NEW SUPPRESSORS OF FRAMESHIFT MUTATIONS IN *SALMONELLA TYPHIMURIUM*

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Manuscript received February 24, 1982
Revised copy accepted September 4, 1982

ABSTRACT

Several new types of suppressor mutants have been isolated. These were identified among revertants of mutants originally generated by mutagens other than the acridine-derived ICR191. The new suppressors correct mutations other than those with runs of C or G which are recognized by the previously described suppressors. Several frameshift mutations are corrected by more than one suppressor type. Apparently, the DNA base sequence near these mutant sites includes sites of action for several distinct suppressor types.

FRAMESHIFT mutations are caused by the addition or removal of bases from a coding sequence such that the frame of reference of translation is disturbed. Such mutations can be corrected by secondary compensating frameshift mutations near the original site (CRICK *et al.* 1961; STREISINGER *et al.* 1966). Certain frameshift mutations can also be corrected by unlinked informational suppressors. These suppressor mutations affect tRNA structure, so as to allow occasional reading of an abnormal number of bases and rephasing of translation (RIDDLE and ROTH 1972; RIDDLE and CARBON 1973). All of the originally described suppressible mutations are +1 mutations in runs of G:C pairs in the DNA. The suppressible mutations fall into two distinct classes, those of the CCCC/U type (suppressed by *sufA, B, C*) and those of the GGGG type (suppressed by *sufD, E, F*) (reviewed by ROTH 1974). The reason for the apparent specificity of suppressors for runs of bases is not clear. It may reflect the fact that the original mutations were all induced by the mutagen ICR191 which is specific for causing +1 and −1 mutations in runs of G:C pairs in the DNA. To obtain novel sorts of frameshift suppressors, we have tested the suppressibility of frameshift mutations induced by a variety of mutagens other than ICR191. From the revertants of these mutants a series of new frameshift suppressors have been obtained.

MATERIALS AND METHODS

Strains used are listed in Table 1; all are derived from *Salmonella typhimurium* strain LT2. Conditions of culture and methods of mutant identification and manipulation have been described previously (RIDDLE and ROTH 1972; KOHNO and ROTH 1974, 1978). Methods for scoring suppressor activity are described in the legends to Tables 2 and 3. All other methods are described in the accompanying paper (BOSSI, KOHNO and ROTH 1982).

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TABLE 1

List of strains[a]

Strain	Genotype	Source
TT522	*his-644 zee-1*::Tn*10*	Lab collection
TR712	*hisO1242 hisF2439*	RIDDLE and ROTH 1970
TR725	*hisO1242 hisF3704*	RIDDLE and ROTH 1970
TR767	*hisO1242 hisD3018*	RIDDLE and ROTH 1970
TR935	*hisO1242 hisD3018 sufB1*	RIDDLE and ROTH 1970
TR947	*hisO1242 hisC3072*	RIDDLE and ROTH 1970
TR964	*hisO1242 hisC3732*	RIDDLE and ROTH 1970
TR966	*hisO1242 hisC3734*	RIDDLE and ROTH 1970
TR1034	*hisO1242 hisD3749*	RIDDLE and ROTH 1970
TR1041	*hisO1242 hisF3041*	RIDDLE and ROTH 1970
TR1430	*hisO1242 hisF2118 suf-30*	RIDDLE and ROTH 1970
TR1435	*hisO1242 hisC3072 sufE35*	RIDDLE and ROTH 1970
TR1441	*hisO1242 hisC3746 sufD41*	RIDDLE and ROTH 1970
TR1457	*hisO1242 hisD3749 sufA6*	RIDDLE and ROTH 1970
TR1713	*hisO1242 hisD3068*	RIDDLE and ROTH 1970
TR2644	*hisO1242 hisD6448 sufH90*	This paper
TR2645	*hisO1242 hisF6527 sufI91*	This paper
TR2675	*hisO1242 hisB6575 sufG70*	This paper
TR2682	*hisO1242 hisB6575*	KOHNO and ROTH 1974
TR2683	*hisO1242 hisF6574*	KOHNO and ROTH 1974
TR2684	*hisO1242 hisD6448*	KOHNO and ROTH 1974
TR2685	*hisO1242 hisF6527*	KOHNO and ROTH 1974
TR2703	*hisO1242 hisB6480*	KOHNO and ROTH 1974
TR2705	*hisO1242 hisD6580*	KOHNO and ROTH 1974
TR2707	*hisO1242 hisC6581*	KOHNO and ROTH 1974
TT2842	*his-644 zee-1*::Tn*10 sufA6*	This paper
TT2846	*his-644 zee-1*::Tn*10 sufD41*	This paper
TT2849	*his-644 zee-1*::Tn*10 sufG70*	This paper
TT2887	*his-644 zee-1*::Tn*10 sufJ128 hisT1529*	This paper
TT2890	*his-644 zee-1*::Tn*10 sufJ128*	This paper
TR3023	*hisO1242 hisC2259*	RIDDLE and ROTH 1970
TR3138	*hisT1504 hisG6608*	J. MCCANN and B. AMES This paper
TR3139	*hisT1504 hisG6609*	J. MCCANN AND B. AMES
TR3144	*aroD5 hisT1529 hisG6609 hisO1242*	This paper
TR3146	*aroD5 hisT1529 hisG6608 hisO1242*	This paper
TR3242	*hisO1242 hisD6610*	E. YAMASAKI and B. AMES
TR3265	*hisT1504 hisG6608 sufJ101*	This paper
TR3791	*hisO1242 hisD6610*	T. KOHNO
TR3794	*hisO1242 hisD6610 sufM95*	This paper
TR6241	*hisA2770*	Lab collection
TR6242	*hisD3040*	OESCHGER and HARTMAN 1970
TR6243	*hisD2780*	Lab collection
TR6244	*hisD3068*	OESCHGER and HARTMAN 1970
TR6245	*hisG3037*	OESCHGER and HARTMAN 1970
TR6246	*hisO1242 hisD3794*	D. L. RIDDLE

[a] All strains are derived from *S. typhimurium* strain LT2.

TABLE 2

Cross-suppression pattern of new frameshift suppressors[a]

Mutation	Origin of mutation	Reversion pattern		Suppression by previously described frameshift suppressors				Suppression by new frameshift suppressors				
		ICR	NG	*sufA*	*sufB*	*sufD*	*sufE*	*sufG70*	*sufH90*	*sufI91*	*sufJ101*	*sufM95*
hisD3018 (CCCU, +1)	ICR	+	+	+	+	−	−	−	−	−	+	+
hisD3749 (CCCU, +1)	ICR	+	+	+	+	−	−	−	−	−	−	−
hisD3068 (GGGG, +1)	ICR	+	+	−	−	+	+	−	−	−	−	−
hisB6480	Proflavin	+	−	+	−	−	−	+	−	−	−	−
hisB6575	Spont.	+	−	+	+	−	−	+	−	−	−	−
hisC6581	Spont.	+	−	+	−	−	−	+	−	−	+	−
hisD6580	Spont.	+	−	−	−	−	−	+	−	−	+	−
hisF6574	Spont.	−	−	−	−	−	−	+	−	−	−	−
hisD6448	Proflavin	−	−	−	−	−	−	−	+	−	−	−
hisF6527	Proflavin	+	−	−	−	−	−	−	−	+	−	−
hisG6608	Mitomycin-C	+	+	−	−	−	−	−	−	−	±	−
hisG6609	Mitomycin-C	+	+	−	−	−	−	−	−	−	±	−
hisD6610	9-amino acridine	+	+	+	+	−	−	−	−	−	−	+

[a] In describing reversion patterns of the mutations used, the + sign signifies that the mutagen in the heading induces His^+ revertants. In testing cross-suppression, transducing phage grown on each suppressor-carrying strain was used to transduce the *his* frameshift mutants selecting His^+ recombinants. Transductants that owed their His^+ phenotype to inheritance of the donor suppressor were identified by their colony morphology as described previously (RIDDLE and ROTH 1970). In the columns describing suppression, the + sign indicates the presence of colonies after 36 to 42 hr incubation at 37°. The − sign indicates that no colonies were seen after the same period. A positive (+) response in this table requires a suppressor efficiency of at least 1%. Plates were rescored after 5 days' incubation. In most cases no changes in the original response were noted. The only exceptions to this were mutations *hisG6608* and *hisG6609* which show a positive response only after the longer incubation; this response is scored as ± in the table. Suppressor *sufJ101* can correct *hisG6608* and *hisG6609* more efficiently if the strain also carries a *hisT* mutation (see text).

TABLE 3

Suppression spectrum of sufJ *compared with the suppression spectra of previously described suppressors*[a]

Mutation	*sufA*	*sufD*	*sufJ*	*sufJ hisT*
hisD2780	+	−	−	−
hisD3018	+	−	+	+
hisD3040	+	−	−	−
hisD3749	+	−	−	−
hisD6610	+	−	−	−
hisC2259	+	−	−	−
hisC3734	+	−	−	−
hisA2770	+	−	+	+
hisG3037	−	+	−	−
hisD3068	−	+	−	−
hisC3072	−	+	−	−
hisC3732	−	+	−	−
hisF2118	−	+	−	−
hisF2439	−	+	−	−
hisF3041	−	+	−	−
hisF3704	−	+	+	+
hisG6608	−	−	±	+
hisG6609	−	−	±	+
hisD3794	−	−	+	+

[a] Transducing phage was grown on each *his* frameshift mutant and used in transduction crosses with recipient strains that each carry the deletion mutation *his-644* and one of the various frameshift suppressors. Recipient strains (TT522, 2842, 2846, 2890, 2887) and donor strains (TR6243, 767, 6242, 1034, 3791, 3023, 966, 6241, 6245, 6244, 947, 964, 1430, 712, 1041, 725, 3146, 3144, 6246) are listed in Table 1. Appearance of His^+ transductants signifies that the donor mutation is corrected by the recipient suppressor. Each donor phage was crossed by a control recipient containing deletion *his-644* but no suppressor mutation; all of these control crosses failed to yield His^+ transductants. The definitions of + and − are as in Table 2.

RESULTS

Source of the mutants

Proflavin was tested as a potential inducer of new frameshift mutations because it is a powerful frameshift mutagen for phage T4, where it has been shown to induce a variety of frameshift types (STREISINGER *et al.* 1966). We found that proflavin is mutagenic for bacteria but is not specifically a frameshift mutagen. Rather, it acts as an inducer of *recA*-dependent, error-prone repair (KOHNO and ROTH 1974). As such, it induces a variety of point mutation types including frameshifts. The array of proflavin-induced mutations is very similar to the array of mutation types that arise spontaneously. Of 100 proflavin-induced mutations, only one is suppressible by the original set of frameshift suppressors (*sufA-F*). Thus, it appears that error-prone repair seldom causes +1 mutations in G:C runs (KOHNO and ROTH 1974). The proflavin-induced frameshift mutations appear to be a diverse group, some of which prove to be correctable by new suppressor types. Since proflavin-induced mutations seem to arise by a mechanism similar to spontaneous mutation, we also checked a

series of spontaneous frameshifts for suppressibility. Several mitomycin-C-induced mutations (obtained from J. McCANN and B. AMES) and one 9-aminoacridine-induced mutation (obtained from E. YAMASAKI and B. AMES) were also tested and proved to be correctable by new suppressor types.

The mutations used are listed in Table 2. (The first three entries of Table 2 present previously described mutations for comparison.) All of the new mutations were preliminarily classified as frameshifts on the basis of genetic criteria. (1) Most are induced to revert by the frameshift mutagen ICR191 but not by the base substitution mutagen, nitrosoguanidine (NG) (see Table 2). (2) None is suppressed by standard nonsense suppressors. (3) None gives rise to revertants that carry nonsense suppressors. (4) All are suppressed by new suppressors that are unable to correct nonsense mutations. (5) All show polar effects on the expression of distal genes in the histidine operon.

Subsequent to the genetic classification of the new mutations, two have been shown to be +1 frameshift mutations by DNA sequencing. Mutation *hisD6580* is the +1 frameshift mutation: ACCG → ACCAG (BOSSI and ROTH 1981); mutations *hisG6608* and *hisG6609* are identical +1 frameshift mutations: CGCC → CGCCC (W. BARNES, personal communication). In addition, two of the new suppressors, whose initial isolation is described here, have been characterized in some detail. The *sufG* suppressor reads the four base codon AAAA (KOHNO and ROTH 1978); the *sufJ* suppressor reads the nonmonotonous codons ACCU, ACCC and ACCA (BOSSI and ROTH 1981).

The new suppressors

The cross-suppression pattern of the frameshift mutations and the new suppressors isolated in this study are listed in Table 2; also included are three of the previously described frameshift mutations and their suppressors, *sufA, B, D and* E. It should be noted that several, but not all, of the mutations suppressed by the new suppressor *sufG* are also corrected by the *sufA* suppressor. Similarly, *sufJ* corrects several mutations that are also suppressed by other suppressors. Mutation *hisC6581* is corrected by *sufA, sufG* and *sufJ*.

To obtain other examples of cross-suppressibility, a series of previously described frameshift mutations were tested for suppressibility by the new suppressor *sufJ* (Table 3). These tests revealed that three of the mutations tested (*hisA2770, hisF3704* and *hisD3794*) are suppressible by *sufJ*. Two of these mutations are also corrected by previously described suppressors (*sufA* or *sufD*).

These results suggest that suppressors with different specificities may correct the same frameshift mutation by acting at distinct sites *near* the actual mutant site. This is similar to the internal compensating frameshift mutations which can correct a frameshift mutation by rectifying the reading frame at various sites slightly removed from the site of the original mutation (CRICK *et al.* 1961). Some frameshift mutations must be located near more than one sequence at which a frameshift suppressor can act. For example, mutation *hisB6480* must be near a site recognized by *sufA*(CCCC/U) and a site for *sufG*. Mutation *hisD3018* must be near a site for *sufA,B* and a site for *sufJ*. Similarly, mutation *hisD6580* must be near sites for *sufG* and *sufJ*. In several cases, existence of

these multiple sites has been directly demonstrated. Mutation *hisD3018* has been shown to be near the sites CCCU (*sufA*) and ACCC (*sufJ*); mutation *hisD6580* is near the suppressible sequences AAAU (*sufG*) and ACCA (*sufJ*) (BOSSI and ROTH 1981).

In Table 3, the activity of the *sufJ* suppressor has been checked in the presence and absence of the secondary mutation *hisT*. The *hisT* mutation leaves the cell unable to form pseudouridine in the anticodon loop of many tRNAs. The *hisT* mutation greatly improves the ability of *sufJ* to correct mutations *hisG6608* and *hisG6609*, the mutations used in the original isolation of *sufJ*. This dependence of *sufJ* activity on the *hisT* mutation was not seen for any of the other mutations that are correctable by *sufJ*. This *hisT* effect will be discussed further in the accompanying paper (BOSSI *et al.* 1982).

The new suppressors have all been genetically mapped. The *sufH* suppressor maps at minute 52 of the Salmonella chromosome; it is 50% cotransducible with the *trz* locus and is dominant to a wild-type *E. coli* copy of this chromosomal region. The *sufI* suppressor maps at minute 12 but shows no cotransduction with the following markers in that region: *purE*, *proAB*, *nag*. The *sufM* suppressor has a suppression pattern that is distinct from that of previously described suppressors; yet, its map position is indistinguishable from the position of *sufA* (12% cotransducible with *xyl* at minute 78 of the map). Probably *sufM* will prove to be a slightly different allele of the *sufA* locus. Detailed mapping and dominance tests of *sufG* and *sufJ* are reported elsewhere (KOHNO and ROTH 1978; BOSSI *et al.* 1982).

The *sufH* and *sufI* suppressors both have a deleterious effect on growth. Both suppressors are unstable and are frequently lost. This apparent instability is probably due to positive selection of revertants that grow faster. The instability is probably not due to the presence of a tandem duplication since the suppressors are still subject to frequent loss after introduction of a *recA* mutation.

Efficiency of several suppressors has been estimated (for one site each) by assaying the ability of the suppressors to relieve polarity and increase the level of distal gene expression. The *sufG* suppressor is approximately 5% efficient; *sufH*, *sufJ* and *sufM* are all approximately 1% efficient.

DISCUSSION

Two conclusions are indicated. First, it seems clear that frameshift suppressors are not limited to the previously described types that act at runs of C or G residues. This apparent site specificity of early suppressors is probably due to the fact that the initially studied suppressors were obtained as revertants of mutants induced by ICR191, a mutagen specific for runs of G:C pairs in DNA. New suppressor types are found when revertants of mutations obtained in other ways are tested.

Second, the nature of frameshift mutations makes it possible for a single mutation to be corrected by two suppressors with distinct sites of action. This can occur if sequences near the mutant site provide sites for both types of suppressors. Some of the suppressed proteins produced by the action of these suppressors would be expected to contain short runs of improper amino acids

due to out-of-phase reading of the message between the site of the mutation and the site of suppressor action.

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Corresponding editor: D. BOTSTEIN