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Interaction between mutations of ribosomes and RNA polymerase: A pair of *strA* and *rif* mutants individually temperature-insensitive but temperature-sensitive in combination

(RNA nucleotidyltransferase/temperature-sensitive mutant/*strA*–*rif* interaction)

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ABSTRACT A temperature-sensitive lethal mutant of *Escherichia coli* has been constructed by combining two temperature-insensitive mutations: a *rif180* mutation that modifies RNA polymerase (RNA nucleotidyltransferase; nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) and a *strA24* mutation that modifies the ribosomal protein S12. The temperature sensitivity is a property of the combination of these two particular alleles; replacement of either of the alleles relieves the temperature sensitivity. An isogenic strain containing a different *strA* mutation (i.e., *rif180 strA11)* is not temperature sensitive. Evidently ribosomes modified by the particular *strA24* mutation are not compatible for growth at 42° with an RNA polymerase altered by the *rif180* mutation, which suggests that *in vivo* there may exist some interaction between structures of ribosomes and the RNA polymerase.

RNA polymerase (RNA nucleotidyltransferase; nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) and the ribosome carry out the two distinct steps, transcription and translation, in the expression of genetic information. The *rif* locus on the chromosome of *Escherichia coli* contains the genes for heavier subunits of RNA polymerase (1–3, 21). Mutants of the *rif* locus are easily recognized by their resistance to rifampicin (4). Several genes for ribosomal proteins have been identified in the *rif* region (5, 6). Many of the ribosomal genes are clustered around the *strA* locus (7, 8); also the gene for the α-subunit of the RNA polymerase has been located in the *strA* region (9). The *strA* gene determines the structure of the ribosomal protein S12; this gene also determines the response of *E. coli* to the drug streptomycin (10–12). The enzyme and the organelle appear to function independently of each other, and it is not known at the present time whether the intermingling of genes of the RNA polymerase and the ribosome is of any specific advantage to the cell.

In a previous report, we have shown that in male strains of *E. coli strA*-mediated permissiveness to bacteriophage T7 may be reversed by some *rif* mutations. The *strA*-mediated permissiveness to T7 and its reversal by specific *rif* alleles could be correlated, respectively, with enhanced and reduced transcription of the T7 genome (13). Thus, although the RNA polymerase and the ribosome carry out different steps in gene expression, it appeared that mutations affecting their structures were interacting somehow.

We are interested in exploring the interaction between *strA* and *rif* mutations. It was known that mutations at the *rif* locus sometimes have a temperature-sensitive effect on growth of the cell, either because the mutated subunits of RNA polymerase may fail to assemble in mature enzymes or because the RNA polymerase containing the mutated subunit is inactive at 42° (14–16). Our approach was to see if the temperature sensitivity caused by a *rif* mutation could also be modified by *strA* alleles. In the course of this attempt, we found that there exists a class of temperature-insensitive *rif* mutation that becomes temperature-sensitive in the presence of a specific temperature-insensitive *strA* mutation in the cell. In this paper we describe the isolation and preliminary characterization of such a temperature-sensitive *strA*–*rif* double mutant, the temperature sensitivity of which depends on the simultaneous presence of both of the *strA* and *rif* alleles in the cell. Removal of either of the alleles from such a double mutant relieves the temperature sensitivity of the strain. Analysis of this temperature-sensitive *rif*–*strA* double mutant suggests that the architecture of the ribosome may have a significant influence on the functioning of the RNA polymerase *in vivo*.

MATERIALS AND METHODS

Bacteria. The strains of bacteria and their derivations are described in Table 1. The isolation of the mutants and the properties of *strA11* and *strA24* alleles have been described previously (13, 17). Minimal medium A (18) was routinely supplemented with 0.2% glucose. L medium (19) was used as rich medium. Streptomycin and rifampicin were used at final concentrations of 500 μg/ml and 100 μg/ml, respectively.

Transduction was mediated by phage P1 according to the procedure of Lennox (19). After transduction, a delayed selection for streptomycin resistance was used (11). For the transduction of *rif* alleles, the recipient strains contained an *argH* mutation; the *rif* transductants were then screened among arg^+ transductants.

All *rif* mutants were spontaneous and were obtained as described before (13). Mapping experiments show that the rifampicin-resistance mutation described in this paper is located in the *rif* locus (20).

Turbidity of the cultures was measured as OD_{490} (Lumetron).

Synthesis of RNA. Synthesis of stable RNA in the culture of bacteria was indicated by the continued accumulation of [2-^{124}C]uracil into trichloroacetic-acid-insoluble precipitate over an extended period of time. The bacteria were pregrown at 30° in medium A containing proline (50 μg/ml) and casamino acids (500 μg/ml). They were then subcultured in the same medium and a portion was shifted to 42°. At the start of the experiment (t = 0 hr) each culture (5 ml) also received 0.5 ml of [2-^{14}C]uracil (specific activity 2.5 μCi/mg). At intervals thereafter, 0.5 ml of culture was withdrawn and added to 2 ml of cold 5% tri-

Abbreviations: Rif^R, rifampicin-resistant; Sm^R, streptomycin-resistant; Arg$^+$, arginine-independent.

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Table 1. List of strains

Strain	Growth at 42°	Source/derivation
F⁻EC-O	+	By curing the episome in EC-O, which is F'_{TS114}-lac^+/(*lac-pro*)$^\nabla$ $sulI^+$ $strA^+$, vitamin-B1-requiring (13)
F⁻EC-O *strA11*	+	Transduction of *strA11* from C1-1 (17)
F⁻EC-O *strA24*	+	Spontaneous streptomycin-resistant mutant of EC-O and subsequent curing of the episome (13)
F⁻EC-O *argH⁻*	+	R. Ludwig
F⁻EC-O *argH⁻ strA11*	+	Transduction of *strA11* from F⁻EC-O *strA11* to F⁻EC-O *argH⁻*
F⁻EC-O *argH⁻ strA24*	+	Transduction of *strA24* from F⁻EC-O *strA24* to F⁻EC-O *argH⁻*
F⁻EC-O *rif180*	+	Spontaneous from F⁻EC-O (see *text*)
F⁻EC-O *rif180 strA11*	+	Transduction of *strA11* to F⁻EC-O *rif180*
F⁻EC-O *rif180 strA24*	−	Transduction of *strA24* to F⁻EC-O *rif180*

All strains are derived from F⁻EC-O: F⁻(*lac-pro*)$^\nabla$ $sulI^+$ $strA^+$ rif^+.

chloroacetic acid. The acid-insoluble precipitate was filtered and dried and its radioactivity was measured in a liquid scintillation counter. From such an experiment, the rate of synthesis of stable RNA during a 30-min pulse was determined as Δ^{14}C-cpm/30 min; Δcpm indicates the amount of [2-^{14}C]uracil accumulated in the macromolecules during the 30-min period prior to withdrawal of the sample.

The rate of synthesis of total RNA was also obtained by determining the amount of [2-^{14}C]uracil incorporated into acid-insoluble form during a shorter pulse of 3 min. The bacteria were pregrown at 30°, subcultured in medium A containing proline (50 μg/ml) and casamino acids (500 μg/ml) and then transferred to 42°. At different times thereafter, 0.5 ml aliquots were withdrawn, added to tubes containing 0.25 μCi of [2-^{14}C]uracil, and maintained at 42°. After 3 min, incorporation of radioactivity was stopped with 2 ml of cold 5% trichloroacetic acid. The samples were stored on ice, filtered, and dried, and the radioactivity was measured in a liquid scintillation counter.

Rate of Synthesis of Proteins. The rate of protein synthesis was measured by determining the amount of radioactive amino acids incorporated into acid-insoluble form during a short pulse. The bacteria were pregrown at 30° and subcultured in medium A containing proline (100 μg/ml). At different times thereafter, 0.5 ml aliquots were withdrawn and added to tubes containing 0.25 μCi of ^{3}H-labeled amino acids maintained at 42°. After 3 min, incorporation of radioactivity was stopped with 2 ml of cold 5% trichloroacetic acid. The samples were filtered and dried, and radioactivity in the precipitate was determined in a liquid scintillation counter.

RESULTS

Temperature-sensitive *rif* mutations defective in the function or assembly of RNA polymerase have been described (14, 15). We attempted to see if the temperature sensitivity caused by a *rif* mutation might be modified by any *strA* mutation. Therefore, a large number of spontaneous rifampicin-resistant (Rif^R) derivatives were obtained at 30° from the wild-type strain F⁻EC-O [F⁻(*lac-pro*)$^\nabla$ $strA^+$ rif^+] and these Rif^R derivatives were screened for growth at 42°. Out of 300 Rif^R derivatives of F⁻EC-O, none was found to be temperature-sensitive for growth at 42°. However, using the same procedure, we did obtain temperature-sensitive Rif^R derivatives quite frequently from isogenic strains containing two different *strA* alleles. Also, we noticed that the temperature-sensitive *rif* mutant strains described earlier already contained *strA* mutations (14, 15). We then tested whether any temperature-insensitive *rif* mutation would cause a strain to become temperature-sensitive in the presence of a suitable *strA* mutation. The following procedure was adopted: (*i*) 50 different temperature-insensitive Rif^R derivatives of F⁻EC-O were grown at 30°. (*ii*) The 50 cultures were then mixed together and the mixed culture, now containing 50 different *rif* alleles, was transduced with a desired *strA* donor. Streptomycin-resistant (Sm^R) transductants were selected at 30°. In the experiments described here either the *strA24* allele or the *strA11* allele was transduced in separate crosses. The isogenic strains F⁻EC-O *strA24* and F⁻EC-O *strA11* were used as donors. (*iii*) 200 Sm^R transductant colonies from each cross were screened for growth on L plates at 42°.

The experiment was repeated three times with different sets of Rif^R derivatives. The *strA* allele would be randomly transduced to the different Rif^R mutants present in the mixture of recipients. Both the donor $strA^-$ rif^+ and the recipient $strA^+$ rif^- strains grow normally at 42°. Hence, if any *strA* transductant from the above cross should fail to grow at 42°, it might be concluded that the transduced *strA* allele in association with the resident *rif* allele of the recipient generated the temperature-sensitive phenotype.

When the *strA24* allele was transduced in the above experiment, a number of the transductants (e.g., 30 of 200) failed to grow on nutrient broth plates at 42°. Therefore, the temperature-insensitive *strA24* allele in combination with several temperature-insensitive *rif* mutations produced a temperature-sensitive phenotype. F⁻EC-O *rif180 strA24* used later for further characterization is one of these 30 temperature-sensitive colonies (see below).

In a separate experiment, the *strA11* allele was transduced to a mixture of the same 50 *rif* mutants. All of the 200 *strA11* transductants from this cross grew well at 42°.

Transduction analyses with F⁻EC-O *rif180 strA24* (Table 2)

First, wild-type F⁻EC-O $strA^+$ rif^+ was used as donor, the temperature-sensitive *rif180 strA24* was the recipient and temperature-insensitive (ts$^+$) transductants growing at 42° were selected. On screening, these transductants were found to have either of two phenotypes: 59 out of 82 transductants tested were rifampicin-resistant and streptomycin-sensitive (genotype presumably *rif180* $strA^+$); the remaining 23 out of 82 transductants were rifampicin-sensitive and streptomycin-resistant (genotype presumably rif^+ *strA24*). No transductant that survived the high-temperature selection was found to be resistant to both drugs. Therefore, the temperature sensitivity of the *rif180 strA24* strain is a property of the combination of *rif180* and *strA24* alleles and not a property of either of the alleles alone.

F⁻EC-O *rif180* $strA^+$, obtained as a rifampicin-resistant streptomycin-sensitive transductant from the above, was used

Table 2. Transduction analysis

Donor × Recipient	Phenotype selected at 30°	Frequency of temperature sensitivity
1. *strA11 rif*$^+$ × *strA*$^+$ *rif180*	Sm^R	0/124
2. *strA24 rif*$^+$ × *strA*$^+$ *rif180*	Sm^R	54/54
3. *strA*$^+$ *rif180* × *argH*$^-$ *strA11 rif*$^+$	Arg$^+$ Rif^R *	0/96
4. *strA*$^+$ *rif180* × *argH*$^-$ *strA24 rif*$^+$	Arg$^+$ Rif^R *	50/50

Sm^R, streptomycin resistant; Arg$^+$, arginine-independent; Rif^R, rifampicin resistant.

* The recipients in transductions 3 and 4 were F$^-$EC-O *argH*$^-$ *strA11* and F$^-$EC-O *argH*$^-$ *strA24*, respectively. Arg$^+$ transductants were selected initially. Approximately 55% of the Arg$^+$ transductants were also Rif^R. Arg$^+$ Rif^R transductants thus obtained were screened for temperature-sensitive phenotype. No temperature-sensitive colony was found among Arg$^+$ rifampicin-sensitive transductants from either cross.

as the recipient in transductions 1 and 2 and was used as donor in transductions 3 and 4 (see Table 2). All the *rif180 strA24* transductants were temperature-sensitive (transductions 2 and 4) but none of the *rif180 strA11* transductants were temperature-sensitive (transductions 1 and 3). In control crosses the *strA11*, *strA24*, and *rif180* alleles were transduced to the *strA*$^+$ *rif*$^+$ recipient. All of the *strA11 rif*$^+$, *strA24 rif*$^+$, and *strA*$^+$ *rif180* transductants were temperature-insensitive. Apparently, the property of the *rif180* is influenced by the *strA24* allele and not by the *strA11* allele. Thus, a given change in the structure of RNA polymerase responds differently to specific changes in the *strA* allele.

In a separate experiment spontaneous streptomycin-resistant derivatives, obtained from F$^-$EC-O *rif180* at 30°, were screened for growth at 42°. Three out of 12 streptomycin-resistant derivatives from F$^-$EC-O *rif180* were temperature-sensitive, whereas none of the 65 streptomycin-resistant derivatives of F$^-$EC-O were temperature sensitive. Thus temperature sensitivity seems to occur much more frequently among *strA* mutants in the presence of the *rif180* allele.

Characterization of F$^-$EC-O *rif180 strA24*

Some preliminary experiments have been aimed at understanding the nature of temperature sensitivity generated by the combination of *rif180* and *strA24* mutations. At 42°, cultures of F$^-$EC-O *rif180 strA*$^+$ and F$^-$EC-O *rif*$^+$ *strA24* grow exponentially as expected (data not shown). In contrast, as is shown in Fig. 1, the double mutant F$^-$EC-O *rif180 strA24* grew linearly for several hours when exposed to 42°, like other temperature-sensitive RNA polymerase mutants (14). During the residual growth, the number of viable cells in the culture of F$^-$EC-O *rif180 strA24* decreased exponentially with time. Thus, exposure to 42° was lethal although some growth continued.

Why did the presence of the *strA24* allele cause temperature sensitivity in the strain containing an RNA polymerase affected by the *rif180* mutation but not in a strain containing the wild-type RNA polymerase (Table 1)? One possible mechanism could be that the *strA24* allele influences in a damaging way the synthesis of RNA by the *rif180* RNA polymerase. To examine this possibility, we measured the synthesis of stable RNA in the three strains *rif180 strA*$^+$, *rif*$^+$ *strA24*, and *rif180 strA24* during growth at 42°. Fig. 2 shows that the accumulation of stable RNA in the *rif180 strA*$^+$ and *rif*$^+$ *strA24* cultures kept pace with increase in cell mass; but in the *rif180 strA24* culture synthesis of stable RNA gradually slowed.

The linear increase of turbidity suggested that the culture of *rif180 strA24* continued to synthesize proteins after being shifted to 42°. This is confirmed by the data in Fig. 3. At 42°, the rate of protein synthesis remained fairly constant for at least 4 hr. This also indicates that the amount of messenger RNA is not affected for some time by exposure to the higher temper-

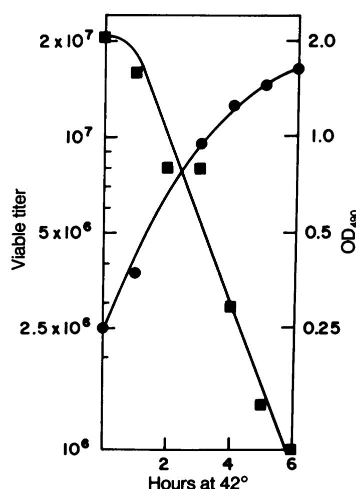

FIG. 1. Growth (●) and viable titer (■) of F$^-$EC-O *rif180 strA24* at 42°. Bacteria were pregrown at 30° in L medium, and subcultured in the same medium at t = 0 hr and transferred to 42°. Turbidity was measured at 490 nm (Lumetron). Viable titer was determined by spreading a suitably diluted aliquot on L agar and counting the number of colonies after incubating the plates at 30°.

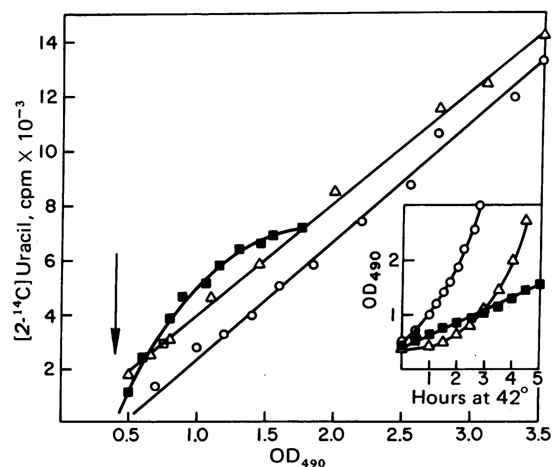

FIG. 2. Growth and synthesis of stable RNA in cultures of F$^-$EC-O *rif180 strA*$^+$ (Δ), F$^-$EC-O *rif*$^+$ *strA24* (○), and F$^-$EC-O *rif180 strA24* (■) at 42°. The strains were pregrown and subcultured in minimal medium supplemented with casamino acids. At OD_{490} = 0.4 indicated by the arrow, the samples were transferred to the same medium at 42° and [2-^{14}C]uracil was added to each. At different times thereafter OD_{490} was monitored and the amount of radioactivity incorporated into macromolecules was determined. The inset shows the growth of the three strains at 42°.

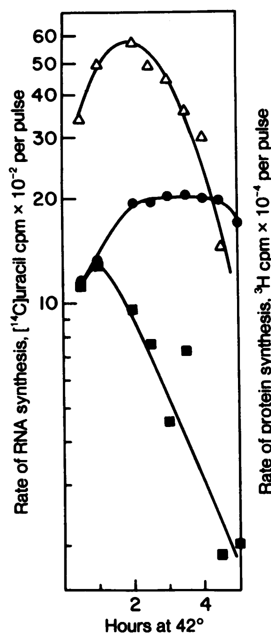

FIG. 3. Rate of protein synthesis (●) and rates of RNA synthesis during a 30 min pulse (■) and during a 3 min pulse (Δ) in a culture of F⁻EC-O *rif180 strA24* at 42°. The data for the rate of RNA synthesis during the 30 min pulse were obtained by a transformation of the data presented in Fig. 2 (see also *Materials and Methods*).

ature. Fig. 3 also shows the rates of RNA synthesis in *rif180 strA24* during a long pulse (30 min) and during a shorter pulse (3 min). RNA synthesis observed during a long pulse essentially represents the synthesis of stable RNA. It is apparent, therefore, that at 42°, the rate of synthesis of stable RNA in the *rif180 strA24* double mutant continues to decrease exponentially. The same phenomenon is observed if the rate of synthesis of total RNA is assayed during the shorter pulse (3 min). In other words, the synthesis of stable RNA is affected earlier and more severely than the synthesis or level of messenger RNA after the *rif180 strA24* strain is exposed to the restrictive temperature.

DISCUSSION

We have shown that a pair of *strA* and *rif* mutations together can produce a new phenotype: temperature sensitivity at 42°. Not all the temperature-insensitive *rif* alleles are identical in this respect, for, when combined with a suitable *strA* allele like *strA24*, only certain *rif* mutations produced temperature-sensitive transductants. *strA* alleles have been previously classified with respect to their capacity to restrict suppression of nonsense mutation (22). We have shown before that the *strA11* and *strA24* alleles may be distinguished by their ability to confer permissiveness to bacteriophage T7 in male strains of *E. coli* (13). The result presented here shows that *strA* alleles may also be distinguished by their behavior towards suitable *rif* alleles.

At the restrictive temperature, the strain *rif180 strA24* continues to synthesize proteins but progressively stops synthesis of stable RNA. This suggests that the transcription process is affected more directly than the translation step. We have noted earlier that in male strains of *E. coli* the *strA24* allele influences the transcription of T7 genome (13). The result presented here shows that the ability of the *strA24* allele to influence transcription may be extended to that of the bacterial genome if the RNA polymerase is suitably sensitized by a mutation like *rif180*.

The data show that some kind of a compatibility is necessary between the *strA* and *rif* alleles even in normally growing *E. coli*. This may imply the existence of an interaction between structures of ribosomes and the RNA polymerase and also indicates, perhaps, that the optimal synthesis of stable RNA is crucially dependent on the cooperation between the RNA polymerase and the ribosome of an appropriate configuration. We have not yet investigated any detailed mechanism of this phenomenon but at least two hypotheses are possible: (*a*) It may be that, at 42°, *strA24* ribosomes overproduce chemicals like guanosine tetraphosphate and that the *rif180* RNA polymerase is hypersensitive to such compounds (23). Thus, the "stringent" effect on stable RNA synthesis would be simulated in spite of the continued protein synthesis in the *strA24 rif180* strain. However, one might then expect quick shut-off of the synthesis of stable RNA rather than the observed progressive decay of the rate, which is typical of the first-order decay of an activity (Fig. 3). Also it would not explain the lethal effect of high temperature. (*b*) Ribosomes may be mechanically "coupled" with RNA polymerase for optimum transcription. The altered configurations of *rif180* RNA polymerase and *strA24* ribosome at 42° may not match each other for effective "coupling" to occur. Such an idea is consistent with the observations that, *in vitro*, the transcription of bacteriophage T4 DNA is stimulated by the presence of the ribosome and ribosomal proteins (24–26) even under conditions in which translation does not take place. Also, a study of streptomycin-dependent strains points in this direction (27). On the basis of the present genetic data we suggest that "coupling" may exist and requires a compatibility between the structures of ribosomes and RNA polymerase.

This paper is dedicated to the memory of Luigi Gorini. S.L.C. deeply mourns the loss of Luigi—a warm friend and a truly great human being.

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