et al.* 1980).

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Note added in proof: Another mutation that affects ribonuclease P, designated *rnpB3187*, was mapped near min 68 of the *E. coli* chromosome (APIRION and WATSON 1980).

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