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THE SYNTHESIS OF SOME PROTEINS IS AFFECTED IN RNA PROCESSING MUTANTS OF ESCHERICHIA COLI

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Summary: The synthesis of E. coli proteins was examined, by two-dimensional O'Farrell gels, in mutant strains defective in all possible combinations of the RNA processing enzymes RNase III, RNase E and RNase P. We found that the synthesis of most proteins was unaffected; however, the synthesis of a significant number of proteins, 21 out of 80 tested, was drastically reduced in the strain defective in all three enzymes. It appears that the two enzymes RNase III and RNase E are responsible for most of these changes.

Processing of RNA in bacteria is a well established feature in the metabolism of rRNA and tRNA (see papers in Refs. 1, 2). It was shown, primarily by the use of mutants defective in RNA processing enzymes, that the three endoribonucleases RNase III, RNase E and RNase P participate in these reactions (3-6).

Since previously it was demonstrated that RNase III strains are nonmotile (7), and that RNase III participates in processing of T7 mRNA (8) and λ mRNA (9), we decided to assess to what extent these enzymes also participate in mRNA metabolism. In order to accomplish this, we compared the synthesis of proteins in the various RNA processing mutants at different temperatures. This was carried out by labeling proteins with ^{35}S-methionine when strains were grown at 30°C or 43°C. The results show that all these enzymes, but primarily RNase III and RNase E, affect mRNA metabolism.

MATERIALS AND METHODS: Differential Induction of β-Galactosidase. Exponentially growing cultures were induced and assayed for β-galactosidase according to the procedure described by Simon and Apirion (10). The differential rate of induction is the number of units of β-galactosidase produced per 1,000 cpm of [^{3}H]leucine incorporated into total protein.

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Electrophoresis in Two-Dimensional Gels. We used the procedures described by O'Farrell (11) and Pederson, Reeh and Neidhardt (12) with the following modifications: Cultures were labeled and induced simultaneously by adding 3 mls of an exponentially growing culture to a test tube containing 75 μCi of [^{35}S]methionine (1250 Ci/mmol), cAMP and IPTG for 30 min. The growth medium contained salts (13), 0.4% glycerol, and was supplemented with the strains' requirements and 0.1% peptone. In all experiments at 43°C, cultures had been transferred to the high temperature 40 min prior to labeling and induction. Two ampholines were used, 1.6% pH 5.0 to 7.0 and 0.4% pH 3.5 to 10.0. The second dimension gel was composed of a 4% (polyacrylamide) stacking and a 10% separation layer.

After drying, gels were treated by the En^{3}Hance procedure as recommended by New England Nuclear in the product literature. Gels were loaded with 0.6 to 2x10^6 cpm/gel and autoradiographed from 4 to 9 hrs at -75°C. To count specific proteins from a gel, the gel was dried and the spots (according to the autoradiogram) were removed with an eighteen gauge needle and incubated in 0.75 ml of 30% hydrogen peroxide containing a drop of sodium perchlorate at 67°C until the gel piece was solubilized. Triton-based scintillant was added and the sample was counted in a Tricarb spectrometer.

One hundred and five spots were selected at random (we chose only spots which seemed readily identifiable) on the autoradiogram. Spots which did not behave in the same manner in the two wild-type strains used (D10 and N2089) at 30°C and 43°C were eliminated as well as spots which later proved difficult to identify reproducibly. The eighty spots which remained, formed the basis for the results shown in Table 1. Density values, 0 to 4 (see Table 1), were assigned to each spot without knowledge of the particular strain.

Identification of β-Galactosidase in Gels. O'Farrell gels were run as above, using labeled wild type cell extracts with or without the addition of 10μg of unlabeled β-galactosidase (Sigma). Gels were stained for proteins and autoradiographed and the position of β-galactosidase noted.

Strains. All strains were *lac*$^+$ derivatives (P1 mediated transductants) of strains described by us previously (14). The wild type strains used were D10 (15) and N2089 which is isogenic with the *rnc* strain N2090 (Table 1) and is very similar to strain N2076 (14,16).

RESULTS: In order to compare protein synthesis in the various mutant strains [^{35}S]methionine was added to cultures incubated at 30°C (a permissive temperature) or to cultures grown at 30°C and transfered to 43°C for 40 min. Labeling was carried out for 30 minutes. Samples of each of the eight strains labeled at 30°C and 43°C were analyzed by electrophoresis in two-dimensional gels (11). Autoradiographs from three strains are displayed in Figure 1. Although most of the proteins appeared at both temperatures in the wild-type strain, some did not. Similar observations have been reported previously (17).

When we compared the proteins in the different mutants we found that most proteins were unaffected; a significant number, however, was affected

Table 1. The level of synthesis of a large number of proteins in mutants defective in RNA processing

Strain	Genotype	No. of proteins exhibiting changes in the level of synthesis at 43°C as compared to 30°C.			
		None	Slight	Medium	Large
D10	+++	46	32	2	0
N2090	*rnc*	52	18	7	3
N3427	*rne*	29[a]	27	9	3
N2015	*rnp*	51	25	3	1
N3524	*rnc,rne*	35	25	13	7
N2017	*rnc,rnp*	47	23	8	2
N3531	*rne,rnp*	38	25	13	4
N3525	*rnc,rne,rnp*	26	33	11	10

[a] Gel was cracked, making some spots unreadable.

The same eighty spots on each gel were assigned a number from 0 to 4 depending on the density of the spot on the autoradiogram. (0, spot completely absent; 4, spot very dark). Size of the spot was not a factor in assigning this measurement as some small spots were very dark. The ratings within each strain at 30°C and 43°C were compared. Thus, this table actually reports the minimum change in the level of synthesis between 30°C and 43°C, since spots which were large and dark at 30°C often appeared smaller and dark at 43°C, but, again, this was not taken into account because of the complications that would have been introduced. (*Slight change* is a difference between the 30°C and the 43°C rating of 1, i.e. 2 to 1 or 3 to 2; *medium change* is a difference of 2, i.e. 3 to 1 or 4 to 2; and *large change* is a difference of 3 or more.)

particularly in the triple mutant. To evaluate these observations we analysed the proteins which could be clearly recognized in the gels from the wild type strains. We concentrated on 80 proteins (spots), which comprised about 20% of the total number of spots observed in the gels. Each spot was assigned a value from 0 to 4, 4 being the darkest to 0 being not visible. (All spots chosen were clearly visible in the wild-type strain.) The relative intensities were assigned in each gel separately. By comparing within each strain spots from proteins synthesized at 30°C and 43°C, we could assess whether each spot was changed or unchanged, and the degree of the change. These observations, for 80 proteins, are summarized in Table 1. It can be seen that the most

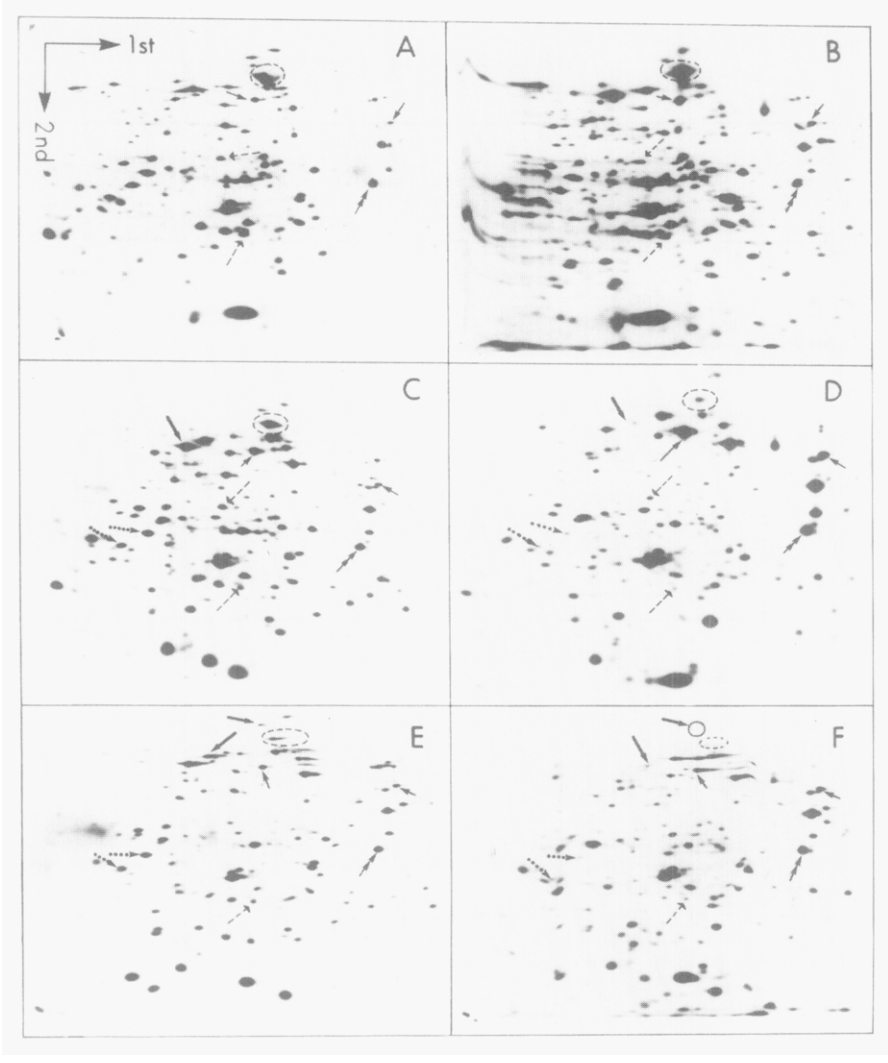

Figure 1. Protein gels of wild type (A, B); double mutant, rnc, rne (C,D); and triple mutant, rnc, rne, rnp (E,F). All strains were induced for β-galactosidase. The position of β-galactosidase is indicated by the broken circle. Panels A, C, and E show proteins synthesized at 30°C, and B, D, F show proteins synthesized at 43°C. The first dimension is isoelectric focusing and the second is electrophoresis in a sodium dodecyl sulfate polyacrylamide gel. For further details see "Materials and Methods". Some of the proteins are indicated by arrows: no change, →; slight change, --→; medium change, ····→; large change ➡. This classification corresponds to that presented in Table 1. The double-headed arrow designates the protein which was used for the normalization in Table 3.

drastic changes took place in the triple mutant where 21 proteins, out of 80, appeared to be synthesized markedly less efficiently or not at all at the non-permissive temperature. A detailed reading of Table 1 shows that the enzymes RNase III and RNase E are primarily responsible for the observed changes.

Table 2. Differential Induction of β-galactosidase in RNA processing mutants

Strain	Genotype	Differential rate of induction		Ratio of 43°C to 30°C
		30°C	43°C	
D-10	wild type	40	39	0.98
N2090	*rnc*	10	4	0.40
N3427	*rne*	30	12	0.40
N2015	*rnp*	14	20	1.43
N3524	*rnc*,*rne*	38	5	0.13
N2017	*rnc*,*rnp*	17	11	0.65
N3531	*rne*,*rnp*	37	32	0.86
N3525	*rnc*,*rne*,*rnp*	42	8	0.19

Differential rate of induction; units of β-galactosidase produced per incorporation of 1000 cpm [^{3}H]leucine into protein. (For further details see "Materials and Methods".)

(When the wild type strain N2089, which is isogenic with the *rnc* strain N2090 used here, was tested, the results obtained were similar to those seen with the wild type strain D10. Therefore, the results obtained with the *rnc* strain N2090, Table 1, are highly significant.)

Since β-galactosidase is a well characterized protein which is easy to assay and which appears in large quantities when cells are induced, we decided to follow the synthesis of this enzyme in all the strains analyzed. To accomplish this, we measured the differential induction of β-galactosidase in all the eight strains at 30°C and 43°C (Table 2). In the triple mutant strain, the level of β-galactosidase synthesis at 43°C is diminished 5-fold from the level at 30°C, whereas in the wild-type strain there is no such diminution. Again RNase III and RNase E were the enzymes implicated in this change. (This effect is not due to a decrease in the functional half life of β-galactosidase mRNA, see Ref. 14).

Since we could identify the spot which corresponded to β-galactosidase in the 2D gels, we compared the amount of radioactivity incorporated into this

Table 3. Synthesis of β-galactosidase in various RNA processing mutants

Strain	Genotype	30°C	43°C	Ratio of 43°C to 30°C
D-10	+++	2319	7495	2.33
N2090	rnc	3514	2513	0.71
N3427	rne	2151	2432	1.13
N2015	rnp	4202	11375	2.71
N3524	rnc,rne	5903	1346	0.23
N2017	rnc,rnp	4856	3616	0.74
N3531	rne,rnp	5290	1987	0.38
N3525	rnc,rne,rnp	1850	618	0.33

The β-galactosidase spot was cut out from the various gels (see Fig.1) and counted. The counts were normalized to that of a protein which did not show significant variations in the ratio of its synthesis at 43°C and 30°C in the various strains tested. This protein is designated in Fig. 1. Each gel was loaded with a different number of counts from 0.6 to 2 x 10^6 cpm. The specific activity of [^{35}S]methionine in the proteins at 30°C and 43°C is not the same.

spot in the various gels (Table 3), and the level of incorporation was normalized to a spot which appeared to be unaltered in all strains at both temperatures. Again we found a large reduction in the level of synthesis in the triple mutant at 43°C, and again this was caused mainly by the rnc and rne mutations.

DISCUSSION: The experiments described here were designed to find out whether or not mRNA in E. coli is affected by RNA processing enzymes. In order to achieve this goal we measured the level of synthesis of a large number of proteins under conditions where the processing enzymes were either active or inactive. It was possible to carry out these experiments since we have constructed strains deficient in the three processing enzymes RNase III, RNase E, and RNase P, in all possible combinations. In the absence of these enzymes, protein synthesis continues and at least some enzymes can be induced. These results are expected, since the absence of RNA processing does not interfere with the preexisting translation machinery.

The experiments presented here show that although the synthesis of most proteins is unaffected by these three RNA processing enzymes, an appreciable number of proteins are affected. We showed that out of 80 proteins examined, the synthesis of 21 was significantly affected by these three enzymes (Table 1). We examined specifically the enzyme β-galactosidase and found that the level of synthesis of this enzyme is significantly reduced in mutants defective in the RNA processing enzymes RNase III and RNase E.

The presentation of the data in Table 1 minimizes the differences in the level of protein synthesis. First, we did not consider size variation among the spots. This will tend to include in class one (no change, first column) those proteins which appear in relatively large quantities. For instance, β-galactosidase, the synthesis of which is clearly affected in the RNA processing mutants is included in column 4 in Table 1 (large change) only for the triple mutant strain. Second, the level of synthesis of a number of proteins increased at 43° in the wild type strain but not in some of the mutants; these alterations were not included.

The level of induction of β-galactosidase varied among the strains; this is most likely due to differences in the genetic make-up of the different strains. The rnc strain N2090, for instance, was induced to only about one-fourth the level of D10; however, when the isogenic rnc^+ strain N2089 was tested, it showed a similarly low level of induction and this level did not change significantly between temperatures (the levels were 11 and 9 in the units of Table 2).

While the experiments described here clearly show that the three RNA processing enzymes RNase III, RNase E, and RNase P (especially the first two) play a significant role in the synthesis of many proteins, they do not permit us to decide what the role of these enzymes is in the synthesis of the affected proteins. One interesting possibility is that a ribosomal binding site becomes more effective, or available, after a processing cleavage is introduced into the message.

However, it is not obvious that all the changes we observed here should be interpreted only in terms of the necessity of mRNA processing. Another possibility is that the processing enzymes affect the level of the mRNA. This could be either an effect on RNA synthesis or on the stability of the mRNA. Both of these possibilities are interesting and should be explored. Elucidation of which, if any, of the mechanisms discussed above is responsible for the phenomenon described here, will require further experiments.

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