10Sa RNA complements the temperature-sensitive phenotype caused by a mutation in the phosphoribosyl pyrophosphate synthetase (*prs*) gene in *Escherichia coli*

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(Received 30 October 1995)

From *Escherichia coli* cells with a deletion in the *ssrA* gene that encodes 10Sa RNA after treatment with a mutagen, we isolated two temperature-sensitive mutants, which we designated TS15 and TS101. The temperature-sensitive (ts) phenotype of the mutants could be overcome by introduction of the wild-type *ssrA* gene but not by the mutants of *ssrA*. By a complementation test using Kohara's mini-set of clones and by subcloning of a fragment from the phage clone 246, we found that both mutations were in the *prs* gene that encodes phosphoribosyl pyrophosphate synthetase. Sequencing of the mutant *prs* gene of TS101 showed that residues 215, cysteine, in the encoded protein had been changed to tyrosine. That such a mutant exists suggests that 10Sa RNA associate with the *prs* gene product in a functional way.

10Sa RNA is one of the small stable RNAs found in *Escherichia coli* (Ray and Apirion, 1979; Chauhan and Apirion, 1989). The gene for 10Sa RNA, *ssrA*, is at 56.5 minutes on the linkage map of the *E. coli* chromosome. An identical species of RNA has been found in *Alcaligenes eutrophus* (Brown et al., 1990), *Mycobacterium tuberculosis* (Tyagi and King, 1991), *Mycoplasma capricolum*, and *Bacillus subtilis* (Ushida and Muto, 1993; Ushida et al., 1994). This small RNA can be arranged in a structure equivalent to a half-molecule of tRNA (Komine et al., 1994; Ushida et al., 1994). In particular, 10Sa RNA from *E. coli* has the determinant G · U pair (G3 · U70) of alanine tRNA. There have been studies of the inactivation of the gene for 10Sa RNA. For example, disruption of the *ssrA* gene in strain JC7623 of *E. coli* by the insertion of the *cat* gene interferes with cell growth at various temperatures (Oh and Apirion, 1991). The absence of functional 10Sa RNA seems to be responsible for growth inhibition of λ-P22 hybrid phage (Retallack et al., 1994) and an increase in the expression of Alp protease (Kirby et al., 1994).

We have isolated a *ssrA* deletion mutant from W3110 (Komine et al., 1994), in which the -10 promoter signal and

about two-thirds of the coding region of *ssrA* has been deleted and replaced by a gene for the enzyme that confers kanamycin resistance. This Δ*ssrA* strain grows normally at 42°C but does not grow well at 45°C. In the present study, we isolated a temperature-sensitive mutation that could be complemented by introduction of the wild-type *ssrA* gene. The Δ*ssrA* strain was treated with the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) (Miller, 1972) and studied by a classical penicillin screening at 42°C for concentration of temperature-sensitive mutants. Colonies that formed on LB plates at 32°C after penicillin treatment were replicated onto three plates with toothpicks: (A) an LB plate at 32°C, (B) an LB plate at 42°C, and (C) a λ plate containing phage λ10SA at 42°C. Because λ10SA includes a fragment of between 2.16 and 2.24-kb of the wild-type *ssrA* gene in the λgt11 vector (Komine et al., 1994), a ts mutant that cannot grow on plate B but can grow on plate C should be the result of complementation by the wild-type *ssrA* gene. Among about 80 mutants isolated independently, we found two mutant strains, which we named TS15 and TS101, which had temperature-resistant growth in the presence of the *ssrA* gene. Figure 1 shows the complementation of TS15 and TS101 by lysogenization with λ10SA. λ phages carrying a mutant *ssrA* gene, in which the encoded G3 · U357 pair of 10Sa RNA was changed to a G3 · C357 or

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Fig. 1. Temperature sensitivity and complementation of strain TS15 and TS101 (from W3110 $\Delta ssrA$) by lysogenization of λ phage carrying the *ssrA* or the *prs* gene. The original $\lambda 10SA$ and λPRS were constructed with phage vector $\lambda gt11$ so that they carried the marker *cI857* and could not be maintain as prophages at 42°C. We obtained λcI^+ derivatives by genetic crossing. Because those phages behave the phage *att* site, the stable lysogens integrated by them could be isolated easily for a test of the immunity of phage λ . One loop of overnight cultures in LB medium at 32°C was streaked on LB plates. After incubation at 32°C (2) and 42°C (1) overnight, the plates were photographed. 1, TS15 ($\lambda 10SA$); 2, TS15 (λPRS); 3, TS15 (λ); 4, TS101 ($\lambda 10SA$); 5, TS101 (λPRS); 6, TS101 (λ); 7, W3110 $\Delta ssrA$ (λ).

G3 · A357 pair, did not complement any of the *ts* mutations (data not shown). These results indicated that, although 10Sa RNA was dispensable in the original $\Delta ssrA$ strain at 42°C, the *ts* mutants needed 10Sa RNA when a certain gene was damaged by mutation.

To locate the *ts* mutation in TS15 and TS101, we used complementation tests for the *ts* characteristic with a mini-set of clones from Kohara's phage library (Kohara et al., 1987). Phage clones 246, 247, 438, 439, and 440 complemented the temperature sensitivity of both TS15 and TS101. Because clones 438, 439 and 440 contained the *ssrA* gene, the gene of interest appeared to be in both clones 246 and 247. A restriction map of the insert DNAs of clones 246 and 247 is shown in Figure 2A. The inserts of clones 246 and 247 were digested with *EcoRI* and subcloned into plasmid Bluescript KS (-), and each plasmid was used to transform the *ts* mutants. Cells with plasmid pBS2.3kb, which carried a 2.3-kb *EcoRI* fragment, complemented the temperature sensitivity of TS15 and TS101, so both *ts* mutations were in the 2.3-kb *EcoRI* fragment of Kohara's phage clones 246 and 247. As shown in Figure 1, when the phage λprs^+ carrying this 2.3-kb *EcoRI* fragment was lysogenized to the *ts* mutants, both mutant bacteria grow at 42°C. Because TS101 was a more stable *ts* mutant than TS15, we used mainly the TS101 mutant in further experiments. We produced a series of deletion mutants of pBS2.3kb (Yanisch-Perron et al., 1985) and examined their ability to complement the temperature sensitivity of TS101 after transformation (Fig. 2B). Among the mutants with deletions from the "left" side, AS50 and AS26 had lost the ability to complement the sensitivity.

Fig. 2. (A) Location of the 2.3-kb *EcoRI* fragment on the map of the *Escherichia coli* chromosome. The restriction map is taken from Kohara et al. (1987). The eight horizontal bars (top) indicate the restriction sites of the following enzymes, in order, from the top: *Bam*HI, *Hind*III, *Eco*RI, *Eco*RV, *Bgl*I, *Kpn*I, *Pst*I, and *Pvu*II. The 2.3-kb *Eco*RI fragment is indicated as a shaded box. The insert DNAs in phage clones 246 and 247 are indicated below the restriction map. (B) Deletion mutants of pBS2.3kb and their ability to complement the temperature sensitivity of TS101. ++, Strong complementation; +, weak complementation; -, no complementation. ORF2 codes for a 32-kDa of unknown function (Post et al., 1993).

Among the mutants deleted from the opposite side, SB26 and SB14 weakly complemented the temperature sensitivity. SB24 and SB78 had completely lost this ability. AS2 seemed to include the entire gene of interest, so we sequenced the insert DNA of this plasmid as described elsewhere (Sanger et al., 1977) and found that this fragment contained only one open reading frame (ORF). This ORF was identified as the *prs* gene from a similarity search in the DNA database (GenBank).

Another mutation(s) may be responsible for the temperature sensitivity of TS101 rather than a mutation in the *prs* gene because TS101 was first isolated after mutagenesis with NTG. To exclude this possibility, we attempted to transfer the mutant *prs* gene of TS101 into another strain by P1 transduction. The recipient strain was LE392 $\Delta hemA$, in which the coding region of the *hemA* gene had been deleted and replaced by a gene for the enzyme that

confers resistance to kanamycin (Nakayashiki et al., 1995). The mutant *prs* gene of TS101 was transduced together with the *hemA* gene: The $\Delta hemA$ mutant formed a tiny colony, so large colonies could be selected on LB plates as *hemA*⁺ transductants and tested for kanamycin resistance (Km^R). Next, we transduced the $\Delta ssrA::km^R$ marker from the original W3110 $\Delta ssrA::km^R$ strain into the LE392 *hemA*⁺ (*prs*_{ts101}) transductant, selecting kanamycin-resistant colonies and identifying the products from the *ssrA* gene by the polymerase chain reaction (PCR) (Saiki et al., 1988). The Km^R transductant of LE392 *hemA*⁺ (*prs*_{ts101}) was temperature-sensitive and its temperature sensitivity was complemented by both the *prs* gene and the *ssrA* gene, indicating that the transductant had *prs*_{ts101} $\Delta ssrA::km^R$. The mutation responsible for the temperature sensitivity of TS101 was therefore a mutation in the *prs* gene.

To identify the ts101 mutation in the *prs* gene, we sequenced a 2.3-kb *EcoRI* fragment of the mutant *prs* gene, amplified by PCR from the genomic DNA of TS101. Base 1,161, guanine, in the *prs* gene had been changed to adenine, as found by comparison with the sequence in the database (accession number M13174). This change caused a change of Cys215 to Tyr215, suggesting the possibility that the ts mutant PRPP synthetase was unable to form an intermolecular disulfide (S-S) bond. This alteration may be the cause of the temperature sensitivity of TS101. We have not identified the TS15 mutation in the *prs* gene.

The *prs* gene is a structural gene for phosphoribosylpyrophosphate (PRPP) synthetase (ribose-phosphate pyrophosphokinase; ATP : D-ribose-5-phosphate pyrophosphotransferase, EC 2.7.6.1) (Hove-Jensen et al., 1986). PRPP synthetase consists of 314 amino acids and catalyzes the reaction ribose-5-phosphate + ATP → PRPP + AMP. PRPP is an important precursor for the synthesis of purine and pyrimidine nucleotides, the pyridine nucleotide coenzyme NAD, and the amino acids histidine and tryptophan. The gene is subject to metabolic regulation in *E. coli* (Hove-Jensen, 1983). A mutant allele in *E. coli*, named *dnaR*, that causes thermosensitive initiation of chromosome replication is an allele of the *prs* gene (Sakakibara, 1992, 1993).

We propose ways in which 10Sa RNA affects the function of the *prs* gene product. One possibility is that 10Sa RNA interacts specifically with PRPP synthetase to increase its activity. When the wild-type PRPP synthetase is present, 10Sa RNA is dispensable. Once the conformation of PRPP synthetase has changed and its activity decreases, however, 10Sa RNA might modify the features of the mutant PRPP synthetase. Another possibility is that 10Sa RNA is a transcriptional factor that up-regulates the expression of PRPP synthetase. Or, 10Sa RNA might act as a heat-shock protein or might up-regulate heat-shock proteins. In this case, 10Sa RNA might suppress the temperature sensitivity of other

proteins in addition to PRPP synthetase.

Tu et al. (1995) discovered that a small population of murine interleukin-6 molecules overexpressed in *E. coli* had a previously unknown kind of C-terminal modifications attached to the "tag" peptide, and that this tag peptide was from the *ssrA* gene. On the other hand, Retallack and Friedman (1995) reported that the 10Sa RNA seems to modulate the action of DNA-binding proteins. The functional role of 10Sa RNA remains unknown.

We have no genetic or biochemical evidence indicating which is correct.

We thank A. Muto and Y. Sakakibara for helpful discussions. This research was supported by a Grant-in-Aid for Scientific Research on Priority Areas (no. 04272104) from the Ministry of Education, Science, Sport, and Culture of Japan.

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