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Genetic Mapping of the F Plasmid Gene That Promotes Degradation of Stable Ribonucleic Acid in *Escherichia coli*

YOSHINARI OHNISHI,[1]* HATSUMI IGUMA,[2] TSUNEKO ONO,[1] HARUKO NAGAISHI,[3] AND ALVIN J. CLARK[3]

Department of Bacteriology, School of Medicine, Tokushima University, Tokushima 770, Japan[1]; Department of Microbiology, School of Medicine, Kyushu University, Fukuoka 812, Japan[2]; and Department of Molecular Biology, University of California, Berkeley, California 94720[3]

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F^+ *Escherichi coli* cells that contain an *srnA* mutant allele degrade their stable ribonucleic acid (RNA) extensively after RNA synthesis is blocked at 42°C. The relevant gene promoting degradation of stable RNA, *srnB*⁺, or its promoter was mapped between 1.7 and 2.8 kilobases on the F plasmid by using deleted F′ plasmids and chimeric plasmids composed of pSC101 and fragments of F plasmid.

Messenger ribonucleic acid (mRNA) in *Escherichia coli* cells is generally unstable (20), although *trp* mRNA synthesized from the P_L promoter of lambda *trp* phage is stable (26). Ribosomal RNA (rRNA) in *E. coli* cells, on the other hand, is generally stable (20), although a mutant of *E. coli* degrades rRNA at 42°C after RNA synthesis is inhibited (18). This Srn^- (stable RNA negative) phenotype is dependent on two components. The first is the *srnA1* allele, located at 9.4 min on the recalibrated map of *E. coli* (2, 16, 17). The second is the F plasmid (17), which may be either autonomous (F^+ or F′) or integrated with the *E. coli* chromosome (Hfr). This indicates that degradation does not occur simply because an autonomous plasmid is present (16, 17), and that at least one specific gene of F is involved. This gene has been called *srnB*⁺. In this paper we describe experiments localizing part or all of *srnB*⁺ to a 2.3-kilobase (kb) region of F.

MATERIALS AND METHODS

Bacterial strains. The strains used are listed in Tables 1 and 2.

Media. Minimal salts-glucose medium is composed of (per liter): Na_2HPO_4, 7 g; KH_2PO_4, 3 g; NH_4Cl, 1 g; Na_2SO_4, 0.8 g; $FeCl_3$, 1.75 mg; $MgCl_2$, 48.8 mg; $CaCl_2$, 2.75 mg; and 0.4% glucose. For genetic studies, required amino acids, purine, pyrimidine, or nucleoside (50 μg/ml, respectively) were added to the minimal salts-glucose medium; 0.0005% thiamine or 0.2% Casamino Acids was added when necessary.

Conjugation. Conjugation and the other genetic manipulations were performed by the procedures of Miller (13).

Transformation of the chimeric plasmid DNA into the *srnA* strains. To select strains that received chimeric plasmid deoxyribonucleic acid (DNA), tetracycline-resistant strains were isolated after mixing the DNA and $CaCl_2$-treated cells of strain YS12 (12). Confirmation of the presence of the plasmid was by agarose gel electrophoresis of plasmid DNA after digestion by restriction endonuclease *Eco*RI (23).

Assay of remaining RNA. The stable RNA of each strain was labeled with [^{3}H]guanine or [^{3}H]uridine for 3 h at 30°C in minimal salts-glucose medium. The cultures were then shifted to 42°C and shaken for 60 min. After the addition of 200 or 500 μg of rifampin per ml (t = 0), 0.2-ml portions of cells were precipitated in ice-cold 5% trichloroacetic acid at the indicated times, and the precipitates were counted in a liquid scintillation spectrometer. Counts in DNA were subtracted from the acid-insoluble counts to give the values of RNA (16).

RESULTS

Effect of R plasmids on degradation of stable RNA. Several R factors were transferred into the F^- *srnA1* strain YS31 to determine how widespread a character the *srnB*⁺ gene is. R386 is in the same incompatibility group as F (IncFI) (15), yet the rRNA of an R386 *srnA1* strain was stable at 42°C after RNA synthesis had been inhibited (Table 3). Two other plasmids, R222 and R100-1, which belong to the IncFII class, also did not carry an active *srnB* gene (Table 3). This result indicates that *srnB* is probably not in the *tra* gene region of F (approximately 62 to 93 kb), since R100-1 is homologous to most of this region of F as judged by heteroduplex mapping studies (21). Thus, the *srnB*⁺ gene is not characteristic of enterobacterial conjugative plasmids.

It should be noted in passing that there is an indication that the R factors may slightly destabilize RNA in an *srnA*⁺ strain (Table 3). This is the opposite of the *srnB*⁺ effect of F. It is not, however, a very large effect, and the overall genetic backgrounds of the *srnA*⁺ R-factor

TABLE 1. *List of basic strains*

Strain	Sex	Partial genotypes	Reference
YS11[a]	F+	*srnA1 leu purE trp metE lys thi ara lacZ xyl str*	(16, 17)
YS12[b]	F⁻	Same as YS11	This paper
YS31	F⁻	*srnA1 pyrB59 argH1 str-9*	(17)
YS105	Hfr	*srnA1 thr-1 leu-6 his-4 str-31 pyrE60 thi-1*	(16)
YS107[c]	Hfr	*tsx-316,* otherwise same as YS105	This paper
AB284	F^+	*thr-1 leu-6 thi-1 sup-49 lacZ4 str-8*	(1)
JC2909[d]	F*lac*$^+$	*metB1 leu-6 his-1 argG6 lacY1,Z4 malA1 xyl-7 mtl-2 gal-6 str-104 recA1*	(22)

[a] Pro^+ *srnA1* transductant of YS607 described without a name by Ohnishi (17).
[b] Recovered after acridine orange treatment of YS11.
[c] Spontaneous $T6^r$ mutant of strain YS105.
[d] Produced by crossing strain JC1553 with strain X314 and selecting for inheritance of F*lac*$^+$. X314 is the F*lac*$^+$ strain AB785. JC2909 could be called JC1553/F*lac*$^+$. Another strain, labeled JC1553/F*lac*$^+$, was obtained from D. R. Helinski.

TABLE 2. *List of plasmid-carrying derivatives of strain YS31 produced by conjugation with plasmid donors*

Derivative	Plasmid	Plasmid donor	Source and reference of plasmid	
YS32	F	YS357		(17)
YS313	R386	JC10561[a]	H. Hashimoto	(6)
YS314	R222	JC10562[b]	T. Miki	(14, 25)
YS315	R100-1	JE2100	T. Miki	(24)
YS316	F210	χ790	E. Ohtsubo	(4, 8)
YS317	F13-4	JE513	E. Ohtsubo	(8)
YS318	FΔ (33–43)	W1655	E. Ohtsubo	(11)
YS319	F14	AB1206	B. J. Bachmann	(19)
YS320	FΔ (0–14.5)	ND3	E. Ohtsubo	(22)

[a] Originally called J53/R386. Since this name does not conform to Recommendation 9 of Demerec et al. (5), we have changed the name in conformity to Recommendation 10 (5).
[b] Originally called CSH-2/R222. We have changed this name for the reasons given in footnote *a*.

TABLE 3. *Effect of R plasmids on stable RNA degradation*

Plasmid	Percent RNA remaining at 60 min[a]		Strains	
	srnA$^+$	*srnA*	*srnA*$^+$	*srnA*
R386	70.2	93.4	JC10561	YS313
R222	83.8	91.6	JC10562	YS314
R100-1	86.3	87.1	JE2100	YS315
F	ND[b]	20.0		YS32
None	ND	95.9		YS31

[a] *srnA*$^+$ and *srnA* strains carrying each plasmid were tested as indicated in Materials and Methods.
[b] ND, Not done.

strains are different from that of YS31. Thus, it is possible this effect may not even be due to the R factors.

Deletion mapping of the $srnB^+$ gene on F plasmids. Since the *srnB* gene appeared not to lie in the *tra* region, we chose to examine mutants of F carrying deletions of segments between 0 and 42.9 kb. Each plasmid was transfered to YS31, and the percentage of RNA remaining was determined after RNA synthesis had been inhibited for 60 min at 42°C (Table 4). FΔ(0–14.5) did not lead to as much RNA loss as did the other plasmids and hence was a candidate to be an *srnB* deletion mutant. FΔ(0–14.5) carries a deletion of 0 to 14.5 kb, a segment that includes the gamma-delta integration sequence from 2.8 to 8.5 kb. Note that F210 carries a deletion of 3.5 kb from this region (8.5 to 12.0 kb) and yet is $srnB^+$. Thus, *srnB* probably lies between 0 and 8.5 kb or between 12.0 and 14.5 kb. Since a mutant of F8 carrying a deletion of 8.5 to 16.3 kb had already been tested (17) and found to be $srnB^+$, this rules out a location for *srnB* between 12.0 and 14.5 kb.

A previous test of F*lac*$^+$ had given us the hope that we had further narrowed the region carrying *srnB* to 2.8 to 8.5 kb. YS31 carrying F*lac*$^+$ showed 33% RNA remaining under the same conditions used for the experiments here (17). This indicated that the F*lac*$^+$ plasmid used was $srnB^+$. Since F*lac*$^+$ had been determined to carry a deletion of 0 to 2.8 kb (22), we concluded that $srnB^+$ could not lie in that region. Later we realized that the F*lac*$^+$ donor we had used to make the strain was the original F*lac*$^+$ strain 200PS (1, 10) and not the strain (JC1553 F*lac*$^+$) from which Sharp et al. (22) had isolated their characterized plasmid. We then transferred F*lac*$^+$ plasmids from JC1553 F*lac*$^+$, obtained from D. Helinski, and from another JC1553

F*lac*$^+$, known as JC2909, into YS12. We found them to produce a level of stability similar to that of FΔ(0–14.5). Thus, F*lac*$^+$, as characterized by Sharp et al., appears to be *srnB* not *srnB*$^+$. This indicates that the deletion of 0 to 2.8 kb observed by Sharp et al. may have been a secondary mutation produced after the original excision event which produced F*lac* (9). Consequently, some or all of the *srnB* gene or its promoter must lie outside of the gamma-delta sequence and between 0 and 2.8 kb.

It should be noted that the *srnA*$^+$ strains carrying the different deletion mutant F plasmids showed a lower RNA stability than the non-plasmid-carrying *srnA* strain. As indicated in the previous section, this may have been due to different genetic backgrounds of the strains.

Does the *E. coli* chromosome have the gamma-delta sequence and show *srnB*$^+$ function? The gamma-delta sequence of 2.8 to 8.5 kb on the wild-type F plasmids is duplicated on F14 (19). Since F14 originated from the Hfr strain AB313, Ohtsubo et al. (19) hypothesized that F had integrated, by recombination, between its own gamma-delta sequence and a chromosomally located one. If this is true, then AB284, the F$^+$ ancestor of AB313, should be at least diploid for the gamma-delta sequence, with one copy on the plasmid and one on the chromosome. We would then expect that curing an *srnA* derivative of AB284 of its F plasmid would not result in stabilizing the rRNA if the chromosomal copy of gamma-delta carried a functional *srnB* gene.

To test this prediction, AB284 was grown into the phenocopy F$^-$ state and was mated with YS107, an HfrH strain transferring *srnA1* early in conjugation. A T6^r Lac$^+$ [His$^+$ Pyr$^+$] recombinant (YS2844) was isolated and determined to be F$^+$ by sensitivity to male-specific phage fd and *srnA* by the instability of its rRNA (Table 5). This strain was then cured of its F plasmid by treatment with acridine orange, and F$^-$ derivative YS2845 was isolated. This strain was Srn$^+$ (Table 5), indicating that with the loss of the F plasmid (and its functional *srnB* gene), the stability of the rRNA had been improved. Thus, unless YS2845 is a back mutant at *srnA* or carries a new suppressor mutation of *srnA1*, it must carry no functional *srnB* gene. To make certain the strain was not some kind of Srn$^+$ revertant, it was reinfected with F from W2241 (7) to produce YS2846. This strain was Srn$^-$ (Table 5), indicating that an *srnB*$^+$ gene can be fully expressed in the YS2845 genetic background. Our conclusion from these experiments is that (i) a chromosomal gamma-delta sequence does not carry a functional *srnB* gene or (ii) the *E. coli* chromosome does not have a gamma-delta sequence or an *srnB* gene.

The Srn phenotypes of AB1206 and YS319 were also determined (Table 5). The latter is an *srnA1* derivative carrying F14, the gamma-delta duplication plasmid. As expected, AB1206, being *srnA*$^+$, was Srn$^+$, whereas YS319 carried *srnB*$^+$ of F14 and *srnA* on the chromosome and was Srn$^-$.

Mapping the *srnB* gene by examining chi-

TABLE 4. *Stability of stable RNA in strains carrying F′ plasmids*

Plasmid	Deletion (kb)	Percent RNA remaining after 60 min		Strains	
		srnA$^+$	*srnA*	*srnA*$^+$	*srnA*
FΔ (0–14.5)	0–14.5	74.6	70.7	ND3	YS320
F210	8.5–12.0	61.2	18.1	χ790	YS316
F13-4	17.6–37.8	54.1	16.3	JE513	YS317
FΔ (33–43)	32.6–42.9	83.4	23.0	W1655	YS318
F*lac*$^+$	0–2.8	91.4	59.7	JC1553/F*lac*$^+$	YS1201
				JC2909	YS1202
None		ND[a]	87.7		YS31

[a] ND, Not done.

TABLE 5. *Relevance of the supposed gamma-delta sequence on the host chromosome to srnB*$^+$ *function*

Strain	*srnA*	Fertility	Percent RNA remaining after 60 min	Srn	*srnB*
YS107	−	HfrH	15.6	−	+
AB284	+	F$^+$	75.8	+	+
YS2844	−	F$^+$	31.8	−	+
YS2845	−	F$^-$	92.8	+	−
YS2846	−	F$^+$	23.6	−	+
W2241	+	F$^+$	62.1	+	+
AB1206	+	F14	88.0	+	+
YS319	−	F14	15.9	−	+

meric plasmids. To supplement the deletion mapping experiments already described, we examined chimeric plasmids for the presence of a functional *srnB* gene. The chimeric plasmids were derived by in vitro recombination of pSC101 and fragments of F produced by endonuclease *Eco*RI partial hydrolysis (23). These plasmids were transformed into *srnA* strain YS12, using their tetracycline resistance character for the selection of transformants. Stability of RNA was determined in the transformants (Table 6 and Fig. 1). pRS31, which is composed of 13.9 kb of F from 82.3 to 1.7 kb on the standard map, was functionally *srnB* as shown by the high stability of RNA. By contrast, pRS7 was $srnB^+$. This plasmid carried a 2.3-kb segment from 1.7 to 4.0 kb in addition to the sequences carried by pRS31. Thus, all or part of the $srnB^+$ gene lies in this additional segment. The other results in Table 6 are consistent with this conclusion. It can be noted that the longest of the chimeric plasmids tested (pRS15) led to less RNA instability than did other $srnB^+$ plasmids (pRS7, pRS26, and pRS8). It is conceivable that there is a gene in the 9.3- to 25.9-kb segment of F that counteracts the $srnB^+$ gene. It is also possible that the $srnB^+$ gene dosage is higher in strains carrying the three smaller plasmids than in the strain carrying pRS15.

DISCUSSION

Using deletion mutant plasmids, we showed that the $srnB^+$ gene lies within the 14.5-kb segment of F between 0 and 14.5 on the standard map of F (22). A discrepancy in the RNA instability caused by two F*lac*$^+$ plasmids led to the further conclusion that $srnB^+$ lies between 0 and 2.8 kb on the map. This was confirmed by using chimeric plasmids which show that the $srnB^+$ gene lies between 1.7 and 4.0 kb on the map. It is thus tempting to conclude that $srnB^+$ lies between 1.7 and 2.8 kb. We cannot, however, from these tests distinguish the exact location of the *srnB* gene because the test used is a functional test. To be functional, each gene that determines an RNA or protein product requires not only its own structural integrity but also a particular association with an RNA polymerase promoter sequence. Thus, it is possible that the *srnB* gene lies in either the 0- to 1.7-kb or the 2.8- to 4.0-kb segment, whereas its promoter lies between 1.7 and 2.8 kb.

In making this analysis, we observed a difference in phenotype determined by the original F*lac*$^+$ plasmid in strain 200PS and the derivative F*lac*$^+$ plasmid in the JC1553 genetic background. The latter F*lac*$^+$ plasmid was used for

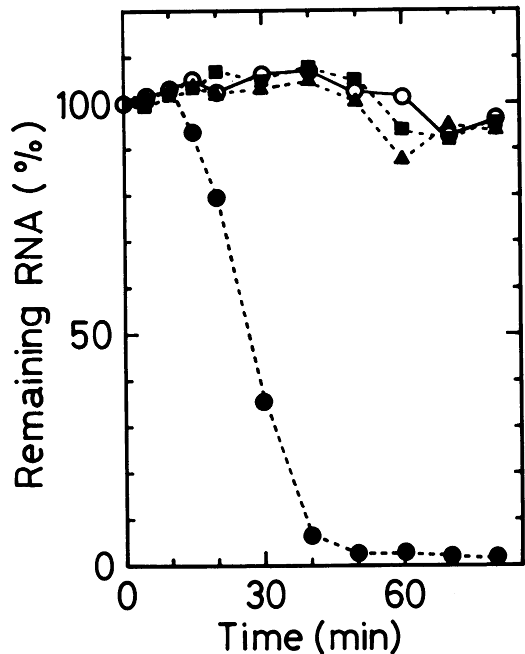

FIG. 1. *Stability of prelabeled RNA of strains YS12, JC5293, JC5294, and JC5295 after treatment with rifampin at 42°C. Stable RNA of cells was labeled with 0.3 μCi of [³H]uridine per ml (26 Ci/mmol; The Radiochemical Centre, Amersham, England) for 3 h at 30°C in the minimal salts-glucose medium containing required nutrients and Casamino Acids. The cultures were then maintained at 42°C and shaken for 60 min. After addition of 500 μg of rifampin per ml, 0.2-ml portions of cells were precipitated and the remaining RNA was assayed as in Materials and Methods. One hundred percent is about 20,000 cpm. Solid line shows the F⁻ strain YS12 (○), and dashed lines show the strains that have the plasmids; JC5293 (▲), JC5294 (■), and JC5295 (●) have plasmids pSC101, pRS31, and pRS7, respectively.*

TABLE 6. *Stability of stable RNA in srnA strains carrying pRS plasmids*

Strain[a]	Plasmid	Map length of F fragment (kb)	Remaining RNA (%)	Presence of $srnB^+$ gene
YS12			90.2	−
YS11	F⁺	0–94.5	17.7	+
JC5293	pSC101		90.0	−
JC5294	pRS31	82.3–1.7	89.7	−
JC5295	pRS7	82.3–4.0	2.7	+
JC5296	pRS2	4.0–9.3	89.2	−
JC5297	pRS26	68.1–4.0	3.8	+
JC5298	pRS8	69.3–9.3	3.8	+
JC5230	pRS15	69.3–25.9	36.5	+

[a] Strains from JC5293 to JC5230 are Tet^r transformants of strain YS12 and for the transformation plasmid DNA are isolated from strains JC10101, JC10127, JC10118, JC10115, JC10123, JC10119, and JC10121 (3, 23).

extensive heteroduplex mapping experiments, which established a deletion of 0 to 2.8 kb on the map of F (22). Since this plasmid is functionally *srnB* by our tests, whereas its presumed ancestor from 200PS is $srnB^+$ (17), we propose that the difference has resulted from a mutation subsequent to excision of F*lac*$^+$ from the original Hfr strain. Heteroduplex mapping techniques may be required to test this proposal.

The role played by the plasmid-carried *srnB* gene in RNA instability is interesting to speculate about. If its effect were demonstrable only on rRNA, then $srnB^+$ might produce an RNA element that binds to ribosomes, rendering their RNA more susceptible to ribonuclease (RNase) digestion. Alternatively, $srnB^+$ might produce a protease that degrades (perhaps highly selectively) ribosomal proteins, thereby rendering rRNA more susceptible to RNase digestion. Alternatively; *srnB* might produce an RNase as previously suggested (16, 17). Recent results (H. Iguma, T. Yamamoto, F. Imamoto, and Y. Ohnishi, unpublished data) support this latter proposal by showing that $srnB^+$ leads to degradation of *trp* mRNA, whose origin is at the P_L promoter of lambda *trp* phage.

Since the *srnB* gene is plasmid carried, we may speculate that it adds a marginal degree of survival or adaptability to cells that carry it by possibly increasing breakdown of RNA components under conditions of nutritional stress. It is unclear whether or not $srnB^+$ affects RNA turnover during normal growth. The conditions we have used to detect the presence of $srnB^+$ are extreme in that we have inhibited RNA synthesis with an antibiotic. In order for $srnB^+$ to increase survival under normal conditions, even marginally, we would expect it, for example, to decrease the half-life of rRNA under conditions of growth or partial starvation. Experiments on the turnover of RNA, as distinguished from its degradation, have not yet been done.

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