GENETIC ANALYSIS OF AN *ESCHERICHIA COLI* MUTANT WITH A LESION IN STABLE RNA TURNOVER

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ABSTRACT

A mutant that rapidly degrades more than 80% of its rRNA and tRNA under defined conditions was genetically analyzed. Two genes, *srnA* and *srnB*, are separately located, and the mutated alleles of both are required for degradation of stable RNA in cultures treated with rifampicin at 42°. *srnA* is closely linked to *tsx* by matings and transduction tests; by P1 transduction, the gene order is *lac* (9 min) *proC* (9.55 min) *tsx* (9.8 min) *srnA* (about 10 min) *purE* (12 min) *rnsA* (14.4 min). *srnB* is not yet completely mapped, but is outside the *lac-rnsA* region, probably in the region between 75 and 90 min.—The product of the *rns*A gene, RNase I, is a potent endonuclease of *E. coli*, and the only one known that can attack ribosomes and tRNA. However, not only are the *srn* lesions genetically separate from *rnsA*, but also, derivatives of an *srn* strain were prepared lacking RNase I, and they retain the Srn⁻ phenotype. Thus, no correlation of rapid RNA turnover and RNase I activity has been found.

ALTHOUGH ribosomal RNA and transfer RNA are very stable in growing *E. coli* cells (HERSHEY 1954; SIMINOVITCH and GRAHAM 1956; DAVERN and MESELSON 1960; NEIDHARDT 1964), they are degraded gradually and to a limited extent in a variety of non-growing states. These include starvation for phosphate (HORIUCHI, HORIUCHI and MIZUNO 1959), magnesium (MCCARTHY 1962; NATORI, NOZAWA and MIZUNO 1966), potassium (ENNIS and LUBIN 1965), or nitrogen (MANDELSTAM 1960; BEN-HAMIDA and SCHLESSINGER 1966), or incubation at high temperature (NOZAWA, HORIUCHI and MIZUNO 1967). These findings made it reasonable that cells would have regulatory mechanisms to stabilize bulk RNA against degradation, and recently a mutant was indeed isolated that grows normally at 30° or 42°, but very rapidly degrades more than 80% of rRNA and tRNA at 42° after RNA synthesis is stopped (OHNISHI and SCHLESSINGER 1972). It is tentatively called an *srn* (*s*table *RN*A) mutant.

The *srn* mutant, strain V64S, provides a potential tool to look at the genetic control of bulk RNA turnover. Here, an initial genetic analysis of this strain and correlative *E. coli* K12 strains is reported. In particular, two lesions responsible for the phenotype have been mapped, especially with respect to the *rnsA* gene. The *rnsA* gene is of special interest because its product, RNase I, is the only known nuclease with extensive activity against ribosomes and tRNA. The results show that there are at least two gene loci involved in regulation, one of which is

TABLE 1

Bacterial strains used

Strain	Markers*	Source and/or reference
AB312	Hfr *thr leu lacZ str thi*	ADELBERG (TAYLOR and ADELBERG 1960)
AT2243	Hfr C *pyrE metB*	TAYLOR via BACHMAN (1972)
AT2535	F⁻ *tsx his purF str argH pyrB*	TAYLOR via BACHMAN
AT2538	F⁻ *thr leu proA lacY galK his str pyrE argE thi*	TAYLOR via BACHMAN
BE110	Hfr C	OTSUJI (OHNISHI 1971)
D10	Hfr H *rnsA met* (λ)	GESTELAND (1966)
GP1	Hfr H *ilv cya metE thi*	YOKOTA and GOTS (1970)
Hfr H57	Hfr H *thi*	.....
HI404	Hfr *lac met*	Product of mating JC12 × AB311 IEHARA and OTSUJI
KG20	F⁻ *pro his argH ampA purA*	NORDSTRÖM, ERIKKSON-GRENNBERG and BOMAN (1968) via BACHMAN
LC607	F⁻ *leu lacZ proC purE trp str metE*	NISHIMURA *et al.* (1971) via MATSUBARA
V64S	Hfr H *thi* λ⁻ *srnA srnB*	OHNISHI and SCHLESSINGER (1972)
AB7N	F⁻ *lacZ* or *lacY tsx metE nal*	Nal^r from AB2067
LC65N	Sex⁻ *his nal str pyrE srnA srnB*	Product of mating Hfr H57 × YD54
LH32	Hfr H *lacZ proC purE str*	Product of mating Hfr H57 × LC607
YD53	Hfr H *srnA srnB rnsA*	RNase I⁻ mutant isolated from YS105
YD54	Sex⁻ *srnA srnB*	Sex⁻ mutant isolated from YS105
YS105	Hfr H *thr leu his str pyrE srnA srnB*	Product of mating V64S × AT2538
YS154	Sex⁻ *purE his nal srnB*	.....
YS273	Sex⁻ *lacZ proC tsx purE his str nal srnB*	Product of mating YSH327 × YS6027
YS275	Sex⁻ *lacZ proC tsx his str nal srnB*	Product of mating YSH327 × YS6027
YS2714	Sex⁻ *lacZ proC tsx str nal srnB*	Product of mating YSH327 × YS6027
YS6027	Sex⁻ *thr his nal str srnA srnB*	Product of mating AB312 × LC65N
YSH327	Hfr H *lacZ proC tsx purE str*	Tsx^r mutant spontaneously isolated from LH32

* Genetic symbols are those used by TAYLOR and TROTTER (1972).

closely linked to *tsx*. No functional or genetical correlation between *srn* and *rnsA* genes, or RNase I activity, was found.

MATERIALS AND METHODS

Bacteria: The bacterial strains used were all derivatives of *Escherichia coli* K-12. They are listed in Table 1. Only relevant markers are shown under genotype. Mutagenesis was performed by the procedure of ADELBERG, MANDEL and CHEN (1965).

Bacteriophages: Male-specific phages fd and R17, and female-specific phages W31 and T7, were used to test for the presence in strains of the sex factor. Phage T6 was also used to determine the *tsx* character. Phage P1kc was used for transduction.

Media: Nutrient broth, complete medium and M3 medium have already been described (OHNISHI 1971; OHNISHI *et al.* 1972). For genetic studies, required amino acids, purine and pyrimidine (50 μg/ml, respectively) were added to M3 medium.

Mating: Mating and strain construction were performed by the procedure of MILLER (1972).

Transduction: Transduction by phage P1kc was performed according to LENNOX (1955).

Scoring of Srn⁻ phenotype: An overnight culture of each recombinant or transductant was

diluted 1:50 into 1 ml of M3 medium containing each required amino acid or base and 0.1–0.6 μCi of ^{3}H-uracil (18 Ci/m mole) or ^{3}H-guanine (16.6 Ci/m mole). The culture was shaken at 30° for 3 hours, and then shifted up to 42° with shaking for 60 min. 200 μg/ml rifampicin were then added (t=0). A 0.2–0.4-ml portion of the culture was immediately taken into cold 8% trichloroacetic acid, and 60 min later the same volume of the culture was again sampled. The relative stability of stable RNA was expressed as the ratio of acid-insoluble counts at 60 min and 0 min. A strain which shows a ratio of less than 0.45 is scored as *srn*. In this paper *srn*+ represents the scoring for *srnA*+ *srnB*+, *srnA*+ *srnB*, or *srnA srnB*+; and *srn* implies the genotype *srnA srnB* (see RESULTS AND DISCUSSION).

Preparation of cell extracts for RNase I enzyme assays: Each culture of strains D10, YD53 or YS105 was grown at 30° to an optical density at 420 nm of 0.8–0.9. The cells were harvested and washed with 10mM tris-HCl buffer, pH 7.5. The cells were opened by cavitation with sonic oscillations from a Biosonick (Bronwill Scientific) instrument 3 times for 20 sec at 20-sec intervals. The extract was centrifuged for 30 min at 30,000 × g, and the supernatant fraction (S30) used as the test source of RNase I enzyme. Protein estimation was by the method of LOWRY *et al.* (1951).

Assay of RNase I activity: Assay B of GESTELAND (1966) was modified by substitution of ^{3}H-polyadenylic acid (1.94 A_{260} units, 5,300 cpm) for *E. coli* tRNA. Incubations were for 40 min at 37°. The clean supernatant fluid after ethanol precipitation was counted by the procedure of SPAHR and HOLLINGWORTH (1961).

RESULTS

Mapping trials with srn *strains:* Genetic analysis of the *Srn*$^-$ phenotype should help in understanding the mechanisms of degradation of stable RNA. In a preliminary study, the degree of breakdown of stable RNA in a variety of *E. coli* strains was tested under comparable conditions described in MATERIALS AND METHODS. No strain comparable to strain V64S was found (that *srn* strain shows a test ratio of less than 0.45). However, other strains fell into at least two classes: some (especially many F$^-$ strains) showed a ratio of 0.9 to 1.0; others (mostly Hfr strains) showed partial breakdown (a ratio of 0.6 to 0.8), similar to that observed in strain GP1, the parental strain of V64S (Figure 1). These results already suggested that several loci might be involved in determining the extent of stable RNA turnover. Also, although the Srn$^-$ phenotype cannot be directly selected for, and must be tested in liquid culture, the tests are quite reproducible. The quantitation of phenotypic classes (Srn$^-$ and Srn$^+$) is thus cumbersome but straightforward.

Mapping trials were carried out both by matings, with or without interruption, and by P1 transduction. The *srn* mutant as isolated was a derivative of Hfr Hayes, which begins to inject DNA clockwise from a point about 88 min on the Taylor map (TAYLOR and TROTTER 1972). Preliminary crosses of the Hfr H *srn* strain with a well-marked F$^-$ strain (AT2538 *thr leu proA lac gal his str pyrE argE*) indicated that at least one allele required for the Srn$^-$ phenotype was located very late on the map, in the region of 75–90 min. For example, among six recombinants of each class of *thr*$^+$*leu*$^+$*str*, *proA*$^+$*lac*$^+$*str*, *gal*$^+$*his*$^+$*str*, *gal*$^+$*str*, *his*$^+$*str*, *thr*$^+$*leu*$^+$*proA*$^+$*lac*$^+$*gal*$^+$*str*, *pyrE*$^+$*str* and *argE*$^+$*str*, only one of the *argE*$^+$*str* recombinants was *srn*. This recombinant, YS105, was used as a donor in further experiments.

As expected for late marker entry, a cross of strain YS105 Hfr H *srn* with strain

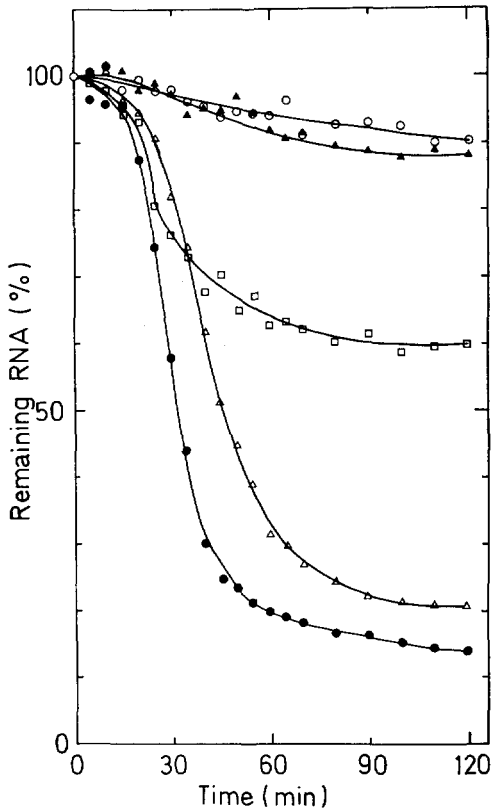

FIGURE 1.—Fate of prelabelled RNA of strains D10, GP1, YD53 and YS105 after treatment with rifampicin at 30° and 42°.

Stable RNA of cells was labelled with 2.5 μ Ci of ^{3}H-guanine (16.6 Ci/m mole) for about 3 hours at 30° in M3 medium containing 50 μg/ml uracil. The cultures were then shifted up to 42° except the another culture of strain YS105 (○) and shaken for 60 min. After adding 200 μg/ml rifampicin, 0.2 ml portions of cells were precipitated in ice-cold 5% trichloroacetic acid at the indicated times, and the precipitate was counted in a liquid scintillation spectrometer. Counts in DNA are substracted from the acid-insoluble c.p.m. to give the values of RNA. 100% is 6,000 c.p.m. ○, YS105 at 30°; ▲, D10 at 42°; △, YD53 at 42°; ●, YS105 at 42°; □, GP1 at 42°.

AB7N F$^-$ *trp nal* for more than 60 min produced *trp*$^+$*nal* recombinants; but among 72, no *srn* recombinant was found. Instead, when *argE*$^+$*str* recombinants were selected, 7 of 98 were *srn*. Also, all seven *srn* recombinants were male, even though 70 of the 98 recombinants were female. Again this suggested that a late marker (*srnB*, see below) was required for the Srn$^-$ phenotype. The yield of *srn* recombinants increased to 21% in a cross in which even later markers were selected (*pyrB*, 85 min and *purA*, 82.5 min; Table 2). Again all the *srn* recombinants were male strains.

Two genes required for Srn$^-$ phenotype: Assuming that expression of the Srn$^-$ phenotype requires two separate mutations in different genes, loss of one of these mutations should give a wild-type phenotype like that of strain GP1. To explore

TABLE 2

Conjugation of YS105 (Hfr H thr leu pyrE srn) × *AT2535 (F⁻* argH pyrB) and *YS105* × *KG20 (F⁻* argH purA) *at 37° for 3 hours**

Recombinant types	YS105 × AT2535		YS105 × KG20		Total no. of recombinants
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	
srn Hfr	10	4	6	5	25
srn F⁻	0	0	0	0	0
srn+ Hfr	8	21	19	19	67
srn+ F⁻	12	5	5	6	28
Total no.	30	30	30	30	120

* Recombinants selected: *pyr*+ *thr*+ *leu*+ in Exp. 1; *pyr*+ *arg*+ *thr*+ *leu*+ in Exp. 2; *pur*+ *thr*+ *leu*+ in Exp. 3; *pur*+ *arg*+ *thr*+ *leu*+ in Exp. 4.

this possibility, an *srn* strain was required that would be phenotypically F⁻. Thus a strain which is resistant to male-specific phages, surface exclusion negative (Sex⁻; ACHTMAN 1973) but still *srn*, was isolated from the Hfr H *srn* strain after mutagenesis by nitrosoguanidine. *his*+*str* recombinants were then selected by mating Hfr H *srnA*+ *srnB*+ strain with this Sex⁻ *srnA srnB* strain for 90 min (Table 3). Although the mating was interrupted after 90 min in this experiment, entry of the donor DNA was apparently very slow, because all recombinants had a recipient marker *pyrE*. Since 24 of 112 recombinants were *srn*+ in this cross, *srnA* might be located between the starting point for DNA injection in Hfr H and *his*, but not closely linked to the *his* region. This indicates that in addition to the late marker (*srnB*), *srnA* is required for the Srn⁻ phenotype.

To check the linkage of the *srnA* gene to other genes, the frequency of unselected markers in 8 *srn* recombinants was measured (Table 4). *thr* is most closely linked to *srn*, but this is probably due to linkage to *srnB*. Another marker closely linked to *srn* is *lac*. Therefore *lac*+*metE*+*nal* recombinants were selected to obtain a high frequency of *srn* recombinants and unselected markers were scored. Table 5 shows that *srn* recombinants were now 78% of the total and that all of them had *tsx*+ and male characters. Also no *tsx*+ female or *tsx*⁻ male recombinants showed the Srn⁻ phenotype. This implies that *srnA* is probably closely linked to *tsx*, and *srnB* to the Hfr H region.

TABLE 3

Interrupted mating of strain Hfr H57 and YD54 Sex⁻ thr leu his str pyrE srnA srnB

Recombinants selected	Recombinant types				No. of recombinants
	thr	*leu*	*srn*	*pyrE*	
	+	+	+	—	17
	+	+	—	—	5
his+ *str*	+	—	—	—	4
	—	—	+	—	7
	—	—	—	—	79
Total no					112

TABLE 4

Genetic characters of srn *recombinants in the cross of V64S Hfr H* srnA srnB and *AT2538 F*⁻ thr leu proA lac gal his str pyrE argE*

Recombinants	Markers and the map positions (min)†								
	thr 0	*leu* 1.5	*proA* 6.5	*lac* 9	*gal* 17	*his* 38.5	*pyrE* 72	*argE* 78.5	F
YS105	0	0	1	1	1	0	0	1	1
VA9	1	0	0	1	1	0	0	1	1
VA36	1	0	0	1	0	0	0	1	1
VA40	1	0	0	1	0	0	0	1	1
VA41	1	1	0	1	0	0	0	1	1
VA63	1	0	1	1	0	0	0	1	1
VA64	1	0	0	1	0	0	0	1	1
VA77	1	0	0	0	0	0	0	1	1

* Recombinants selected: *argE*+ *str*.
† The alleles of markers derived from the donor are represented by 1, and those derived from the recipient, by 0.

Support for the existence of the *srnA* gene was obtained by interrupted mating of an Hfr C (strain BE110) and a Sex⁻ *srnA srnB thr leu str* strain (YD54). 77% of the *leu*+*str* recombinants or *thr*+*str* recombinants were converted to Srn+ phenotype after interrupted matings for 20 min and 25 min, respectively. Since the *rnsA* gene for RNase I is not transferred early by Hfr C (REINER 1969), *srnA* cannot be *rnsA*.

To permit finer mapping of gene *srnA*, a strain with the markers *lac proC tsx srnA*+ *srnB* was constructed by interrupted mating of strain YSH327 Hfr H *lacZ proC tsx* × strain YS6027 Sex⁻ *thr nal srnA srnB*. Transductions were then carried out by using phage P1kc grown on strain YS105 *srnA srnB*. The recipient strains were YS273, YS275 and YS2714, which have the markers *lacZ proC tsx srnB*. *proC*+ transductants were selected, and were analyzed for cotransducibility of *lac*+, *tsx*+ and *srnA*. Table 6 shows that *srnA*, compared to *proC*, is located on the far side of *lac* (column A), linked closely to *tsx* and more distal

TABLE 5

Conjugation of strain YS105 (*Hfr H* thr leu his str pyrE srnA srnB) × *AB7N* (*F*⁻ proA lac tsx gal trp his nal str pyr metE thi) *to select* lac+ nal metE+ *recombinants*

Recombinants selected	Recombinants types*			No. of recombinants
	tsx	sex	*srn*	
	s	m	—	77
	s	m	+	1
lac+ *nal metE*+	s	f	+	6
	r	m	+	10
	r	f	+	5
Total no				99

* s, r, m and f mean sensitive, resistant, male and female, respectively.

TABLE 6

Transductions of strain YS273, YS275 and YS2714 which have the markers lacZ proC tsx srnB *with phage P1kc grown on strain YS105* srnA srnB *and selection of* proC+ *transductants*

	Classes of unselected markers	Recipient strains YS273	YS275	YS2714	Total no.	Percent
A	*lac*+ *srn*	0	3	0	3	1.3
	lac srn	24	20	18	62	26.0
	lac+ *srn*+	14	8	11	33	13.9
	lac srn+	62	39	39	140	58.8
	Total no	100	70	68	238	100.0
B	*tsx*+ *srn*	22	19	18	59	24.8
	tsx srn	2	4	0	6	2.5
	tsx+ *srn*+	18	8	23	49	20.6
	tsx srn+	58	39	27	124	52.1
	Total no	100	70	68	238	100.0
C	*lac*+ *tsx*+ *srn*	0	2	0	2	0.8
	lac+ *tsx*+	1	3	6	10	4.2
	lac+	14	11	11	36	15.2
	tsx+	40	27	41	108	45.4
	srn	24	23	18	65	27.3

than *tsx* (column B). Since one of 120 *purE*+ transductants was *srn* in the transduction of strain YS154 *purE srnB* with phage P1kc grown on strain YS105 *srnA srnB*, the order of genes is *lac* (9 min) *proC* (9.55 min) *tsx* (9.8 min) *srnA purE* (12 min) *rnsA* (14.4), and the *srnA* locus is at about 10 min on the Taylor map (Table 6, column C).

Lack of correlation between degradation of rRNA and tRNA and activity of RNase I: The genetic analysis already indicated that neither *srn* gene was identical with *rnsA*, the gene for RNase I. Among the RNases discovered in *E. coli* (BARNARD 1969), RNase I is a most reasonable candidate for degradation of rRNA and tRNA to acid-soluble products. One direct test for its involvement can be carried out by isolating RNase I⁻ derivatives from a *srn* mutant. This is possible according to the procedure of GESTELAND (1966). Such a derivative was made, and the specific activity of RNase I and stability of stable RNA were assayed (Figure 1). The *srn* mutant has a wild-type allele for *rnsA*, which is the structural gene for RNase I; its activity is about 9.37 μmole/h/mg protein in the standard assay system. Strain D10, which was isolated as an RNase I⁻ mutant by GESTELAND (1966), has an RNase I activity less than 0.04 μmole/h/mg. The RNase I⁻ *srn* mutant degrades stable RNA like the parental RNase I+ *srn* strain, but has only residual RNase I activity (0.04 μmole/h/mg). One might therefore suggest that the degradation of stable RNA in an *srn* mutant is not mainly attributable to RNase I (see DISCUSSION).

DISCUSSION

The genetic analysis thus far strongly indicates that at least two loci, *srnA* and *srnB*, are involved in the regulation of stable RNA breakdown in *E. coli*. *srnA*

is closely linked to *tsx*; *srnB* is located much later on the Coli map, and shows a strong linkage with maleness. Until further mapping is carried out, this correlation cannot be further analyzed, but it is conceivable that *srnB* is itself directly allied to maleness, perhaps because of a feature of surface structure in male strains that affects the accessibility of RNA to nuclease action.

In partial extension of the correlation of *srnB* and maleness, I have noted in tests like those of Figure 1 that a number of Hfr strains, including strains GP1, Hfr H57, AB312, AT2243 and HI404, showed a ratio of 0.6 to 0.8 for the relative stability of RNA, while F^- strains all fell in the range 0.9 to 1.0. However, the correlation is not perfect; strain BE110, for example, showed a relatively high value (0.8–0.9). Thus, it is still possible, for example, that *srnA*, *srnB* and maleness are all required—or that the linkage of *srnB* and maleness is simply close. Also, it is possible that total breakdown in the strain *srnA srnB* might be caused by summation of partial breakdowns respectively due to *srnA* and *srnB*.

The initial biochemical characterization of the Srn^- phenotype in relation to RNase I and the rnsA gene is disconcerting. Perhaps the low levels of RNase I still in the *srn* strain may be adequate for breakdown of stable RNA. For example, severe reductions of the enzymes DNA polymerase I and RNase III in strains *polA* and AB105, respectively, permit fair residual function of the enzymes (OKAZAKI, ARISAWA and SUGINO 1971; NIKOLAEV, SILENGO and SCHLESSINGER 1973), even with amber mutations in the *polA* gene. Further biochemical and genetic analysis, for example of deletions of the *rnsA* gene, may give a decisive answer. Should RNase I not be involved in the observed massive degradation of stable RNA, an uncharacterized nuclease(s) or unknown potent form of a characterized one must be involved.

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