PROPERTIES OF BACTERIOPHAGE T4 MUTANTS DEFECTIVE IN DNA POLYMERASE

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GENE *43* of bacteriophage T4 resides at approximately 320° on the standard map (STAHL, EDGAR and STEINBERG 1964). Studies of temperature-sensitive (*ts*) and amber (*am*) mutants reveal that gene *43* is the structural gene for the viral DNA polymerase (DE WAARD, PAUL and LEHMAN 1965; WARNER and BARNES 1966; NOSSAL 1969). The T4 DNA polymerase resembles the *Escherichia coli* DNA polymerase in its requirements for a template, a primer, all four deoxyribonucleoside triphosphates, and magnesium (APOSHIAN and KORNBERG 1962; RICHARDSON, SCHILDKRAUT and KORNBERG 1963; GOULIAN, LUCAS and KORNBERG 1968; ENGLUND *et al.* 1968). The two enzymes also have very similar and large molecular weights (about 110,000 daltons). The viral polymerase is distinguished from the corresponding host enzyme, however, by its amino acid composition, its immunological specificity, its strong preference for single-stranded template DNA, and its lack of 5′ exonuclease activity (COZZARELLI, KELLY and KORNBERG 1969).

It is not yet clear whether the T4 DNA polymerase is specifically responsible for the normal semiconservative replication of viral DNA. The corresponding host cell enzyme exhibits several properties to be expected of a repair enzyme (KELLY *et al.* 1969), and can be mutationally inactivated without impairment of the replicative ability of the cell, although with increased sensitivity to ultraviolet irradiation and to alkylating agents (DE LUCIA and CAIRNS 1969). The viral enzyme, however, is absolutely required for the initiation and maintenance of viral DNA replication (EPSTEIN *et al.* 1963; WARNER and HOBBS 1967; I. R. LEHMAN, personal communication). The viral enzyme has also been implicated in the repair of ultraviolet damage (BALDY 1968) and in genetic recombination (BERNSTEIN 1967; BERGER, WARREN and FRY 1969), but its role in repair remains equivocal (SPEYER and ROSENBERG 1968). The viral enzyme may be periodically required in some initiating aspect of DNA replication without actually functioning as the replication enzyme itself, or it may function both as a DNA replicase and as a repair enzyme (in contrast to the host enzyme).

T4 DNA polymerase is deeply involved in the determination of spontaneous mutation rates, and also in the response of the virus to chemical mutagens (SPEYER 1965; SPEYER, KARAM and LENNY 1966; FREESE and FREESE 1967; DRAKE and ALLEN 1968; SPEYER 1969; DRAKE 1969, 1970; DRAKE *et al.* 1969).

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Our interest in this facet of polymerase activity led us to survey the properties of many gene *43* mutants. These mutants originated in the Caltech collection, and were generously donated by R. S. EDGAR; they are now available from this laboratory.

MATERIALS AND METHODS

Strains: The *ts* and *am* mutants were produced in bacteriophage T4D in a variety of experiments (EDGAR and LIELAUSIS 1964; EDGAR, personal communication). Mutants recovered after treatment with 2-aminopurine carry *A* and *E* prefixes; with 5-bromodeoxyuridine, *B*, *CB*, *G* and *L* prefixes; with nitrous acid, the *N* prefix; with proflavin (but presumably not frameshift mutations), the *P* prefix; and spontaneous mutants carry the *S* prefix. The mapping experiments frequently placed two or more mutants at a single site. When such mutants carry the prefixes *CB*, *G* or *L*, they are likely to be members of a clone. Mutants which carry the prefixes *A*, *B*, *E*, *N*, *P* and *S* were produced under conditions where clone formation was unlikely.

Escherichia coli strains have been described by BRENNER and BECKWITH (1965), KAPLAN (1967 and personal communications), and KRIEG and STENT (1968). BB cells are nonpermissive for amber mutants. CR63, C600, CA265 and CA273 carry amber suppressors, while CA165 carries an ochre suppressor.

Stocks and assay conditions: Stocks were grown at 32°C on a rotary shaker using L broth and either BB cells (for *ts* mutants) or CR63 cells (for *am* mutants). The corresponding cells were used for plaque assays of total phages. Wild-type revertants or recombinants were selectively plated on BB cells at 42–43°C (for *ts*+) or on BB cells at 32°C (for *am*+).

Complementation tests: Log phase BB cells were infected with an average of five of each parental phage (at 43°C when either parent was a *ts* mutant, and at 37°C when both parents were *am* mutants). The mixtures were held on a rotary shaker, and antiphage serum was added at five min to inactivate unadsorbed particles. A 2,500-fold dilution into broth was made at ten min. Lysis was completed at 40 min by shaking with chloroform. Burst sizes were then measured under permissive conditions, assuming all cells to have been infected. Single-parent controls were always run in parallel.

Recombination spot tests: Virus stocks were adjusted to 5×10^9/ml, and one drop of each parent was mixed with two drops of cells at 5×10^8/ml (BB cells for *ts* mutants, and CR63 cells for *am* mutants). After 30 min at room temperature, the mixtures were spotted with sterile paper strips onto plates previously poured with BB cells. The plates were then incubated at 43°C (for *ts* mutants) or at 37°C (for *am* mutants). Single-parent controls were always run in parallel. A lysed spot indicates recombination.

Standard crosses: Crosses were performed at 32°C in a permissive host (BB cells, or CR63 cells if one or both parents carried an *am* marker), using an average multiplicity of five of each parent. Lysis was completed with chloroform at 40 min. Wild-type recombinants were detected on BB cells at 32°C for *am*+, and at 42–43°C for *ts*+. Recombination frequencies were estimated by doubling the frequency of wild-type recombinants, and were corrected for the efficiency of plating of the wild type under permissive *vs.* nonpermissive conditions. Data were accepted from crosses when the burst size was at least 50, and when 100 or more plaques were counted in both the mutant and wild-type assays. Different representatives of a given site were sometimes used in different crosses, but are identified throughout by the mutant name chosen to identify the site unless otherwise indicated. In general, two or more crosses were performed and the values obtained were averaged.

Burst size determinations: Burst sizes produced by amber mutants were measured by (nearly completely) adsorbing an average of about 0.1 particle per BB cell (nonpermissive conditions) or per CR63 cell (permissive conditions), incubating at 32°C, and terminating lysis at 40 min with chloroform. When BB cells were used, residual unadsorbed virus was extensively inactivated with antiserum, which was then removed by dilution. The wild type was always included as a control. Burst sizes were calculated per input particle.

Burst sizes produced by *ts* mutants were measured by adsorbing an average of about 10 particles to BB cells at 43°C (nonpermissive conditions), or about 0.1 particle to BB cells at 32°C (permissive conditions), incubating at the corresponding temperature, and terminating lysis at 40 min with chloroform. In the experiments at 43°C, residual unadsorbed virus was extensively inactivated with antiserum, which was then removed by dilution. The wild type was always included as a control. Burst sizes were calculated per input particle at 32°C, or per infected cell at 43°C.

Base analogue spot tests: Approximately 10^8 *ts* particles (or less for leaky or for rapidly reverting mutants) were plated with BB cells. The plates were spotted with small drops of 2-aminopurine and 5-bromouracil at 10 mg/ml each, and were allowed to stand for 30 min at room temperature. They were then incubated at 42–43°C, and scored for clusters of revertant plaques around the spots; the unspotted regions constituted the background.

Suppression tests: Suspensions of amber mutants at about 10^6/ml were streaked with sterile paper strips onto plates poured with various suppressor cells, and with a CR63 control. The plates were incubated overnight at 32°C and scored for the presence and appearance of plaques.

RESULTS AND DISCUSSION

Gene *43* mutants were initially identified in complementation tests, using *tsL56*, *tsL141* and *amB22* as standard representatives (EPSTEIN *et al.* 1963; EDGAR and LIELAUSIS 1964). A total of 49 *am* and 41 *ts* mutants were identified. Many tests were performed to detect intracistronic complementation among gene *43 ts* mutants, but no significant positive results were encountered (DRAKE *et al.* 1969). This result suggests that the enzyme does not normally function as an oligomer (FINCHAM 1966). The *E. coli* DNA polymerase is already reported to be fully active as a monomer (JOVIN, ENGLUND and BERTSCH 1969).

The mutants were mapped into sites by recombination spot tests, and these assignments were confirmed by standard quantitative crosses. Members of the same site could usually be shown not to recombine at frequencies greater than 0.001%, except for occasional mutants exhibiting high spontaneous reversion rates. The members of each site are listed in Table 1. Note that the conditions under which these mutants were isolated sometimes promoted the recovery of clones. The multiple occurrences which are observed at many of the sites (particularly at *tsL91*, *tsG39*, *tsL42* and *tsL141*) are therefore unlikely to have resulted from high mutation rates (hot spotting).

A member of each site was mapped using two-factor crosses. The map of the *am* mutants appears in Figure 1. The recombination frequencies are averages of two or more crosses. The map order of four pairs of closely linked sites could not be determined from two-factor crosses. Several examples of marker effects can be observed among the *am* mutants, particularly when a member of one of the close unordered pairs consistently appears "farther" than the other member from most of the more distant sites, regardless of whether the more distant sites are to the left or the right (Table 2). The map in Figure 1 averages out these differences, however, since it treats the pairs as a unit. The data of Table 2 do not reveal whether one of the markers stimulates, or the other depresses, recombination frequencies. Furthermore, since the markers were obtained from mutagenized stocks but were never backcrossed, the apparent marker effects may be due to fortuitous mutations elsewhere in the genome.

TABLE 1

Assignments of mutants to sites

Amber mutants		Temperature-sensitive mutants	
Site	Other members	Site	Other members
E4302	*E4303, E4310, E4311, E4312, E4319, E4325, E4329, E4333, E4340, E4341*	*L91*	*L74, L106*
		L33	*N24*
		G39	*G40, G42, G43*
B22	*BU21, E4323, N020, N036*	*P36*	*P39*
C125	*E4315, E4336*	*L42*	*CB79, CB82, CB86, CB87, CB88*
E4317	*E4328, E4330, E4335, E4337*		
BU23	*E4301, E4305, E4307, E4316*	*L141*	*CB90, CB120, CB121, CB122, CB123, CB124, CB126*
E192	*E4321, E4326*		
E4304	*E4313, E4339, N101*	*L88*	
E4309	*E4318, E4320, E4331*	*P25*	
E4322	*E4324, N019*	*S9*	
E4314	*E4332, E4334*	*P26*	
E4306	*E4327, E4338*	*L56*	
		L97	
		A68	
		A60	
		A69	
		A73	
		L98	
		G37	*A71*
		L107	
		A58	

The map of the *am* mutants can be compared with the corresponding polypeptide by means of the mapping function of STAHL, EDGAR and STEINBERG (1964). A total of seven different paths were traced across the map, using all of the distances at least once, and two distances twice. The corresponding numbers of base pairs were determined from the mapping function, and were multiplied

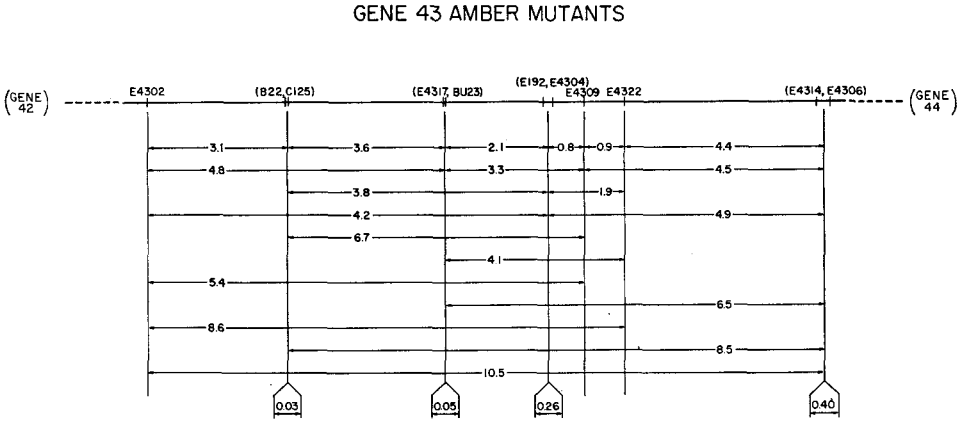

FIGURE 1.—Map of the amber mutants of gene *43*.

TABLE 2

Marker effects in crosses between amber mutants: recombination frequencies

Site of first parent	Site of second parent							
	B22	*C125*	*E4317*	*BU23*	*E192*	*E4304*	*E4314*	*E4306*
E4302	3.0	3.1	5.5	4.3	6.1	3.5	7.9	11.1
B22	..	..	2.8	3.5	4.3	4.8	..	..
C125	..	..	3.4	4.0	5.0	2.5	..	..
E4317	2.8	3.4	..	..	3.1	1.0	..	..
BU23	3.5	4.0	..	..	3.1	2.1	6.7	7.0
E192	4.3	5.0	3.1	3.1	..	..	..	..
E4304	4.8	2.5	1.0	2.1	..	..	4.2	4.5
E4309	7.2	6.2	2.8	3.6	0.8	0.7	4.0	4.6
E4322	..	..	3.6	4.4	..	..	..	..
E4306	8.3	8.6	6.1	7.0	5.7	4.5	..	..

The entries are recombination frequencies averaged from several crosses; more than one member of a site was frequently used. No entry is given when data are available for only one member of a pair.

by 110/3 to convert them to polypeptide molecular weights. The average molecular weight was 117,000 daltons (range 84,000–165,000). This value agrees well with the molecular weight of 112,000 daltons determined by GOULIAN, LUCAS and KORNBERG (1968) using purified T4 DNA polymerase. The terminal *am* sites therefore encompass most of the cistron. The length of the *am* map also agrees with estimates obtained by MOSIG (1968 and personal communication) using a combination of physical and genetic measurements. Her data place the size of the corresponding polypeptide within the 92,500–152,000 dalton range, with the lower value being preferred.

Purified T4 DNA polymerase contains an inseparable 3′ exonuclease activity (GOULIAN, LUCAS and KORNBERG 1968). When grown in nonpermissive hosts, *am* mutants from the *B22* and *C125* sites still produce the nuclease, although the polymerase activity is of course abolished; mutants at the *E4302*, *E4317*, *E4304*, *E4322* and *E4306* sites fail to produce nuclease under nonpermissive conditions (NOSSAL 1969 and personal communication). The orientation of the gene (see below) indicates that gene *43* polypeptides missing approximately 20% of their C-terminal ends are uniquely capable of expressing nuclease activity.

POGOSOV and ALIKHANIAN (1968) have also constructed a map of gene *43*, using *amB22* plus 41 independently isolated *am* mutants induced by hydroxylamine. The 41 mutants were assigned to approximately 27 sites, and the sites themselves appear to be grouped into three clusters. When analyzed by means of the T4 mapping function, the map of these 27 sites is only 21% as long as the map in Figure 1, which is composed of 11 sites. Furthermore, the map of POGOSOV and ALIKHANIAN locates *amB22* as a terminal marker, whereas it appears about one fifth of the distance into the map shown in Figure 1. It is not yet clear whether the differences between the two maps arise from the different origins of the mutants studied, or from differences in methodology.

The map of the *ts* mutants proved extremely difficult to construct. Whenever possible, three markers were ordered by placing on the outside that marker pair which exhibited the largest of the three recombination frequencies. This rule was occasionally violated, however, when it led to serious inconsistencies when several additional markers were considered. The map appears in Figure 2. We confirm the order, and approximately the scale, of *tsL91*, *tsL141*, *tsL97*, and *tsL107* as determined by EDGAR and LIELAUSIS (1964).

In addition to being much longer than the *am* map, the *ts* map contains many more anomalies. Crosses between *ts* mutants were frequently observed to be very poorly reproducible, and many of the entries of Figure 2 represent averages of 4 to 8 crosses. Furthermore, certain crosses between *ts* mutants could never be performed completely acceptably because very low burst sizes were consistently obtained. These difficulties seems to be characteristic of the *ts* mutants, since similar difficulties were rarely encountered among crosses between *am* mutants. Another factor which may sometimes confuse the construction of internally consistent one-dimensional maps is the phenotype of the double mutant recombinants. These have been assumed to be temperature sensitive, but might nevertheless sometimes grow at the nonpermissive temperature, thus doubling the apparent recombinant frequency. For these reasons, and because of the additional complexities to be discussed below, the correspondence between the *ts* map and the arrangement of the markers on the chromosome is likely in some regions to be imperfect.

All of the mutants employed in this study were induced by chemical mutagens. It is therefore likely that some of them are double mutants (see Chapter 6 of DRAKE 1970). This possibility is particularly obvious in the case of *ts* mutants, first because many potential sites seem to be available, and second because double mutants are likely to exhibit increased temperature sensitivities. (Note that *ts* mutants are often incompletely inactive at the nonpermissive temperature; see Table 5.) One unequivocal example of a double *ts* mutant has already been encountered. The *L42* site contains a series of five additional mutants which probably constitute isolates from a single clone. Another mutant, *tsCB78*, recombines with *tsL42* but not with *tsCB79*, *tsCB82*, *tsCB86*, *tsCB87*, or *tsCB88*. It seems likely that the double mutant arose during a single chromosome replication during 5-bromodeoxyuridine mutagenesis, multiplied further, and then recombined with a wild-type chromosome. The *tsCB78* marker produces 9.8% recombinants with *tsL42* and appears to be located to the left of gene *43*, since it produces 8.1% recombinants with *tsP36*, and 12.6% with *tsL88*. Amber mutants in genes *42* and *43*, however, are efficiently complemented by *tsCB78*. The composition of the double mutants was confirmed by backcrossing *tsCB87* to T4D and isolating *ts* mutants which mapped at the *tsL42* site or at the *tsCB78* site, but not at both. The *tsCB87* mutant was shown previously to be strongly antimutagenic (DRAKE and ALLEN 1968; DRAKE *et al.* 1969). We therefore tested the antimutagenicity of its individual components (Table 3). Only the component which resides in gene *43* exhibits antimutagenicity.

Both *tsL91* and *amB22* have been reported to be recombinogenic, particularly

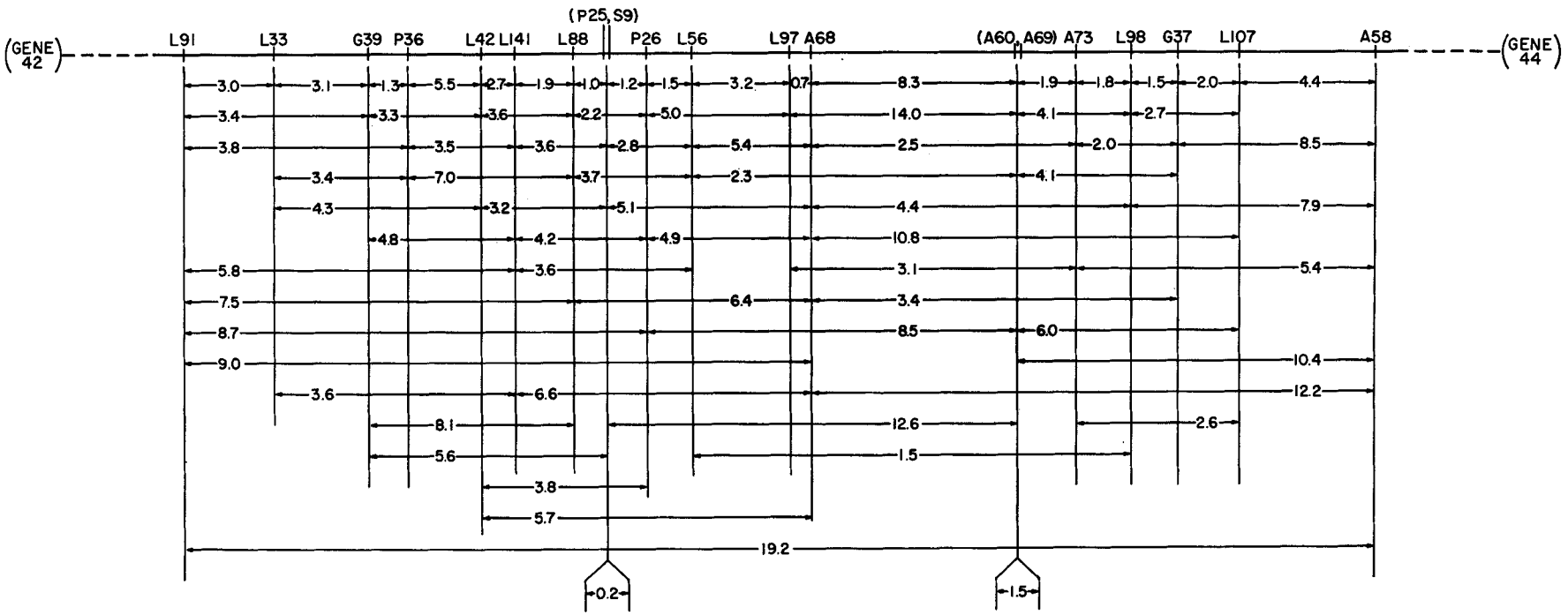

FIGURE 2.—Map of the temperature-sensitive mutants of gene *43*.

TABLE 3

Antimutagenicity of the components of CB87

r^+ revertants per 10^8 *rUV199* particles in the presence of the indicated *ts* allele					
Experiment 1			Experiment 2		
ts+	*tsL42*	*tsCB78*	*ts*+	*tsL42(CB87)*	*tsCB78(CB87)*
63	0.2	66	50	1.3	424
86	0.5	86	142	..	556
92	0.6	113	...	..	...
137	1.0	223	...	..	...

The *ts-r* double mutants were constructed by recombination, individual stocks were grown in BB cells at 32°C, and $r+$ revertants were assayed on KB cells at 32°C. The notation *tsL42(CB87)* indicates that the *tsL42* allele was recovered from a backcross of *tsCB87*. The difference between the two *tsCB78* experiments is not considered to be significant.

under semipermissive conditions but also under apparently permissive conditions (BERNSTEIN 1967; BERGER, WARREN and FRY 1969). Considering the likely role of repair processes in genetic recombination, both *am* and *ts* DNA polymerase mutants might be expected to exhibit marker effects. The *ts* mutants, however, are more likely to do so than are *am* mutants, because *ts* mutants are selected initially for their destabilizing effects upon the polypeptide, and because many *ts* mutants are not fully functional even at a "permissive" temperature such as 32°C (see Table 4). On the other hand, *am* mutants growing under permissive conditions will frequently produce polypeptides identical to, or at least functionally indistinguishable from, the wild-type polypeptide. It is therefore not very surprising that the *ts* map is much longer than the *am* map, and that the *ts* map contains serious anomalies.

Even under permissive conditions, of course, *am* mutants produce approximately equal numbers of aborted and completed polypeptide chains (STRETTON, KAPLAN and BRENNER 1966). Coincidentally, *amB22* is nearly unique among gene *43 am* mutants in exhibiting a very strong nuclease activity under nonpermissive conditions (NOSSAL 1969). This nuclease activity is probably responsible for the recombinogenicity of *amB22* under permissive conditions, in which case most other gene *43 am* mutants would probably not be particularly recombinogenic in the tests employed by BERGER, WARREN and FRY (1969).

The *am* and *ts* maps were oriented both with respect to each other, and with respect to the nearby (but not adjacent) genes *42* and *44*. Despite the difference in map lengths, the left terminal *am* and *ts* markers are quite close to each other, and the right terminal markers are similarly closely aligned (Figure 3). This result strengthens the conclusion that the terminal markers encompass nearly all of the cistron.

The orientation of gene *43* with respect to genes *42* and *44* was established by the crosses described in Figure 4, and is the same as the orientation determined by EDGAR and LIELAUSIS (1964). The directions of transcription and translation of the gene can therefore be deduced. GUHA and SZYBALSKI (1968) have shown that virtually all of the genes which are transcribed early in the T4 growth cycle pro-

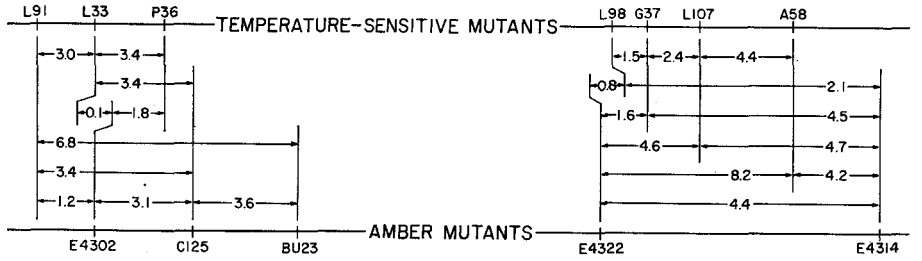

FIGURE 3.—Orientation of the ends of the maps of amber and of temperature-sensitive mutants of gene *43* with respect to each other.

duce mRNA complementary to only one of the strands of the DNA duplex. All such "early" genes must therefore be transcribed in the same direction. The *rII* region, which is transcribed early, is oriented in the anticlockwise direction (CRICK *et al.* 1961), whereas numerous "late" genes are oriented in the clockwise direction (STREISINGER *et al.* 1968). Gene *43* is therefore transcribed (and translated) in the direction from gene *44* towards gene *42*.

POGOSOV and ALIKHANIAN (1968) measured the burst sizes produced when nonpermissive cells were infected with 5 *am* and 5 wild-type particles, and observed a gradient of decreasing burst sizes across the cistron. Within their report, however, and also within a later version (ALIKHANIAN and POGOSOV 1969), gene *43* is oriented differently in different maps with respect to outside markers. In a third version (POGOSOV and ALIKHANIAN 1969) the two maps have become congruent, and the smaller burst sizes are associated with the end of the cistron closest to gene *42*. The mechanism producing the gradient is unknown, but is not likely to consist of "negative complementation" in which a mixed oligomer is inactivated by its mutant components (FINCHAM 1966), since intracistronic complementation could not be detected in gene *43* (DRAKE *et al.* 1969). We have repeated these tests using representatives of the first and last three sites in the cistron (*amE4306*, *amE4314*, *amE4322*, *amC125*, *amB22*, and *amE4302*). The average relative burst sizes from three experiments using B cells (compared to a value of 1.0 in single-parent controls using am^+ particles) were 0.8, 1.0, 1.0, 0.7, 1.3, and 1.4, respectively. No indication of a gradient was observed. In their

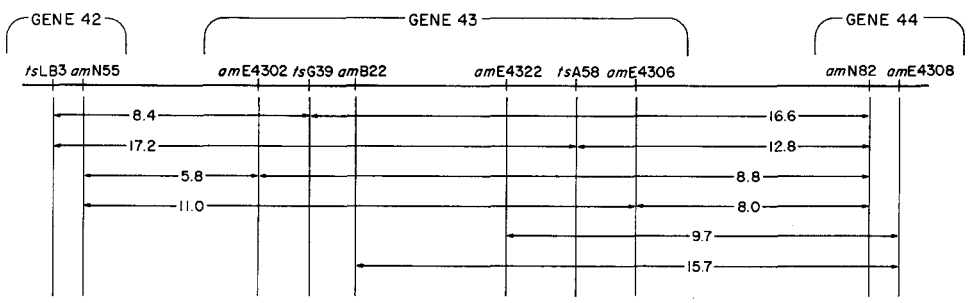

FIGURE 4.—Orientation of gene *43* with respect to gene *42* and gene *44*.

TABLE 4

Properties of gene 43 amber mutants

am site	Relative burst size		Suppression*				
	CR63	BB	ser	gln	tyr	lys	ochre
E4302	0.17	0.00002	+	—	+	—	—
B22	0.37	0.00007	+	+	+	+	—
C125	0.71	0.0001	+	+	+	+	+w
E4317	0.69	0.00006	+	+	—	—	—
BU23	0.75	0.00003	+	+	—	+	—
E192	0.56	0.00006	+	+	+	+	+w
E4304	0.066	0.00006	+	+w	+w	—	—
E4309	0.58	0.0002	+	+	+	+	+w
E4322	1.63	0.0002	+	+	+	+	+w
E4314	0.85	0.00007	+	+	+	—	—
E4306	0.022	0.00005	+	+w	+	—	—

* The suppressor strains, according to the amino acid inserted, the name and genotype of the strain, and the approximate efficiency of chain propagation, were: serine, CR63*suI*+, 67%; glutamine, C600*suII*+, 33%; tyrosine, CA265*suIII*+, 50%; lysine, CA273*suIV*+, 9%; unknown, CA165*suB*+, less than 5%. The notation +w indicates that the plaques which did appear were small and hazy.

TABLE 5

Properties of gene 43 temperature-sensitive mutants

ts site	Relative burst size		Reversion		Effect on mutation
	32°C	43°C	2AP	5BU	
L91	0.15	0.02	+	+	mutator
L33	0.78	0.01	+	—	mutator
G39	0.70	0.02	+	+	.....
P36	0.29	0.01	—	—	.....
L42	0.55	0.005	+	+	strong antimutator
L141	0.29	0.15	+	—	strong antimutator
L88	0.35	0.006	+	—	strong mutator
P25	0.54	0.02	—	—	mutator
S9	0.63	0.03	+	+	.....
P26	0.24	0.02	—	—	mutator
L56	0.58	0.01	—	—	strong mutator
L97	0.014	0.02	—	—	mutator
A68	0.55	0.004	+	—	mutator
A60	0.097	0.01	+	—	.....
A69	0.057	0.01	+	—	.....
A73	0.053	0.01	—	—	.....
L98	0.15	0.006	—	+	strong mutator
G37	0.82	0.05	—	—	antimutator
L107	0.76	0.02	—	—	.....
A58	0.066	0.02	+	+	mutator

two 1969 reports, POGOSOV and ALIKHANIAN reported relative burst sizes of about 0.2 and 0.4 using *amB22*, compared to our value of approximately 1.3.

A number of additional properties of the *am* and *ts* mutants are presented in Tables 4 and 5. The *am* mutants are harshly restricted in the nonpermissive host, the largest burst sizes being about 10^{-4} of the wild-type control. (The nature of the released particles was not investigated. They may have included am^+ revertants.) Most of the *am* mutants grew well in the permissive host CR63, which inserts serine into the UAG codon. (The *am* mutants were first defined by their ability to grow in CR63 but not in BB.) Mutants at sites *E4304* and *E4306*, however, produced very low burst sizes and small plaques on CR63. Inspection of Table 2 indicates that *amE4304* produced generally lower recombinant frequencies than did its close neighbor *amE192*, whereas *amE4306* produced somewhat higher frequencies than did its neighbor *amE4314*. No obvious relationship between gene *43* function and recombination is evident, although BERNSTEIN (1967) and BERGER, WARREN and FRY (1969) observed increased recombinant frequencies with defective DNA polymerases.

All of the amber mutants except *amE4302* also propagate on C600 cells, which insert glutamine into the UAG codon. Two mutants, however, produce small and hazy plaques ($+^w$). Since most of these mutants were induced by mutagens which produce transitions, *amE4302*, *amE4304* and *amE4306* probably arose from the UGG tryptophan codon rather than from the CAG glutamine codon. The suppressor which inserts tyrosine into the UAG codon fails to propagate two of the mutants, and propagates a third poorly. The suppressor which inserts the amino acid (lysine) most distantly related to the probable wild-type amino acid (glutamine or tryptophan), and does so inefficiently, propagates only six of the eleven mutants. Finally, the ochre suppressor, whose amino acid insertion is unknown, propagates only four of the gene *43* amber mutants. These all produce small and hazy plaques, probably because of the low chain propagating efficiency of the ochre suppressor.

The *E4304* site contains an anomalous mutant, *N101*. KRIEG and STENT (1968) reported that *N101* grows on certain *E. coli* strains which are nonpermissive for all other T4 amber mutant tested, and fails to grow on several other strains which are permissive for nearly all other amber mutants. They concluded that *N101* carries a missense rather than a UAG codon. This result is consistent with the observations of WARNER and BARNES (1966), who reported that *N101* is leaky and induces a small amount of DNA synthesis in nonpermissive B cells, while producing a small burst size in CR63 without the production of detectable levels of DNA polymerase. In our hands *N101* produces tiny plaques on B, pinpoint plaques on BB, and small plaques on CR63 cells. Its relative burst size on CR63 was only 1.4% of the wild-type value.

The *ts* mutants (Table 5) exhibit relatively large burst sizes under nonpermissive conditions. These mutants frequently produce variable numbers of pinpoint plaques at 43°C which must be distinguished from ts^+ recombinants in crosses. Some also exhibit sharply reduced burst sizes at the arbitrarily chosen permissive temperature of 32°C. The five mutants exhibiting burst sizes below

10% of the wild-type value at 32°C (*A58*, *A60*, *A69*, *A73*, *L97*) all also produced anomalous recombinant frequencies (Figure 2).

Surprisingly, 8 of the 20 *ts* mutants could not be shown to revert in base analogue spot tests (2AP = 2-aminopurine; 5BU = 5-bromouracil), although these tests are very sensitive in the T4*rII* system. A number of factors probably conspire to produce the negative results. Mutator activity acting upon the marker itself may mask reversion induced by base analogues (FREESE and FREESE 1967), while antimutator activity may suppress base analogue reversion (DRAKE and ALLEN 1968; DRAKE *et al.* 1969). Some of the mutants may be double mutants, since in two cases (*tsP36*, *tsL56*) no spontaneous revertants could be observed; however, many of the mutants were so leaky that plates seeded with the 10^5 to 10^8 particles required in the test were completely lysed. The data on mutator and antimutator activity of the *ts* mutants was taken from DRAKE *et al.* (1969) and from SPEYER, KARAM and LENNY (1966). Many of the tests were of a preliminary nature, so that absence of an entry does not necessarily mean absence of mutator or antimutator activity.

The genetic and chemical properties of the *ts* and *am* mutants show certain correlations when compared with their map locations. (Map locations here will be estimated as percentages from the origin of the cistron at the end closest to gene *44*, *amE4314* being at 0% and *tsL91* being at 100% of the known length of the cistron.) Except for *tsL98*, the mutants which thus far exhibit the strongest effects on mutation rates (*tsL42*, *tsL141*, *tsL88*, and *tsL56*) cluster in a region between approximately 60% and 75% of the cistron. This region is close to the point (at about 80%) at which chain cleavage preserves nuclease activity (NOSSAL 1969), hinting at a possible relationship between nuclease activity and accuracy. The chemical properties of *ts* mutants were studied by DE WAARD, PAUL and LEHMAN (1965). They observed that *tsL91* (near 100%) grown at 37°C produced a very labile enzyme, that *tsL141* and *tsS9* (in the 60–75% cluster) grown at 37°C produced a stable enzyme, and that *tsL97* (at about 50%) and *tsL107* (at about 10%) produced little enzyme at 37°C, but produced an enzyme at a lower temperature which was stable at 37°C.

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SUMMARY

Maps are presented summarizing the results of two-factor crosses among amber and temperature-sensitive mutants of bacteriophage T4 affecting gene *43*, the structural gene for the viral DNA polymerase. The *am* map contains 11 sites, and its length agrees with the size of the polypeptide. The *ts* map contains 20 sites and is several times longer than the *am* map, although the ends of the two maps are similar; this difference is attributed to the recombinogenic effect of gene *43* *ts* mutations. The gene has been oriented on the map of the chromosome,

and its direction of transcription deduced. Growth patterns of the mutants, the susceptibility of *ts* mutants to reversion by base analogues, and the ability of *am* mutants to grow on various suppressors are also described.

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