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Genetic Mapping of a Mutation That Causes Ribonuclease III Deficiency in *Escherichia coli*

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The mutation that causes ribonuclease III (RNase III) deficiency in strain AB301-105 of Kindler et al. (1973) has been mapped by use of F′ merodiploids, Hfr matings, and P1 transduction. This mutation, *rnc-105*, lies close to *nadB*, near 49 min on the genetic map of *Escherichia coli*. The *rnc-105* mutation has been transferred from its original genetic background by transduction and conjugation, and these new strains have the same defects in ribonucleic acid processing reported previously for AB301-105. Strains that carry *rnc-105* grow more slowly than parental *rnc*$^+$ strains, but the difference in growth rate seems to depend on the genetic background of each strain. Bacteriophage T7 grows about equally well in RNase III$^+$ and III$^-$ female strains of *E. coli*, even though the specific cuts that RNase III makes in T7 ribonucleic acid are not made in the RNase III$^-$ strains. A low-phosphate defined medium in which most *E. coli* strains seem to grow well was developed. This medium is equally useful for labeling ribonucleic acids with $^{32}PO_4$ and as a selective medium for genetic manipulations. It was used to determine the growth requirements of strain AB301-105, which are biotin and succinate in addition to the methionine and histidine requirements of the parental strain. The biotin mutation lies near the position expected from known mutations of *E. coli*, but the succinate mutation apparently does not. The possibility that the succinate requirement could be due to the RNase III deficiency is discussed. A *uraP* mutation was isolated for use in transferring *rnc-105* between strains by conjugation. It lies near 47 min, somewhat removed from the commonly accepted position for *uraP*.

Ribonuclease (RNase) III of *Escherichia coli* was originally identified as a nuclease that specifically degrades double-stranded ribonucleic acid (RNA) (23). Subsequently, it has been shown that this enzyme has a role in processing both *E. coli* ribosomal RNAs and bacteriophage T7 messenger RNAs, cutting the primary transcripts at specific sites (5, 6, 18). Studies on the intracellular role of RNase III became possible when an RNase III-deficient mutant of *E. coli*, AB301-105, was isolated by Kindler et al. (12). This mutant was derived from an RNase I-deficient Hfr strain, A19, that had been isolated by Gesteland (8). Both strains A19 and AB301-105 were selected after heavy mutagenesis, and both acquired unknown nutritional requirements in addition to their RNase deficiencies.

It seemed desirable to obtain well-characterized RNase III$^-$ strains so that the effects of RNase III deficiency in vivo could be studied in the absence of possible secondary effects. In addition, female strains were needed to study effects of RNase III deficiency on T7, since T7 does not grow on male strains of *E. coli* (16). The mutation conferring RNase III deficiency has now been mapped near *nadB* on the genetic map of *E. coli* and has been transferred out of the original strain by P1 transduction and by conjugation.

MATERIALS AND METHODS

Strains. The *E. coli* strains used in this work are listed in Table 1 and Fig. 1. Strains A19 and AB301-105 were obtained from P. H. Hofschneider; a λ-cured derivative of AB301-105 was from D. Schlessinger; PR7 was from R. Gesteland; W3110 was from A. D. Kaiser; the Hfr strains of Low (14) were from M. Riley; and the F′ strains of Fig. 1, as well as AT2092, PA3306, and X′121, were from B. Bachmann at the *E. coli* Genetic Stock Center at Yale.

The male-specific bacteriophage R17 (20) was obtained from R. Gesteland, and P1CM*clr*100 (24) was obtained from J. L. Rosner. The wild-type T7 is that described in reference 26.

Nomenclature. Following the nomenclature proposed by Apirion and Watson (2), the gene affecting RNase III is referred to as *rnc* and the specific *rnc* mutation of AB301-105 as *rnc-105*. Likewise, the gene affecting RNase I is referred to as *rna* rather than

TABLE 1. *E. coli K-12 strains*[a]

Strain	Genotype	Derivation	Reference
AB301	Hfr PO21 *metB1 rel-1* (λ)	From P4x (chart 5 of reference 4)	8
A19	Hfr PO21 *metB1 his-95 rna-19 rel-1* (λ)	Nitrosoguanidine-induced mutant of AB301	8, 12
BL15	F⁻ *rna-19 rel-1* (λ)	Sequential P1 transductions of A19; F⁻ by R17 selection	
AB301-105	Hfr PO21 *metB1 his-95 bio-3 suc? rna-19 rnc-105 rel-1* (λ)	Nitrosoguanidine-induced mutant of A19	12
BL103	F⁻ *metB1 his-95 bio-3 suc? rna-19 rnc-105 rel-1* λ⁻	AB301-105 cured by λ by UV (Schlessinger); F⁻ by R17 selection	
BL114	F⁻ *suc? rna-19 rnc-105 rel-1* λ⁻	Sequential P1 transductions of BL103	
BL117	F⁻ *metB1 his-95 bio-3 suc? rna-19 rnc-105 rel-1 str-132* λ⁻	*str* transductant of BL103 using P1 grown in PR7 (22)	
BL119	F⁻ *thy his-95 bio-3 rna-19? rnc-105 rel-1? str-132* (sugars?) λ⁻	Recombinant from KL983 × BL117; *thy* by trimethoprin selection (17)	
BL214	Hfr PO45 *thi-1? uraP119 rnc-105 rel-1?* λ⁻	F'-mediated chromosome transfer, F15/BL119(*uraP*)→KL16	
BL311	F⁻ *thi-1 argH1 nadB4 rnc-105 str-9 tonA2 supE44* (sugars) λ⁻	F15/BL119(*uraP*)→BL15 to give BL229 (*rnc uraP*); P1·BL229→AT2092 to give BL304 (*pheA*⁺ *rnc*); P1·BL304→PA3306	
KL16	Hfr PO45 *thi-1 rel-1* λ⁻		4 (chart 6), 14
KL983	Hfr PO53 *xyl-7 lacY1 mglP1* λ⁻		14
W3110	F⁻ λ⁻		4 (chart 8)
AT2092	F⁻ *thi-1 argH1 his-1 pheA2 purF1 str supE44* (sugars) λ⁻?		28
PA3306	F⁻ *thi-1 argH1 nadB4 purI66 str-9 tonA2 supE44* (sugars) λ⁻		30
X'121	F⁻ *thi-1 pyrD34 tyrA2 his-68 trp-45 str-118* (sugars) λ⁻	Brenner collection	

[a] Specific allele numbers and PO numbers (Hfr point of origin) are those of the *E. coli* Genetic Stock Center at Yale. The points of origin of KL16 and KL983 are shown in Fig. 1; PO21 is similar to Hfr H (PO1). The genotype (sugars) indicates several different mutations affecting metabolism of different sugars.

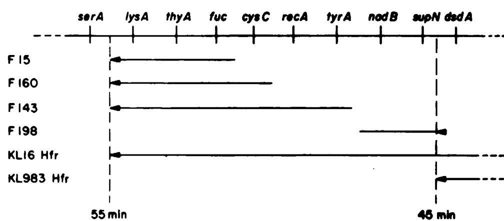

FIG. 1. *Location of F' factors and Hfr points of origin. Map positions of genetic markers, location of F' factors, and Hfr points of origin are given in references 29, 15, and 14, respectively. The F' donor strains were obtained from the E. coli Genetic Stock Center and were: F15/KL110, NF306 (for F160), KLF43/KL259 (for F143), and F198/FF7040.*

rnsA. All other genetic symbols are those given in Taylor and Trotter (29); the specific allele numbers are those of the *E. coli* Genetic Stock Center (4).

Growth media. Tryptone, yeast extract, and agar were obtained from Difco. Agar plates contained 1% agar. The general purpose medium for growth of stocks and for matings was TB (1% tryptone, 0.5% NaCl). LB medium (TB plus 0.5% yeast extract) was used in some experiments.

Two low-phosphate minimal media were used for selective plates and for labeling with $^{32}PO_4$. Both contain 0.16 mM phosphate and 20 mM buffer and will support logarithmic growth to approximately 5 × 10^9/ml (absorbance at 600 nm of 0.5 to 0.6) before lack of phosphate starts to limit growth. B2 medium contains (per liter): bis(2-hydroxyethyl)imino-tris-(hydroxymethyl)methane (Bis-Tris; Sigma Chemical Co., no. B-9754), 4.2 g; NH_4Cl, 1 g; NaCl, 2.5 g; KCl, 1.5 g; 1 M $MgSO_4$, 1 ml; 1 mM $FeCl_3$, 1 ml; enough concentrated HCl to give pH 7 (about 0.4 ml); glucose, 4 g; and 64 mM phosphate buffer (3 g of KH_2PO_4 plus 6 g of Na_2HPO_4 per liter), 2.5 ml. M2 medium is the same as B2 except that 20 mM sodium maleate (pH 7) replaces the Bis-Tris and HCl. The glucose and phosphate are autoclaved separately from each other and from the rest of the medium, which can be made up 20× concentrated.

Maleate has a very high absorbance in the ultraviolet (UV), so M2 medium is not used for experiments in which the culture is irradiated with UV light. Bis-Tris is transparent to UV but costs 70 times as much as maleate (about $0.65 per liter of B2 medium at current prices). Therefore, M2 medium is usually used for making plates and screening of mutants, and B2 medium is used for experiments in which the culture is irradiated with UV or where results are

to be compared with those from UV-irradiated cultures. For example, bacteriophage RNAs and proteins are usually labeled in B2 medium.

Required growth supplements were added to M2 or B2 medium at the following concentrations (per milliliter): 20 μg of L-amino acids, purines, or pyrimidines; 1 ng of biotin; 50 ng of nicotinic acid; and 100 ng of thiamine. To improve growth rates, selective media usually contained 20 μg of each of the 20 usual L-amino acids (except for any involved in selection) per ml; such media are referred to as M2a or B2a. Streptomycin was used at 100 μg/ml, and 6-azauracil was used at 20 to 40 μg/ml.

Mating and transduction. Mating and general manipulation were done essentially as described by Low (14) or Miller (17). The P1 strain used for transduction was P1CM*clr*100 (24). Lysates were grown in liquid culture at 41 C in LB plus 5 mM $CaCl_2$. After lysis, surviving bacteria were killed with chloroform, and the lysate was centrifuged to remove debris. Recipient cells were grown at 37 C in M2a medium containing four times the usual concentration of phosphate plus any necessary growth requirements. When the cells reached about 3×10^8 to 5×10^8/ml, an equal volume of an appropriate dilution of P1 lysate (in 10 mM $MgSO_4$, 5 mM $CaCl_2$, 100 μg of gelatin per ml) was added, and incubation was continued for another 20 to 30 min. Samples were then mixed with 0.2 ml of 1 M sodium citrate followed by 2.5 ml of top agar and then plated on selective plates. In some experiments, the transduction frequency was improved by irradiating phage P1 with UV light immediately before it was added to the cells (3). For LB lysates diluted 20-fold (in 10 mM $MgSO_4$, 5 mM $CaCl_2$, 100 μg of gelatin), 1,000 to 2,000 ergs of light per mm^2 from two germicidal lamps produced the maximum stimulation of transduction, approximately fivefold. (A 20-fold dilution of LB medium in the above solvent had an absorbance of 1.3 at 260 nm.)

Assay for RNase III deficiency. The following test was used to assay for RNase III deficiency in different *E. coli* strains. A 50-μl sample of a culture of cells growing in appropriately supplemented M2a or B2a medium at a concentration about 3×10^8 to 5×10^8/ml was added to 0.5 μCi of $^{32}PO_4$ and labeled for 10 min at 30 C. The pulse was stopped with a cyanide-phosphate mixture, the sample was centrifuged, and the cells were suspended in buffer that contained 1% sodium dodecyl sulfate (27). The sample was then heated for 2 min in a boiling-water bath and analyzed by electrophoresis for 2 h at 70 V on a gel of 2% polyacrylamide plus 0.5% agarose, all as described previously (27). RNase III-deficient cells are defective in processing ribosomal RNA, and the autoradiogram of the gel thus gives a distinctive pattern (6).

RESULTS

Defined medium for growing AB301-105. For mapping and transduction experiments with AB301-105, it was necessary to be able to grow the strain in defined medium. It was also desirable that the medium be low in phosphate so that RNA could be labeled efficiently with $^{32}PO_4$. In the first attempt to find such a medium, growth supplements were added to the low-phosphate minimal medium used previously (27). However, it was discovered that the high concentration of Tris (0.1 M) used to buffer this medium interfered with growth. This seems to be true for almost any strain but is particularly apparent for AB301-105, which grows slowly even in a rich medium (12). Reducing the buffer concentration improved growth, but Tris (pK 8.1) provides little buffering at pH 7 against the acid produced during growth of *E. coli* in glucose-containing media. Therefore, several buffers with pK's in the range of 6 to 7 were tested to find one that would allow good growth and provide good buffering capacity. Two buffers were selected: maleate (pK 6.6) and Bis-Tris (pK 6.5). A buffer concentration of 20 mM was chosen because it gives little inhibition of growth rate, and cells can grow to about 5×10^8/ml before the pH drops below pH 6.5. (The composition and some properties of the media made with these buffers are summarized above.) Both M2 and B2 media (appropriately supplemented for auxotrophic strains) support the growth of all *E. coli* strains tested, including some that grow poorly or not at all in the 0.1 M Tris medium.

Growth requirements of A19 and AB301-105. Strain AB301-105 was derived from A19, which was itself derived from AB301 (Table 1). A19 was isolated because it was deficient in RNase I, but it also acquired one or more new growth requirements in addition to the methionine requirement of AB301 (8). The first step in determining the growth requirements of AB301-105 was to define the growth requirements of A19; these turned out to be methionine and histidine. This was surprising, since it was reported originally (8) that the new growth requirement of A19 was not an amino acid. It is possible that the A19 strain used by Kindler et al. (12) is different from the A19 originally isolated but, in any case, the A19 strain that gave rise to AB301-105 seems to require only methionine and histidine.

In addition to the methionine and histidine requirements of strain A19, AB301-105 acquired a requirement for biotin and will grow in minimal medium supplemented with biotin plus the usual 20 amino acids. By removing individual amino acids from this medium, it can be shown that the methionine and histidine requirements were retained and that lysine is also needed for growth. As discussed below, the lysine requirement can be replaced by succinate and is best regarded as a succinate requirement.

Selection of a female derivative of AB301-105. A female derivative of strain AB301-105 was needed in order to study the effects of RNase III deficiency on the growth of T7 and for use as a recipient in conjugation experiments. The female strain was isolated by R17 selection, starting with a derivative of AB301-105 that had been cured of λ by UV treatment. The λ^- strain was isolated and kindly provided by D. Schlessinger; it seems to grow somewhat better than AB301-105 itself.

Selection was on TB plates in the presence of 4×10^{11} R17 phage. Since this phage grows on male cells but does not adsorb to F^- cells (20), all male cells should be infected and killed, and any female cells should survive and form colonies. Approximately 0.04% of the cells could form colonies in the presence of R17. Eighteen colonies, all that grew on one R17 selection plate, were purified and tested further. Each strain was completely resistant to R17 and each remained RNase III^-. T7 grew normally on 12 of the strains, giving normal plaques at the same efficiency as on B, but grew poorly on the remaining six strains, giving small plaques at 5% the efficiency as on B. (The R17-resistant derivative of AB301-105, isolated by Apirion and Watson [1] seems to be of the latter type, since this strain [N938] and its derivatives all plate T7 with low efficiency [Table 6 of reference 1].)

It was somewhat surprising to find that T7 grows normally on RNase III-deficient strains, since both early and late T7 messenger RNAs are processed by RNase III (5, 7). Apparently this processing is not needed for growth of T7, at least in K-12 strains. The partial restriction of T7 growth by six of the R17-resistant strains could be explained most simply as being due to incomplete loss of F. The 12 R17-resistant strains on which T7 grows normally are assumed to be truly F^-; one of them, BL103, was used for further work.

Succinate requirement of BL103. P1 transduction was used to remove growth requirements from BL103. Since RNase III-deficient strains grow slowly, the selective plates for these experiments (and wherever practical throughout this work) contained a mixture of those amino acids not involved in the selection. Transductants to His^+ and Bio^+ were found easily, with no background of His^+ or Bio^+ colonies on control plates. Lys^+ colonies appeared about as frequently when no P1 was added as when it was present, indicating that this mutation either reverts with high frequency or is easily suppressed. However, no transductants to Met^+ were obtained.

The inability to obtain Met^+ strains seemed unusual since A19 is easily transduced to Met^+. The reason became clear when further transductions were attempted: Bio^+ and His^+ derivatives of BL103 could not be transduced to Met^+, but a Lys^+ derivative could. Apparently the requirement for lysine is actually a requirement for lysine plus methionine. Several types of mutation in *E. coli* that lead to a requirement for lysine plus methionine have been described, including some in which the requirement can be replaced by succinate (10). BL103 can grow in B2a (plus biotin) medium, in which the lysine is replaced by succinate, and Met^+ transductants can be selected on B2a (plus biotin) plates containing 5 mM succinate but lacking methionine. Therefore, this mutation should be thought of as a requirement for succinate.

To study further the properties of the succinate requirement, BL103 was transduced in sequence to Bio^+, His^+, and Met^+, leaving the strain RNase III deficient and succinate requiring. This strain, BL114, exhibits different growth requirements depending on whether it is grown on minimal plates plus individual growth supplements or on plates supplemented with all but one of the 20 amino acids. On plates without extra amino acids, it will grow in the presence of succinate alone, methionine alone, or isoleucine alone, but not lysine alone. This is consistent with a requirement for succinate, since degradation of methionine and isoleucine (but not lysine) gives rise to succinyl coenzyme A (reference 13, p. 448). However, as noted for strain AB301-105 itself, BL114 is unable to grow without lysine on plates supplemented with the 19 other amino acids (unless succinate is also added). This seemed strange, since BL114 grows in the presence of methionine alone or isoleucine alone, both of which are present in the mixture.

By testing individual amino acids for ability to inhibit the growth of BL114 on minimal plates containing methionine, it was found that aspartic acid (but not asparagine or any other amino acid) creates the requirement for lysine. This observation too can be rationalized in terms of a succinate requirement. Aspartic acid is the starting point toward synthesis of lysine, and succinyl coenzyme A is required at an intermediate step. In the presence of aspartic acid, a succinate deficiency might lead to accumulation of 2,3-dihydropicolinic and tetrahydropicolinic acids (reference 13, p. 549). If these intermediates are toxic, degradation of methionine, valine, and isoleucine might not produce enough succinyl coenzyme A to permit the cell

to grow in the presence of aspartic acid. Addition of succinate would relieve the block; addition of lysine would prevent the conversion of aspartic semialdehyde to these intermediates, by feedback inhibition.

Approximate mapping of the bio, his, and suc mutations. A set of rapid mapping experiments was done by the procedures of Low (14) to determine whether the *bio*, *his*, and *suc* mutations of strain AB301-105 lie near the positions known for such mutations on the genetic map of *E. coli* (29). For this purpose, BL117, a streptomycin-resistant derivative of BL103, was constructed by transduction from a streptomycin-resistant donor. Twelve different Hfr strains were replica plated onto a lawn of BL117 on different selective plates so that patterns of transfer could be observed. The growth patterns on this set of plates were consistent with the *bio* mutation being near 17 min and *his* being near 39 min, as expected. However, the *suc* mutation seemed to map near *his* and not near 16 min, where the previously characterized *sucA* and *sucB* genes have been mapped. This raised the possibility that the Suc^- (or Lys^-) phenotype of AB301-105 and its derivatives might be due to the RNase III deficiency rather than to an independent mutation. None of the Lys^+ strains that arose in the presence or absence of P1 in transduction experiments appeared to be RNase III^+, as judged by growth rate, and none of those tested was RNase III^+. This could be explained if mutations that suppress the Suc^- phenotype arise fairly frequently. Because of the uncertainty about the cause of the Suc^- phenotype, the genotype for the succinate requirement of AB301-105 and those derivatives known to require succinate (or lysine) is indicated by "*suc?*" in Table 1.

Approximate mapping of rnc-105 by F′ factors. RNase III deficiency cannot be scored directly on plates, so to map the *rnc* marker individual strains must be isolated and tested for RNase III activity. For each test, a growing culture was exposed to $^{32}PO_4$ for 10 min, and then the labeled RNAs were analyzed by electrophoresis on gels. RNase III-deficient strains are defective in processing ribosomal RNA, and their labeled RNAs give a distinctive pattern on gels (6).

The first indication of the location of the *rnc-105* mutation came from the rapid mapping experiments described above. A character influencing colony size seemed to be transferred early by KL16 but late by KL983, which would place it between 45 and 55 min on the genetic map of *E. coli* (Fig. 1). It seemed possible that the change in colony size might be due to the RNase III deficiency, and tests for RNase III activity in recombinant strains isolated on the rapid mapping plates supported this idea.

Low (15) described a set of F′ factors that covers the region between 45 and 55 min, and several of these (Fig. 1) were used to refine the position of *rnc-105*. For this purpose the donor strain should be *recA*, so that only the F′ factor and none of the chromosome will be transferred. However, the F143 F′ factor carries the $recA^+$ allele, so this donor strain is able to transfer the chromosome by recombination with the F′ factor. The recipient in these matings was BL119, a *thyA*, *rnc-105* strain (Table 1). The *thyA* marker made it possible to select directly for transfer of each of the F′ factors except F198. The transfer of F198 could not be selected for directly but, when a mating mixture was plated under conditions where the donor strain could not grow, 5 of 30 colonies tested were susceptible to R17, indicating that they had received the F′. These five strains plus 8 to 10 of the largest colonies from each of the other three matings were tested for RNase III, susceptibility to R17, and the presence of outside markers (an indication of chromosomal transfer or contamination). All of the strains that had received F15, F160, and F198 were RNase III^-, were susceptible to R17, and had not received any outside markers. This strongly suggests that the *rnc* locus is not carried by any of these F′ factors. On the other hand, 8 of 10 strains that received F143 were RNase III^+, were susceptible to R17, and had not picked up outside chromosomal markers. Therefore, it is likely that the *rnc* locus is on F143. The combined results suggest that *rnc-105* lies between *nadB* and *cysC* on the genetic map (Fig. 1).

Transfer of rnc-105 by chromosome mobilization. The approximate position deduced for *rnc-105* is within a segment of the genetic map that is continuously linked by P1 transduction (29). However, initial attempts to map *rnc-105* by co-transduction with nearby markers were hampered because the P1 lysates produced from RNase III^- strains had rather low frequencies of transducing particles, perhaps 5% or less the frequency in lysates from RNase III^+ strains. Since it was not clear whether the difference in transduction frequency was due to the *rnc-105* mutation or to some other mutation of AB301-105, it was decided to move the *rnc-105* mutation from its original background by F′-mediated chromosome transfer, map it by conjugation experiments, and then verify this position by P1

transduction.

To move *rnc-105* to another strain, it was desirable to have a nearby selective marker. *uraP* (resistance to 6-azauracil) lies in this region and mutants can be selected easily (17). Therefore, a spontaneous *uraP* derivative of F15/BL119 was isolated and used for F′-mediated chromosome transfer. This *uraP* mutation, designated *uraP119*, was the only one used in the experiments reported in this paper.

The recipients for the F′-mediated chromosome transfer were F^- strains W3110 and BL15 and Hfr strain KL16. KL16 was chosen as a recipient because an *rnc-105* recombinant that retained the Hfr point of origin of KL16 would transfer *rnc-105* early and would be useful for mapping by conjugation. The KL16 culture was starved for 6 h at 37 C in minimal medium without glucose to make it a better recipient (17). Mating was for 25 min, and the mating mixture was plated on M2a medium containing no histidine or biotin so that the donor strain could not grow. After 80 min at 37 C, the plates were overlayered with 5 ml of the same agar containing 500 μg of 6-azauracil to prevent the growth of recipients that had not received *uraP*. (A subsequent experiment indicated that full expression of resistance to 6-azauracil may require 3 h or more.) Recombinant colonies were picked at random, taking all colonies on a plate or all colonies from one section of a plate, and tested for RNase III. Seven of eight *uraP* recombinants with W3110, 19 of 24 with BL15, and 29 of 40 with KL16 were RNase III^-. This would indicate that *rnc-105* is probably fairly close to *uraP*. BL214, one of the recombinants from the cross with KL16, was used as an Hfr donor for *rnc-105* in further work.

In all three of these crosses, the RNase III^- colonies grew more slowly than the RNase III^+ colonies, a distinction apparent when recombinants were streaked onto fresh plates and incubated at 37 C overnight. This difference in growth rate between RNase III^+ and III^- strains seems to hold for essentially all of the recombinants tested, not only from these crosses but from the Hfr matings and P1 transductions discussed below. Thus, it seems likely that the *rnc-105* mutation causes a decrease in growth rate in most strains, and this difference in growth rate can provide a reliable preliminary screening for RNase III deficiency of recombinants. However, the magnitude of the difference in growth rate seems to be a function of the genetic background of the strain, being very large in some cases and rather small in others. It is not yet clear what genetic factors might influence growth rate of RNase III-deficient strains or how the different genetic elements might interact.

Mapping of rnc and uraP by conjugation. With BL214 as the donor in conjugation experiments, the positions of *rnc-105* and *uraP* were mapped relative to nearby nutritional markers. A cross was made between BL214 (Hfr *rnc-105 uraP*) and AT2092 (F^- *pheA purF str*), from which $purF^+$ *str* recombinants were selected. Since the origin of transfer in BL214 is at 55 min and *purF* is at 44 min, the entire region of interest was transferred to each recombinant. A total of 224 recombinants from two matings was tested for *pheA* and *uraP* (Table 2), and the results indicate that *uraP* falls between *pheA* and *purF*. Fifty-three of the recombinants of Table 2 (all of the colonies on a single plant) were also tested for RNase III. The results (Table 3) are consistent with the order *pheA*, *rnc*, *uraP*, *purF*. An indication of whether the remaining 171 recombinants of Table 2 were RNase III^+ or III^- could be obtained from the relative growth rates of the recombinants on the control plates of the test for *pheA* and *uraP*; 35 of these were also tested for RNase III. These additional data are entirely consistent with, and reinforce, the data of Table 3. In particular, the class of recombinants that would require three crossovers between the outside markers (for the order shown in Table 3) contained no recombinants, whereas if *rnc* were between *uraP* and *purF* the triple crossover class would contain 25

TABLE 2. *PurF⁺ str recombinants of BL214 × AT2092*[a]

A B C D
BL214 Hfr ◁—— *uraP* ——-----
55 50 44 64
AT2092 F^- —— *pheA* —— *purF* — *str*

Recombinant genotype[b]		No.	Minimum crossovers	Crossovers between *pheA* and *purF*
pheA	*uraP*			
+	0	140	A, D	0
0	+	45	C, D	1
0	0	27	B, D	1
+	+	12	A, B, C, D	2

[a] The numbers on the map indicate approximate position on the genetic map of *E. coli* (29); the capital letters identify intervals between markers.

[b] +, Wild type; 0, mutant.

TABLE 3. *Rnc composition of purF⁺ str recombinants of BL214 × AT2092*[a]

A B C D E

rnc *uraP*

BL214 Hfr

55 50 44 64

AT2092 F⁻

pheA *purF* *str*

Recombinant genotype[b]			No.	Minimum crossovers	Cross-overs between *pheA* and *purF*
pheA	*rnc-105*	*uraP*			
+	0	0	33	A, E	0
0	+	+	11	D, E	1
0	+	0	3	C, E	1
0	0	0	2	B, E	1
+	0	+	1	A, C, D, E	2
+	+	+	2	A, B, D, E	2
+	+	0	1	A, B, C, E	2
0	0	+	0	B, C, D, E	3

[a] The numbers on the map indicate approximate position on the genetic map of *E. coli* (29); the capital letters identify intervals between markers.

[b] +, Wild type; 0, mutant.

recombinants, and if *rnc* were to either side of *pheA-purF* the triple crossover class would contain 11 recombinants.

To position these markers more precisely, a second cross was made, this time between BL214 (Hfr *rnc-105 uraP*) and PA3306 (F⁻ *nadB purI str*). A total of 225 *purI⁺ str* recombinants from a single mating was tested for *nadB* and *uraP* (Table 4, experiment 1), and each of the 38 recombinants that had one or more crossovers between *nadB* and *uraP* was tested for RNase III (Table 5). Seven hundred fifty-six recombinants from a second mating were also tested for *nadB* and *uraP* (Table 4, second experiment). The data of Table 4 indicate that *uraP* is not between *nadB* and *purI* and is probably to the *purI* side. The data of Table 5 fit best with the order *rnc-105 nadB purI uraP*, but might also be compatible with *rnc-105* being between *nadB* and *purI*, considering the relatively few recombinants tested and the effects of negative interference. Thus, the combined data from the crosses with strains AT2092 and PA3306 indicate that *rnc-105* is quite close to *nadB* and that the order of markers is *pheA rnc-105 nadB purI uraP purF*, with some uncertainty in the order of *rnc-105* and *nadB*.

Mapping of rnc-105 and uraP by transduction. As mentioned previously, P1 lysates grown on RNase III⁻ strains gave relatively poor transduction for several different widely spaced markers, usually 5% or less the frequency given by lysates grown on RNase III⁺ strains. Under standard conditions, the number of transductants was usually fewer than five per plate; irradiation of the phage particles with UV light before mixing with the recipient cells (3) seemed to increase the transduction frequency two- to fivefold. The low transduction frequency may be due to the *rnc-105* mutation itself, since all *rnc-105* strains tested, including those produced by recombination or transduction, showed this behavior.

P1 lysates were grown on several different RNase III-deficient strains and used to transduce different nutritional markers. Transductants were selected on defined plates, a culture of each transductant was grown in the selective medium, and each culture was tested for RNase III and for outside markers of the recipient strain. Transductants were chosen randomly; usually all colonies on a plate were tested. RNase III deficiency was found to co-transduce with *pheA*, *tyrA*, *nadB*, and *purI* (Table 6) and with *glyA* (not shown). The results (Table 6) indicate that *rnc-105* is close to *nadB*, probably between it and *purI*. A more detailed analysis of the transductants of PA3306, a strain that carried mutations in both *nadB* and *purI*, is given in Table 7.

The *nadB* and *purI* markers of PA3306 are

TABLE 4. *PurI⁺ str recombinants of BL214 × PA3306*[a]

A B C D

uraP

BL214 Hfr

49 48.5 64

PA3306 F⁻

nadB *purI* *str*

Recombinant genotype[b]		No.		Minimum cross-overs	Cross-over *nadB*-*uraP*
nadB	*uraP*	Expt 1	Expt 2		
+	0	187	634	A, D	0
+	+	25	97	A, C	1
0	0	6	17	B, D	1
0	+	7	8	B, C	2

[a] The numbers on the map indicate approximate position on the genetic map of *E. coli* (29); the capital letters identify intervals between markers.

[b] +, Wild type; 0, mutant.

closely linked, 55% co-transduction having been reported when selection is for *nadB*⁺ and 28% when selection is for *purI*⁺ (30). The experiments of Table 7 are in good agreement with the published values, 48% co-transduction between *nadB* and *purI* being found when selection was for *nadB*⁺ and 21% when selection was for *purI*⁺. (It is not clear why the frequency of co-transduction of these two markers should depend on which is the selected marker.) In the

TABLE 5. *Rnc composition of the 38 purI⁺ recombinants of experiment 1, Table 5, that must have had at least one crossover between nadB and uraP*

Possible order

A B C D E — *rnc* ... *uraP* / *nadB* *purI*

A B C D E — *rnc* ... *uraP* / *nadB* ... *purI*

Recombinant genotype			No.	Minimum crossovers	Crossovers between *rnc-purI*	Minimum crossovers	Crossovers between *nadB-purI*
rnc	*nadB*	*uraP*					
0	+	0	Not tested[a]	A, E	0	A, E	0
0	+	+	21	A, D	0	A, D	0
+	0	0	4	C, E	1	C, E	1
+	0	+	6	C, D	1	C, D	1
+	+	0	Not tested[a]	B, E	1	A, B, C, E	2
+	+	+	4	B, D	1	A, B, C, D	2
0	0	0	2	A, B, C, E	2	B, E	1
0	0	+	1	A, B, C, D	2	B, D	1

[a] 187 recombinants were *nadB*⁺ *uraP*, none of which were tested for *rnc*.

TABLE 6. *Co-transduction of rnc and uraP with nutritional markers*

Donor	Recipient	Selected marker	Co-transduction			
			rnc		uraP	
			No.[a]	%	No.	%
rnc-105 uraP (several)	AT2092	*pheA*⁺	6/34	18	0/34	<3
rnc-105 uraP (several)	X'121	*tyrA*⁺	17/103	17		
rnc-105 uraP (BL214)	PA3306	*nadB*⁺	32/58	55	1/58	2
rnc-105 uraP (BL214)	PA3306	*purI*⁺	15/56	27	3/56	5
rnc-105 uraP (BL214)	PA3306	*nadB*⁺*purI*⁺	29/32	91	0/32	<3
W3110	BL311 (*nadB rnc-105*)	*nadB*⁺	28/44	64		

[a] The number of *rnc* transductants/total number tested is given, except for the *nadB*⁺ transductants of BL311, where it is the number of *rnc*⁺ transductants/total.

TABLE 7. *Distribution of transductants from P1·BL214 (rnc) → PA3306 (nadB purI)*

Selected marker	Subclass	Co-transduction							
		nadB-purI		*rnc-nadB*		*rnc-purI*		*rnc-(nadB purI)*	
		No.	%	No.	%	No.	%	No.	%
nadB⁺		28/58	48	32/58	55				
purI⁺		12/56	21			15/56	27		
nadB⁺	*rnc*					24/32	75		
purI⁺	*rnc*			11/15	73				
nadB⁺	*purI*⁺							24/28	86
purI⁺	*nadB*⁺							11/12	92
nadB⁺ *purI*⁺								29/32	91

same experiments, the *rnc-105* marker showed 55% co-transduction with *nadB* and 27% with *purI*. Approximately 90% of the transductants that received both *nadB* and *purI* also acquired the *rnc* locus. Further analysis indicates that among those transductants that were selected for *nadB* and that also brought in *rnc*, approximately 75% were also transduced for *purI*; and among those that were selected for *purI* and that also brought in *rnc*, approximately 73% were also transduced for *nadB*. This suggests a closer linkage among these markers than that found by simple co-transduction. Perhaps the difference reflects a poor survival of transductants that acquire the *rnc-105* marker; however, co-transduction of *nadB* and *rnc-105* (when selecting for $nadB^+$) is about the same whether the transducing particles are rnc^+ (64%) or rnc^- (55%) (Table 6).

The conjugation experiments discussed in the previous section indicate that the *uraP* marker is located between *purI* and *purF*. Co-transduction was found between *uraP* and *purI* (3 of 56) and between *uraP* and *nadB* (1 of 58) but not between *uraP* and *pheA* (0 of 34). This would also place the *uraP* marker between *purI* and *purF*, probably near 47 min on the genetic map of *E. coli*. Taylor and Trotter (29) had placed it near *pheA*, based on unpublished data, but indicated uncertainty about its exact position. The *uraP* marker used in my experiments could represent a second locus, but it seems more likely that there is only one *uraP* locus and that it should be placed near 47 min rather than 50 min.

DISCUSSION

The original RNase III^- strain of Kindler et al. (12), AB301-105, retains the methionine and histidine requirements of its parent, A19, and has acquired two new growth requirements. One of these is for biotin, and this mutation probably lies in the known biotin cluster near 17 min on the genetic map of *E. coli*. The other new requirement is for succinate, although it can be satisfied by various amino acids. This mutation is probably not at one of the *suc* loci previously mapped near 16 min.

The succinate requirement may be due to an independent mutation that arose in AB301-105, but it could conceivably be due to the *rnc-105* mutation itself. If, for example, RNase III cleavage were needed for efficient translation of some messenger RNAs (as has been found for at least one T7 mRNA[7]), the RNase III deficiency itself could produce growth requirements. Preliminary experiments provide some support for this idea. Several transductants to RNase III deficiency that were selected and cultured in M2a medium (which contains all 20 amino acids) are not able to grow on minimal plates. However, colonies arise on minimal plates at rather high frequency, as was also noted for the succinate requirement of AB301-105. These colonies are not revertants to RNase III^+, and at least some of them give a pattern of pulse-labeled RNAs that is different from the patterns observed in either the wild type or in the RNase III-deficient parental strain. Perhaps the strains that are able to grow in minimal medium carry suppressor mutations that eliminate the requirement for RNase III cleavage of the critical messenger RNAs. Obviously, further work is needed to determine whether these speculations are correct. However, these experiments do indicate that it may be possible to use RNase III-deficient strains to select mutations in other genes that affect RNA metabolism. It is also possible that some of the physiologically compensating mutations identified by Apirion and Watson (1) in derivatives of AB301-105 may not have been present in the original strain but might have been selected by the growth conditions used to isolate the derivatives.

The *rnc-105* mutation lies close to *nadB*, near 49 min on the genetic map of *E. coli*. Transduction experiments place it between *nadB* and *purI*; mapping by conjugation would be consistent with this position, although the data would favor a location on the other side of *nadB*; and experiments with F′ factors place *rnc* on the opposite side of *nadB* from *purI*. Transduction experiments of Apirion and Watson (2) also place it between *nadB* and *purI*. The transduction experiments are probably the most reliable, but further experiments are needed to determine conclusively on which side of *nadB* the *rnc-105* mutation lies.

The *uraP* mutation analyzed in the course of this work lies between *purI* and *purF*, probably near 47 min. Since *uraP* mutations are isolated easily, are quite close to *rnc*, and can be used as a selective marker, they are convenient for moving the *rnc-105* mutation to new strains by conjugation. Unfortunately, *uraP* and *rnc-105* are not close enough to be co-transduced with a reasonable frequency, and the background of spontaneous mutation to *uraP* is too high to use it as a selective marker in transduction experiments.

Precise mapping of the *rnc-105* mutation now makes it possible to construct isogenic RNase III^+ and III^- strains in virtually any genetic background. These strains can be used to study the role of RNase III in RNA metabolism of both

E. coli and bacteriophage T7. Preliminary experiments have detected no differences in E. coli or T7 RNA metabolism whether the RNase III-deficient strain is a derivative of AB301-105 or whether the *rnc-105* mutation is introduced into a new strain by conjugation or transduction. Somewhat surprisingly, T7 grows about equally well on RNase III^+ and III^- strains, even though little, if any, processing of T7 RNA occurs in the RNase III^- strains (7). Apparently, processing of T7 RNAs is not needed for growth, at least in the normal laboratory hosts.

Knowing the location of *rnc-105* and having nutritional markers that are closely linked to it should make it possible to isolate new mutations in the *rnc* gene by localized mutagenesis (11, 19). It may also be possible, using specialized techniques (9, 21, 25), to isolate a transducing phage that carries the *rnc* gene and that could be used to greatly increase the yield of RNase III for preparing large quantities of pure enzyme.

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