Phenotypic Suppression by Streptomycin of Amber Mutants in the Ribonucleic Acid Bacteriophage Coat Protein Cistron

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After mutagenesis with nitrosoguanidine or ultraviolet light, 298 streptomycin high-resistant and 98 streptomycin high-dependent mutants were isolated from HfrC Su⁻. They were tested for their ability to phenotypically suppress five different amber ribonucleic acid (RNA) bacteriophage mutants in the presence of streptomycin. The phage mutants are all in the coat protein, which is 129 amino acids long; the uracil-adenine-guanine codons were at the following positions: *sus3* and *amb2*, 6; *amb11*, 50; *amb21*, 54; *sus11*, 70. Only *sus3* and *amb2* could be phenotypically suppressed by streptomycin; this was clearly demonstrated in nine mutant strains, seven *str*-HR and two *str*-HD. The suppression was always dependent upon added streptomycin and was dose-dependent in all cases. None of the mutants showed measurable suppression in absence of the drug. Among revertants to streptomycin independence from streptomycin-dependent strains that could show phenotypic suppression, most of those that were still resistant to streptomycin (10 μ g or more) retained the capacity to show phenotypic suppression; whereas among those revertants sensitive to 10 μ g of streptomycin or less, none retained the capacity. Eight different amber polar mutants (strong and weak) in gene 34 of phage T4 were also tested for pleiotypic suppression by streptomycin in all the streptomycin-resistant and -dependent strains isolated. No suppression was found in any of the 396 strains tested.

Since the pioneering work of Gorini and Kataja (5), many studies of phenotypic suppression by streptomycin were carried out (6, 18). The most systematic work showed that not all amber mutations could be phenotypically suppressed by streptomycin. We decided to set up a system to find out what could account for the range of alleles suppressed. For this purpose, well-characterized ribonucleic acid (RNA) bacteriophages were chosen as the material for study. We tested RNA phage mutants at positions 6, 50, 54, and 70 of the coat protein. Only the amber mutants at position 6 were phenotypically suppressed by streptomycin. These findings are discussed in terms of streptomycin action in the cell and in terms of mechanisms of suppression.

MATERIALS AND METHODS

Bacteria, strains and phages. Bacterial strains K10, S26RIE, and S26RID are SU⁻, SUI⁺, and SUII⁺, respectively. (For nomenclature, see reference 9). The RNA phages employed are listed in Table 1. T4 phage amber mutants at gene 34 were supplied by A. Nakata (14).

Mutagenesis. Mutagenesis with nitrosoguanidine was carried out according to the method of Clark and Margulies (3). When ultraviolet (UV) light was the mutagenic agent, mutants were isolated from the survivors after 80% of the cells had been killed by irradiation from a 15-w germicidal lamp.

Media. Media were prepared as described by Gussin (7), with the modification that CaCl₂ was used at a final concentration of 5×10^{-3} M.

Drugs. The *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (nitrosoguanidine) was purchased from K & K Laboratories (Jamaica, N.Y.). Dihydrostreptomycin was from Takeda Pharmaceutical Co. (Japan).

RNA phage plaque assays. The plaque assays were performed according to the method of Gussin (7), except that 5×10^{-3} M CaCl₂ rather than 2×10^{-3} M was added to the soft agar. Streptomycin-resistant strains were grown in absence of streptomycin, whereas streptomycin-dependent strains were grown in the presence of 400 μ g of streptomycin per ml. When streptomycin was used, the bottom layer contained the final streptomycin concentration tested; an equivalent amount per milliliter was added simultaneously to the soft agar, host strain, and bacteriophage.

RESULTS

Streptomycin high-resistant (*str*-HR) and streptomycin high-dependent (*str*-HD) strains

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TABLE 1. *RNA phages used*

Mutant	Parental phage	Amber site at coat cistron	Polarity
<i>sus</i> 3 ^a	f2	6	+
<i>sus</i> 11 ^a	f2	70	—
<i>amb</i> 2 ^b	R17	6	+
<i>amb</i> 11 ^b	R17	50	—
<i>amb</i> 21 ^c	R17	54	—

^a Supplied by N. D. Zinder (10).^b Supplied by G. N. Gussin (7).^c Supplied by J. Tooze (16).

were isolated from K10 HfrC which carries no amber suppressor. (For nomenclature, *see* reference 1) After nitrosoguanidine mutagenesis, 248 *str*-HR strains and 98 *str*-HD strains (all independent isolates) were obtained; another 50 *str*-HR strains were isolated after treatment with UV irradiation. They all were tested for plating efficiency of the various amber mutants (Table 1) in the absence and in the presence of streptomycin. Streptomycin was added at a final concentration of 500 to 1,000 μ g/ml.

Only seven *str*-HR and two *str*-HD mutants, all isolated after treatment with nitrosoguanidine, suppressed *amb*2 and *sus*3 mutants in the presence of streptomycin. The plating efficiencies obtained with all five amber mutants from two *str*-HR strains and one *str*-HD strain are listed in Table 2. Furthermore, no mutants that could suppress genotypically in the absence of streptomycin were obtained.

The efficiency of phenotypic expression by streptomycin is a function of its concentration (Fig. 1). At first it would appear from the shape of the response curve that some critical site is being saturated with streptomycin, but the curves are often complex (Fig. 1, 2) and, therefore, permit no simple interpretation.

Since among the amber RNA phage mutants only the polar mutants *sus*3 and *amb*2 were phenotypically suppressed, we wanted to find out whether phenotypic suppression by streptomycin is a property correlated with polar mutants. We tested phenotypic suppression by streptomycin of six strong polar mutants of T4 bacteriophage, (B25, A454, B265, N58, A459, B285) and two weak polar mutants (B258, A455) all in gene 34 (14). None of the 396 bacterial mutant strains tested showed any degree of suppression of any amber codons in gene 34 in the presence or in the absence of streptomycin.

We also examined whether the capacity to sup-

FIG. 1. Effect of streptomycin on the suppression of *amb*2 in two *str*-HR strains. Phage *amb*2 was titrated in presence of various levels of streptomycin (as indicated) in two *str*-HR strains, SK314 and SK200. The values given were normalized against those obtained for the standard suppressor strain S26R1E (*SU*⁺*I*).

TABLE 2. *Plating efficiency of amber mutants on str-HR and str-HD strains^a*

Strain tested	Streptomycin phenotype	Amber <i>SU</i>	<i>Sm</i> ^b	<i>amb</i> 2	<i>sus</i> 3	<i>amb</i> 11	<i>amb</i> 21	<i>sus</i> 11	R17
S26R1E	Str-S	<i>SU</i> I ⁺	—	1	1	1	1	1	1
K10	Str-S	<i>SU</i> [—]	—	5×10^{-4}	2×10^{-3}	3×10^{-4}	2×10^{-3}	7×10^{-3}	1
SK152	Str-HD	<i>SU</i> [—]	+	0.5 ^c	0.3 ^c	3×10^{-4}	2×10^{-3}	8×10^{-3}	0.4
SK532	Str-HR	<i>SU</i> [—]	—	4×10^{-4}	1×10^{-3}	3×10^{-4}	2×10^{-3}	7×10^{-3}	0.8
			+	2×10^{-2c}	1×10^{-2c}	2×10^{-4}	9×10^{-4}	6×10^{-3}	0.7
SK314	Str-HR	<i>SU</i> [—]	—	8×10^{-4}	2×10^{-3}	6×10^{-4}	2×10^{-3}	7×10^{-3}	0.4
			+	0.1 ^c	6×10^{-2c}	7×10^{-4}	1×10^{-3}	1×10^{-2}	0.5

^a Values for plating efficiency were obtained by plating phages in the bacterial strains and normalizing the ratio of plaques on each indicator strain to those on S26R1E (*SU*⁺*I*).

^b Symbols: +, streptomycin added (1,000 μ g/ml); —, no streptomycin added.

^c Plaques are smaller than those on the S26R1E strain.

press phenotypically is retained in revertants of *str*-HD strains that had this capacity. Streptomycin-independent revertants from one of the permissive *str*-HD strains (SK152) were isolated after treatment with nitrosoguanidine by plating cells on streptomycin-free plates. Among the revertants, two subgroups were tested for phenotypic suppression of RNA phage amber mutants. One group included clones which could resist 20 to 50 μ g of streptomycin per ml; the other was comprised of those sensitive to 10 μ g/ml. Among 34 members tested from the first group, all but one could still phenotypically suppress; whereas Of 15 tested members of the second group, none showed phenotypic suppression. Again, all the five amber RNA phage mutants were tested, but only the two polar mutants (*sus3* and *amb2*) could be phenotypically suppressed.

The dose response of phenotypic suppression to streptomycin was tested in two strains, the parental strain SK152 (*str*-HD) and one of its streptomycin-independent derivatives, no. 29. The suppression in the streptomycin-independent derivative is efficient even at low levels of streptomycin (Fig. 2; cf. Fig. 1, 2).

DISCUSSION

Phenotypic, streptomycin-mediated suppression of well-characterized coat amber mutants of

RNA phages occurred only with *sus3* and *amb2*, those carrying the mutation site at position 6. Other RNA phage mutants at position 50, 54, and 70 were not suppressed.

To understand the apparent allele specificity of streptomycin-mediated suppression, more work is required; the experiments are feasible since the system developed here is susceptible to considerable detailed study. At present, at least some alternative explanations can be listed. (i) Perhaps the simplest possibility is that streptomycin does insert amino acids at the amber codons of the other mutants, but that the coat protein produced cannot function and accumulates in a non-functional form. Since the preparation of coat protein and the sequencing of its amino acids has been accomplished (17), it should be possible to determine in the other, nonsuppressed phage mutants, which amino acids, if any, are inserted in the coat protein. (ii) It is also possible that streptomycin fails to suppress mutations at later locations in the gene. Streptomycin might modify the ribosome more easily when it is located near an initiator codon. Some evidence suggests that streptomycin has a selective effect at or near initiation of protein synthesis (11). More complicated suggestions can also be made, based on "antisuppressive" effects of some codons neighboring the amber codons.

The failure of amber mutants in T4 phage gene 34 to be suppressed at all by streptomycin raises another curious problem, since streptomycin-mediated suppression of some T4 mutants has been observed (8, 15). Since only one of four mutant sites in the RNA phage coat protein could be repaired, it is possible that all eight T4 mutants in gene 34 tested are again members of a very fastidious class of alleles. However, since phage-directed protein synthesis is rather resistant to streptomycin action in whole cells (4), it is also possible that streptomycin simply cannot enter some strains of *Escherichia coli* that are infected with T4 phage.

It is of interest that the phenotypic suppression of the RNA phage mutants required high levels of drug in *Str*-HR or *Str*-HD mutants, whereas lower levels were sufficient in many revertants from high dependence. One possibility is that the parental *str*-HD SK152 (Fig. 2) could phenotypically suppress even at low levels of streptomycin; however, since it cannot grow at such low levels, the potential is not realized. The other more interesting possibility is that the second mutations introduced into strain SK152 have a dual effect: they could abolish the requirement for streptomycin and increase the flexibility of the ribosome, permitting the ribosome to respond to lower levels of streptomycin in producing miscoding. If the original mutation to streptomycin dependence is

FIG. 2. Effect of streptomycin on the suppression of five amber mutants of RNA phages by the *str*-HD strain, SK152, and one of its streptomycin-independent derivatives. The efficiency of plating of phages R17, *amb2*, *amb11*, *amb21*, *sus3*, and *sus11* on strain SK152 *str*-HD and its derivative no. 29 was tested in the presence of various levels of streptomycin; an amount of 400 μ g of streptomycin per ml was the lowest level tested for streptomycin-mediated phenotypic suppression in strain SK152, since this strain would not grow well in these media at lower levels of streptomycin.

intact and the mutation to streptomycin independence falls in a second ribosomal gene, this could be another example of the interactions between ribosomal elements discussed in other publications (1, 2, 9, 12, 13; Apirion et al., *J. Mol. Biol.*, *in press*).

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