Anomalously revertible r_{II} mutants of phage T_4

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SUMMARY

A class of r_{II} mutants revertible by both base analogues and acridines is described. The members of this anomalous class are base-substitution mutants suppressed by a phase-shift mutation in a phage gene mapping outside the r_{II} region.

In bacteriophage T_4 mutagenesis with acridines induces phase-shift mutations exclusively. Base substitution mutants are specifically induced by base-analogues such as 5-bromouracil. A phase-shift mutant can suppress another phase-shift mutant and a base-substitution mutant can be reverted or suppressed by another base-substitution, but a base-substitution is not ordinarily expected to cancel the consequences of a phase-shift (Barnett *et al.* 1967). It follows that a given mutant is not expected to be revertible by both acridines and base-analogues. In fact there are a number of r_{II} mutants with just such properties (Orgel & Brenner, 1961; Kreig, 1963). This anomaly was troublesome because it suggested either that there were occasional non-triplet readings in protein synthesis in phage infected cells or that the theory of mutagenesis by acridines (Brenner *et al.* 1961) was wrong. In this paper we show that neither explanation is necessary.

$T4_Br263$, a spontaneous mutant mapping near the middle of the r_{II} B cistron, was the first anomalously revertible mutant reported. As shown by Orgel & Brenner (1961), when this phage is plated on *Escherichia coli* strain KB, which does not permit the growth of r_{II} mutants, two kinds of revertants are found. One makes wild-type plaques on KB, the other makes minute plaques. The first type of revertant is induced by mutagenesis with 5-bromouracil. Proflavin mutagenesis specifically induces the minute revertant.

The minute revertants of r263 show a peculiarity not mentioned in the original report. They form minute plaques not only on KB but also on BB and non-λ-lysogenic K strains. It will be recalled that phages deleted for the entire r_{II} region make wild-type plaques on BB and $K12\lambda^s$, and so does r263, the parent of the minutes.

This suggested to us that the minute revertants have a suppressor for r263 which can also inhibit phage multiplication. To isolate this suppressor we backcrossed a spontaneous minute revertant of r263 with $T4r^+$ and plated the cross lysate on strain B, which grows the minute as a minute R. Two classes of recombinants were seen. One made normal R plaques and was assumed to be r263. The other made

Table 1. *si+ suppression of members of ambivalent subset III*

Mutant	Location (segment)	si+ suppression on KB
rAP129	A 1	+
rAP80	.	+
rHB6	A 3	+
r106	A 6	+
r556	B 2	−
r263	B 4	+
r1948	B 7	+
rN38	.	+
rEM16	.	+
rA84	.	+
rSD80	B 8–9	−
rNT284	.	+
rEM34	.	+

Table 2. *si+ suppression of members of ambivalent subset II*

Mutant	Location (segment)	si+ suppression on KT	
rSN86	A 1	+	
rHB309	A 2	+*	
r154	A 3	+	
rN40	B 1	+	poor
rN89	.	+	
rHB33	B 8	+	

* HB309 si+ was not isolated.

minute R+ plaques. We identified this recombinant as the segregated suppressor. The recombinants amounted to 20% of the cross progeny. This frequency corresponds to the segregation of a marker farther from r263 than the ends of the r_{II} region. We deduce that r263 is a base-substitution mutant, and that its anomalous reversion is due to the induction of an extra-cistronic suppressor. We have named the active allele of this suppressor si+.

1. THE RANGE OF si+ SUPPRESSION

r638 si+ and r1272 si+ were constructed by crossing r638 and r1272 with r263 si+. r638 is a deletion overlapping all rIIB mutants. r1272 deletes the entire rII region. The recombinants make minute R plaques on B. They do not plate on KB – a finding anticipated from the general failure of extended rII deletions to revert.

We studied the range of si+ suppression by crossing r1272si+ mutants and searching for suppressed recombinants on selected K strains. Our findings, which are set out in Tables 1–5, may be summarized as follows.

(1) si+ suppression is allele specific. It can work for mutants of both rII cistrons (Tables 1–4).

(2) si+ suppressed most of the members of ambivalent subset III (Benzer & Champe, 1961) to grow on KB (Table 1). Ambivalent subset III, which includes

Table 3. *Growth properties of amber mutants combined with si+*

Mutant	Location (segment)	Bacterial suppressor*				
		Su_I^+	Su_{II}^+	Su_B^+	Su_C^+	Su^-
rN97	A 3	.	.	+	+	−
rS116	.	−	−	−	.	.
r2074	B 1	.	.	.	+	−
rNT332	.	.	.	.	+	−
rX237	B 4	.	.	+	.	−
rX417	B 7	.	.	+	+	−

* The suppressing strains are those described by Brenner & Beckwith (1965). Su_I^+ and Su_{II}^+ are amber suppressors. Su_B^+ and Su_C^+ are ochre suppressors. The Su^- strain is KB.

Table 4. *Growth properties of ochre mutants combined with si+*

Mutant	Location (segment)	Bacterial suppressor				
		Su_I^+	Su_{II}^+	Su_B^+	Su_C^+	Su^-
rN55	A 1	−	−	.	.	.
rX20	A 2	−	−	.	+	−
rX220	.	−	−	.	.	.
rX372	A 3	−	−	.	.	.
rX352	A 4	−	−	.	.	.
rN31	.	−	−	.	.	.
rX25	A 5	−	−	.	.	.
rX170	.	−	−	.	.	.
rX319	.	−	−	.	.	.
rX337	.	−	−	.	.	.
rX358	.	−	−	.	.	.
rX164	A 6	−	−	.	+	−
rX558	.	−	−	.	.	.
rN21	.	−	−	.	+	−
r360	B 1	−	−	.	+	−*
rUV375	.	−	−	.	.	.
rX27	.	−	−	.	.	.
r375	.	−	−	.	.	.
rN24	.	−	−	.	.	.
rN17	B 4	.	.	.	.	.
rX528	.	−	−	.	.	.
rN7	.	−	−	.	.	.
rX321	.	−	−	.	+	−
rX234	B 7	−	−	.	.	.
rX191	.	−	−	.	.	.
rN12	.	−	−	.	+	−
rN29	B 8	−	−	+	+	−

* 360 si+ was not isolated.

r263, is a set of slightly leaky mutants distinguished by their inability to grow on KB and their ability to grow on the KB derivative KB-1. Most of them are known to be base-analogue revertible (Champe & Benzer, 1962; Kreig, 1963; J. W. Drake, personal communication). A double of two si+ suppressible mutants, r106 + r263, is

Table 5. *si+ suppression in double mutants*

Phage	Bacterial suppressor	
r263 + rN97 si+	Su_I^+	Parallel amber and si+ suppression
r263 + rN11 si+	Su_I^+	Parallel amber and si+ suppression*
r263 + rS172 si+	Su_I^+	Parallel amber and si+ suppression†
r263 + rX417 si+	Su_I^+	Parallel amber and si+ suppression
rN21 + rP53B′r+si+	Su_0^+	Synergistic si+ and ochre suppression of N 21: translocation suppression of P 53 (Freedman & Brenner, 1972)

* rN11 is an amber located in segment A 4.
† rS172 is an amber located in segment A 5.

not si+-suppressible. We have not been able to isolate more powerfully suppressing variants of si+ as single step revertants of this double mutant.

(3) Members of ambivalent subset II (Benzer & Champe, 1961), a second leaky subset, are suppressed by si+ to grow on strain 112-12(λh), which grows the subset very poorly (Table 2).

(4) si+ enables some ambers, and also some ochres, to grow on ochre-suppressing strains which would otherwise restrict them (Brenner & Beckwith, 1965) (Tables 3, 4).

(5) No ochre mutant is si+-suppressed to growth on an amber suppressing strain (Table 4). No nonsense mutant, amber or ochre, is suppressed to growth on the su⁻ stain KB (Tables 3, 4).

No extracistronic suppressor has ever been recovered as a suppressor of a phase-shift mutant, although the mutation rate to si+ is higher than the reversion rates of many phase-shift mutants from which revertants have been isolated, and over 200 suppressed doubles have been examined (Barnett *et al.* 1967).

In sum, si+ suppression of an r_{II} mutant seems to require the persistence of some trace of r_{II} function. Deletions, phase-shift mutants and nonsense mutants, none of which can make complete r_{II} proteins (McClain & Champe, 1967), are not suppressed by si+. The mutants we have found suppressed by si+ are all either leaky, or nonsense mutants growing on su+ bacterial strains, and both classes are expected to make some complete, if altered, r_{II} product.

Clearly si+ cannot provide a complete replacement for r_{II} function. Since si+ suppression of nonsense mutants is a function of the su status of the host, it seems unlikely that si+ suppresses by changing the genetic code. Other mechanisms are compatible with the scope of si+ suppression. si+ may increase the amount or activity of available r_{II} products, or reduce the demand for r_{II} function. Our experiments have not discriminated between these possibilities.

Altogether we have identified 29 r_{II} mutants suppressible by si+, and no doubt a more extensive search would turn up others. The anomalously revertible mutants described by Kreig (1963) are obviously candidates for this kind of suppression, and EM34, the only one we have tested, is a si+-suppressible member of ambivalent

Table 6. *Burst sizes of si$^+$ phage on B*

	Phage	Burst size
Ambivalent subset III	rAP129 si$^+$	2·6
	rAP80 si$^+$	2·5
	r263 si$^+$	4·3*
	r1948 si$^+$	5·5
	rA84 si$^+$	4·9
	rNT284 si$^+$	7·3
	rEM34 si$^+$	4·5
Amber	rN97 si$^+$	2·7, 2·1*
	rEM84 si$^+$	5·7, 2·7*
	rAP164 si$^+$	2
	rX417 si$^+$	4·3
Ochre mutants	rX358 si$^+$	7·3
	rX164 si$^+$	4·3
	rN97 + r263 si$^+$	7*
	rEM84 + r263 si$^+$	2·5*
	rX417 + r263 si$^+$	3·8*
	r$^+$ si$^+$	3·6, 8

Phages were adsorbed at low multiplicity to strain B growing exponentially in tryptone broth. After 7 min complexes were diluted into tryptone at 37°; 80 min later they were treated with chloroform. Burst sizes are normalized to r$^+$ si$^-$ = 100, with the exception of the asterisked (*) results, which are normalized to si$^-$ phages carrying identical r markers, and represent lysis after 45 min.

subset III. Most of the si$^+$-suppressible mutants are positively identified as revertible by base-analogues, though we only know the mutant codons for the ambers and ochres. In any case, it is clear that by the appropriate choice of K strains a large class of r$_{II}$ mutants could be shown to be revertible by both acridine and base-analogue mutagenesis, because of the possibility of si$^+$ suppression. Some other r$_{II}$ mutants and multiple mutants show this dual revertibility for a different reason. In these cases a base substitution can suppress a nearby phase-shift in the r$_{II}$ by introducing a signal for the reinitiation of the polypeptide chain (Sarabhai & Brenner, 1967). The point we wish to emphasize is that no substantiated exception to the theory of mutagenesis is known (Brenner *et al.* 1961).

2. PROPERTIES OF THE si LOCUS

All si$^+$ phages we have studied grow poorly. Burst sizes of si$^+$ recombinants growing in strain B are shown in Table 6. Because of the sickness of si$^+$ phage, mutants arising in phage growth are powerfully selected, unless si$^+$ is maintained by making growth dependent on si$^+$ suppression. When this is not done si$^-$ mutants may constitute several per cent of si$^+$ low-titre stocks.

To map the si locus we first transferred r263 into a T4$_D$ background by multiple crosses, and then selected a new spontaneous si$^+$ revertant. This si$^+$ was mapped with respect to the T4$_D$ markers ac41 (Edgar & Epstein, 1961) and amber B262 (Epstein *et al.* 1963). The crosses described in Table 7 establish the order r$_{IIA}$, r$_{IIB}$ (r263) ... ac$_{41}$ ... si ... gene 38 (B262). They also show that the leaky

Table 7. *Mapping of si⁺ in* T4$_D$

Cross	Recombinants scored	Yield
r263 ac 41 × H17	r⁺ac 41 H17⁺/total r⁺ H17⁺	72/104
r263 ac 41si⁺ × T4$_D$	r263 si⁻/total phage	295/3410
r263 ac 41si⁺ × H17	r263 si⁻ H17⁺/total si⁻ H17⁺	227/240
r263 ac 41si⁺ × B262	r263 si⁻ B262⁺/total si⁻ B262⁺	40/190
r263 ac 41si⁺ × r263 H17	si⁻ ac 41 H17⁺/total si⁻ H17⁺	79/100
r263 ac 41si⁺ × r263 B262	si⁻ ac B262⁺/total si⁻ B262⁺	17/100

amber mutant H17 (Edgar & Wood, 1966) lies between the r$_{II}$ region and si. The recombination frequency between r263 and si was found to be 17 %.

r263 reverts to minutes with indices of around 10^{-5}. Most of these minutes are si⁺ revertants: 36 out of 37 minute revertants selected from two stocks of r263 made minutes plaques on B. This low mutation rate, but more particularly the indifferent induction of si⁺ by 5-bromouracil mutagenesis (Orgel & Brenner, 1961), suggests that mutation to si⁺ does not represent the inactivation by a phase shift of a cistron encoding a functional protein in wild-type T4. Nor does it appear to be a reversion of a cistron inactivated in T4⁺, since si⁻ mutants isolated from r263si⁺ did not delineate an extended gene. Twelve such mutants were picked as large R plaques on B from a 5-bromouracil mutagenized stock of r263si⁺, and spot crossed in pairs, selecting for si⁺ recombinants on KB. Each mutant was also crossed with r263. One mutant gave recombination appreciably above the background due to reversion with three others, but no other crosses gave recombinants. A second batch of si⁻ mutants has been studied by Mrs Leslie Barnett, who crossed four 2-amino-purine induced mutants, a 5-bromodeoxyuridine induced mutant, and a spontaneous mutant in pairs. None of these crosses gave recombinants.

We do not know how si⁺ suppression works. The t cistron of T$_4$ maps in the same region as si, between genes 38 and 52. Amber mutants of t show lysis inhibition in the absence of superinfection (Joslin, 1970), and this phenotype is suppressed by r$_{II}$ mutants, including deletions (Joslin, 1971). It may be that si⁺ has its direct effect on the expression of t cistron function. However, it is unlikely that this is the only immediate consequence of si⁺, since phages with no t function, unlike si⁺ phages, are not inhibited in intracellular growth.

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