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Genetic recombination and complementation between bacteriophage T7 and cloned fragments of T7 DNA

(physical mapping/*Escherichia coli*/pMB9 plasmid)

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ABSTRACT **Fragments of phage T7 DNA have been cloned in *Escherichia coli* by using the plasmid pMB9. Such cloned fragments are able to recombine with infecting phages, thus providing a means to integrate the physical and genetic maps of T7 DNA. Approximately 65% of the T7 DNA molecule has been found in clones so far, and analysis of these clones has mapped genes *12–17* with an accuracy of about 1% the total length of T7 DNA. At least some cloned segments can supply T7 functions to infecting phages.**

Molecular cloning of DNA is a powerful tool for analyzing the structure and function of both prokaryotic and eukaryotic DNAs, and for amplifying specific gene products (1). We are applying molecular cloning techniques to the analysis of phage T7 DNA in order to refine the structural and genetic map of T7 DNA and to explore the usefulness of these techniques for studying the biochemical relationships between T7 and its host. T7 DNA is well suited for such analysis because most T7 genes have been identified and mapped genetically, and a well-characterized collection of mutants is available (2, 3).

MATERIALS AND METHODS

Phage and Bacterial Strains. Wild-type T7, T7 mutants, *Escherichia coli* B, suppressing strain *E. coli* 011′ (Su^+), and *E. coli* 011′L1 have been described previously (2, 4). The Su^- recipient for transformations, *E. coli* HMS174 (r_{K12}^- m_{K12}^+ *recA1* rif^R Su^-), was derived from the *E. coli* K-12 strains W3110 (*thy*$^-$) and KL16-99 (5). The Su^+ recipient was HB101 (r_B^- m_B^- *pro*$^-$ *gal*$^-$ str^R *recA1* Su^+) (6).

Cloning Procedures. Plasmid pmB9 (7) was used as the cloning vehicle. Fragments of T7 DNA were inserted at the single *Eco*RI cleavage site of pBM9 using poly(dA·dT) connectors (8–11). Fragments of T7 DNA were generated either by cleavage with *Hpa* I (12) or by shearing the DNA to an average size of 4% its full length. Shearing was carried out by stirring a DNA solution (5 μg/ml in 1 M NaCl/50 mM Tris·HCl/1 mM EDTA, pH 8) at full speed in a Lourdes homogenizer at 0°. The recombinant plasmids were introduced into recipient bacteria essentially as described by Wensink *et al.* (13). The pMB9 plasmid confers resistance to tetracycline, and this property was used to select transformants. Clones prepared from *Hpa* I fragments are designated "HP," those prepared from shear fragments, "JC."

Recombination and Complementation Tests. Plasmid-containing bacteria were grown in tryptone broth supplemented with the components of M9 medium (2). Overnight cultures grown in tubes at 37° without shaking were used directly (0.2 ml per plate) for testing the plating efficiency of T7 mutants. For complementation tests, overnight cultures were diluted 100-fold into fresh medium and incubated at 37° without shaking for 3.5 hr (10^8 cells per ml). Infective T7 particles (15 per cell) were added, and incubation was continued at 37° until lysis. Progeny were titered on *E. coli* 011′ (Su^+) and *E. coli* B (Su^-) to measure the burst size of mutant and wild-type T7.

Preparation and Analysis of DNA. T7 DNA was prepared by phenol extraction of purified phage particles. Plasmid DNA was prepared, after amplification in the presence of chloramphenicol, by gentle lysis of the cells with lysozyme and detergent, followed by centrifugation to remove chromosomal DNA (14). Further treatments included phenol extraction, ethanol precipitation, or banding in CsCl gradient containing ethidium bromide at 200 μg/ml with subsequent removal of the ethidium bromide. Heteroduplexes between plasmid and T7 DNA were formed and analyzed by the method of Davis *et al.* (15) using deletion mutants to mark the left end of T7 DNA. Gel electrophoresis was carried out on agarose slab gels (12).

Containment. All procedures were carried out under P1 physical containment with EK1 host–vector as specified by the National Institutes of Health Guidelines for Recombinant DNA Research.

RESULTS

Cloning Random Fragments of T7 DNA. T7 is a strictly virulent phage, and certain portions of T7 DNA carry information lethal to the host cell. We sheared wild-type T7 DNA to fragments of 4% its full length, hoping to separate lethal from nonlethal regions and thereby to be able to clone a large portion of the genome. The fragments were inserted in pBM9 using poly(dA·dT) connectors, and a set of 210 tetracycline-resistant transformants was isolated. More than 80% of the plasmids contained more DNA than the parental pMB9 plasmid, as analyzed by electrophoresis in 1% agarose gels.

To demonstrate that fragments of T7 DNA were contained within the plasmids, five different plasmid DNAs that appeared to have large insertions were isolated and subjected to heteroduplex analysis: pJC13, 15, 19, 27, and 32. Each of the five DNAs formed a heteroduplex with T7 DNA, and the location of each fragment within full length T7 DNA was determined (Fig. 1). Each cloned fragment is unique, and at least four different regions of the T7 DNA molecule are represented in these five clones.

Recombination with Cloned Fragments of T7 DNA. Recombination between a cloned fragment and an infecting phage

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Abbreviations: Su^+ and Su^-, suppressing and nonsuppressing, respectively.

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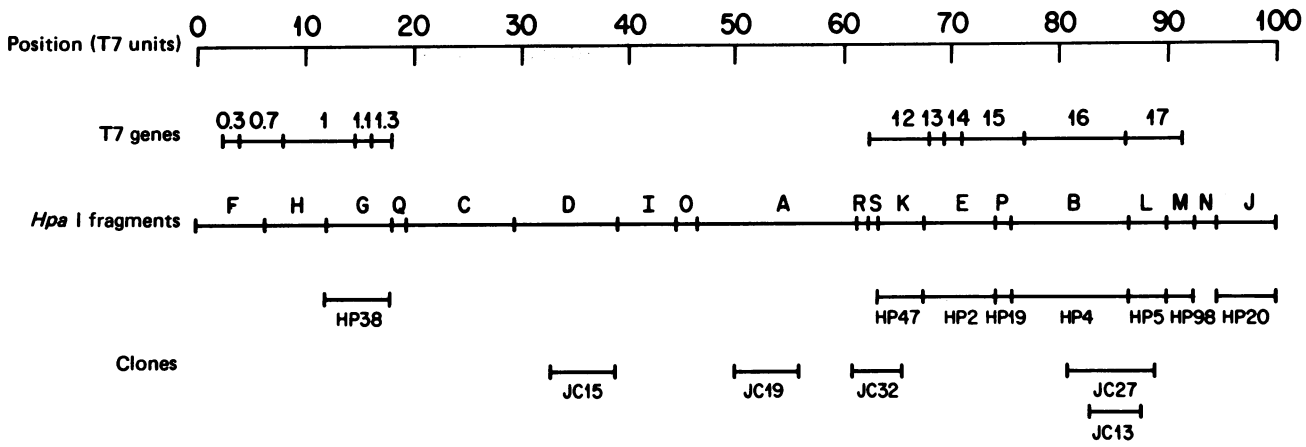


FIG. 1. Physical map of cloned fragments and T7 genes in T7 DNA. The map of *Hpa* I fragments is from ref. 12. Identification of cloned *Hpa* I fragments is described in the text. Random fragments are positioned on the basis of heteroduplex mapping modified by recombinational analysis (Fig. 2). Positions for T7 genes *0.3–1.3* are from refs. 12 and 16. Positions for genes *12–17* are from the present work (see text).

can be detected when the infecting phage is a mutant and the cloned fragment is wild type. If the T7 mutant used for testing recombination does not grow on the host that carries the cloned fragment of T7 DNA, recombination between the infecting phage and a cloned fragment covering the site of the mutation can be observed directly by the increase in plating efficiency that results from the production of wild-type recombinants. This provides a powerful and simple way to map the location of cloned fragments and to integrate the genetic and physical maps of T7 DNA.

T7 amber mutants are an ideal type of infecting phage, because the mutations have been found in most T7 genes and such phage do not plate efficiently on Su^- hosts (2). Heteroduplex analysis suggested that the cloned fragments of pJC13 and pJC27 should lie in the gene *16–17* region of the genome, and that the fragment in pJC13 should lie entirely within the fragment in pJC27 (Fig. 1). (Ref. 12 gives a physical map of T7 genes, based on sizes and relative locations of T7 proteins.) As shown in Table 1, the plating efficiency of a contiguous set of gene *16* and *17* amber mutants increases 2–5 orders of magnitude when the host contans pJC13 or pJC27 instead of the parental plasmid pMB9 and, as predicted by the heteroduplex analysis, the set of mutations covered by pJC13 lies entirely within the set covered by pJC27. Furthermore, each of the five cloned fragments that had been mapped by heteroduplex analysis was found to give wild-type recombinants with a contiguous set of T7 mutants in the expected region of the genome (Fig. 2), as expected if a single continuous fragment of T7 DNA had been cloned in each.

Although the test for increased plating efficiency is a simple way to map cloned fragments relative to genetic markers, it may not work for mutations in every gene. Because little if any of the mutant protein is produced in an Su^- host, an increase in plating efficiency may not be observed if the mutation is in a gene essential for recombination, or if cells in which wild-type recombinants can form are unable to produce phage particles. Therefore, until it is shown that mutants in a particular gene are able to increase their plating efficiency by recombination with a cloned fragment, negative results must be confirmed by testing recombination in an Su^+ host. As shown in following sections, the plating efficiency test has been shown to work for mutations in genes *4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,* and *19* (Fig. 2). Gene *1* mutants are the only ones for which this test has been shown not to work (see below). No clones that cover genes *2, 3, 3.5,* or *18* have yet been identified.

Mapping of Random Clones by Recombination. The results above suggested that a simple spot test could be used for rapid screening of a large population of cloned fragments. Samples of as many as 45 different phage stocks, representing any portion of the T7 genome, are applied to a lawn of each host strain to be tested. The pattern of clearing of the spots indicates the probable location of the cloned fragment, which can then be checked by direct plating of individual mutants.

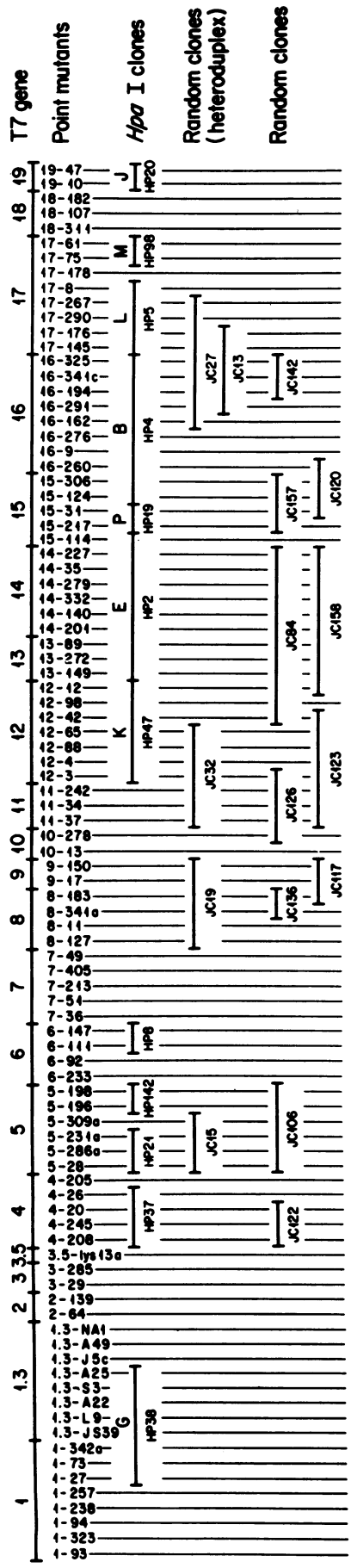
Spot tests were used to screen 124 of the random clones of sheared fragments of T7 DNA, and regions identified by spot test were then mapped by testing the plating efficiency of individual amber mutants. Cloned fragments representing parts of genes *4* and *5*, and most of the region between genes *8* and *17*, were identified among the random clones. Positions of a number of these cloned fragments relative to the T7 amber mutations are given in Fig. 2.

Cloning of *Hpa* I Fragments of T7 DNA. Having shown that much of the T7 DNA molecule can be cloned in pMB9, we turned to cloning fragments of T7 DNA produced by the restriction endonuclease *Hpa* I. *Hpa* I cuts wild-type T7 DNA into 19 specific fragments; the size and location of each are known (Fig. 1). Thus, recombination with cloned *Hpa* I fragments can give precise information about the physical location of T7 amber mutations and therefore the T7 genes.

The DNA used for cloning *Hpa* I fragments was from a deletion mutant of T7, C74, that lacks all of gene *0.7* (16). The deletion eliminates the leftmost *Hpa* I cleavage site in T7 DNA,

Table 1. Plating efficiency of T7 amber mutants on plasmid-carrying HMS174 (Su^-) strains

T7 strain	Plating efficiency relative to 011′ (Su^+)		
	HMS174/ pMB9	HMS174/ pJC13	HMS174/ pJC27
Wild type	1	1	1
16-276	11×10^{-6}	12×10^{-6}	10×10^{-6}
16-162	1×10^{-6}	1×10^{-6}	0.13
16-291	25×10^{-6}	0.065	0.17
16-194	2×10^{-6}	0.19	0.24
16-325	5×10^{-6}	0.25	0.38
17-145	2×10^{-6}	0.008	0.02
17-176	8×10^{-6}	0.05	0.05
17-290	5×10^{-6}	4×10^{-6}	0.006
17-267	12×10^{-6}	12×10^{-6}	0.004
17-8	33×10^{-6}	29×10^{-6}	49×10^{-6}



and the resulting fusion fragment contains parts of fragments F and H; the remaining 17 *Hpa* I fragments of C74 DNA are the same as those from wild-type DNA. The mixture of fragments produced by cutting C74 DNA with *Hpa* I was cloned in pMB9 using poly(dA-dT) connectors, and a set of 152 tetracycline-resistant transformants was isolated. Of the tested clones, 70% contained more DNA than did the parental pMB9 plasmid.

Clones from the *Hpa* I digest of T7 DNA were screened by spot test, and the location of cloned fragments was determined by their ability to increase the plating efficiency of individual T7 mutants. As described below, *Hpa* I fragments G, K, E, P, B, L, M, and J have been identified in this set of clones, plus four fragments that have not yet been assigned (Fig. 2). We estimate that 65% of the T7 DNA molecule is represented in the combined *Hpa* I and random shear clones of Figs. 1 and 2.

Integration of Physical and Genetic Mapping Using Cloned *Hpa* I Fragments. A series of adjacent fragments covering genes *12–17* could be identified as *Hpa* I fragments K, E, P, B, L, and M by knowledge of the sizes of the proteins (3) and amber peptides (unpublished observations) covered by each fragment (Figs. 1 and 2). With the exception of fragment M, which was found only twice in the first 100 clones, each of the above fragments was found relatively frequently. Integration of the physical and genetic data determines the physical location of genes *12–17* with an uncertainty of about 1 T7 unit (Fig. 1).

The only break in the continuous string of amber mutations covered by the above set of cloned fragments was *17*-178, which appears not to be covered by either L or M. This could indicate that a small portion at the end of L or M was not included in the clones tested, but it is also possible that *17*-178 carries a secondary mutation that prevents the formation of wild-type recombinants with either plasmid.

Cloning of the Right End of Phage T7. Another type of clone, found at least 10 times in this set of *Hpa* I clones, covers both of the available gene *19* mutants and presumably contains the right end of the T7 DNA, fragment J. These clones are unusual because they restrict the growth of T7, wild-type phage giving small plaques at low efficiency. It should be possible to identify the region of the J fragment responsible for the restriction of T7 growth by cloning subfragments of J. It would be particularly interesting if the critical region is the terminal repetition of T7 DNA (18).

Clones Carrying Genes Involved in Phage DNA Metabolism. At least 11 representatives of a clone that covers the right end of gene *6* were also identified in this set of clones. However, three other types, covering parts of genes *4* and *5*, were each represented only once. Further work will be required to identify what parts of T7 DNA were carried by these clones, but the three types found only once might contain only parts of *Hpa* I fragments, a minor fraction of the digest. Presumably clones of these entire fragments are lethal.

Cloning of Fragment G— the Origin of T7 Replication. Previous work (12) showed that fragment G contains the right

FIG. 2. Location of cloned fragments relative to T7 mutations. The T7 mutants are described in refs. 2 and 4, and mapping of cloned fragments was based on ability to recombine with infecting phages, as described in the text. Gene 7 mutants are able to grow on *E. coli* K-12 strains (17), including *E. coli* HMS174. Therefore recombination of cloned fragments with gene 7 mutants was tested by looking for an increase in wild-type progeny after growth on the plasmid-carrying strain rather than by direct plating of gene 7 mutants. In this way pHP8, pJC19, and pJC135 were all shown not to cover any of the gene 7 mutations.

end of gene *1*, all of gene *1.1*, and the left end of gene *1.3*, and that fragment Q contains the right end of gene *1.3*. Clones of fragments G or Q in HMS174 (Su^-) could not be detected by direct plating of phage mutants because gene *1* mutants are too defective, and gene *1.1* and *1.3* mutants are able to plate on HMS174. However, gene *1.3* specifies T7 ligase, and gene *1.3* mutants are unable to plate on ligase-deficient hosts (4). Therefore, plasmids from 48 different clones from the *Hpa* I digest were screened for fragments of gene *1.3* by transferring them to a ligase-deficient host (011'L1) and then testing for increased plating efficiency of gene *1.3* mutants. Clone 38 was found to contain fragment G, but no clone containing fragment Q was identified.

Recombination between the cloned G fragment and gene *1* mutants could not be detected by increased plating efficiency of gene *1* mutants on HMS174/pHP38, presumably because gene *1* mutants are too defective to form recombinants and/or produce progeny [gene *1* specifies the T7 RNA polymerase (19)]. Confirmation that the cloned fragment is fragment G was obtained by transferring pHP38 to the Su^+ host, HB101, and testing for recombination between the cloned fragment and different gene *1* and *1.3* mutants under permissive conditions. In this test a large increase in the fraction of wild-type T7 in the progeny indicates that recombination has occurred. As expected, the cloned fragment covers both the right end of gene *1* and the left end of gene *1.3* (Fig. 2), the entire G fragment. This plasmid should carry the origin of replication of T7 DNA, which has been mapped near position 17 in the T7 DNA (20).

Complementation by Cloned Fragments of T7 DNA. Those cloned fragments that cover all of the amber mutations available for a particular gene (Fig. 2) were tested for their ability to complement an infecting mutant. The burst size of individual amber mutants is measured on an Su^- host that carries a cloned fragment of T7 DNA. If the cloned fragment can provide the function needed by the mutant, the burst size should represent an appreciable fraction of that observed with wild-type T7. Such tests show that pHP2 and pJC84 can provide the needed functions to gene *13* and gene *14* mutants, and that pJC158 can provide the gene *13* function (Table 2). Mutants that can be complemented give a burst 35–70% of that given by wild type in the same strain, and the progeny are amber mutants (wild-type recombinants making up only 10–25% of the total). On the other hand, mutants covered by the cloned fragment, but not complemented by it, show no increase in burst size over that observed for pMB9 (for example, *14*-140 on pJC158 and *15*-114 on pHP2 in Table 2). Even mutants that plate with efficiencies of 0.2 to 0.4, such as *16*-194 on HMS174/pJC27 in Table 1, give no detectable increase in burst size (data not shown).

The observation that the cloned fragment of pJC158 covers all available gene *14* mutations (Fig. 2), but does not complement a gene *14* mutant (Table 2), suggests that not quite all of gene *14* has been cloned in this plasmid. Other cloned fragments that cover all available markers in a gene (Fig. 2), but do not appear to complement mutants in that gene (data not given), include pJC106 (gene *5*), pJC32, pJC123, and pJC126 (gene *11*), and pHP4 (gene *16*). The cloned fragment of pJC126 is the only one of these to cover markers in both adjacent outside genes and, although the lack of complementation by the other cloned fragments could be explained by the lack of a complete gene, the lack of complementation by pJC126 is presumably for some other reason.

As would be expected, the plating efficiency of an amber mutant on a complementing Su^- host is the same as that on an Su^+ host (Table 3), and the plaques are large and uniform. In contrast, when the cloned fragment covers the amber mutation but does not complement it, the plaques are variable in size, and the plating efficiency rarely reaches as high as 0.3 (Tables 1–3).

Table 2. Complementation of T7 amber mutants by cloned fragments of T7 DNA, as measured by burst size in plasmid-carrying HMS174 (Su^-) strains

Host	T7 strain	Burst size on indicator strain Su^-	Burst size on indicator strain Su^+
HMS174/pMB9	Wild type	35	46
	13-149	<0.01	1.7
	14-140	<0.01	1.2
	15-114	<0.01	1.2
HMS174/pHP2	Wild type	25	35
	13-149	2.9	25
	14-140	1.4	12
	15-114	<0.01	1.5
HMS174/pJC84	Wild type	19	21
	13-149	2.8	11
	14-140	2.0	8.2
	15-114	<0.01	1.2
HMS174/pJC158	Wild type	31	32
	13-149	1.4	12
	14-140	<0.01	0.6
	15-114	<0.01	0.9

Phage obtained after infecting plasmid-carrying strains of HMS174 were titered on *E. coli* B (Su^-) and *E. coli* 011' (Su^+) in order to determine the number of wild-type and amber progeny. The small apparent burst of amber mutants on HMS174/pMB9 and on other noncomplementing hosts is probably due to unadsorbed phage. See Fig. 2 for the location of the cloned fragments carried by these plasmids.

Complementing hosts would be very useful if they could serve as helper strains for isolating or propagating mutants that are completely defective in the complemented gene. However, recombination between the cloned fragment and the mutant strain would also be occurring, and there is a danger that wild-type recombinants would overgrow a plaque. To determine how serious a problem this might be, we investigated the composition of plaques made by *13*-149 and by *14*-140 on

Table 3. Plating efficiency of T7 amber mutants on plasmid-carrying HMS174 (Su^-) strains

T7 strain	Plating efficiency relative to 011' (Su^+): HMS174/pMB9	HMS174/pHP2	HMS174/pJC84	HMS174/pJC158
12-55	2×10^{-5}		2×10^{-5}	2×10^{-5}
12-42	20×10^{-5}		0.009	20×10^{-5}
12-98	80×10^{-5}		0.12	50×10^{-5}
12-12	1×10^{-5}	2×10^{-5}	0.3	0.14
13-149	2×10^{-5}	1.0	0.9	0.9
13-272	1×10^{-5}	1.2	0.7	0.7
13-89	3×10^{-5}	1.2	0.8	1.1
14-201	1×10^{-5}	1.4	1.1	0.05
14-140	1×10^{-5}	1.2	1.4	0.03
14-332	15×10^{-5}	1.0	0.9	0.01
14-279	1×10^{-5}	1.1	1.0	0.01
14-35	16×10^{-5}	1.1	1.0	0.01
14-227	9×10^{-5}	0.8	0.8	0.003
15-114	2×10^{-5}	0.006	2×10^{-5}	2×10^{-5}
15-217	2×10^{-5}	2×10^{-5}	2×10^{-5}	1×10^{-5}

HMS174/pHP2, a complementing host. Two of the largest plaques made by each mutant were isolated after incubation at 37° for 2.3 hr, and two more after 3.5 hr. The composition was analyzed by suspending each plaque in growth medium, plating out a dilution on the Su^+ host, and determining whether the plaques that grew represented amber mutants (by picking individual plaques onto a lawn of an Su^- host). Amber mutants represented 11–35% of the phages in the 2.3-hr plaques, and 1–17% in the 3.5-hr plaques, levels high enough to encourage further exploration of the use of complementing strains as helpers.

DISCUSSION

Genetic recombination between cloned fragments of T7 DNA and infecting phage mutants provides a uniquely powerful way to integrate the genetic and physical maps of T7 DNA. The availability of a large collection of genetically mapped mutants of phage T7 makes possible the rapid screening of cloned fragments, and virtually any degree of resolution should be attainable simply by cloning and analysis of well-defined fragments of T7 DNA produced by restriction endonucleases.

Our experiments suggest that *Hpa* I fragments C, D, and A cannot be cloned intact, presumably because they carry information that is lethal to *E. coli*. This might explain the failure of our early attempts to clone the large fragments of T7 DNA produced by *Mbo* I (12). With an appropriate set of fragments, it should ultimately be possible to pinpoint all the lethal region of T7 DNA and perhaps to clone every part of the T7 genome.

Recombination between cloned fragments of T7 DNA and infecting phages is apparently due to a T7 recombination system. It occurs in *recA*$^-$ hosts, but not when the phage mutant is defective in gene *1*, which specifies the RNA polymerase needed to transcribe most T7 genes. Therefore, it was somewhat surprising to find that T7 mutants defective in genes *4*, *5*, or *6*, all of which have been implicated in T7 recombination (21, 22), were able to recombine with cloned fragments. Apparently these functions are not absolutely required for recombination during T7 infection.

Only a few of the recombinant plasmids analyzed here could have carried entire T7 genes. Certain clones carrying genes *11*, *13*, or *14* also carried markers from both adjacent genes and therefore should have contained entire genes. Of these, clones carrying gene *13*, or both genes *13* and *14*, produced products that could be used by infecting mutants, whereas the clone carrying gene *11* did not. This difference might be explained if there were a promoter for T7 DNA polymerase ahead of gene *13* but not gene *11*. However, previous work (23) has suggested that promoters for T7 RNA polymerase lie ahead of both gene *11* and gene *13*. Other factors that might affect the ability of a particular cloned gene to complement an infecting mutant include the number of protein molecules needed for efficient complementation, how well the T7 gene can be transcribed by *E. coli* RNA polymerase, the number of copies of the plasmid DNA, and whether the plasmid DNA is degraded after infection.

The availability of cloned fragments of T7 DNA, and their ability to recombine with and complement infecting phages, provide several novel opportunities for genetic and biochemical analysis of T7. An appropriate set of clones would make possible rapid mapping of new T7 mutants. Mutagenesis could be restricted to a specific region of the T7 DNA by mutagenizing the cloned fragment and then allowing wild-type T7 to pick up the mutations by recombination during infection. Complementing hosts might provide the means to isolate and propagate completely defective strains. Cloned fragments could serve as model systems for studying phage recombination, the mechanism by which T7 degrades host DNA but not its own DNA after infection, the basis for mutual exclusion between T3 and T7, and the exclusion of superinfecting phages. Plasmids that contain the origin of T7 DNA replication or the terminal repetition of T7 DNA are now available and should be extremely useful in studies involving these two important regions in the T7 DNA molecule. Finally, if T7 proteins such as DNA polymerase, DNA ligase, DNA binding protein, gene *4* protein, and gene *6* exonuclease can be synthesized in the uninfected host cell, it may be possible to substitute one or more of them for *E. coli* counterparts having a similar function, thus providing a novel approach to studying the role and specificity of both the T7 and *E. coli* enzymes.

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