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Bacteriophage S13: A Seventh Gene

Abstract. *Genetic complementation tests of ochre-type suppressible mutants of bacteriophage S13 have revealed a seventh gene. A mutation of this gene has no significant effect on synthesis of the phage replicative form DNA, but like five other phage genes it prevents the appearance of single-stranded DNA. With the new gene, it seems likely that approximately 70 to 100 percent of the phage genes are accounted for.*

An exhaustive analysis was initiated by E. S. Tessman (*1*) to identify all the genetic functional units of phage S13, a functional unit (or gene) being defined as equivalent to a genetic complementation group. With the new gene described here, we estimate that 70 to 100 percent of the functional units are accounted for.

As a result of isolating many temperature-sensitive (*t*) and amber-type suppressible (*su*) mutants, six complementation groups have been found and designated I, II, IIIa, IIIb, IV, V. Groups I through IV were identified in the original experiments on S13 complementation groups (*1*). At that time IIIa and IIIb were recognized as separate categories by complementation tests between *su* mutants, but those tests gave low burst sizes, suggesting that there was intragenic complementation. However, the low burst sizes could be due to a polarity effect (*2*) because subsequent complementation tests with *t* mutants showed unambiguously that IIIa and IIIb mutants strongly complement and therefore represent separate complementation groups (*3*). Mutants of group V affect lysis and show properties similar to those of the gene identified in the closely related bacteriophage ϕX174 (*4*).

The new group was found by isolating ochre-type *su* mutants that are suppressible by the ochre suppressor *Escherichia coli* CA165 (*5*). The ochre mutants were induced by treatment with hydroxylamine (*6*) to a survival of 0.45. The treated phage were plated on a derivative of strain CA165. This strain is normally not sensitive to S13, presumably because of failure to adsorb the phage, so the sensitive derivative CA165.2 was isolated by a method previously applied to other suppressing hosts (*7*, *8*).

We used ochre-type *su* mutants that grew on CA165.2 but not on the *Escherichia coli* strain C600.1 (an S13-sensitive derivative of the amber suppressor C600) nor on C122 (nonsuppressing).

Complementation tests with C122 as the host were performed by the method of E. S. Tessman (*1*). The ochre mutants were tested against amber mutants from each of the known complementation groups. In unmixed infection, none of the bacteriophage used can multiply in C122 except for group V mutants. The group V mutants not only multiply intracellularly but are also difficult to use in complementation tests because they frequently produce large bursts when infected cells are diluted for plating. Therefore, decisive complementation tests could be done only with groups I through IV. Among 13 ochre mutants, only

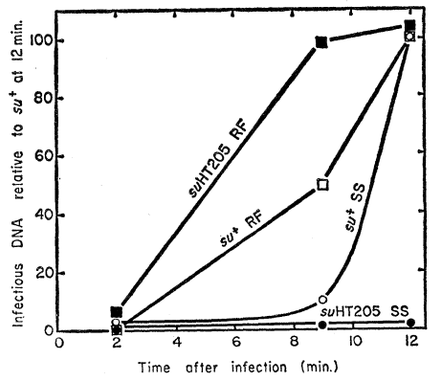

Fig. 1. Synthesis of double-stranded replicative form (RF) and single-stranded (SS) DNA as a function of the time after infection of the nonpermissive host C122 with the group VI ochre mutant *su*HT205, compared to infection with wild type phage. Data on RF and SS are relative to the respective su^+ values at 12 minutes, which are normalized to 100. Assays of infectious DNA were made with spheroplasts of CA165.2.

*su*HT205 (Table 1), complemented amber mutants in each of five previously known groups tested (omitting group V).

When assayed on the indicator strains C600.1 and CA165.2, the ochre mutant *su*HT205 plates only on CA165.2 while all the ambers plate with a much higher efficiency on C600.1. Thus, on each indicator strain essentially only one of the two parent types was assayed, and so the true burst sizes are larger than those shown (except for tests involving group IV mutants for which there is a phenomenon of poor rescue, discussed below).

Although tests with group V mutants

Table 1. Burst sizes in complementation tests of *su*HT205.

Mutant tested against *su*HT205	Complementation group	Burst size			
		Assayed on amber suppressor C600.1		Assayed on ochre suppressor CA165.2	
		Mutant alone	Mixed infection	Mutant alone*	Mixed infection
*su*HT205	?			.28	
*su*HS 52	I	0.9	30		
*su*HS129	II	< 0.1	50		
*su*HS 94	IIIa	0.3	35		
*su*HS132	IIIb	0.2	35		
*su*HS113	IV	0.6	1.6		20
*su*HT205	?	< 0.01		0.7	
*su*HS 52	I	2.2	19	0.2	90
*su*HS 86	II	1.2	54	0.4	114
*su*HS 62	IIIa	2.9	37	< 0.01	99
*su*HS 66	IIIb	3.7	16	< 0.01	72
*su*HS100	IV	2.5	1.8	0.3	49

* The discrepancy between the values for the burst sizes of amber mutants assayed on strain C600.1 versus CA165.2 is due to a low efficiency of plating for these amber mutants on the ochre suppressor, CA165.2.

Table 2. The seven known genes of phage S13.

Complementation group	Function
I	Coat protein structure
II	Unknown—possibly SS DNA synthesis
IIIa	Unknown—possibly SS DNA synthesis
IIIb	Coat protein structure
IV	RF DNA synthesis
V	Lysis
VI	Unknown—possibly SS DNA synthesis

give equivocal results, *su*HT205 is clearly in a separate functional unit from group V because, unlike group V mutants, it does not produce mature phage intracellularly. Furthermore, cultures of C122 multiply-infected with *su*HT205 clear at the normal time, but when infected with a group V *su* mutant the culture remains turbid.

The test with the group IV mutant *su*HS113 shows the poor-rescue phenomenon discovered by E. S. Tessman (*1*, *9*). Mutant *su*HT205 and *su*HS113 clearly complement each other, but the burst consists almost entirely of the *su*HT205 type, the group IV mutant being very poorly rescued. The fact that *su*HT205 is rescued in all the complementation tests is additional evidence that it is in a functionally different group from IV.

The existence of a new complementation group has been confirmed by another set of tests with a temperature-sensitive false revertant of *su*HT205, labeled *ts*HT205.26.1. This mutant complements *su* mutants in groups I, II, IIIa, IIIb, and IV, but it does not complement nor recombine with *su*HT205.

The functions of some of the complementation groups are known in a general way. Groups I and IIIb determine, at least in part, the phage coat (*1*, *8*). Group IV controls the replication of the replicative form (RF) DNA (*9*). Group V mutants produce intracellular phage normally, but cell lysis is delayed (compare ϕX174, *4*).

The function of VI might involve DNA replication. This possibility was studied by measuring the synthesis of infectious double-stranded RF and single-stranded (SS) phage DNA in the nonpermissive host C122 after infection with *su*HT205 at a multiplicity of infection of 3. The RF DNA was measured by the hydroxylamine inactivation method of Howard, Tessman, and Howard (described in *9*), and SS DNA was assayed by the heat treatment of I. Tessman (described in *10*) which releases DNA from the phage coat so that both naked and encapsulated SS DNA are measured together. In comparison with the parent *su*+, *su*HT205 produces essentially normal amounts of RF DNA, the differences being within the range of variation observed from experiment to experiment for the wild-type phage (Fig. 1). But no SS DNA is made by the group VI mutant. For both RF and SS DNA, the *su*+ results are consistent with previous results (*9*).

With the addition of the new complementation group there are now five functional units of S13 (groups I, II, IIIa, IIIb, VI) of which mutants have no significant effect on RF DNA synthesis but prevent the appearance of SS DNA. Since groups I and IIIb appear to be structural genes for coat proteins (*8*) their roles in the control of SS DNA synthesis must be indirect; perhaps the unprotected SS DNA is degraded or is converted into RF DNA. Thus, if a part of the phage genome has the function of specifying one or more enzymes for synthesizing SS DNA, then groups II, IIIa, and VI are likely candidates for that function (Table 2).

With the addition of the seventh gene, have all the genes in S13 (and also ϕX174) been identified by mutation? The fact that only one mutant site in group VI has been found so far makes it obvious that other groups might not yet be represented at all. And some undiscovered genes could be nonessential and thereby escape detection by conditional-lethal mutants.

However, a rough coding calculation gives an idea of how close we may be to accounting for all the phage genes. Given an RF DNA length of 1.64 ± 0.11 μ (*11*) and assuming a spacing of 3.4 Å of the nucleotides along the chain (*12*) there are 4800 ± 320 nucleotide pairs per RF molecule. If we assume a triplet code, the phage can specify approximately 1600 amino acids. Because 160 amino acids seems to be a low average number of amino acids for a protein subunit (compare *13*), we estimate that ten polypeptide species are a generous maximum limit to what can be coded by the phage; the one phage protein analyzed (in ϕX174, *14*) has 225 amino acids per subunit. Thus, seven complementation groups should account for at least 70 to 100 percent of the phage genes.

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Optical Differentiation of Amoebic Ectoplasm and Endoplasmic Flow

Abstract. *Optical activity differentiating the flowing and nonflowing amoebic cytoplasm was detected. This evidence indicates molecular alignment in the flow stream and can be used to provide data on the direction of alignment. The results were obtained by utilizing a dynamic polarized-light detection system which is sensitive only to specimens which possess a preferred axis.*

The application of engineering-systems techniques has led to the development of a system capable of detecting low-level optical activity in living specimens which has heretofore escaped detection (*1*, *2*). Optical activity is defined here as any polarized light phenomenon. The system was developed to study the mechanisms of amoeboid motility and is now capable of differentiating cytoplasm optical activity found in the flowing endoplasm from