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F Factor Promotes Turnover of Stable RNA in Escherichia coli

Abstract. *Male bacteria that contain an* srnA− *mutant allele degrade their "stable" RNA massively after RNA synthesis is blocked at 42°C; a normal F− female strain shows no such RNA breakdown unless both the* srnA− *allele and maleness (F factor) are introduced.*

In some conditions ribosomal RNA (rRNA) and transfer RNA (tRNA) are extensively degraded, but they are usually called "stable" because unknown regulatory mechanisms protect them against degradation in growing cells (*1, 2*). To try to clarify the mechanism of bulk RNA turnover, Ohnishi and Schlessinger isolated a mutant that grows normally at 30° or 42°C, but very rapidly degrades more than 80 percent of its rRNA and tRNA at 42°C after RNA synthesis is stopped (*2*). The *srn*− mutant was genetically analyzed, and the results suggested that two loci are involved in degradation of stable RNA in the mutant (*3*). One of them, *srnA*−, was closely linked to the *tsx*+ gene and mapped at about 10 minutes on the Taylor and Trotter map (*3, 4*). The other locus, *srnB*− or *srnB*+, was not completely mapped, but was probably in the region between 75 and 90 minutes (*3*). I now report that the *srnB* allele is associated with the F factor that produces maleness, and that a female strain can become *srn*− by the successive introduction of the *srnA*− allele and the F factor.

The original *srn*− mutant was a derivative of the male strain Hfr. All of the *srn*− strains obtained after conjugations and transductions were also found to be male strains, and in the course of mapping of *srnB*+, its site was always close to that of the integrated F DNA. Since an F− *srn*− strain never was found, three possibilities seemed open for the specification of the *srn*− phenotype: (i) *srnA*− and *srnB*− are necessary for the *srn*− phenotype, and *srnB*− is closely linked to integrated F DNA; (ii) *srnA*−, *srnB*−, and F DNA are all necessary, and *srnB*− is closely linked to integrated F DNA; (iii) *srnA*− and F DNA are necessary, and the F DNA bears one of the genes required for the extensive breakdown of stable RNA. In other words, alternative (iii) is that *srnB* is an F-specified or -regulated gene.

One of the lines of evidence suggesting the third possibility is shown in Table 1. The parent of the mutant, strain GP1 (*3*), already contains F DNA and, if it is thereby *srnB*+, should be able to donate it to recipients that contain *srnA*−, to produce strains capable of massive RNA breakdown. Consistent with this notion, not only were *srn*− recombinants always male, but the parental strain GP1 *srnA*+ was able to donate the *srn*− phenotype to a recipient F− *srnA*− strain (phenotypically *srn*+) after uninterrupted mating (Table 1). Thus the parental strain GP1 truly has one of the genetic loci required for the degradation of stable RNA.

Stronger evidence for the correlation of F factor and the capacity of strains for RNA breakdown was then obtained. Many Hfr strains show a less extreme, partial breakdown (*3*), and I checked to see whether F+ or F′ strains show a similar phenotype. Strains YS357 F+ *srn*+ (*5*) and W2241 F+ *srn*+ (*6*) did indeed show partial breakdown of stable RNA after treatment with rifampicin at 42°C, but not at 30°C (Fig. 1, a and c). When the F factor of strain YS357 or W2241 was put into the strain YS142 F− *srnA*− (which is phenotypically *srn*+), the strain showed the massive breakdown of stable RNA characteristic of the *srn*− phenotype (Fig. 1, b and c). The frequency of *srn*− and F factor donation to the F− *srnA*− strain was the same: 49 of 70 recipient clones.

Table 1. Conjugation of strain GP1 Hfr H (*srn*+) × YS142 F− *pyr*− *str*r *srnA*− (*srn*+) to select *pyr*+ *str*r recombinants.

Markers		Recombinants (No.)
srn	Sex	
−	M	16
−	F	0
+	M	15
+	F	19

Since the recipient strain YS142 was a recombinant after uninterrupted conjugation between strain YS105 Hfr H *srn*− and AB7N F− *srn*+ [table 5 in (*3*)], it was still possible that more than two mutant loci were involved in specifying the observed degradation of stable RNA. Another F− *srnA*− recipient therefore was constructed by crossing strain V64S Hfr H *srn*− (*2, 3*) with AT2535 F− *his*−*str*r*srn*+ (*3*) at 37°C for 80 minutes to permit selection of *his*+*str*r recombinants which contained *srnA*−; these were still phenotypically *srn*+. Typical was the strain YS31 F− *srnA*−: it showed complete stability of stable RNA, but the stable RNA broke down massively after an F factor was introduced from strain YS357 (Fig. 1d).

Proof that some feature of the F factor itself gives the *srn*− phenotype was obtained by introducing (or curing) various F factors in a YS31 F− *srnA*− (phenotypically *srn*+). When F *lac*+, F8, or KLF 12 (*7*) were introduced, all of the F′ recipients checked (4, 3, and 4 F ductants, respectively) showed massive degradation of stable RNA in the test condition (Fig. 1e). But when the newly isolated strains YS31/F *lac*+ were spontaneously cured of F *lac*+, the resultant derivatives showed no degradation of stable RNA (Fig. 1e). Therefore, the F factor is required for the *srn*− phenotype.

To show that only two loci are required for the *srn*− phenotype, I transferred both F factor and *srnA*− allele to an indifferent wild-type female starting strain, LC607 F− *proC*− *srnA*+ (phenotypically *srn*+) (*3*). First, the

F factor of strain YS357 was transferred to the strain LC607, and it was designated strain YS607 (F+ *proC*− *srnA*+). Next, phage P1kc was grown on strain YS31 F− *srnA*−(*srn*+), and used to transduce *proC*+ into YS607 [*proC*+ is located very close to *srnA*− (*3*)]. In two independent experiments, 18 of 70 transductants (approximately 25 percent) and 21 of 70 transductants (30 percent) showed the *srn*− phenotype (Fig. 1f). These frequencies of cotransduction of *srnA*− allele with *proC*+ allele are completely compatible with previous data [27.3 percent; table 6 in (*3*)]. I infer that the *srn*− phenotype results from the combined presence of the F factor and the *srnA*− allele.

Involvement of the F factor in degradation of stable RNA was unexpected, but lysogenic conversion or phage conversion often confer specific properties on the host (*8*). Lysogenic phages and sex factors all belong to the class of episomes or plasmids, and a term like "episome conversion" or "plasmid conversion" would cover all these types of phenomena.

The gene or genes on the F factor required for degradation of stable RNA (*srnB*+) must be located in a region other than those between 0 and 3 thousand bases, and 8.5 thousand and 15.8 thousand bases, and probably also outside the *traJ* gene of the F factor. This can be inferred because F *lac*+ and F8 both could donate the *srn*− phenotype to an F− *srnA*− (*srn*+) strain, even though they delete those small regions (*9*); also the surface-exclusion negative Hfr strain, which is *traJ*− (*10*), had the same capacity.

There are two possible mechanisms that might protect stable RNA against degradation. The first of these supposes that active ribonuclease (or ribonucleases) is compartmentalized (that is, that there is an inactive or latent ribonuclease). The second possible scheme requires the existence of a protein factor (or factors) which would bind RNA, protecting it (perhaps by a change in conformation) and resulting in stabilization of stable RNA.

A special location or conformation of RNA vis-à-vis ribonuclease is consistent with the earlier results that showed that drugs that interfere with ribosome function in polysomes block degradation (*2*); that the massive turnover in the mutant extends to tRNA, but is selective for RNA, with no effect on protein turnover (*2*); that ribosomes seem morphologically normal in mutant cells sectioned after massive breakdown begins (*11*); and that total intactness of rRNA is not necessary for ribosome function (*12*). However, it is not yet clear which of *srnB*+ or *srnA*− promote nuclease activity or substrate accessibility, or how. Alternative models could be evaluated by reproducing the degradation of stable RNA in vitro and purifying the ribonucleases or proteins involved.

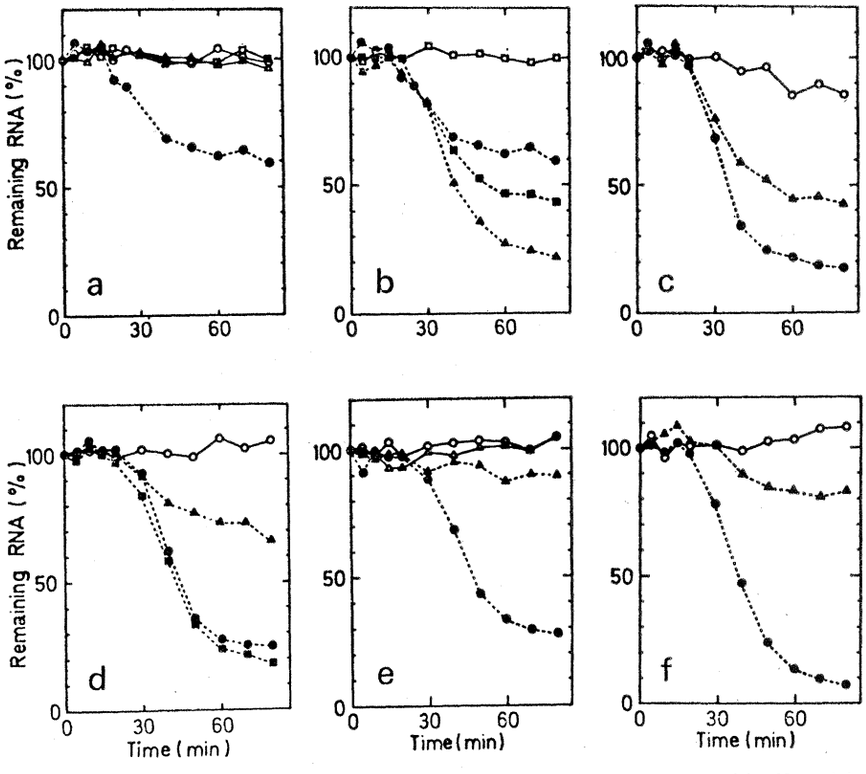

Fig. 1. Fate of labeled RNA of various kinds of strains after treatment with rifampicin at 42°C. Stable RNA of cells was labeled with [^{3}H]guanine (0.3 μc/ml; 16.6 c/mmole) or [^{3}H]uracil (18 c/mmole) for about 3 hours at 30°C in minimal salts glucose medium (*3*) containing required amino acids, purine, pyrimidine (50 μg/ml, each) or thiamine (5 μg/ml). The cultures were then maintained at 42°C, except one culture in (a), and shaken for 60 minutes. After addition of rifampicin (200 μg/ml), 0.2-ml portions of cells were precipitated in ice-cold 5 percent trichloroacetic acid at the indicated times, and the radioactivity in the precipitate was counted in a liquid scintillation spectrometer. The number of counts in the DNA was subtracted from the number in the acid-insoluble portion to give the values of RNA. Solid lines show the F− strains, except one culture at 30°C in (a); dashed lines show the strains which have F DNA. (a) ○, YS357 F+ at 30°C; △ and □, YS357 F− (F+ was cured from strain YS357 F+); and ●, YS357 F+. (b) □, YS142 F−; ●, YS357 F+; ■ and ▲, YS142 F+ (F factor from strain YS357 F+). (c) ○, YS142 F−; ▲, W2241 F+; ●, YS142 F+ (F factor from strain W2241 F+). (d) ○, YS31 F−; ▲, YS357 F+; ● and ■, YS31 F+ (F factor from strain YS357). (e) ○, YS31 F−; △, YS31 F− (F′ was cured from YS31/F *lac*+); ▲, 200PS/F *lac*+; ●, YS31/F *lac*+ (F′ factor from strain 200PS/F *lac*+). (f) ○, YS31 F− *srnA*−; ▲, YS607 F+ *srnA*+; ●, YS607 F+ *srnA*−.

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