AN ESCHERICHIA COLI K12 MUTANT CARRYING ALTERED RIBOSOMAL PROTEIN (S10)

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SUMMARY: An Escherichia coli mutant (JE14373) carrying decreased stability of stable RNA species was found to have altered electrophoretic mobility of a 30S ribosomal protein (S10). Recombinants covering str gene (76 min on E. coli linkage map by Bachmann, Low and Taylor, 1976 (ref. 1)) obtained from a cross of CSH64 x JE14373, restored normal S10 protein. The size analysis of RNAs labeled for 15 min with [^{3}H]uridine showed 50 to 60 % decrease of 16S RNA in this mutant strain, but almost no decrease of 23S RNA at 10 or 40 min after addition of rifampicin. On the other hand, no change was observed in the stability of both rRNA pieces in its parental PA3092 strain even at 40 min after addition of rifampicin.

Remarkable progress has been made in the elucidation of protein - RNA interaction within the Escherichia coli ribosome by biochemical study on nuclease digests of the complexes formed between ribosomal RNA (rRNA) and proteins (r-proteins) (2, 3, 4). To clarify interaction of r-protein with rRNA, genetic mutants of ribosome (see ref. 5) could be utilized as powerful tool. On the other hand, stable RNA including rRNA and transfer RNA is known to turn over slowly during steady state growth of Escherichia coli (6, 7, 8, 9). However, regulatory mechanisms to stabilize rRNA or transfer RNA were suggested to exist in E. coli (10). Two genes (srnA and srnB) were responsible for promoted turnover of stable RNA at 42°C when RNA synthesis was stopped (11), and also that these genes seem not to link to the structural genes for r-proteins or rRNA. Thus, we decided to isolate E. coli mutants with increased rate of turnover of stable RNA. In this report, we will describe correlation of rRNA stability with an altered 30S r-protein.

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MATERIALS AND METHODS

Bacterial strains

Screening of mutants carrying altered turnover of RNA among about 1,200 temperature sensitive E. coli K12 mutants will be described elsewhere (Kuwano, Ono, Endo, Hori, Hirota, Nakamura and Ohnishi, manuscript in preparation). The parental strain is PA3092 (F^-, thr, leu, lacY, trp, his, thy, strA, malA, xyl, argH, supE), from which a mutant strain JE14373 was derived. For the cross experiment, a nalidixic acid resistant strain was isolated spontaneously from JE14373 strain and CSH64 (Hfr, thi, with injection order from argA, strA) (12) was used as donor strain.

Assay for RNA degradation and analysis of RNA size by sucrose density gradient centrifugation

Exponentially growing cells (2 to 4 x 10^8 cells/ml) in minimal medium were labeled for 2 or 15 min with 1 μCi/ml [^{3}H]uridine (27 Ci/mmole, New England Nuclear, Boston) at 30°C, and then transferred to 37°C immediately after RNA synthesis was stopped by addition of 500 μg/ml rifampicin and 200 μg/ml uridine. After addition of rifampicin, aliquots (0.2 ml) withdrawn from the cultures at the indicated times were added to 2 ml of ice-cold 10 % trichloroacetic acid. The radioactivities collected on glass fibre filter were counted by Beckman Liquid Scintillation Counter. The highest value of [^{3}H]-labeled RNA remaining on the filter was expressed as 100 %. To prepare radioactive RNA, the cells (30 ml culture) were labeled at 30°C for 15 min with 1 μCi/ml [^{3}H]uridine and then RNA synthesis was stopped by rifampicin (500 μg/ml) to follow fate of size distribution of [^{3}H]labeled RNA at 37°C. [^{3}H]RNA extracted from the cells at 0, 10 and 40 min after addition of rifampicin was placed on a 10 to 30 % sucrose density gradient containing 0.1 M sodium acetate (pH 5.1)-1 mM EDTA-0.01 % sodium dodecyl sarcosinate as described previously (13) and was spun for 4hr at 39000 rpm with a Spinco L-2 centrifuge.

Preparation of r-proteins and gel electrophoresis

Ribosomal proteins of 70S ribosome from 2 to 4g cells were extracted with acetic acid after the method of Waller and Harris (ref. 14). Some 400 to 500 μg r-proteins were applied to two-dimensional polyacrylamide gel electrophoresis developed by Kaltschmidt and Wittmann (ref. 15) and staining was done with coomassie brilliant blue.

RESULTS

The mutant strain JE14373 which shows temperature sensitive growth, was isolated by screening of stability of pulse-labeled RNA at 42°C from nitrosoguanidine mutagenized E. coli K12 strain (PA3092). As shown in Fig. 1, we followed the fate of labeled RNA after RNA synthesis was blocked by addition of rifampicin. Wild type strain PA3092 which had been pulse-labeled at 30°C for 2 min showed 50 to 55 % breakdown of RNA within 20 min at 37°C (Fig. 1a). Therefore 45 to 50 % of pulse-labeled RNA during steady state of growth was thought to correspond to stable RNA fraction. By contrast, some 70 % of the pulse-labeled RNA of JE14373 strain degraded within 20 min after rifampicin addition to the culture (Fig. 1a). To locate a mutation affecting RNA stability of JE14373 strain, various Hfr strains were crossed with this mutant.

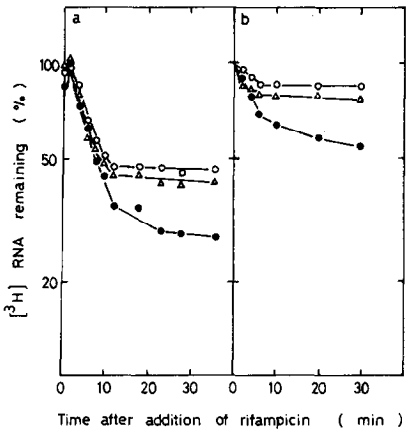

Fig. 1. Comparison of RNA decay curves. The cells of PA3092 (○), JE14373 (●) and a Str-S recombinant (△) cultured to exponential phase at 30°C were labeled for 2 min (a) or for 15 min (b) with 1 μCi/ml [^{3}H]uridine at 30°C and then the fate of labeled RNA was examined at 37°C after addition of rifampicin. The value of 100 % corresponds to the highest radio-activity in each experiment.

It was found that all 12 recombinants (Str-S) among Nal-R Mal$^+$ colonies obtained from a cross (CSH64 x JE14373) showed the decay curve of RNA as comparable to that of parental PA3092 strain, and the representative decay curve of recombinants covering str locus was shown in Fig. 1a. However, all these Str-S recombinants were found to show still temperature sensitive growth and this temperature sensitive gene was found to be located close to argH gene (unpublished data). On the other hand, when RNA was labeled with [^{3}H]uridine for 15 min at 30°C, only 10 to 15 % of RNA of PA3092 strain degraded within 30 min at 37°C after addition of rifampicin, while breakdown of 40 to 45 % of labeled RNA was observed in the mutant strain within 30 min (Fig. 1b). Even when labeled for 15 min, 20 % of the RNA in the recombinants covering str region degraded within 30 min after rifampicin addition. Thus, under either labeling condition (Fig. 1a and b), some 15 to 20 % RNA among stable fraction of bulk RNA degraded in the mutant strain, whereas only little, if any, degradation was observed in wild type strain and Str-S recombinants. However, unstable RNA (presumably mRNA) degraded at similar rate in both

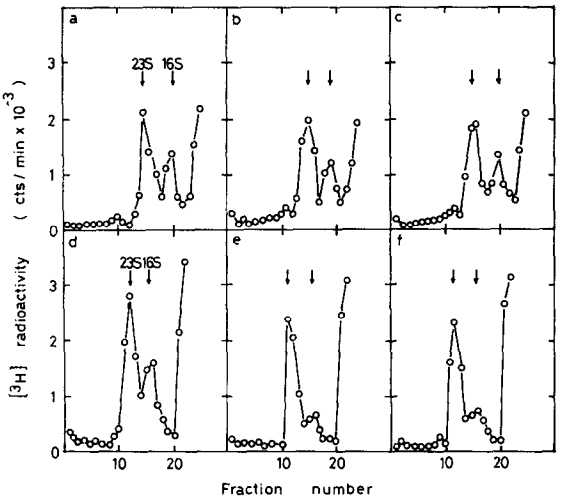

Fig. 2. Sedimentation profiles of ribosomal RNA of PA3092 and JE14373. [^{3}H] RNA's extracted from PA3092 treated with rifampicin for 0 min (a), 10 min (b) and 40 min (c) or from JE14373 with rifampicin for 0 min (d), 10 min (e) and 40 min (f) were analysed by centrifugation on a sucrose density gradient containing sodium dodecyl sarcosinate. Two peaks from left to right indicate 23S and 16S rRNA, respectively.

parental and the mutant strain (see Fig. 1a). Almost identical data on the RNA stability were also obtained when RNA decay was analyzed at 30°C or 42°C (data not shown).

Different from the wild type strain, this mutant was found to show more extensive degradation of stable RNA (Fig. 1). However it must be noticed that the extent of degradation of stable RNA within 30 min after addition of rifampicin varied from 10 to 20 %, but never reached to 80 to 100 % as described previously (10). Thus, the degradation of ribosomal RNA was examined by sucrose density gradient analysis. After the exponentially growing cells of PA3092 and JE14373 were labeled for 15 min at permissive temperature, the temperature for culture was shifted up to 37°C and [^{3}H]RNA was extracted at 0, 10 and 40 min after rifampicin addition. The sucrose-density gradient patterns of [^{3}H]RNA showed that both 16S and 23S rRNA were stable in the parental strain even at 40 min after RNA synthesis was stopped (Fig. 2a, b and c) and the ratio (2 : 1) of 23S : 16S rRNA seemed not to be affected during incubation with

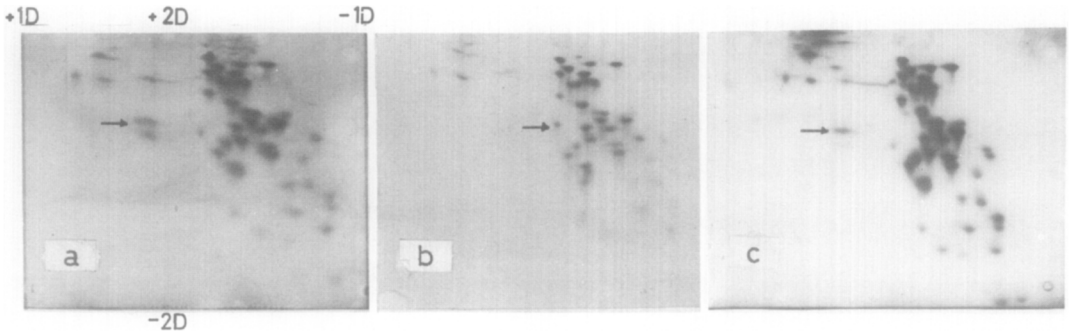

Fig. 3. Separation of 70S ribosomal proteins by two-dimensional polyacrylamide gel electrophoresis (15). 70S r-proteins were derived from PA3092 (a), JE14373 (b) and a $\underline{str}^+$ recombinant (c), respectively. Arrows indicate 30S protein-S10.

rifampicin. In contrast with the parent cells, in the mutant, smaller rRNA corresponding to 16S appeared to be unstable at 10 min as well as 40 min after addition of rifampicin (Fig. 2d, e and f) and 50 to 60 % of 16S rRNA degraded, while little, if any, degradation of 23S rRNA occurred, showing the differential stability of 16S and 23S rRNA in the mutant strain.

To investigate a possible alteration of r-proteins in correlation with the changes of rRNA stability, the analysis of r-proteins was done with two-dimensional gel electrophoresis (15). The electrophoretic pattern of 70S ribosomal proteins from PA3092 and JE14373 strains were shown in Fig. 3a and b. As indicated by arrow in Fig. 3a and b, an acidic r-protein S10 according to the nomenclature $\underline{E.\ coli}$ r-proteins by Wittmann (ref. 16) was found to clearly alter its electrophoretic mobility in this mutant strain. This change in the mobility may be ascribed to amino acid substitution of S10 protein. The electrophoretic pattern of 70S r-proteins extracted from the recombinants covering $\underline{str}$ region showed the restoration of normal S10, as shown in Fig. 3c, which may suggest that $\underline{str}$ region is the gene for S10 mutation in this mutant strain as described previously (17, 18).

DISCUSSION

With respect to regulatory mechanism for stabilizing stable RNA, a mecha-

nism for coordinate balancing of both 16S and 23S rRNA as well as transfer RNA was suggested previously (10, 11, 19). We reported here that an Escherichia coli mutant having altered stability of 16S rRNA. This is a case that turnover or stability of rRNA might be limited by its own surrounding protein elements. Since ribonuclease I is the only known nuclease which shows extensive activity against ribosomes and transfer RNA, one may infer that this enzyme might be involved in partial digestion of 16S rRNA. Proteins S1, S9, S13 and S19 among 30S ribosome proteins remain bound to 3'-proximal region of 16S rRNA (3). Another study by Yuki and Brimacombe (ref. 4) showed that the isolated ribonucleoprotein fragment contained S7, S9, S10, S14 and S29 and 3'-proximal pieces of 16S rRNA. Further analysis on protein-RNA interactiaon suggested that S7 protein of these five proteins is the primary target of cross-linking to the RNA molecule (20). Recently, Sommer and Traut (ref. 21) identified neighboring protein pair in the 30S ribosomal subunit and demonstrated the existence of a cross-linked dimer S3-S10. Thus the lability of 16S RNA in this mutant strain might be ascribed to the environmental indirect effect of altered S10 on protein S7, if S7 is one of main proteins to maintain the structure of 30S ribosomal subparticles. Concerning genetics of ribosome mutation affecting rRNA stability, only a few mutants were analysed. An altered S4 protein from the E. coli which is reverted from streptomycin dependence to independence bound weakly to 16S rRNA (22), which might be correlated with a biochemical finding that S4 binds to 5' proximal half of 16S rRNA fragment (2). On the other hand, a mutant of altered S1 protein was isolated (23). Since S1 is shown to bind to 3' end of 16S rRNA (24, 25), it must be interesting to know if a S1 mutant shows altered stability of 16S rRNA.

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