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Altered mRNA Metabolism in Ribonuclease III-Deficient Strains of *Escherichia coli*

VENUGOPAL TALKAD, DANIEL ACHORD, AND DAVID KENNELL*

Department of Microbiology and Immunology, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri 63110

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The metabolism of mRNA from the lactose (*lac*) operon of *Escherichia coli* has been studied in ribonuclease (RNase) III-deficient strains (*rnc-105*). The induction lag for β-galactosidase from the first gene was twice as long, and enzyme synthesis was reduced 10-fold in one such mutant compared with its isogenic *rnc*$^+$ sister; in the original mutant strain AB301-105, synthesis of β-galactosidase was not even detectable, although transduction analysis revealed the presence of a normal *lac* operon. This defect does not reflect a loss of all *lac* operon activity; galactoside acetyltransferase from the last gene was synthesized even in strain AB301-105 but at a rate several times lower than normal. Hybridization analyses suggested that both the frequency of transcription initiation and the time to transcribe the entire operon are normal in *rnc-105* strains. The long induction lag was caused by a longer translation time. This defect led to translational polarity with reduced amounts of distal mRNA to give a population of smaller-sized *lac* mRNA molecules. All these pleiotropic effects seem to result from RNase III deficiency, since it was possible to select revertants to *rnc*$^+$ that grew and expressed the *lac* operon at normal rates. However, the *rnc-105* isogenic strains (but not AB301-105) also changed very easily to give a more normal rate of β-galactosidase synthesis without regaining RNase III activity or a faster growth rate. The basis for this reversion is not known; it may represent a "phenotypic suppression" rather than result from a stable genetic change. Such suppressor effects could account for earlier reports of a noninvolvement of RNase III in mRNA metabolism in deliberately selected *lac*$^+$ *rnc-105* strains. The ribosomes from *rnc-105* strains were as competent as ribosomes from *rnc*$^+$ strains to form translation initiation complexes in vitro. However, per mass, β-galactosidase mRNA from AB301-105 was at least three times less competent to form initiation complexes than was A19 β-galactosidase mRNA. RNase III may be important in the normal cell to prepare *lac* mRNA for translation initiation. A defect at this step could account for all the observed changes in *lac* expression. A potential target within a secondary structure at the start of the *lac* mRNA is considered. Expression of many operons may be affected by RNase III activity; *gal* and *trp* operon expressions were also abnormal in RNase III$^-$ strains.

Ribonuclease (RNase) III is an endoribonuclease of *Escherichia coli* specific for double-stranded RNA (10, 38, 42). With the isolation of an RNase III$^-$ strain, AB301-105 (26), the enzyme was soon found to play a role in the maturation of rRNA (12, 32) as well as in the processing of bacteriophage T7 early mRNA (12, 15) by promoting site-specific cleavages (13). Recent studies have extended our understanding of the role of RNase III in rRNA maturation (16, 19) and have shown that it cleaves the early leftward mRNA product of bacteriophage λ during infection (29). The terminal 5′ and 3′ sequences released upon cleavage have been identified for several targets (27, 36, 37, 39, 45). The specificity seems to involve more than the primary base sequence (19) which is not identical in these cases (reviewed in 36).

RNase III seemed a good candidate for an enzyme that might initiate degradation of *E. coli* mRNA, possibly causing the intercistronic cleavages that occur in polycistronic molecules (1, 9). Silengo et al. (43) reported that total pulse-labeled RNA decayed slower in AB301-105 but that tryptophan operon (*trp*) and β-galactosidase messages were inactivated at approximately normal rates. However, Apirion and Watson, working with isogenic strains, reported no effect

of RNase III deficiency on either β-galactosidase mRNA or total pulse-labeled RNA degradation (3, 7).

The work reported here began several years ago with AB301-105 and its parent A19 and continued with the more defined isogenic RNase III +/− strains constructed by Studier (46) when they became available. Results with both pairs will be presented since it seems likely that in both cases the effects are caused by RNase III deficiency. Whereas there is no indication that this enzyme is responsible for a normal degradation step, its absence can result in an apparently abnormal decay because it appears to affect the efficiency of translation of certain *E. coli* mRNA's.

MATERIALS AND METHODS

Strains. *E. coli* strain A19 [Hfr PO21 *metB1 his-95 rna-19 rel-1* (λ)] and its RNase III⁻ derivative, AB301-105 [Hfr PO21 *metB1 his-95 bio-3 suc rna-19 rnc-105 rel-1* (λ)], were from P. H. Hofschneider (26). The following strains were constructed and kindly provided by W. Studier. *E. coli* strain BL309 (F⁻) is a *tyrA*⁺ *rnc*⁺ constructed by P1 transduction of X'121 which is *thi-1 pyrD34 tyrA2 his-68 trp-45 str-118 malA1 galK35 mtl-2 xyl-7*. Strain BL308 (F⁻) is the *tyrA*⁺ *rnc-105* P1 transductant of X'121. Strain BL227 is the *rnc-105 uraP* recombinant from F15/BL119 (46)-mediated chromosome transfer into W3110. Strain BL15 (46) is F⁻ *rna-19 rel-1* (λ), and BL229 is an *rnc-105 uraP* recombinant from F15/BL119 chromosome transfer into BL15.

E. coli strain 310 contains the deletion *lacX74* of the *lac* operon and was derived by curing strain RV *thi* F'*lac*.

Growth conditions. The various media used will be given in each experiment, but the results are not generally media dependent. M9 salts (2) was the base for defined minimal media. It was supplemented with glycerol (0.2%), casein hydrolysate (0.8%), uracil (50 μg/ml), tryptophan (100 μg/ml), and thiamine (1 μg/ml); it will be referred to as M9S. In some experiments M9S was further supplemented with biotin (6 μg/ml), sodium succinate (100 μg/ml), methionine (100 μg/ml), histidine (100 μg/ml), and lysine (100 μg/ml); this is called X to give M9X or M9SX. Unless stated otherwise, all experiments were done at 37°C, at which the doubling times in M9S were: 55 min for BL309 and 95 min for BL308; 58 min for W3110 and 67 min for BL227; 50 min for BL15 and 74 min for BL229. In the same medium BL309 doubled every 75 min at 30°C and every 45 min at 42°C, whereas BL308 doubled every 145 min at 30°C and every 88 min at 42°C. Strain BL309 grew with a 34-min, and BL308 with a 70-min, doubling time in antibiotic medium no. 3 (Difco) supplemented with X, uracil (50 μg/ml), and tryptophan (100 μg/ml). Strain A19 doubled every 30 min in Trypticase soy broth (BBL), whereas AB301-105 slowed from a 50- to a 100-min doubling time from early to late exponential growth. Strain A19 doubled every 38 min and AB301-105 doubled every 90 to 100 min in M9 salts-glycerol (0.2%) plus casein hydrolysate (0.8%) supplemented with X.

Agar plates contained 1.5% agar (Difco) and the components indicated. Tetrazolium-lactose plates contained, per liter: nutrient broth (4 g), peptone (3.5 g), yeast extract (3.0 g), 2,3,5-triphenyltetrazolium chloride (Sigma; 50 mg), and lactose (1%).

Enzyme assays. β-Galactosidase (EC 3.2.1.23) and galactoside acetyltransferase (transacetylase) (EC 2.3.1.18) were released from bacteria and assayed by published procedures (23). UDP galactose 4-epimerase (EC 5.1.3.2), galactose 1-phosphate uridyltransferase (EC 2.7.7.10), and galactokinase (EC 2.7.1.6) assays have been described (1).

Translation initiation assay. The assay conditions follow the basic procedures of Revel et al. (34). The reaction is performed either with low-salt washed ribosomes (containing initiation factor) or with initiation factor-dependent high-salt washed ribosomes. The former are prepared in 60 mM $(NH_4)Cl$ rather than 0.5 M.

The initiation reaction contained, in 100 μl, about 300 μg of ribosomes and 110 μg of RNA plus 56 μg of ^{35}S-labeled f-met tRNA. The other components included: tris(hydroxymethyl)aminomethane-hydrochloride (50 mM), pH 7.5; NH_4Cl (100 mM); dithiothreitol (1 mM); GTP (0.5 mM); and magnesium acetate (5 mM). After 10 min at 37°C the contents were centrifuged for 55 min through a 5-ml, 5 to 20% sucrose gradient at 50,000 rpm, and fractions were collected on membrane filters (type HA, Millipore Corp.). Procedures to optimize the reaction specificity will be discussed later (Lim and Kennell, in preparation). A major consideration is that the amount of [^3H]RNA bound to a Millipore filter can include ^3H trapped nonspecifically—presumably to cell proteins. The amount in the initiation complex can only be measured by isolating the 70S complex (by sucrose gradient in this paper). From each filter a 1/8 sector was counted for ^{35}S and ^3H binding, and the remaining filter was reacted for hybrid formation with a DNA filter for 3 days at 52°C.

Although it shows the specificity more clearly, the initiation factor-dependent reaction is more difficult to use. Not only must the correct ratio of ribosomes to RNA be determined for maximum specificity but also the correct amount of initiation factor fraction for each preparation must be determined. Excess initiation factor fraction can lead to a reduced 70S complex and an increased amount of non-70S-bound [^3H]RNA (Lim and Kennell, in preparation). The assays were optimized for specificity.

Other procedures. Radioactive labeling and counting procedures and preparation of RNA for hybrid analyses are described in Blundell and Kennell (9). Preparation of 2.7% polyacrylamide gels and electrophoresis of RNA to determine size distributions are also described (1). The ethylenediaminetetraacetate treatment of *E. coli* (28) was used to increase permeability to actinomycin D (23, 28).

RESULTS

Variable expression of the *lac* operon. All the *rnc-105* strains can show a reduced yield of induced β-galactosidase. In fact, the original RNase III⁻ strain AB301-105 (26) cannot be

induced (Fig. 1). This is not a defect in total *lac* operon expression, since galactoside acetyltransferase from the last gene is induced, although to a level about four times lower than is the parent strain A19; also, transduction analysis shows a normal β-galactosidase gene in AB301-105 (see Discussion). Note the significantly longer induction lag for galactoside acetyltransferase appearance in strain AB301-105 compared with strain A19 (about 6 compared to 3 min) and a somewhat slower functional decay of its message—derived by plotting capacity remaining with time (25).

A short induction of BL308 features a similar but not identical pattern of expression (Fig. 2). There is a very low but detectable induction of β-galactosidase with a threefold-longer induction lag over that of the parent strain BL309. Recently, we have discussed cases in which the transformation of capacity to decay rate is not justified (24), and this example may be a case in point. As can be seen, the decay rate appears to be very slow but increases progressively with time to approach the normal rate. This unusual capacity curve could result from a heterogeneity of transcription or translation rates and have little to do directly with degradation events. Its correct interpretation would require more knowledge of the underlying molecular processes.

Galactoside acetyltransferase synthesis is also reduced in BL308 (about 12-fold) compared with BL309, although the lag time is only increased about 50% (Fig. 2c and d). This means that the lag times for β-galactosidase and galactoside acetyltransferase are about the same in the mutant, whereas the galactoside acetyltransferase lag is twice as long as the β-galactosidase lag in the parent; i.e., in mutant cells the first ribosomes reach the end of the galactoside acetyltransferase message about the same time as they complete the β-galactosidase message. Finally, those galactoside acetyltransferase messages that are translated have a normal functional decay rate. Thus, a greatly reduced level of induced *lac* enzymes and longer induction lags are characteristics of both *rnc-105* strains, although the strains differ with respect to degree.

These observations (Fig. 2) were repeated many times, but after a few months, to our chagrin, strain BL308 showed enzyme bursts more similar to those of the parent (Fig. 3). The

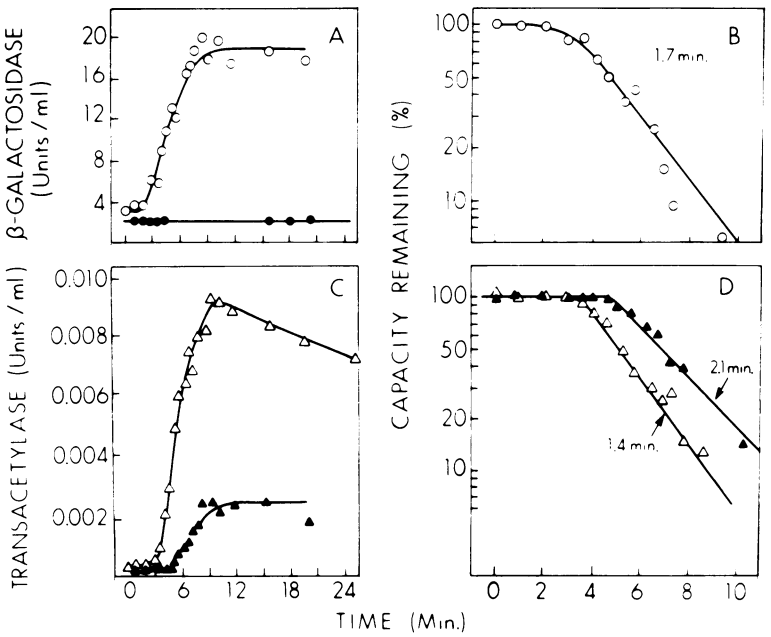

FIG. 1. *Synthesis and decay of a 90-s time set of induced lac messages in strains A19 and AB301-105. Bacteria were grown in Trypticase to 3 × 10⁸ cells per ml and induced at time zero with isopropyl-β-D-thiogalactoside (to 0.5 mM). Cyclic AMP was added to 1.5 mM at −5 min to reduce catabolite repression. Rifampin (Sigma) (to 300 μg/ml) was added at 90 s to inhibit transcription initiation. Samples were taken at the indicated times into chloramphenicol (to 50 μg/ml) plus sodium azide (to 1 mM) at 0°C. (A) β-Galactosidase activities; (B) β-galactosidase message capacity derived from the data in (A); (C) Galactoside acetyltransferase activities; (D) capacity derived from the data in (C). Symbols: A19 (○, △); AB301-105 (●, ▲).*

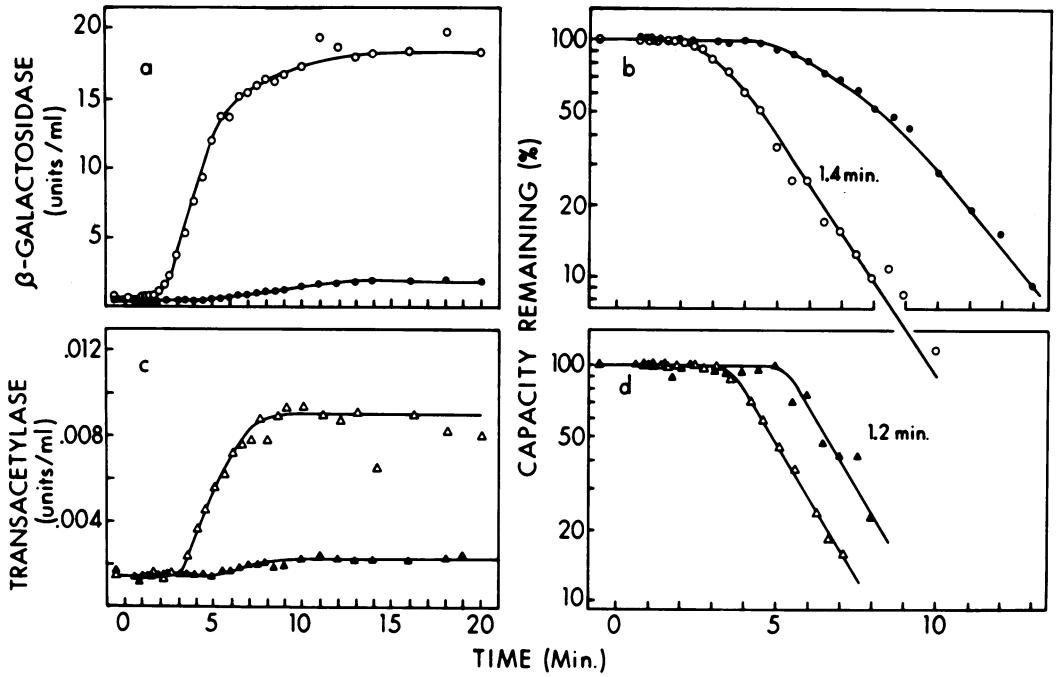

FIG. 2. *Synthesis and decay of a 0.5-min time set of induced lac messages in strains BL309 and BL308. Bacteria were grown in M9S medium to about 2.8 × 10⁸ cells per ml and induced at time zero with isopropyl-β-D-thiogalactoside (to 0.5 mM) 2 min after adding cyclic AMP (to 10 mM). Rifampin (to 300 μg/ml) was added at 30 s to inhibit transcription initiation. Samples were taken at the times indicated into chloramphenicol (to 100 μg/ml) plus sodium azide (to 10 mM) at 0°C. (a) β-Galactosidase activities; (b) β-galactosidase message capacity derived from the data in (a); (c) galactoside acetyltransferase activities; (d) capacity derived from the data in (c). Symbols: BL309* (○, △); *BL308* (●, ▲).

induction lags continued to be longer than those of the parent, but the yields of enzyme were about the same as were the half-lives of the messages. These latter results are more in agreement with those reported for a *lac*⁺ derivative of AB301-105 (43) and by Apirion and co-workers, who observed similar half-lives for β-galactosidase message in closely related (7) as well as in isogenic (3) RNase III +/− strains.

Evidence for a high frequency of suppression. We attempted to determine the basis for this variability. Strain BL308 was labeled with [³H]uridine to test for RNase III activity while a parallel culture was tested for *lac* expression. Irrespective of the level of *lac* expression, the 30S rRNA precursor accumulated, thus indicating the absence of RNase III activity; the more normal expression does not result from its reappearance. Also, the altered strains continued to grow at the same slower than normal rate (given in Materials and Methods) even though *lac* expression had become nearly normal.

A sample from the original glycerol stock of BL308 that had been stored at −20°C was plated on tetrazolium-lactose plates and gave rise to slow-growing pink colonies; they had light-red centers and white peripheries, suggesting weak lactose fermenters. Of 10 such colonies, one gave a β-galactosidase burst similar to the original phenotype shown in Fig. 2a. However, in subsequent experiments it too changed with respect to the *lac* phenotype. The basis for this peculiar phenomenon is being investigated. It can occur from one day to the next, suggesting an adaptation by the population rather than a mutant selection process. The ratio of *lac* expression in mutant/parent is independent of media (all listed in Materials and Methods), growth temperature (30, 37, 43°C), degree of aeration, or cell density, although the strains may differ more in mid-log phase (3 × 10⁸ cells per ml) than at very low cell densities. The results reported for BL308 in the remainder of this paper were obtained with a strain only if it gave a two-to threefold-lower amount of induced β-galactosidase than does BL309 in the experiment. The *rnc-105* members of the other isogenic pairs listed in Materials and Methods gave lower levels of β-galactosidase than their *rnc*⁺ sisters but never as different as the original strain

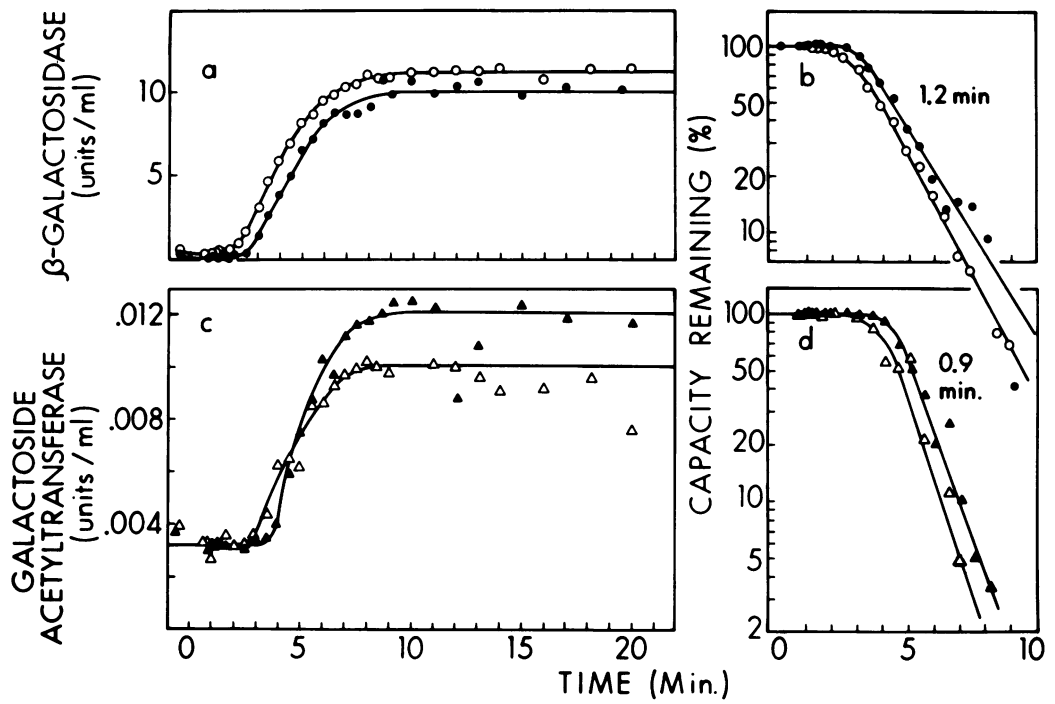

FIG. 3. *Synthesis and decay of a 30-s time set of induced lac messages in strains BL309 and BL308. The same strains used in Fig. 2, 4 months later, after storage on an agar slant at 4°C. The experimental protocol, figure panels, and symbols are the same as in Fig. 2.*

BL308/BL309, indicating that they were already modified when first received.

Evidence that the reduced *lac* expression is caused by RNase III deficiency. Apirion and Watson (5) concluded that the original RNase III$^-$ strain of Kindler et al. (26) contained several other mutations. One of these mutations (*ran*) maps close to the *rnc* locus, and both *ran* and *rnc* confer temperature sensitivity in rich media (6). Although strain BL308 was derived by transducing the *rnc* mutation into a recipient strain, it seemed possible that the effect on mRNA metabolism could still be caused by some other mutant allele, such as *ran*.

Apirion et al. (4) exploited this temperature sensitivity to select revertants that could grow at 45°C. Whereas most of these revertants were suppressors, *rnc*$^+$ true revertants could also be selected with relative ease. We plated about 2×10^7 cells of strains BL308 or BL309 onto AM3SX agar plates and incubated them at 45°C. BL309 gave a confluent lawn in 18 h, whereas the mutant strain gave a slight background growth, with a few very large plus many intermediate-sized colonies on each plate. Several of the very large colonies were picked and restreaked, and all gave uniformly large colonies at 45°C. That these were stable revertants was shown by the fact that they continued to grow rapidly at 45°C even after a return to 37°C for more than 50 generations of growth.

The revertants also regained the ability to grow at the characteristic growth rate of the parent and gave an induced β-galactosidase burst identical to that from BL309. Also, they do not accumulate 30S rRNA (Fig. 4). Thus, these strains appear to be true revertants of *rnc* to *rnc*$^+$, and at the same time they regain the phenotypes of the *rnc*$^+$ parent; it follows that these phenotypes must result from the *rnc* allele.

The following experiments attempt to clarify a possible role of RNase III in mRNA metabolism.

Longer induction lag. The longer induction lag for β-galactosidase in BL308 could result from any one of several causes: (i) a delay before the initiation of transcription, (ii) a slower net rate of RNA polymerase movement, (iii) a delay in initiation of translation or in rate of ribosome movement, or (iv) a delay in assembly of the finished polypeptides into active tetrameric β-galactosidase.

The first possibility is excluded directly since β-galactosidase message accumulates at a maximal rate very soon after induction of strain BL308 (Fig. 5). The fraction of synthesized RNA

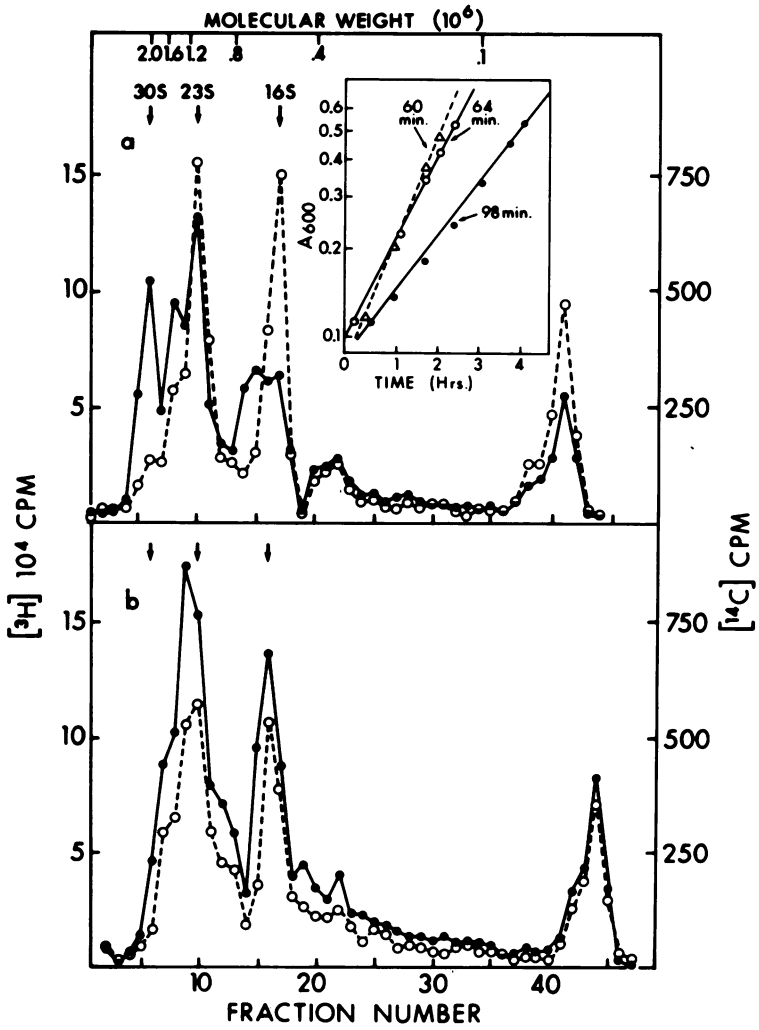

FIG. 4. *Characteristics of revertant strain L3. Polyacrylamide gel electrophoresis of ^{14}C-long-labeled RNA (○) and ^{3}H-pulse-labeled RNA (●). Bacteria were long-labeled by exposure to a limiting amount of [2-^{14}C] uridine three generations before harvest and pulse-labeled by addition of [5-^{3}H]uridine (to 30 μCi/ml and 10 nmol/ml) 10 min before bringing to 0°C with sodium azide (to 10 mM) plus chloramphenicol (to 100 μg/ml). Electrophoresis through 2.7% gels was for 4 h at 4°C at 5 mA/gel. The gel slices were made soluble in 0.3 ml of concentrated NH_4OH overnight and then counted in 3 ml of an aqueous scintillation mix (9). (a) Strain BL308; (b) revertant strain L3 from BL308. The inset shows the growth of strains BL309 (○), L3 (△), and BL308 (●) prior to harvesting.*

in BL308 that is β-galactosidase message specific is about two-thirds that in the parent. At the same time we examined expression from the distal half of the *lac* operon as measured by hybrid to φ80p*lac*20 DNA. As seen, very little of this distal RNA can be detected in BL308; a very low level has been detected in other experiments (shown below). Even more spectacular, strain AB301-105 also accumulates almost as much β-galactosidase mRNA as does strain A19 (Table 1), even though it synthesizes no β-galactosidase (Fig. 1). The mass decay of this β-galactosidase mRNA in AB301-105 occurs at a constant exponential rate slightly slower ($t_{1/2}$ = 2.1 min) than that of the β-galactosidase mRNA of parent strain A19 ($t_{1/2}$ = 1.7 min); mass decay of the distal-labeled *lac* mRNA is slower ($t_{1/2}$ = 3.0 min) than that of the parent ($t_{1/2}$ = 1.4 min) (data not shown), consistent with the different functional decay rates of galactoside acetyltransferase message (Fig. 1).

That RNA polymerase transit time is close to normal (possibility ii) in AB301-105 is shown by using actinomycin D in the kind of experiment

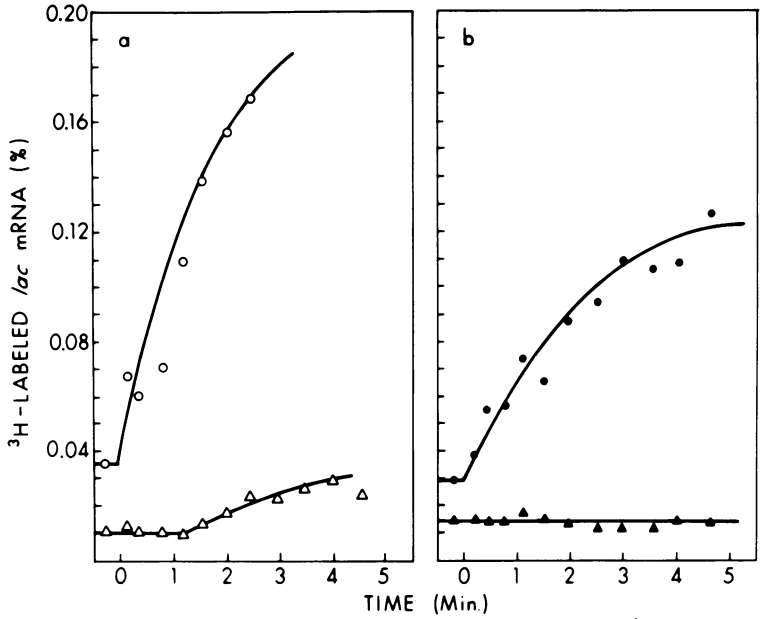

FIG. 5. *Induction of lac mRNA in strains BL309 and BL308 (percentage of [^{3}H]RNA that is lac specific). Growth and induction are the same as in Fig. 2 except that [5-^{3}H]uridine (to 33 μCi/ml and 2 nmol/ml) was added at −1 min and no rifampin was added. Five-milliliter samples were taken at the indicated times into chloramphenicol (to 100 μg/ml) and azide (to 10 mM) at 0°C. The RNA was partially purified (9) and reacted for 3 days at 52°C with denatured λplac5 (○, ●) or φ80plac 20 (△, ▲) DNA. The former carries about the proximal 70% of the operon and the latter about the distal 54%. (a) BL309; (b) BL308.*

TABLE 1. *Synthesis of proximal and distal parts of lac mRNA during steady-state induction of strains BL309 and BL308 and of strains A19 and AB301-105*[a]

Expt	Strain	^{3}H-hybrid[b]		
		λ*plac*5	φ80*plac*20	*lac*5/*lac*20
1	BL309	0.24	0.09	2.7
	BL308	0.21	0.04	5.3
2	BL309	0.15	0.05	3.0
	BL308	0.12	0.02	6.0
3	A19	0.33	0.11	3.0
	AB301-105	0.30	0.07	4.3
4	A19	0.31	0.11	2.8
	AB301-105	0.28	0.06	4.7

[a] Bacteria were grown in M9S and induced with isopropyl-β-D-thiogalactoside for 15 min before addition of [^{3}H]uridine (to 40 μCi/ml and 2 nmol/ml, respectively, for 30 s for strains BL308 and BL309 and 90 s for A19 and AB301-105). RNA was purified for hybridization.

[b] The values refer to the percentage of [^{3}H]RNA that forms stable hybrid to the DNA from the listed transducing phages in two separate experiments. All values are above those obtained on mouse or calf DNA. The last column gives the ratio of percentages for the proximal 2/3 to the distal 1/2 of the *lac* mRNA.

done by Jacquet and Kepes (22). (This experiment could not be performed with strain BL308, because for unknown reasons ethylenediaminetetraacetate treatment was lethal to both BL308 and BL309 strains). The time for completion of the *lac* mRNA in strain AB301-105 is about 2.5 to 3 min, the interval seen in wild-type strains, (e.g., see Fig. 1-3), but galactoside acetyltransferase from the last message does not increase until 6 min (Fig. 6). This demonstrates that the delay occurs at translation or assembly. The latter alternative is unlikely, because the lag time is the same when the bacteria are further incubated at 37°C in the presence of chloramphenicol before preparation of lysates (data not shown).

Other observations suggest a translation delay in *rnc-105* strains. For example, as noted above, whereas β-galactosidase appearance upon induction is delayed twofold or more, there is little further delay before the rise in galactoside acetyltransferase activity in strain BL308. In fact, in the experiment shown in Fig. 2 the times of increase of the two enzymes differ by only 0.5 min in the RNase III$^-$ strain. The corresponding interval in the parent strain is 1.5 min. Since ribosomes initiate independently at the start of each message, a slower peptide elongation rate concurrent with a normal rate of RNA polymerase movement would increase the β-galactosidase lag much more than it would the galactoside acetyltransferase lag, since the galactoside acetyltransferase monomer is only one-fourth the length of the β-galactosidase polypeptide.

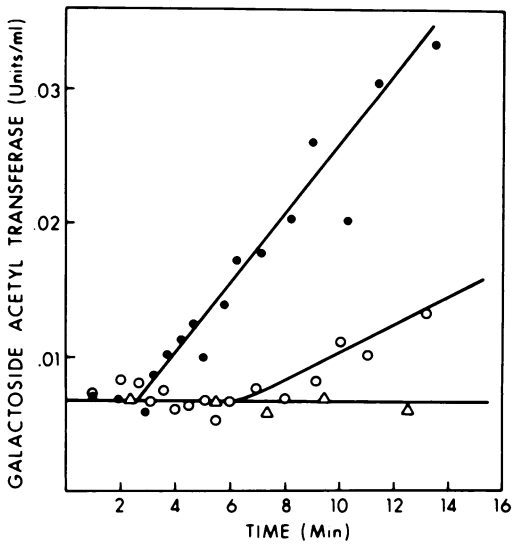

FIG. 6. *Delay in translation of transacetylase message in parallel with a normal transcription time in strain AB301-105. Bacteria were grown in Trypticase soy broth. When at 2.5 × 10⁸ cells per ml, the bacteria were washed on a filter and treated in 0.1 volume of ethylenediaminetetraacetate (EDTA) for 5 min to make permeable to actinomycin D (23, 28). The EDTA treatment was ended with addition of 10 volumes of Trypticase containing cyclic AMP (to 2 mM). After 5 min, one-third of the culture was removed to a separate flask and actinomycin D (to 10 μg/ml) was added 1 min before isopropyl-β-D-thiogalactoside (IPTG) (to 0.5 mM) to show the efficiency with which the inhibitor blocks induction (△). To the remaining two-thirds culture, IPTG (to 0.5 mM) was added at zero time, and samples were taken with time to measure transacetylase activity (○). Other samples were taken and added at the indicated times to 25-ml secondary flasks containing actinomycin D (to 10 μg/ml) and further incubated for an additional 15 min to allow full expression of only completed lac molecules (●).*

Carried to the extreme, a small distal polypeptide could increase sooner than a more proximal larger one if the "uncoupling" of transcription and translation were sufficiently severe.

Further evidence for translational polarity. A sufficiently strong translational block will generate polarity with a decreased yield of mRNA from the distal part of the gene as well as from distal genes in the operon. Further evidence for a translational defect in the *rnc-105* bacteria is shown in Table 1, which gives the syntheses of proximal and distal parts of the polycistronic *lac* mRNA during steady-state induction of strains BL309/BL308 and A19/AB301-105. Such experiments indicate that β-galactosidase mRNA from the proximal two-thirds of the operon is only reduced about 10 or 20% in both *rnc-105* strains compared with the parent strain, whereas mRNA from the distal half is two- to threefold lower. Initiation of transcription is undoubtedly normal, but many RNA molecules are not completed.

Translational polarity results in the synthesis of smaller-sized molecules of *lac* mRNA (33). Wild-type strains have a characteristic size distribution (9), with about 20 to 25% of the molecules full-length at the completion of *lac* mRNA synthesis (Lim and Kennell, unpublished data). This pattern is seen in Fig. 7 for strain A19, whereas the original RNase III⁻ strain AB301-105 has a population of much smaller sizes. Silengo et al. (43) also observed smaller-sized *trp* mRNA in strain AB301-105. We believe this results from translational polarity with the consequent premature termination of transcription rather than from changed activities of specific degradative enzymes in the mutant. The defect in rRNA processing can account for the decreased ratio of rRNA/tRNA in the mutant (Fig. 7) as well as the accumulation of the 30S rRNA seen with short-term labeling (^{3}H).

Translation initiation in vitro. The longer translation time in the face of a normal rate of transcription suggests a defect in some component in the translation process. Ribosome maturation is abnormal in RNase III⁻ strains (12, 19, 32). Although rRNA molecules of normal size can be found (4, 16) and the 3′ end of the 16S rRNA has a normal sequence in strain AB301-105 (45), it is possible that the ribosomes could also include abnormal components that interfere with translation. Ribosomes from strains BL309 and BL308 were compared for capacity to form initiation complexes with either polyuridylic acid-polyguanylic acid [poly(UG)] (Fig. 8) or mRNA from wild-type bacteria. Ribosomes from the RNase III⁻ bacteria are indistinguishable from those from RNase III⁺ cultures.

The marked defect in *lac* expression has remained stable in strain AB301-105 for many transfers, so we compared the ribosomes and β-galactosidase mRNA from strains AB301-105 and A19 for competence to form in vitro initiation complexes in "mixing" experiments. Any combination of ribosomes and total mRNA from parent and mutant strains forms initiation complexes in vitro which can be detected by retention of ^{35}S-labeled f-met tRNA and [^{3}H]RNA in 70S complexes on nitrocellulose filters (Fig. 9). The capacity to form an initiation complex with a specific message can be assessed by measuring the fraction of that message in the total RNA present in the initiation complex. In the case of β-galactosidase message this fraction is determined by ^{3}H-hybrid to λp*lac*5 DNA in the final 70S complex and compared to ^{3}H-hybrid in total RNA reacted in the initiation assay.

The results of a typical mixing experiment are

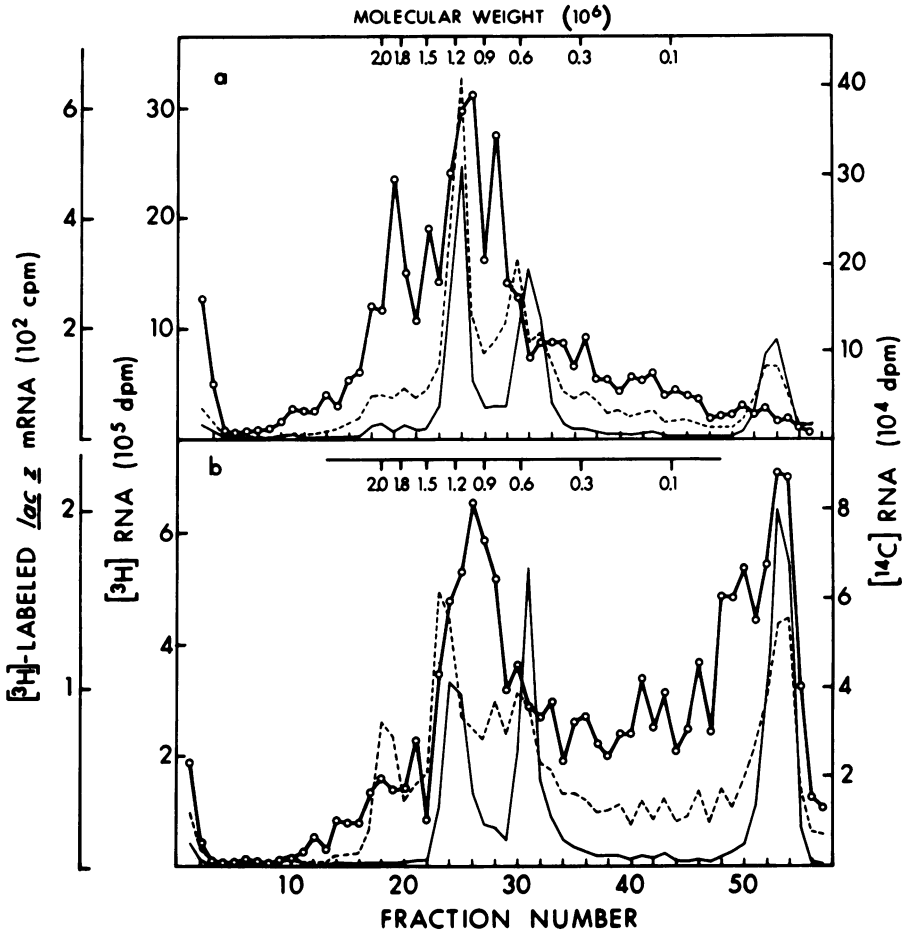

FIG. 7. *Size distributions after gel electrophoresis of lac mRNA, containing β-galactosidase mRNA in E. coli A19 (a) and AB301-105 (b). Bacteria were grown in M9X plus casein hydrolysate (0.8%) and induced as in Fig. 1, with [³H]uridine (to 33 μCi/ml and 2 nmol/ml) added at −1 min and rifampin (to 300 μg/ml) added at +1 min. [2-¹⁴C]uridine (to 0.1 μCi/ml) was added two generations earlier to label only stable RNA. The cultures were harvested at 4 min onto ice with chloramphenicol and azide. RNA from A19 shows clearly the 1.7 × 10⁶-dalton, full-length lac molecule as well as the 5/6 (zy) and 2/3 (z) fragments released by cleavage at the ya and zy junctions, respectively. The 30S rRNA precursor molecule, as well as other rRNA intermediates possibly, can be seen in the pulse-labeled RNA of AB301-105. Symbols: ¹⁴C-long-labeled RNA (——); [³H]RNA (-----); ³H-hybrid to λplac5 DNA* (○).

presented in Table 2. Ribosomes from AB301-105 are as competent as those from A19 to form initiation complexes with β-galactosidase message, e.g., 20 versus 26% using A19 β-galactosidase mRNA. This is true regardless of the origin of the message. In fact, in several experiments the mutant strain ribosomes have been somewhat more active. However, this is not true for the β-galactosidase message from the mutant strain. Regardless of the source of ribosomes, the β-galactosidase message from strain AB301-105 is about three times less competent (per β-galactosidase mRNA mass) to form initiation complex. It is probably even less competent since, as shown in Fig. 7, the AB301-105 β-galactosidase mRNA has a smaller than average size, and this results from the absence of distal parts of the mRNA (Table 1). This means that this population of β-galactosidase mRNA molecules is enriched for the proximal end that includes the ribosome loading site. This would increase the initiation capacity per unit of β-galactosidase mRNA mass compared with the parent RNA. Thus, threefold lower may actually be eightfold or more in terms of activity per loading site.

RNase III affects expression of other operons. We examined expression of the *gal* operon in AB301-105 by measuring synthesis and

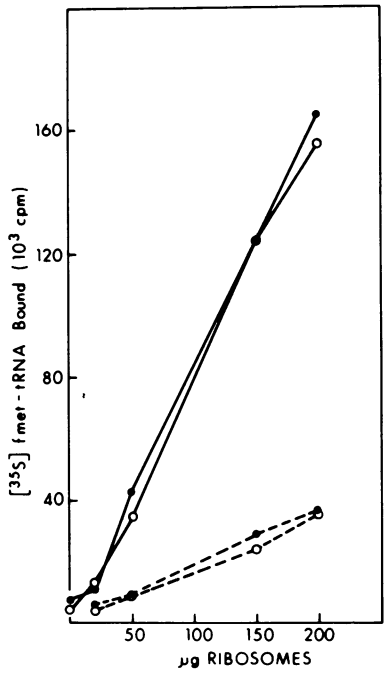

FIG. 8. *Competence of ribosomes from strains with or without RNase III activity to form an initiation complex with poly(UG). The initiation reaction mix contained 10 μg of poly(UG) in 100 μl. ^{35}S-labeled f-met tRNA in complex was trapped on a nitrocellulose filter. Strain BL309 ribosomes with (—○—) or without (- -○- -) poly(UG); strain BL308 ribosomes with (—●—) or without (- -●- -) poly(UG).*

decay of *gal* mRNA from different DNA segments by hybridization analysis. The results are summarized in Fig. 10. Only half as much *gal* mRNA accumulates in the mutant as in the parent. At the same time the mutant RNA is decaying half as fast. Synthesis of the *gal* mRNA and enzymes are greatly depressed in the mutant.

Silengo et al. (43) observed a reduced fraction of *trp* mRNA in AB301-105 as well as a slower than normal mass decay of only a minor fraction but a normal functional decay of two *trp* messages. We measured the level of *trp* mRNA in strains BL309 and BL308 which differ only in the *rnc* allele. In this case both proximal E and distal A mRNA from the *trp* operon are reduced about twofold in the mutant compared with the parent, although the very low level of hybrid A RNA from the mutant (0.07%) may be as low as can be observed (Table 3).

Further studies will be necessary to define the

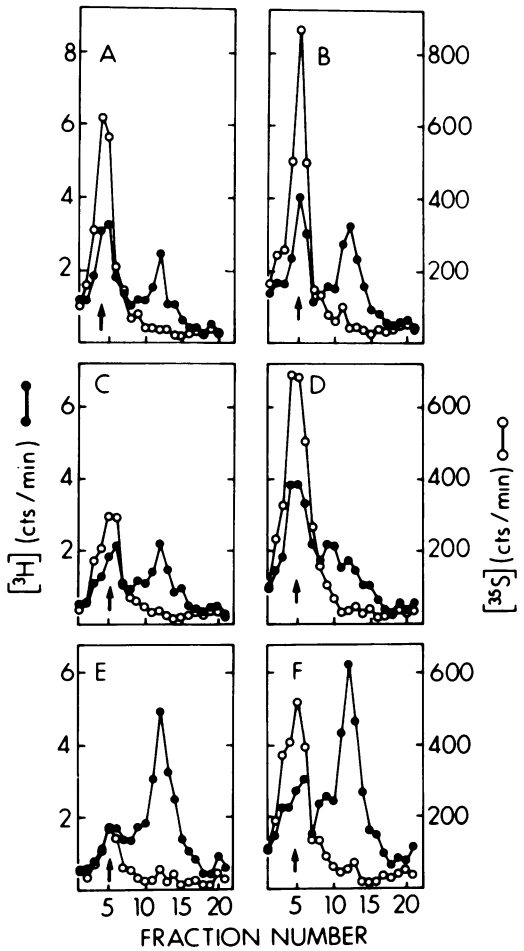

FIG. 9. *Formation of translation initiation complexes with ribosomes and [^3H]RNA from strains A19 and AB301-105. Bacteria were grown in M9SX to 3 × 10^8/ml and induced with isopropyl-β-D-thiogalactoside as in Fig. 1, except that rifampin was not added. After 15 min of induction, [5,6-^3H]uridine (to 60 μCi/ml and 2 nmol/ml) was added, and the cultures were harvested 90 s later. RNA was purified and reacted with ribosomes and ^{35}S-labeled f-met tRNA (same input counts per minute and micrograms in each) to form an initiation complex and put directly on 5-ml 5 to 20% sucrose gradients. Fractions were collected on Millipore filters, and 1/8 sectors were cut from each to obtain the pattern of total ^3H and ^{35}S shown. The ^3H counts per minute input was slightly different for different reactions but was all normalized to the same input counts per minute for the figure. The 7/8 sectors from the 70S peak (arrow) fractions (about 3 through 6) were reacted for hybrid formation to determine the amount of β-galactosidase mRNA in the initiation complex (as shown in an experiment in Table 2). (A) A19 RNA plus A19 ribosomes; (B) A19 RNA plus AB301-105 ribosomes; (C) AB301-105 RNA plus A19 ribosomes; (D) AB301-105 RNA plus AB301-105 ribosomes; (E) 310 RNA plus A19 ribosomes; (F) 310 RNA plus AB301-105 ribosomes. Symbols: ^{35}S (○); ^3H (●).*

TABLE 2. *Initiation complex formation with ribosomes and β-galactosidase mRNA from strains A19 and AB301-105*[a]

Ribosomes[b] prepared in:	Strain[c]	Total β-galactosidase mRNA in initiation reaction (cpm)	β-Galactosidase mRNA bound to 70S complex (cpm)	
			A19[d]	AB105[d]
High salt	A19	51,000	7,325 (14.4)[e]	
	AB105	38,650	1,952 (5.0)	
	310	5,637	624	
Low salt	A19	17,110	3,465 (20)	4,451 (26)
	AB105	8,994	937 (10)	1,218 (13)
	310	2,008	154	140

[a] Bacteria were grown in M9S in 6-liter cultures for the preparation of ribosomes and in 50 ml for preparation of [^{3}H]RNA. [^{3}H]RNA was from cultures induced for 15 min with [5,6-^{3}H]uridine present during the last 90 s. RNA was prepared as described (9); diethylpyrocarbonate, added to inhibit nucleases, does not interfere in the assay.

[b] Ribosomes were prepared either in low salt (60 mM NH_4Cl) or in high salt (0.5 mM NH_4Cl). In the latter case initiation factors in the salt wash were added during the reaction.

[c] Source of [^{3}H]RNA.

[d] Source of ribosomes.

[e] Numbers in parentheses are percentages. The fractions from a 5 to 20% sucrose gradient were collected onto 24-mm Millipore HA filters, and the filters containing the 70S fractions were pooled and reacted with λ*plac*5 DNA filters.

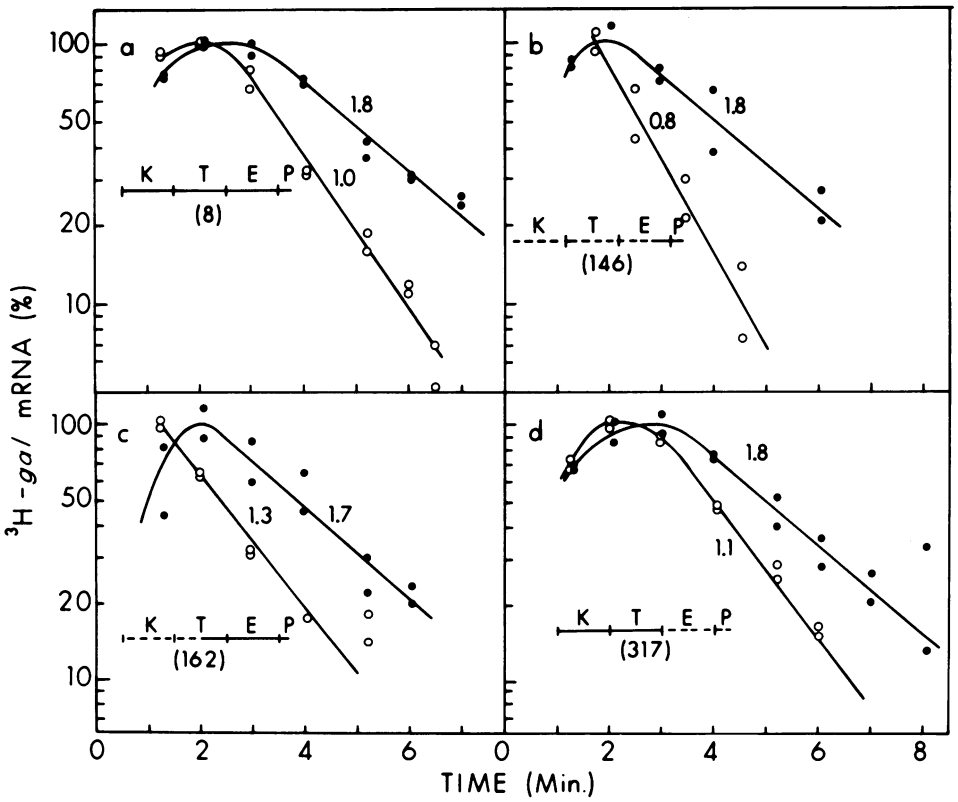

FIG. 10. *Decay of mRNA from specific parts of the gal operon in strains A19 and AB301-105. Bacteria were grown in M9X plus 0.8% casein hydrolysate. At −2 min cyclic AMP was added to 10 mM, [5-^{3}H]uracil was added at −0.5 min (to 33 μCi/ml, 2 nmol/ml), and fucose was added at 0 min (to 10 mM). Rifampin was added (to 300 μg/ml) at 1 min, and [^{12}C]uracil (to 100 μg/ml) was added at 5 min. Samples were taken at the times indicated to purify RNA for hybrid reaction to the gal DNA segments shown by the solid bars in each panel. P, E, T, and K stand for the promoter, epimerase, transferase, and kinase genes of the gal operon, respectively, and the numbers in parentheses are strain numbers for the transducing bacteriophages (kindly provided by M. Gottesman and S. Adhya; see reference 1) carrying these DNAs. Symbols: RNA from strain A19 (○); RNA from strain AB301-105 (●).*

TABLE 3. *Derepression of the tryptophan (trp) operon in RNase III + and − strains*[a]

[3H]RNA	Strain	Derepression (%)	
		trpE[b] DNA	*trpA*[b] DNA
Proximal labeled	BL309	0.23	0.11
	BL308	0.10	0.06
Distal labeled	BL309	0.009	0.12
	BL308	0.005	0.07

[a] Strains BL309 and BL308 were grown to 2 × 10^8/ml in M9S, washed by filtration, resuspended into M9S lacking tryptophan at 37°C, and divided into two equal parts. The proximal-labeled portion was further derepressed by addition of indoleacrylic acid (to 20 μg/ml) at time zero, [3H]uridine (to 33 μCi/ml and 2 nmol/ml) was added at 2 min, and the culture was harvested at 3 min. To the distal-labeled portion indoleacrylic acid was added at time zero, rifampin (to 300 μg/ml) and tryptophan (to 100 μg/ml) were added to block further initiation of transcription at 2 min, [3H]uridine was added at 3 min, and the cells were harvested at 5 min.

[b] φ80p*trp*E and φ80p*trp*A transducing bacteriophages were provided by Charles Yanofsky. E is the first structural gene of the operon and A is the fifth and last.

effects of RNase III deficiency on expression of the *gal* and *trp* operons, in particular, their relationship to the defect in *lac* operon expression, but these results show that their expression is not normal. Thus, the activity may be important for expression of many operons.

DISCUSSION

Why was RNase III reported to have no effect on *E. coli* mRNA metabolism? Apirion and Watson concluded that the original RNase III$^-$ strain, AB301-105 (26), contains at least seven mutations (5). However, it now seems possible that some of its phenotypic characters may not reflect independent and specific mutations, but rather may be unique and separable phenotypes that result from the pleiotropic *rnc-105* genotype. It follows that, if any of these phenotypes can be suppressed independently, then the progressive elimination of these deficiencies could represent the progressive addition of suppressor mutations.

For example, the first step in construction of an isogenic pair was to create a strain from AB301-105 that could grow in minimal medium containing only methionine plus histidine (5). This step, and most succeeding ones, were effected by transduction from strain D10 into a given *rnc-105* strain. Studier (46) showed that these amino acids were required by the parent strain A19 and that the only unique auxotrophic requirements of AB301-105 were for biotin and succinate. However, Studier noted the peculiar fact that this *suc* mutation mapped near 39 min, which is far from the *sucA* and *sucB* loci at 16 min, and "revertants" to *suc*$^+$ from AB301-105 occur at a very high frequency (46). He proposed that this succinate requirement might reflect the RNase III deficiency. However, the revertants to *suc*$^+$ were still RNase III$^-$, so that the reversion would have to have resulted from the expression of a specific suppressor mutation.

Other ill-defined phenotypes were selected in these experiments (5). These included the ability to grow in minimal media at 43°C (*dig*$^+$ for defect in growth) and to overcome a general defectiveness for growth on a variety of sugars (5). In this latter connection the deliberate selection of *lac*$^+$ strains (3, 5, 43) from an *rnc-105* parent could have been for a suppressor that eliminated the requirement for RNase III activity and led to the conclusion that the activity had no role in normal expression of the *lac* operon (3, 7, 43). The defect in β-galactosidase gene expression (Fig. 1) does not reflect a defect in the gene or its *lac* control elements. Apirion and Watson (5) concluded that the β-galactosidase gene was normal in AB301-105 from transductions into *lac* point and partial deletion mutants. We have verified this conclusion by transduction from AB301-105 into a strain with a complete deletion of the *lac* operon (strain 310) and selection of normal *lac*$^+$ transductants at a reasonable frequency (unpublished data). Furthermore, the β-galactosidase mRNA is synthesized at close to normal levels in both AB301-105 and BL308 (Table 1). Other observations reported here show that it is the expression of this mRNA, rather than its synthesis, that accounts for the poor expression of the *lac* operon.

Taken together, the preceding observations suggest that some of the several phenotypes observed in strain AB301-105 may not result from a collection of independent mutations, but rather are pleiotropic effects of the single *rnc-105* mutation. Selection of apparent revertants for a specific defect could represent selection of a specific suppressor for that defect and thus obscure the physiological role of RNase III in these specific functions.

Several other examples are consistent with this view, including even possible compensating activities for the maturation and degradation of rRNA in RNase III$^-$ strains (3, 5, 16). Recently, Apirion and Watson reported that *rnc-105* strains are nonmotile but that >60% of "revertants" to motility remained RNase III$^-$ (8); again, this points to a high frequency of suppression of yet another specific phenotype. Also, there has been some disagreement about the importance of RNase III for the development of phage, such as T7, with some studies suggesting a very marked decrease in phage expression (7, 20)

while other papers report very little effect on efficiency (14, 46). Some of the differences could result from different degrees of suppression in the *rnc-105* strains used.

Nature of the "suppressors." We find it very difficult to maintain the original phenotype for defective *lac* expression in the *rnc-105* mutants of the isogenic pairs. The bacteria quickly adapt in some way to give a less marked reduction in *lac* operon expression (Fig. 2 and 3). At this time we do not know if this change occurs by selection of a suppressor genotype or by a "phenotypic suppression," i.e., by some nongenetic adaptation such as the induction of a normally repressed gene. The change can occur in cultures stored overnight at 4°C with subsequent regrowth at 37°C, which suggests that the latter process may be involved. Until clarified, the change in phenotype should be considered a suppressor effect, recognizing that it may not result from a suppressor mutation.

Possible role for RNase III in mRNA translation. There is an extremely broad spectrum of physiological processes influenced by RNase III activity. This suggests some sensitive component common to all of them. A likely candidate would be the ribosome, since its maturation is altered. However, the evidence discussed here (Fig. 9 and 10, Table 2) argues against this possibility. Some other molecule could be responsible, but another distinct possibility is that this enzyme is involved in the processing of many *E. coli* mRNA's to account for the multiple pleiotropy of AB301-105.

We suggest that a direct effect of the *rnc-105* mutation is on translation initiation of the first gene of the *lac* operon and decreased yields from distal genes can be explained by the generation of translational polarity (21, 31), although there may also be a direct effect on expression of galactoside aceytltransferase message (Fig. 6). The nucleotide sequence of the early part of the *lac* operon (11, 18) shows palindromic regions in the operator DNA. Whereas most discussion has focused on the possible function of these regions for DNA-protein binding, it is possible that they play a role in the expression of the transcription product—the mRNA. Since part of the operator is transcribed to provide the 5′ end of the mRNA (30), two double-stranded configurations before the AUG start of β-galactosidase mRNA are possible (30). Recently, we (Cannistraro and Kennell, submitted for publication) have measured the β-galactosidase message half-lives and the frequencies of translation initiation in the 11 *lac*O^c mutants of Smith and Sadler (44) whose base changes were identified later by Gilbert et al. (17). Of the 11, only 2 have significantly slower initiation frequencies than the parent, and they map at the junction of the loop and stem in mRNA residues 7 and 8 of the structure that is potentially the more stable (30). Cleavage between residues 6 and 7 of the *lac* mRNA would liberate U-U-G-U$_{OH}$ and pG-A-G-. These compare closely with the terminal fragments released from the four of six T7 RNase III sites which have been identified in most detail and are also at loop-stem junctions (36, 39); these fragments are all U-U-A-U$_{OH}$ plus pG-A-U-.

If this secondary structure impeded initiation complex formation, then the absence of RNase III activity, which disrupts it in part, could restrict translation of β-galactosidase message. These speculations are probably best put to the test in in vitro experiments.

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LITERATURE CITED

1. **Achord, D., and D. Kennell.** 1974. Metabolism of messenger RNA from the *gal* operon of *Escherichia coli*. J. Mol. Biol. **90**:581–599.
2. **Anderson, E. H.** 1946. Growth requirements of virus-resistant mutants of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **32**:120–128.
3. **Apirion, D., J. Neil, and N. Watson.** 1976. Consequences of losing ribonuclease III on the *Escherichia coli* cell. Mol. Gen. Genet. **144**:185–190.
4. **Apirion, D., J. Neil, and N. Watson.** 1976. Revertants from RNase III negative strains of *Escherichia coli*. Mol. Gen. Genet. **149**:201–210.
5. **Apirion, D., and N. Watson.** 1974. Analysis of an *Escherichia coli* strain carrying physiologically compensating mutations one of which causes an altered ribonuclease III. Mol. Gen. Genet. **132**:89–104.
6. **Apirion, D., and N. Watson.** 1975. Mapping and characterization of a mutation in *Escherichia coli* that reduces the level of ribonuclease III specific for double-stranded ribonucleic acid. J. Bacteriol. **124**:317–324.
7. **Apirion, D., and N. Watson.** 1975. Unaltered stability of newly synthesized RNA in strains of *Escherichia coli* missing a ribonuclease specific for double-stranded RNA. Mol. Gen. Genet. **136**:317–326.
8. **Apirion, D., and N. Watson.** 1978. Ribonuclease III is involved in motility of *Escherichia coli*. J. Bacteriol. **133**:1543–1545.
9. **Blundell, M., and D. Kennell.** 1974. Evidence for endonucleolytic attack in decay of *lac* messenger RNA in *Escherichia coli*. J. Mol. Biol. **83**:143–161.
10. **Crouch, R. J.** 1974. Ribonuclease III does not degrade deoxyribonucleic acid-ribonucleic acid hybrids. J. Biol. Chem. **249**:1314–1316.
11. **Dickson, R. C., J. Abelson, W. M. Barnes, and W. S. Reznikoff.** 1975. Genetic regulation: the *lac* control region. Science **187**:27–35.
12. **Dunn, J. J., and F. W. Studier.** 1973. T7 early RNAs

and *Escherichia coli* ribosomal RNAs are cut from large precursor RNAs *in vivo* by ribonuclease III. Proc. Natl. Acad. Sci. U.S.A. **70**:3296–3300.

13. **Dunn, J. J., and F. W. Studier.** 1973. T7 early RNAs are generated by site specific cleavages. Proc. Natl. Acad. Sci. U.S.A. **70**:1559–1563.
14. **Dunn, J. J., and F. W. Studier.** 1975. Effect of RNase III cleavage on translation of bacteriophage T7 messenger RNAs. J. Mol. Biol. **99**:487–499.
15. **Dunn, J. J., and F. W. Studier.** 1975. Processing, transcription and translation of bacteriophage T7 messenger RNAs. Brookhaven Symp. Biol. **26**:267–276.
16. **Gegenheimer, P., N. Watson, and D. Apirion.** 1977. Multiple pathways for primary processing of ribosomal RNA in *Escherichia coli*. J. Biol. Chem. **252**:3064–3073.
17. **Gilbert, W., J. Gralla, J. Majors, and A. Maxam.** 1975. Lactose operator sequences and the action of *lac* repressor, p. 193–210. *In* H. Sund and G. Blauer (ed.) Protein-ligand interactions. Walter de Gruyter & Co., Berlin.
18. **Gilbert, W., and A. Maxam.** 1973. The nucleotide sequence of the *lac* operator. Proc. Natl. Acad. Sci. U.S.A. **70**:3581–3584.
19. **Ginsburg, D., and J. A. Steitz.** 1975. The 30S ribosomal precursor RNA from *Escherichia coli*. J. Biol. Chem. **250**:5647–5654.
20. **Hercules, K., M. Schweiger, and W. Sauerbier.** 1974. Cleavage by RNase III converts T3 and T7 early precursor RNA into translatable message. Proc. Natl. Acad. Sci. U.S.A. **71**:840–844.
21. **Imamoto, F.** 1970. Evidence for premature termination of transcription of the *trp* operon in polarity mutants of *Escherichia coli*. Nature (London) **228**:232–235.
22. **Jacquet, M., and A. Kepes.** 1971. Initiation, elongation and inactivation of *lac* messenger RNA in *Escherichia coli* studied by measurement of β-galactosidase synthesizing capacity *in vivo*. J. Mol. Biol. **60**:453–472.
23. **Kennell, D., and C. Simmons.** 1972. Synthesis and decay of messenger ribonucleic acid from the lactose operon of *Escherichia coli* during amino-acid starvation. J. Mol. Biol. **70**:451–464.
24. **Kennell, D., and V. Talkad.** 1976. Messenger RNA potential and the delay before exponential decay of messages. J. Mol. Biol. **104**:285–298.
25. **Kepes, A.** 1963. Kinetics of induced enzyme synthesis. Determination of the mean life of galactosidase-specific messenger RNA. Biochim. Biophys. Acta **76**:293–309.
26. **Kindler, P., T. U. Keil, and P. H. Hofschneider.** 1973. Isolation and characterization of a ribonuclease III deficient mutant of *Escherichia coli*. Mol. Gen. Genet. **126**:53–69.
27. **Kramer, R. A., M. Rosenberg, and J. A. Steitz.** 1974. Nucleotide sequences of the 5′ and 3′ termini of bacteriophage T7 early messenger RNAs synthesized *in vivo*: evidence for sequence specificity in RNA processing. J. Mol. Biol. **84**:767–776.
28. **Leive, L., and V. Kollin.** 1967. Synthesis, utilization and degradation of lactose operon mRNA in *Escherichia coli*. J. Mol. Biol. **24**:247–259.
29. **Lozeron, H. A., P. J. Anevski, and D. Apirion.** 1977. Antitermination and absence of processing of the leftward transcript of coliphage λ in the RNase III deficient host. J. Mol. Biol. **109**:359–365.
30. **Maizels, N. M.** 1973. The nucleotide sequence of the lactose messenger ribonucleic acid transcribed from the UV5 promoter mutant of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **70**:3585–3589.
31. **Morse, D. E., D. Mosteller, and C. Yanofsky.** 1969. Dynamics of synthesis, translation and degradation of *trp* operon mRNA in *E. coli*. Cold Spring Harbor Symp. Quant. Biol. **34**:725–740.
32. **Nikolaev, N., L. Silengo, and D. Schlessinger.** 1973. Synthesis of a large precursor to ribosomal RNA in a mutant of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **70**:3361–3365.
33. **Pastushok, C., and D. Kennell.** 1974. Residual polarity and transcription/translation coupling during recovery from chloramphenicol or fusidic acid. J. Bacteriol. **117**:631–640.
34. **Revel, M., M. Herzberg, and H. Greenshpan.** 1969. Initiator protein dependent binding of messenger RNA to the ribosome. Cold Spring Harbor Symp. Quant. Biol. **34**:261–275.
35. **Richardson, J. P., C. Grimley, and C. Lowery.** 1975. Transcription termination factor Rho activity is altered in *Escherichia coli* with SuA gene mutations. Proc. Natl. Acad. Sci. U.S.A. **72**:1725–1728.
36. **Robertson, H. D., E. Dickson, and J. J. Dunn.** 1977. A nucleotide sequence from a ribonuclease III processing site in bacteriophage T7 RNA. Proc. Natl. Acad. Sci. U.S.A. **74**:822–826.
37. **Robertson, H. D., and J. J. Dunn.** 1975. Ribonucleic acid processing activity of *Escherichia coli* ribonuclease III. J. Biol. Chem. **250**:3050–3056.
38. **Robertson, H. D., R. E. Webster, and N. D. Zinder.** 1968. Purification and properties of ribonuclease III from *Escherichia coli*. J. Biol. Chem. **243**:82–91.
39. **Rosenberg, M., and R. Kramer.** 1977. Nucleotide sequence surrounding a ribonuclease III processing site in bacteriophage T7 RNA. Proc. Natl. Acad. Sci. U.S.A. **74**:984–988.
40. **Rosenberg, M., R. A. Kramer, and J. A. Steitz.** 1974. T7 early messenger RNAs are the direct products of ribonuclease III cleavage. J. Mol. Biol. **89**:777–782.
41. **Schwartz, T., E. Craig, and D. Kennell.** 1970. Inactivation and degradation of messenger ribonucleic acid from the lactose operon of *Escherichia coli*. J. Mol. Biol. **54**:299–311.
42. **Schweitz, H., and J. P. Ebel.** 1971. A study of the mechanism of action of *E. coli* ribonuclease III. Biochimie **53**:585–593.
43. **Silengo, L., N. Nikolaev, D. Schlessinger, and F. Imamoto.** 1974. Stabilization of mRNA with polar effects in an *Escherichia coli* mutant. Mol. Gen. Genet. **134**:7–19.
44. **Smith, T. F., and J. R. Sadler.** 1971. The nature of the lactose operator constitutive mutations. J. Mol. Biol. **59**:273–305.
45. **Sprague, K. U., and J. A. Steitz.** 1975. The 3′ terminal oligonucleotide of *Escherichia coli* 16S ribosomal RNA: the sequences in both wild type and RNase III⁻ cells is complementary to the polypurine tracts common to mRNA initiator regions. Nucleic Acid Res. **2**:787–798.
46. **Studier, W.** 1975. Genetic mapping of a mutation that causes ribonuclease III deficiency in *Escherichia coli*. J. Bacteriol. **124**:307–316.