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Heteroduplex Mapping of Heat-Resistant Deletion Mutants of Bacteriophage T5

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The bacteriophage T5 is known to spontaneously generate deletion mutants (*st* mutants) exhibiting enhanced resistance to heat inactivation in citrate buffer. A series of such mutants has been isolated and the deletions visualized by electron microscopy of heteroduplex molecules. The deletions are found to cluster in one region of the chromosome.

Heat-stable mutants of bacteriophage T5 (*st* mutants) were first described by Adams and Lark (2). The *st* mutation confers on the phage the ability to survive elevated temperatures in saline citrate solutions. Hertel et al. (9) and Lark (12) showed that *st* mutants exhibit a lower density than wild type in CsCl gradients, and Rubenstein (16) correlated this lowered density with a loss of DNA relative to wild type. The heat sensitivity of the phage is directly proportional to its DNA content.

The *st* mutations are of interest because they alter the pattern of single-chain interruptions which occur in T5 DNA (19). It has been suggested that one of these *st* mutations, T5*st*(0), deletes one of the sites where single-chain interruptions normally occur (8). Since the T5*st*(0) deletion has not been shown to tightly link any of the published markers, it was decided to study *st* mutants by physical rather than genetic methods. The experiments reported here represent an analysis by heteroduplex mapping of the DNA of T5*st*(0) and several newly isolated heat-stable mutants.

Wild-type bacteriophage T5*st*(+) and the heat-stable mutant T5*st*(0) used in this study have been described previously (1). Additional heat-stable mutants were isolated from wild type by the method of Hertel et al. (9). Native DNA was labeled and isolated as described by Rhoades and Rhoades (14). The molecular weights of the DNAs of the new mutants were estimated by zone sedimentation in neutral sucrose gradients (3) in the presence of either T5*st*(+) (75.9×10^6) or T5*st*(0) (70.9×10^6) DNA. The molecular weights obtained for the three mutants chosen for further study are given in Table 1.

Heteroduplex molecules were formed by denaturing and annealing a mixture of two native DNA preparations according to the procedure of Davis and Parkinson (5). In all cases single-chain interruptions were repaired by pretreatment with polynucleotide ligase (10). DNA was mounted for electron microscopy as described in the legend of Fig. 1. A double-stranded circular marker DNA, from bacteriophage PM2 (6.3×10^6 [7]), or the double-stranded replicative form of bacteriophage ϕX174 (3.2×10^6 to 3.4×10^6, [18]), was included on all grids. Length measurements were converted into marker units, where one unit equals the length of PM2 DNA or 1.87 lengths of ϕX174 double-stranded replicative form DNA.

When T5*st*(+) DNA was annealed with DNA from the deletion mutants, both fully paired linear homoduplex and heteroduplex molecules were seen in the electron microscope. The heteroduplexes contained a single internal loop of unpaired DNA located approximately eight marker units or 66% from one end of the molecule (Fig. 1A). In some preparations a significant number of molecules were observed with single-stranded material at one or both ends. Structures of this type would be expected since complete repair of the single-chain interruptions in T5 DNA is not generally achieved (10). The position of the deletion loop was measured on all molecules with one intact end regardless of whether the other end was fully duplex. The results of these measurements are shown in Table 1.

One or both deletion end points of the pairs T5*st*(0)-T5*st*(105) and T5*st*(0)-T4*st*(124) fall very close to each other. A sensitive test of their identity or nonidentity is provided by examining their DNAs in heteroduplex form. All pairwise combinations of deletion mutants

TABLE 1. *Physical dimensions of st mutants*

Bacteriophage	Mol wt[a] × 10[6]	Distance of loop from left end of molecule[b]		No. measured	Distance of loop from right end of molecule		No. measured
		Marker units[c]	Wild type length (%)		Marker units	Wild type length (%)	
T5*st*(+)	75.9						
T5*st*(124)	72.5	3.14 ± 0.12[d]	26.0	18	8.36 ± 0.15	69.4	14
T5*st*(0)	70.9	3.23 ± 0.07	26.8	24	8.14 ± 0.21	67.6	21
T5*st*(105)	70.9	3.31 ± 0.10	27.5	11	8.14 ± 0.20	67.6	10
T5*st*(102)	69.8	2.61 ± 0.17	21.6	21	8.21 ± 0.19	68.1	16

[a] The molecular weight for wild-type DNA was obtained by direct measurement in the electron microscope with PM2 and φX174 RFII closed circular DNAs present on the same grid. The length of wild-type DNA is 12.05 ± 0.25 marker units (28 molecules). Molecular weights for the other mutant DNAs were obtained by zone sedimentation in neutral sucrose gradients as described in the text.

[b] The left end of the molecule is defined as the end nearest the heteroduplex loop.

[c] Tracings of DNA molecules were measured with a Keuffel and Esser map measurer and converted to marker units, where one marker unit is one length of PM2 DNA or 1.87 lengths of φX174 RFII DNA.

[d] Standard deviation.

were tested in this way. Overlapping deletions with one common end point or no common end points where one deletion lies completely within the other will form a loop of the type shown in Fig. 1A. Deletions with no common end points, but which do not completely overlap, must form a bubble of single-stranded DNA.

Figure 1B shows a T5*st*(124)-T5*st*(0) heteroduplex, with the unpaired DNA forming a bubble. These structures were also seen in T5*st*(0)-T5*st*(102), T5*st*(105)-T5*st*(124), and T5*st*(105)-T5*st*(102) heteroduplex preparations. Heteroduplexes between T5*st*(102) and T5*st*(124) show the type of loop illustrated in Fig. 1A. When T5*st*(0) and T5*st*(105) DNAs were mixed, denatured, and reannealed, no mismatched DNA could be observed in the electron microscope, although a mismatch of less than 150 bases cannot be ruled out using this technique. From this we conclude that all the *st* deletions lie in the same region of the chromosome, that T5*st*(0) and T5*st*(105) are probably identical, and that the deletions in T5*st*(102) and T5*st*(124) have no common end point with the deletion in T5*st*(0). Figure 2 shows the relative position of the deletions.

Heat-stable deletion mutants have been found to arise spontaneously in bacteriophages T1, T3 (15), T7 (15, 17), and λ (13). The deleted regions include functions supplied by a wild-type host (T7 ligase), functions not necessary for vegetative growth (λ att), and regions having no apparent essential function (T7 genes 0.7 and 1.1, λb519, b506, and b515, and the T5*st*'s). Except for the special case of λ deletions arising from mistakes in excision (13), the mechanisms for generating these deletions are not known.

One possibility is that *st* deletions might arise by excision of a loop of DNA after intramolecular pairing (4). Alternately, a series of deletions of different sizes within a restricted region of the chromosome might arise from unequal crossing over within a repetitive sequence. If this were the case in T5, then the short deletions should produce a heteroduplex loop that wanders due to branch migration, whereas the position of the longest deletion should be more stable. Table 1 shows that this is not the case. This observation does not rule out the possibility that these deletions are generated by illegitimate pairing followed by recombination between two genomes, as sequence homology cannot be said to play a major role in such events (6). Attempts to isolate phage with a larger than wild-type complement of DNA, which would be produced by either type of unequal crossing over, have not been successful.

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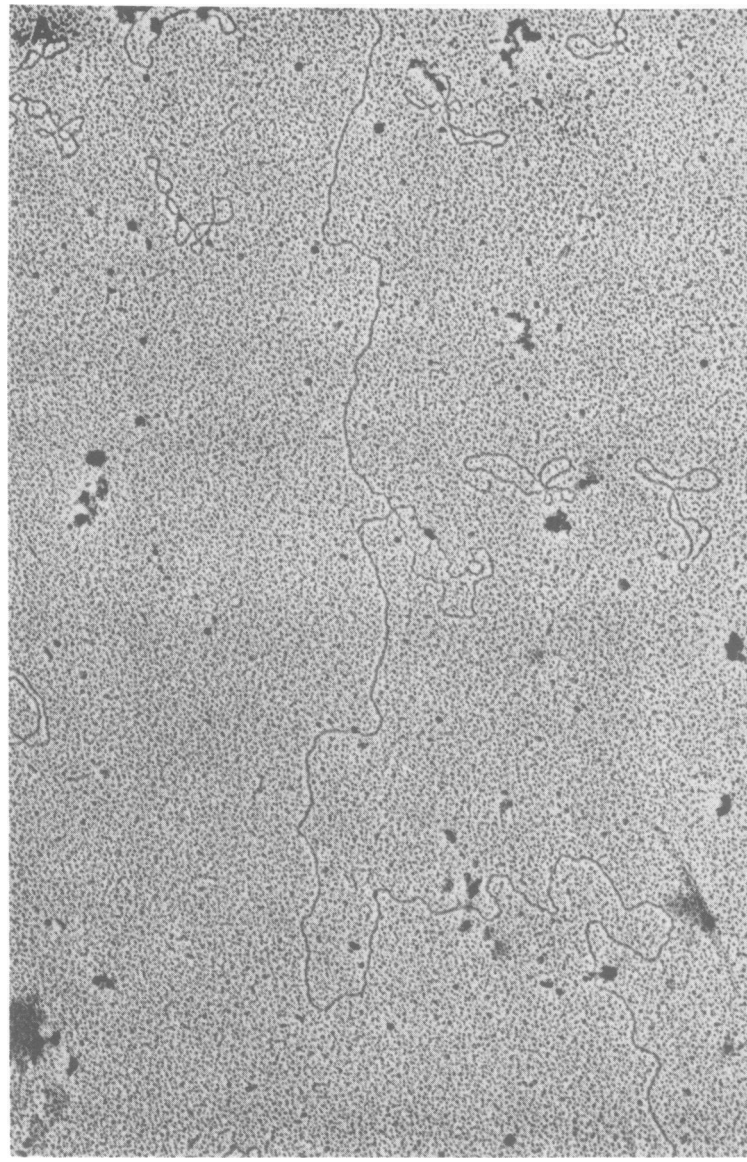

FIG. 1. *After repair of single-chain interruptions with polynucleotide ligase, two native DNAs were mixed, denatured, and annealed to form homo- and heteroduplex molecules as described by Davis and Parkinson (5). DNA was spread by the protein film technique of Kleinschmidt (11) as modified for use with formamide (20) and mounted on a carbon film supported by a Formvar-coated copper grid. Grids were rotary shadowed with platinum at an 8:1 angle. (A) A T5st(+)-T5st(0) heteroduplex molecule. Wild-type DNA forms a single-stranded loop when paired in a heteroduplex with each of the deletion mutants in this study. The circular molecules are φX174 RFII DNA. (B) A T5st(124)-T5st(0) heteroduplex, with the unpaired DNA forming a bubble. Heteroduplexes formed with T5st(0) or T5st(105) DNA and either of the two mutant DNAs show this type of structure.*

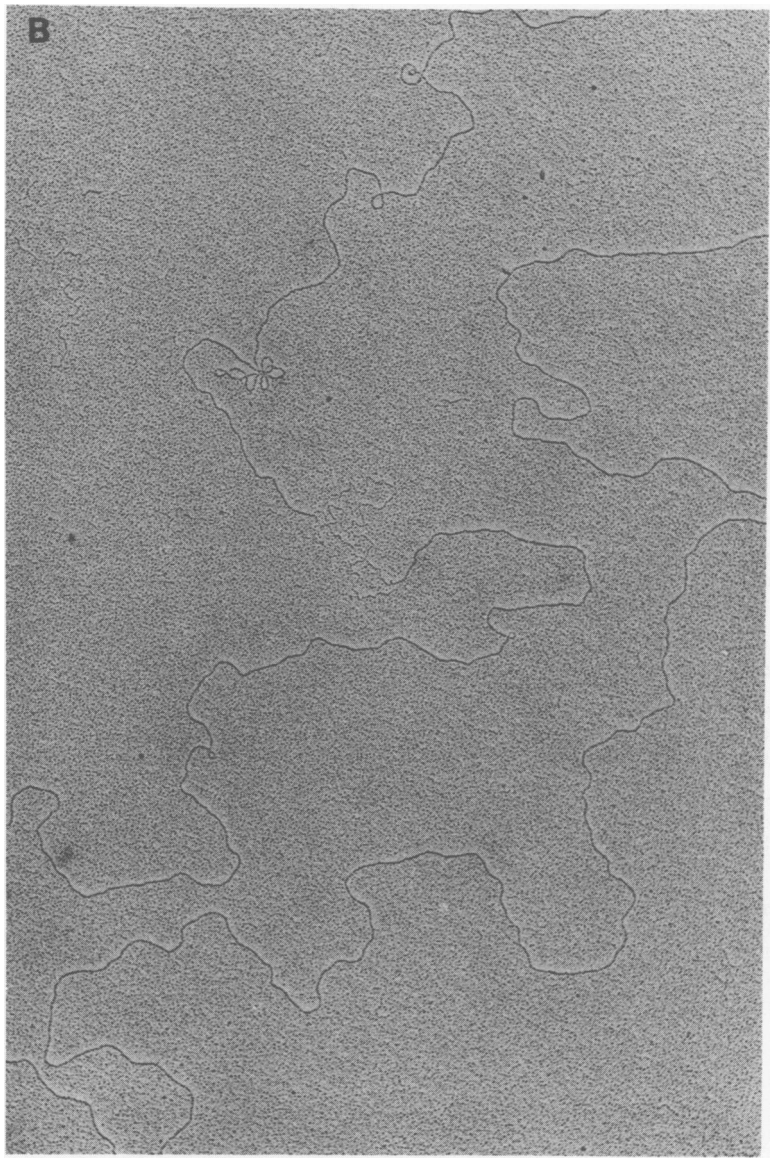

FIG. 1B.

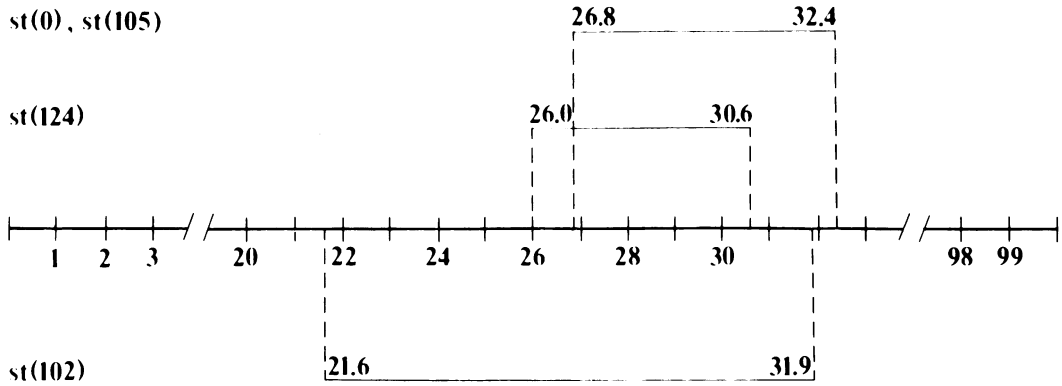

FIG. 2. *Location of st deletions given as percentage of distance from one end of the T5st(+) genome, which is 12.05 marker units long.*

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