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Recombinants Between Bacteriophages T7 and T3 Which Productively Infect F-Plasmid-Containing Strains of *Escherichia coli*

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Recombinant phages between T7 and T3 have been isolated that grow well on strains of *Escherichia coli* that contain the F factor. One phage that has been characterized physically and genetically is predominately of the T7 genotype. Within this hybrid phage, two separate regions of T3 DNA have been located which are necessary for the phenotype of productive growth on F-containing strains. One of these, designated M1, contains the right part of gene 1 and continues through gene 1.3; the second, M2, appears to lie between gene 3 and gene 4.

Bacteriophage T7 is a lytic phage whose growth on female cells has been extensively characterized (20, 36). When T7 infects cells that harbor the F factor (F^+, F′, or Hfr; herein referred to as "male"), the host is still killed, but phage production is inhibited (13). This abortive infection in male cells is also characteristic of a number of phages related to T7, namely, φII (21), W31 (37), and H (12), but in particular is not characteristic of T3. Early reports that growth of T3 is inhibited by F (27, 38) were based on work with strains that were actually T7 (33).

The F-induced abortive infection of T7 has been extensively studied and has generated, in the process, a great deal of controversy over the precise mechanisms of inhibition by the F factor (10). Adsorption and penetration of DNA are normal, as is the synthesis of class I proteins; however, the later stages of infection are grossly aberrant. Host DNA is not degraded, but the parental phage DNA is poorly, if at all, replicated, and the synthesis of class II and class III proteins is severely inhibited. Overall, the abortive infection is characterized by reduced plaque and burst size, low plating efficiency, and inefficient lysis of the host cell (13).

Mutations both in the F factor and in the *Escherichia coli* chromosome that allow T7 growth in males have been isolated. Studies with these mutant strains have resulted in two different hypotheses for the mechanism of F-mediated restriction, i.e., the inhibition by two F gene products of a T7 translational control mechanism (24) or a host-catalyzed transcriptional block (6, 7). A third hypothesis, based on studies with isogenic strains with and without the F factor, has invoked abnormal cell membrane permeabilities as responsible for the abortive infection (5, 9). These hypotheses are largely incompatible with each other.

The above studies have compared wild-type T7 infections of pairs of *E. coli* strains isogenic except for their ability to support phage growth. However, they have been markedly unsuccessful in providing a single molecular mechanism that can explain all of the various phenomena associated with the abortive infection. We have therefore initiated the complementary analysis to those mentioned above, i.e., the isolation of mutants or variants of T7 that can overcome F-mediated restriction. The extensive knowledge that has been accumulated on the biology of T7, as it pertains to infections of female hosts (20, 36), can then also be applied to a precise understanding of the abortive infection.

This report describes the isolation of hybrid T7-T3 phages that grow well on male strains and an analysis of their physical and genetic characteristics. A schematic diagram summarizing these data is shown in Fig. 5. The physiology of these hybrid phages after infection of a male strain of *E. coli* is described in the accompanying paper (29).

MATERIALS AND METHODS

Phage and bacterial strains. Wild-type T3, T3-HR2 (amber mutation in gene 0.3 [35]) T7 and all T7 mutant strains are originally from the collection of F. W. Studier. A map of the T7 genome and the mutants employed in this work is shown in Fig. 1. *E. coli* B and O11′ are the standard permissive hosts for wild-type and amber mutant T7 strains, respectively. *E. coli* BR3 is restrictive primarily for 0.7^- mutants; these strains were from F. W. Studier (30, 31). *E. coli* N2216 [F^- Str^r Thy^- *sup*$^+$ *lig-7*(Ts)], from M. Gellert is nonpermissive for both T7 amber mutants and T7 ligase

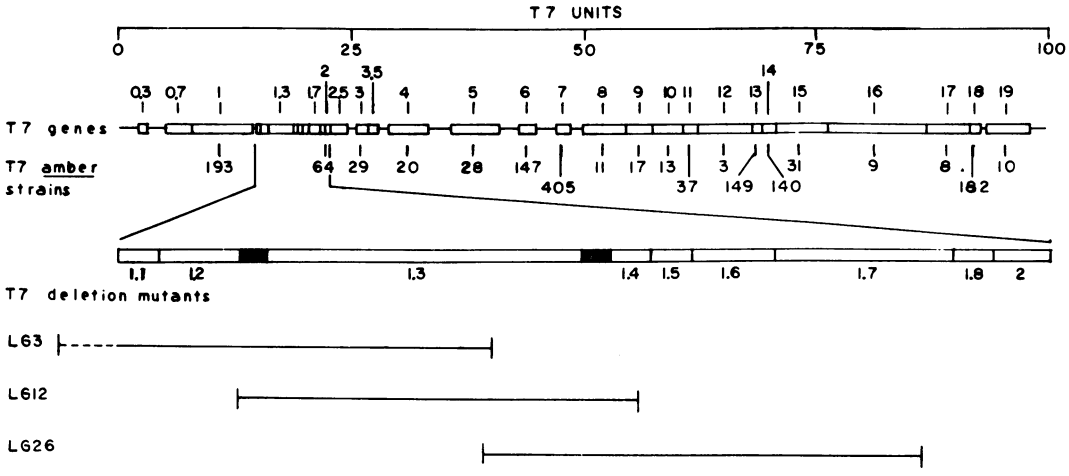

FIG. 1. Genetic map of bacteriophage T7, modified from Studier and Rosenberg (36). The specific amber mutant alleles used in this work are presented underneath each gene. The nomenclature used for T7 is that recommended by Studier (31): the first number indicates the gene in which the mutation is located, and the last number is that given the mutation upon isolation. The amber mutations are as described by Studier (30); the deletions are as described by Studier (31, 32) and Simon and Studier (28).

deletions. *E. coli* LS473 and LS475, from L. Soll, are an Lac$^-$ *sup*$^+$ and Lac$^-$ *supE*44, respectively, isogenic pair of F$^-$ strains that are also T3^r (apparently adsorption mutants). F42 (F' *lac* [19]) was also introduced into these strains from RV/F' *lac*. The resulting male strains are thus nonpermissive for infections by both T3$^+$ and T7$^+$. *E. coli* 993, provided by S. Linn, is an F$^+$ derivative of *E. coli* 803 (1) that is extremely restrictive for T7$^+$, but is sensitive to T3$^+$. For clarity in this report, these male and female strains are referred to as 993F$^+$ and 993F$^-$, respectively.

Media. All in vivo phage work was performed at 30°C in T broth (1% tryptone [Difco Laboratories], 0.5% NaCl) or M9 medium supplemented with 0.4% glucose and necessary nutritional requirements. Solid medium contained 1.3% Difco agar.

Chemicals. Carrier-free [^{35}S]methionine was purchased from Amersham Corp. The restriction enzymes *Mbo*I and *Hpa*I were from New England Biolabs and were used as directed. Agarose was from Bethesda Research Laboratories, and acrylamide was from Miles Laboratories, Inc.

Methods. Genetic recombinations and phage manipulations were performed as described by Studier (30). Concentrated phage stocks were made by differential centrifugation and resuspension or by polyethylene glycol precipitation and banding in CsCl (33). DNA fragments from restriction enzyme digests were analyzed on agarose gels essentially as described by McDonell et al. (23) by using a Tris-borate-EDTA (pH 8.3) buffer or on acrylamide gels in the same buffer (22). DNA was visualized by staining with ethidium bromide. Proteins were labeled with [^{35}S]methionine as described by Studier (32). Briefly, *E. coli* cells growing exponentially in minimal medium at 30°C were irradiated with UV light to suppress host protein synthesis. Cells were further incubated for 15 min at 30°C before infecting with phage at multiplicity of infection of 10. Samples of 0.2 ml were then pulse-labeled with 4 μCi of [^{35}S]methionine. Labeling was stopped by the addition of 1 ml of T broth, and the incubation was continued for 4 min before the sample was chilled and centrifuged. The pellet was suspended in a reducing buffer containing 1% sodium dodecyl sulfate and electrophoresed in a Laemmli gel system as described by Studier (32). Labeled protein bands were visualized by salicylate fluorography (8) or by autoradiography.

RESULTS

T7 variants that grow on male strains of *E. coli*. Direct mutagenesis of T7 or of T7-infected cells with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine failed to yield mutants of T7 that would exhibit large plaques on male strains of *E. coli* with a high plating efficiency. A number of independent mutants were obtained that would plate moderately well on some male strains (e.g., LS473/F' *lac*, efficiency of plating, 0.1 to 0.4 relative to the isogenic female strain), but plaques were small and frequently irregular. In addition these mutants plated relatively poorly on a male host, 993F$^+$, that is extremely restrictive for T7$^+$ (efficiency of plating, 10^{-7}). A second mutagenesis of these isolates also failed to improve significantly either their plaque morphology or their plating efficiency. These phage mutants have not been studied further. It is of course possible that either T7 cannot be mutated to fully overcome F-mediated restriction or that, for whatever reason, this class of mutants is extremely rare.

An alternative approach to obtaining T7 phage that grow on male cells is to isolate recombinants between T7 and T3 which retain a high proportion of T7 DNA, but which contain the T3

genetic elements necessary for growth on male cells. This approach was taken since mutants of T3, which fail to grow on males, had not previously been described, although as a result of this work this class of T3 mutants has now been isolated (manuscript in preparation).

Recombination between the related bacteriophages T7 and T3 is very low. The phages exhibit coinfection exclusion, they are only partially homologous, and an even number of cross-over events is required so that the terminal redundancies are of homologous phage origin (3). To select against the left end of T3 (and therefore also the right end), T3-HR2 (amber mutant in gene 0.3, the left-most gene) was crossed with $T7^+$ in the restrictionless strain *E. coli* C. Unmodified T3 0.3^- strains are severely restricted in hosts that contain an active type I restriction enzyme (35). Progeny phage was plated on LS473/F′ *lac* cells at a high concentration (10^5 to 10^6 phage particles per plate). LS473 and its derivatives are not killed by T3; they appear to be adsorption mutants and they are also phenotypically r_k^+. Selection against parental T3 was both via the leftmost gene and via gene 17, the tail fiber protein, since viable plaque-forming progeny phage must contain T7 adsorption specificity. Selection against parental $T7^+$ was simply by the use of a male strain. Rare large plaques, which were superimposed on a heavy background of small or minute plaques, were then purified several times on LS473/F′ *lac* cells and finally on $993F^+$ cells. Individual plaques were then grown and screened by DNA restriction enzyme analysis.

Restriction enzyme analyses of the recombinant phages. Few restriction enzyme cutting sites are preserved between T3 and T7 (2; see Fig. 3), and by use of these enzymes it is thus possible to at least tentatively ascribe major regions of the recombinant phage genome to one or the other lineage. The enzyme *Mbo*I was initially employed to screen recombinants, since it leads to relatively few fragments with either phage DNA, and these fragments are largely separable from each other by electrophoresis in agarose gels (Fig. 2). Among the large plaque-forming phage isolated there were three major classes. The first class analyzed lacked the rightmost fragment of T3, i.e., T3-E and had acquired a band with the same mobility as T7-C. Class I phage, however, lacked any other T7-sized fragments and were not further studied. The second class of hybrid phage isolated appeared to lack both T3-H and T3-A in addition to T3-E, but did not exhibit T7-sized fragments other than T7-C and T7-F. One of these phages, designated 4.2, was used as a parent in crosses with standard T7 strains containing amber mutations. In a stepwise fashion, amber mutations in T7 genes 18, 17, 16, and 15 were introduced into and then crossed out from this phage in the hope that continued growth of the hybrid phage in the presence of normal T7 strains would lead to the continued replacement of T3 genetic material with T7. This expectation was not realized. *Mbo*I and *Hpa*I restriction enzyme analyses indicated that all of the derivative phages were identical to the parent (data not shown). It did not prove possible to introduce any T7 amber mutations to the left of T7-15 *am*31, and it therefore appeared that in the absence of strong selective pressure these hybrid phages were stable. The inability to introduce a T7 amber mutation in a given gene may be considered at least suggestive evidence for that gene being of an origin other than pure T7, since restriction enzyme analyses had also indicated that only the right end of this hybrid phage was T7.

The most rare but most useful class of phage isolated is represented by phage BO1 in Fig. 2 and Table 1. *Mbo*I analysis (Table 1) showed that this phage contained, in particular, T7-A, the largest fragment, which is more than 50% the size of intact T7. Since this phage also exhibits bands with the same mobility as T7-C, -E, and -F, this analysis indicates that the region from 28.76 to 100% (25) or from gene 3.8 (14) to the genetic right end is derived from T7. Two DNA fragments with estimated sizes 7,450 and 3,330 base pairs (bp) together presumably cover the left end of the phage to gene 3.8. These fragments are not of unique T7 or T3 origin and must therefore have resulted from crossovers between the two phages, eliminating some *Mbo*I recognition sites.

As is described in more detail below, the phage designated BO2 was derived from BO1 by successive recombinations with normal T7 strains. In contrast to BO1, BO2 contains a functional gene 0.7; concomitant with the expression of this gene product was the increase in overall DNA content of the hybrid phage. This extra DNA is found as an increase in the

TABLE 1. Sizes of *Mbo*I restriction fragments of hybrid phage DNAs

Estimated size[a] (bp)		T3 or T7 band of equivalent size
BO2	BO1	
21,400	21,400	T7-A
8,100	7,450	
3,820	3,820	T7-C
3,330	3,330	
2,840	2,840	T7-E
410	410	T7-F

[a] Sizes were estimated from the data of Fig. 2A with T7 DNA fragments as calibrating markers (23).

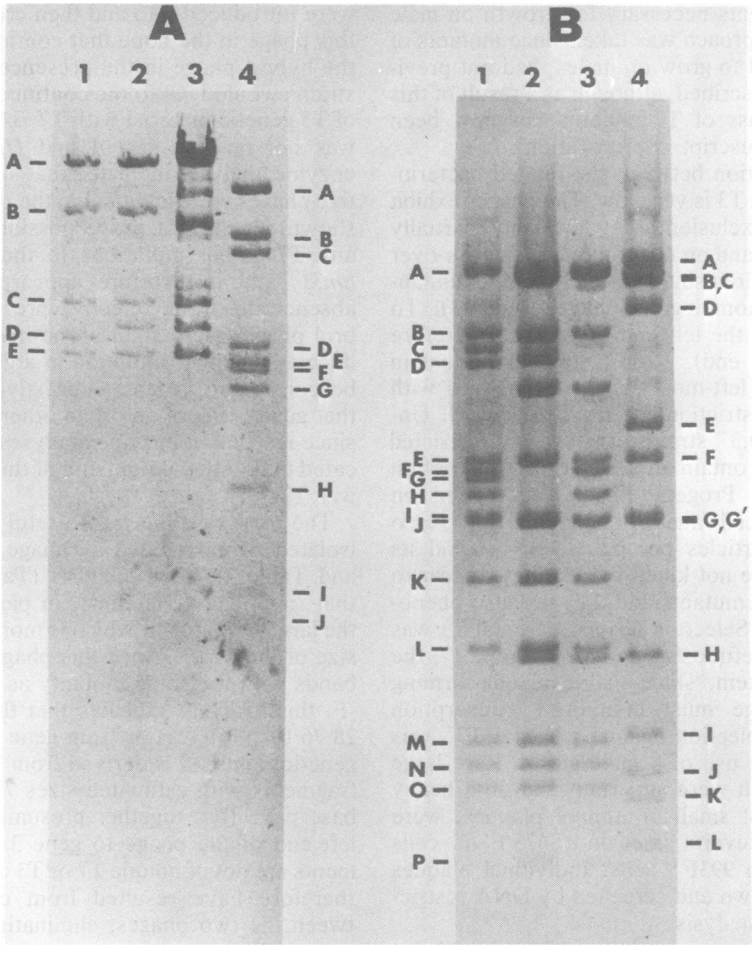

FIG. 2. Restriction enzyme digestion of DNA from T7, T3, and two recombinants, BO1 and BO2, analyzed after electrophoresis in 1% agarose gels. (A) *Mbo*I digest: lane 1, $T7^+$; lane 2, BO2; lane 3, BO1; lane 4, $T3^+$. (B) *Hpa*I digest: lane 1, $T7^+$; lane 2, BO1; lane 3, BO2; lane 4; $T3^+$.

second largest *Mbo*I fragment (Table 1). This indicates that this fragment, which in BO2 is approximately 8,100 bp, is related to the *Mbo*I-B fragment of T7 itself. This fragment of T7, which is 8,311 bp, extends from the left end of the phage through gene 0.7 into gene 1.7 (14, 25). It is thus likely that the 8,100-bp fragment of BO2 also extends from the left end of the phage through gene 0.7, but that a crossover event between T7 and T3 DNA sequences to the right of gene 0.7 results in a T3 *Mbo*I recognition site in or near gene 1.3 (2). Since no fragment corresponding to *Mbo*I-F of T3 can be detected in BO2, a crossover back to T7 DNA sequences must have occurred between gene 1.3 and gene 3.8 (the left end of T7 *Mbo*I-E), resulting in the 3,330-bp fragment of BO2. The order of these fragments is shown in Fig. 3.

A more rigorous analysis by the restriction enzyme *Hpa*I (Table 2) is in general agreement with the foregoing. Digestion of BO1 DNA leads to the appearance of T7 bands from gene 6 to the right end, but no T7-sized bands to the left of gene 5 were detected. However, only two T3-sized fragments were also apparent; these fragments are contiguous in T3 itself, spanning part of gene 1 through gene 1.3 (2).

The *Hpa*I analysis of BO1 DNA (Fig. 2B and Table 2) shows in particular that T3 bands R, O, and N are absent. These fragments lie at the left end of the phage (Fig. 3; data from Bailey et al. [2]) and suggest that the terminal redundancy of BO1 is of T7 origin, a result to be expected from the selection technique used in its isolation. This analysis also shows that at least the N-terminal region of the phage RNA polymerase is not of pure T3 origin, since the T3 *Hpa*I fragments F, K, and L are not present, and these fragments

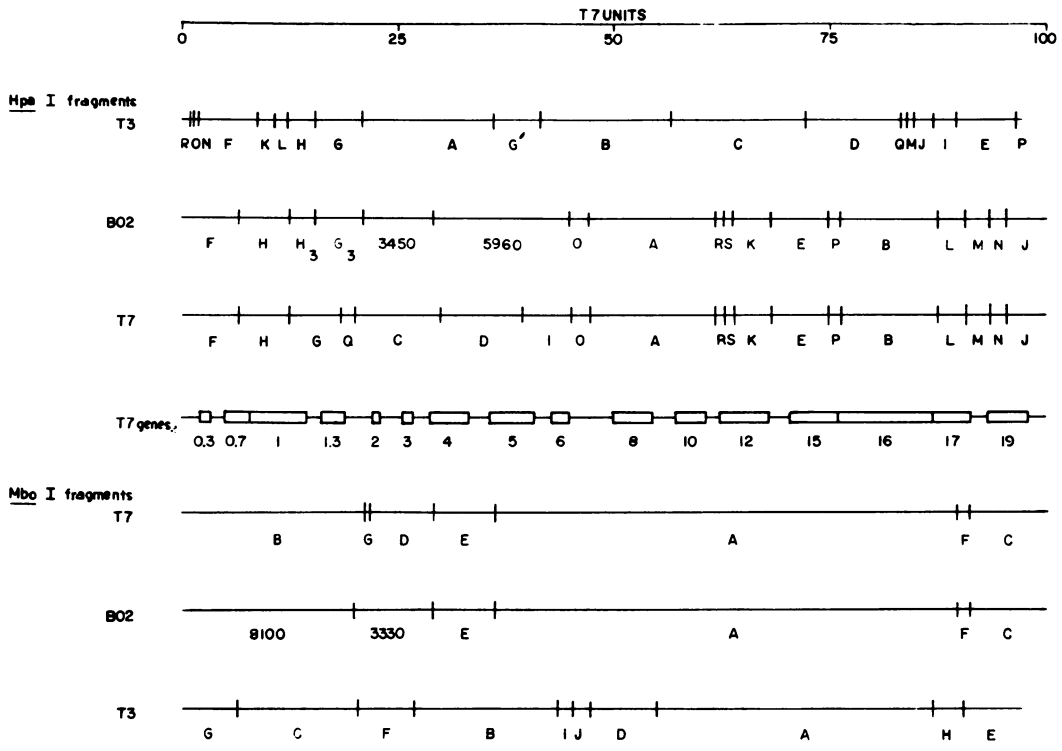

FIG. 3. Restriction map of the T7-T3 recombinant phage BO2. Fragments are of T7 origin except where a subscript 3 is written. These are derived from T3. Fragments that contain both T7 and T3 sequences are given by size in bp. The published maps of T7 and T3 DNAs are shown for comparison. Those of T7 are taken from Rosenberg et al. (25); those of T3 are from Bailey et al. (2), modified to include the additional 2,100-bp *Hpa*I fragment that is present in the T3-Luria strain (see text and reference 33).

encode this part of the enzyme (2). However, since fragments that correspond to the T3 *Hpa*I fragments H and G are apparent in a digest of BO1 DNA, this is indicative that the C-terminal region of the RNA polymerase may be derived from T3.

The above T3-sized fragments are maintained in the derivative hybrid phage BO2, which was constructed as follows. Phage BO1 was crossed with T7 amber mutants, and hybrid phage that retained the ability to grow on male cells, but that also contained the T7 amber mutation, were isolated. T7 amber mutations in genes 18, 16, 14, 12, 10, 8, 6, 5, 4, 3, and 2 (see Fig. 1 for specific alleles used) were introduced into BO2 as easily as recombinations with normal T7 strains. Restriction enzyme analyses of these amber mutation-containing strains showed that they were identical to the parent hybrid phage BO1 (data not shown). This implies that these hybrid phages do not usually lose heterologous DNA in subsequent recombinations with T7. However a gene 1 amber mutation, *am*193, was crossed into the phage only after screening several thousand potential recombinants. Restriction enzyme analysis of this strain, 1^{am}BO1, and a subsequent derivative, BO2, that had regained (again by recombination) a normal gene 1 showed a remarkable result (Fig. 2 and Table 2). Not only did BO2 have a different pattern of fragments from its parent BO1, but also it was a larger phage, containing approximately 800 bp of additional DNA. More importantly, BO2 appears to contain the *Hpa*I fragments T7-F and T7-H, which lie at the left end of the phage. These fragments span the region from the left end into the central portion of T7 gene 1, the T7 RNA polymerase. The conclusion that these fragments are indeed equivalent to T7-F and T7-H is reinforced by the disappearance of a 4,010-bp band seen in BO1, the increase (from BO1) in apparent size of the complete phage by 800 bp, and the simultaneous appearance of the two fragments with a combined size of 4,810 bp. Furthermore, whereas BO2 plates normally on strain BR3, which is a nonpermissive host for 0.7^- mutants (31), BO1 is severely restricted (Table 3). This is consistent with the acquisition,

TABLE 2. Sizes of *Hpa*I restriction fragments of hybrid phage DNAs

Estimated size[a] (bp)		T3 or T7 band of equivalent size
BO2	BO1	
5,960	5,960	T7-A
5,960	5,960	
4,370	4,370	T7-B
	4,010	
3,450	3,450	
2,640	2,640	T7-E
2,500		T7-F
2,310		T7-H
2,120	2,120	T7-J
2,100	2,100	T3-G
1,760	1,760	T7-K
1,380	1,380	T7-L
1,320	1,320	T3-H
995	995	T7-M
890	890	T7-N
840	840	T7-O
605	605	T7-P
410	410	T7-R
285	285	T7-S

[a] Sizes were estimated from data of Fig. 2B by using T7 DNA fragments as calibrating markers (23). The presence of the small fragments was established after electrophoresis through acrylamide gels. The largest band of both BO1 and BO2 was determined to be a doublet after densitometry tracing.

in BO2, of DNA sequences that contain, in part, the 0.7 gene that is carried on the *Hpa*I fragment H of T7 DNA.

It is noteworthy that BO2 lacks only five *Hpa*I fragments of T7 DNA, and these are contiguous (Fig. 3). These fragments, G, Q, C, D, and I, span the central region of gene 1 through to an internal region of gene 6 (36). Only two T3-sized bands, the G and H fragments, can be detected, and these too are contiguous. These fragments contain the right end of gene 1 to gene 1.3 (2). It is therefore likely that BO2 contains a hybrid RNA polymerase, possibly one that has the ability to recognize both T7 and T3 promoters (3, 4). In the original derivation of BO1, a crossover event from T7 into T3 must have occurred at, or close to, the junction of the *Hpa*I fragments T7-H and T7-G and the corresponding T3-L and T3-H junction (Fig. 3). It is of interest that a *Hpa*I site in gene 1 may have been conserved in the evolution of the phages T7 and T3 (2). This site may be part of a larger sequence that has also been conserved and across which recombination between T7 and T3 could occur, leading to the formation of this class of hybrid phage.

There are two *Hpa*I fragments of BO2 DNA that have no immediate T7 or T3 counterpart. In addition, the doublet band with an estimated size of 5,960 bp (Table 2) could not be clearly distinguished from the T3-B or T3-C fragments (6,035 and 5,900 bp, respectively [2]) by size alone. A similar lack of separation exists between T7-I and T7-J and T3-G. These fragments in particular were identified and ordered on a physical map of BO2 DNA by digestion with both *Hpa*I and *Mbo*I either simultaneously or by recutting an isolated, individual fragment with the second enzyme. This identification and ordering (Fig. 3) is based upon the physical maps of T7 and T3 as established by McDonell et al. (23), Rosenberg et al. (25), and Bailey et al. (2). Analyses of BO2 always included parallel digestions and analyses of both T7 and T3. Most of these double or sequential digestions of BO2 DNA gave rise to fragments that correspond to the equivalent digests of T7 DNA (data not shown) and confirm that the hybrid phage is predominately of T7 origin. In particular however, *Hpa*I digestion of the isolated *Mbo*I-A fragment gave rise to a new fragment of 3,410 bp in addition to the expected fragments of T7 itself. This new fragment corresponds directly to the n2I fragment resulting from a partial *Hpa*I digestion of the *Mbo*I-A fragment of T7 (Table 3 of reference 23). There is no evidence that the 3,410-bp fragment of BO2 DNA is however a partial digestion product; a more probable conclusion is that the *Hpa*I site that separates T7-D from T7-I has been replaced by T3 sequences. This experiment also confirmed that one of the

TABLE 3. Plating efficiencies of T7, T3, and the recombinants BO1 and BO2 on various hosts at 30°C

Phage	Relative plating efficiency[a] on						
	B	BR3	N2216	LS473	LS473F'*lac*	993F$^-$	993F$^+$
T7$^+$	1.0	1.1	1.1	1.1	2×10^{-3}	1.2	$<10^{-7}$
BO1	0.5	4.5×10^{-6}	1.0	1.0	0.6	1.1	0.5
BO2	0.7	0.8	1.2	0.9	0.8	1.2	0.7
T3$^+$	1.0	1.0	$<10^{-7}$[b]	$<10^{-7}$[b]	$<10^{-7}$[b]	1.1	1.0

[a] Relative to 011'. With the exception of T7$^+$ in LS473F'*lac*, which leads to ragged plagues ca. 1 mm in diameter, all plaques were clear, round, and ≥2.5 mm in diameter. Plaques of BO1 or BO2 were consistently smaller than those of T7$^+$ or T3$^+$ on the same host. This was especially true on *E. coli* B strains.

doublet bands due to *Hpa*I digestion is in fact T7-A and that neither T3-B nor T3-C is represented in BO2. This is because the sequential digest results in fragments that correspond to T7 *Hpa*I fragments O, R, S, and K in particular. The last three of these are part of the same region of T7 as is represented by *Hpa*I-C of T3 (Fig. 3). Similarly, since the *Hpa*I-O fragment of T7 is present, yet the corresponding bands of T3 (*Hpa*I-I, -J, and -D) are absent, neither of the 5,960-bp bands of Fig. 2 and Table 2 can be T3-B.

If one of the 5,960-bp bands resulting from *Hpa*I digestion is equivalent to T7-A, what is the origin of the second band? This question was answered by a *Hpa*I digest of the isolated *Mbo*I-E fragment of BO2. This leads to the same pattern as for T7 itself, i.e., the appearance of two bands (n6 and n1 of McDonell et al. [23]). The larger of these bands, a fragment of 2,550 bp, is the right-hand fragment of this digestion and abuts the *Mbo*I-A fragment. The combined length of this fragment and the 3,410-bp species that is the leftmost product of *Hpa*I digestion of *Mbo*I-A is 5,960 bp. This is in excellent agreement with the single *Hpa*I digest of BO2, which also gave rise to a 5,960-bp fragment and thus gives further credence to the suggestion that the *Hpa*I site separating T7 fragments D and I is missing in the hybrid phage.

Since both *Mbo*I sites that delineate the E fragment of T7 are present in the hybrid phage, but the *Hpa*I-D–*Hpa*I-I junction is absent, we infer that a crossover between T7 and T3 sequences must have occurred in the region of gene 5. Since the *Hpa*I-I–*Hpa*I-O junction of T7 is present in BO2, a second cross back to T7 occurred within DNA sequences at the beginning of or before gene 6.

On the basis of identical electrophoretic mobilities, the 2,100- and 1,320-bp fragments of BO2 (Table 2) were designated T3-G and T3-H, respectively. The designation of T3-G was further indicated by the appearance of the expected fragments (2) resulting from digestion of BO2 DNA with *Mbo*I and *Hpa*I, both at the same time and sequentially. In addition, Southern blot analyses (data not shown) indicated that the *Hpa*I-G fragment of T3 hybridized to the 2,100-bp fragment of BO2. Similar analyses with the *Hpa*I-H fragment of T3 showed that it hybridized not only to the 1,320-bp fragment of BO2 but also to the T7 *Hpa*I-G fragment. This therefore indicates that the C-terminal parts of T7 and T3 RNA polymerases have a high degree of homology, a result that is consistent with recombinations between T7 and T3 occurring in this region.

A complication arose in the analysis of *Hpa*I-G fragment of T3 and BO2. The *Hpa*I digestion pattern of the T3 used here is slightly different from that reported by Bailey et al. (2) in that the G fragment is in fact a doublet. This, as was reported by Studier (33), is a property of the Luria strain of T3 when compared with the Hausmann strain employed by Bailey et al. Studier (33) also stated that this second G-sized fragment originated from an extra *Hpa*I site that exists in the A fragment described by Bailey et al. (2). Thus, the size of the *Hpa*I-A fragment, seen by Studier (33) and in this work, is 6,325 bp, i.e., 2,100 bp less than that described by Bailey et al. (2). We have confirmed the location for this second 2,100-bp fragment and by means of double and sequential digestions with *Mbo*I and *Hpa*I have further defined it as existing at the right end of the *Hpa*I-A fragment of the Hausmann strain. In the Luria T3 strain this fragment, designated G′ in Fig. 3, separates the *Hpa*I fragments A and B.

The only other significant differences in the *Hpa*I restriction enzyme pattern between the T3 (Luria) strain and the Hausmann strain are the larger sizes of the *Hpa*I-H fragment (1,320 versus 1,200 bp) and of *Hpa*I-F (2,640 versus 2,515 bp).

The only other *Hpa*I fragment of BO2 not assigned a specific origin is the 3,450-bp fragment. By elimination this must lie between the 2,100-bp fragment of T3 origin (*Hpa*I-G, Table 2 and Fig. 3) and the 5,960-bp fragment. Furthermore, this 3,450-bp fragment is cut once by *Mbo*I to give a fragment that comigrates with that designated n6 by McDonell et al. (23) and must be positioned as shown in Fig. 3.

It is not clear from this restriction enzyme analysis exactly where, in the class II region, the crossover back to T7 sequences occurs. It must be to the left of gene 3.8 since this region of BO2 contains T7 restriction enzyme sites. As shown below, this crossover may have occurred in gene 2.5, the single-stranded DNA-binding protein (14, 34).

Figure 3 is a physical map that summarizes our restriction enzyme analyses of the recombinant phage BO2. The size of BO2, estimated from *Hpa*I digestion, is very close to 40,000 bp, which is the same as T7. Two double-crossover events between T7 and T3 are required to form this map, one initiating within gene 1 and terminating before gene 3.8, the second initiating near gene 5 and terminating before gene 6. At least four crossovers between T7 and T3 thus occurred in the formation of this hybrid phage.

Proteins encoded by phage BO2. Phage-encoded proteins have been visualized after [^{35}S]methionine labeling and acrylamide gel electrophoresis. Early in infection, the host RNA polymerase transcribes the leftmost 19% of the T7 genome (14), resulting in four major

protein species. A similar observation has been made with phage T3 (35). The apparent molecular weights of some of the corresponding T3 and T7 gene products are sufficiently different that the early proteins encoded by the hybrid phage BO2 can be unambiguously assigned. BO2 appears to encode a T7-sized RNA polymerase (gp1); it is clearly larger than the T3 enzyme (Fig. 4A). Electrophoresis of labeled proteins by using different acrylamide concentrations or different electrophoretic conditions has also not detected any difference in mobility between the RNA polymerases encoded by T7 and by BO2 (data not shown). This is not in conflict with the restriction enzyme analysis above, since hybrid T7-T3 RNA polymerases, which are the same size as the pure T7 enzyme, have been isolated previously (16).

It is not surprising that the first protein to be made, gp0.3, is of T7 origin since this was selected for in the original cross between T7 and T3. The second major gene product expressed is gp0.7, but the autoradiogram of Fig. 4A does not resolve these T7 and T3 proteins. Other experiments (not shown) indicated that the gp0.7 protein of BO2 has a mobility similar to that of T7 gp0.7. This is also to be expected since, in the formation of BO2, DNA sequences corresponding to the 0.7 gene of T7 were recombined into the original hybrid phage BO1 (concomitantly with an amber mutation in gene 1). Consistent with the restriction enzyme analysis of BO2, the fourth major class I product, gp1.3 (DNA ligase), appears to be of T3 origin. Figure 4A shows that BO2 encodes a protein with the same electrophoretic mobility as T3 DNA ligase, and the corresponding protein of T7 is absent.

Some of the class II proteins have been identi-

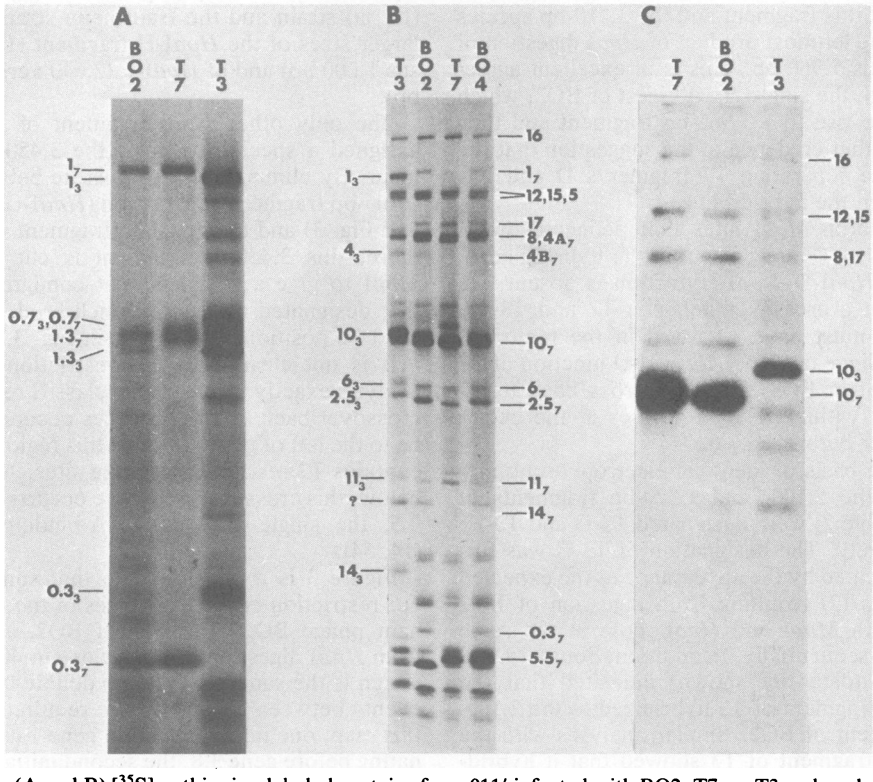

FIG. 4. (A and B) [^{35}S]methionine-labeled proteins from 011′ infected with BO2, T7, or T3 and analyzed after gel electrophoresis on a 10 to 20% gradient of acrylamide. Label was present 6 to 9 min (A) or 12 to 15 min (B) after infection. BO2-encoded proteins are inefficiently labeled after infection of *E. coli* B strains for reasons that are not understood. The use of 011′ (*supE*44) as host for these experiments precluded the use of amber mutants to positively identify some T7 or T3 proteins; in particular we have not yet been able to identify the T3 band, denoted by a question mark in (B) that is represented in both BO2 and BO4. Gene products that are designated on the sides of the autoradiogram have been identified from other experiments and by published analyses of T7 and T3 proteins. Subscripts next to the gene number refer to the specific phage, e.g., 1.3_3 is the gene 1.3 protein of T3. (C) Proteins of mature phage particles labeled with [^{35}S]methionine and analyzed after electrophoresis on a 12.5% acrylamide gel.

fied in Fig. 4B. BO2 apparently encodes a T7-sized primase (gene 4) and probably also the gene 6 exonuclease, but the band corresponding to gene 5 is not separable between T7 and T3. The single-stranded DNA-binding protein of BO2 is aberrant. This protein, which in T7 is the product of gene 2.5 (14), but which in T3 may overlap with gene 3 (15), in BO2 migrates on polyacrylamide gels as a band that corresponds to neither parent (Fig. 4B) and appears to be of hybrid origin.

A band that probably corresponds to T7 gp5.5 (34) is also marked in Fig. 4B. It is not known whether T3 encodes a similar protein; however, neither T3 nor BO2 encodes a protein with the same electrophoretic mobility as the T7 species. Restriction enzyme analysis of BO2 had indicated that the gene 5–gene 6 region is of T3 origin, which is consistent with the apparent absence of T7 gp5.5 in BO2.

Figure 4B also shows the proteins encoded by a further derivative of BO2, namely, BO4. This latter phage resulted from a cross between 5-28, BO2, and 4-20. am^+ recombinants were screened for their *Hpa*I restriction pattern (data not shown); they had lost a 5,960-bp fragment, but had acquired two fragments, one of 3,820 bp and the other of 2,140 bp. The latter corresponds to *Hpa*I-I fragment of T7 (23). All of these recombinants, which should now contain T7 sequences in the gene 5–gene 6 region, retain the complete ability to productively infect male strains; a representative has been designated BO4. In agreement with the restriction enzyme analysis, BO4 has also acquired a T7-sized gp 5.5 (Fig. 4B). Thus, genes 5 through 6 do not have to be of T3 origin for the hybrid phages to grow on male strains of *E. coli*.

Some of the class III proteins encoded by BO2 are also identified in Fig. 4B. Where the equivalent proteins of T7 and T3 separate in this gel system, the class III proteins of the hybrid phage appear to be of T7 origin. Figure 4C shows the proteins of the mature particles of T7, T3, and BO2; in particular it is obvious that the major head protein of T7, the product of gene 10, is represented in BO2.

Genetic characterization of the T7-T3 recombinants. Table 3 shows the plating efficiencies of the recombinant phages BO1 and BO2 and also of the parental phages. The only significant difference between BO1 and BO2 is that BO1 (and all other original hybrids of the class III type) is restricted on strain BR3. This strain was originally selected as being restrictive for 0.7^- mutants of T7, but also is somewhat restrictive for ligase mutants (31). BO1 and BO2 both plate well on the ligase-defective host N2216 and thus carry a functional phage ligase. These data, together with those presented above, are consistent with BO1 lacking gene 0.7 and BO2 having acquired, by recombination, this genetic material.

It is not clear why the recombinant phages consistently plate with lowered efficiency on *E. coli* B strains. Those plaques that do appear are also much smaller than those of $T7^+$ or $T3^+$ plated under identical conditions. It is not due to a type I restriction system, since serial passage of BO2 through *E. coli* B strains has no effect, and unmodified phage (i.e., grown in *E. coli* C) plates on the restriction-proficient strains *E. coli* B and 011′ as efficiently as phage that were previously grown in those hosts (data not shown). This reduced plaque size and plating efficiency possibly relate to our inability to efficiently label BO2-encoded proteins in UV-irradiated *E. coli* B (see legend to Fig. 4).

Both BO1 and BO2 plate on the T3-resistant strains N2216 and LS473 (and its F′*lac* derivative), showing that T7 adsorption specificity is present in these phages. The most important result shown in Table 3 is that both BO1 and BO2 plate on male strains of *E. coli* and, in particular, with high efficiency on $993F^+$, a strain that severely restricts $T7^+$. This result, together with the foregoing physical analyses, shows that we have isolated a phage that is predominately of T7 origin, but that possesses the ability (derived from T3) to grow well on male strains of *E. coli*.

As mentioned above, T7 amber mutations in genes 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, and 18 were introduced by recombination into the original hybrid phage BO1. In addition, the same mutations have all been recombined into BO2. However, we have been unable to recombine BO1 or BO2 with the ligase-deficient strains HA13, LG3, LG12, and LG26 to generate a ligase-deficient phage that grows on males. Both restriction enzyme and protein analyses have shown that the ligase gene of BO2 is of T3 origin, and it is thus not surprising that these T7 mutations could not be recombined into BO2. Conversely, the fact that T7 mutations that span almost the entire genome can be recombined, nonselectively, into BO1 or BO2 (or both) is at least indicative that most of the genetic information in these hybrid phages is of T7 origin. Apart from the specific examples described here, all of the standard T7 mutant strains that we have used have successfully, and relatively easily, been recombined with the hybrid phages to yield progeny that contain the T7 mutation and yet grow on male strains of *E. coli*. These amber mutant derivatives of BO1 or BO2, with only one exception, plated normally with respect to both the presence of the amber mutation and the ability to grow on males. The exception was 2-64, BO2, which produced small or minute

plaques at high frequency when plated on *sup*$^+$ strains. The reason for this is not clear, but it should be noted that gene 2 mutants of T3 have not been described. It is possible that in T3 this gene is not completely essential and by extension may also be nonessential in the hybrid T7-T3 phage BO2. This "leakiness" precluded full use of this mutant in the recombinational analysis described below.

In addition to the ligase mutants mentioned above, crosses of BO2 with 3-29, 6-147 double amber mutants of T7 have been unsuccessful in simultaneously introducing both amber mutations into a phage that grows on male strains. Amber mutant-containing derivatives of BO2 resulting from these crosses possessed, as determined by complementation analysis, either one of the two parental amber mutations, but not both. The most likely reasons for this failure are discussed below.

Preliminary mapping experiments have identified two essential regions of T3 necessary for the hybrid phage BO2 to productively infect male cells. This mapping proved difficult, at least in part because T7 and T3 share only partial, variable homology in the region between gene 1 and gene 6 (11), which is the region that appears to be of hybrid origin in BO2. When BO2 derivatives are crossed with amber mutants of T7, where the selected markers are in genes 1 through gene 5, recombination frequencies to *am*$^+$ were generally 2- to 10-fold lower than the control crosses with homologous phages (either T7 or BO2 derivatives). Recombination frequencies involving markers in gene 6 rightward to gene 19 were the same as control crosses, further indicating that in BO2 there are no extensive regions of T3 DNA to the right of gene 6.

TABLE 4. Genetic analysis of the functions responsible for growth of BO2 in a male strain of *E. coli*

Hybrid phage[a]	Recombined with T7 strain	% of *am*$^+$ phages that grow in 993F$^+$[b]
18^{am}BO2	19-10	30
	17-8	44
16^{am}BO2	17-8	40
	15-31	22
14^{am}BO2	15-31	48
	13-149	24
12^{am}BO2	13-149	37
	11-37	52
10^{am}BO2	11-37	51
	9-17	28
8^{am}BO2	9-17	45
	7-405	39
6^{am}BO2	7-405	43
	5-28	49
5^{am}BO2	6-147	41
	4-20	92 (138/150)
	3-29	74
4^{am}BO2	5-28	6 (14/250)
	3-29	75 (149/250)
	1-193	36 (72/200)
3^{am}BO2	4-20	6 (19/300)
	LG26	2 (19/300)
	1-193	1 (2/300)
1^{am}BO2	LG3	100 (250/250)
	LG12	100 (250/250)
	LG26	97 (242/250)
	2-64	75 (150/200)
	3-29	34 (67/200)

[a] See Fig. 1 for specific amber mutant alleles.

[b] Except where noted, 100 plaques were picked and tested for growth on 993F$^+$

To circumvent the problems of reduced recombination frequencies, BO2 derivatives that contained a standard T7 amber mutation were crossed with standard T7 mutants. Progeny phage that produced plaques on *E. coli* B (or, in the case of crosses involving T7 ligase mutants, on N2216) and were thus *am*$^+$ (and where relevant, also ligase positive) were then screened for their ability to grow on 993F$^+$. Thus, the segregation of the genetic regions responsible for growth on male cells could be measured without any selective pressure for their maintenance. Furthermore, this frequency of segregation is independent of the actual recombination frequency between selected markers.

The segregation of the regions responsible for growth on males is shown in Table 4. Ideally, if these regions are unlinked from the selected genetic markers, then 50% of the progeny wild-type recombinants should grow on 993F$^+$. In practice, all of the crosses involving genes 5 through 19 were somewhat lower than 50%. Aside from statistical fluctuations, these variations from random segregation may be due to the fact that the efficiency of plating of BO2 on *E. coli* B is less than unity (Table 3). Furthermore, we show that there are two independent regions necessary for growth on males, and these probably can segregate from each other. Loss of either region may lead to the formation of a female-specific phage. Crosses involving selectable markers to the left of and including gene 5 lead to frequencies of segregation of the "growth on males" phenotype that are however significantly different from 50%, in some cases being 100%, in others 2%.

When 1-193, BO2 is crossed with the ligase deletion mutants LG3 and LG12, all of the *am*$^+$ *lig*$^+$ recombinants were capable of growth on a male cell, implying that the region required for growth on males lies close to gene 1.3. This conclusion is reinforced by the cross with LG26, which deletes only the right end of the ligase gene (Fig. 1) and by the crosses with 2-64 and 3-29, where female-specific phage recombinants

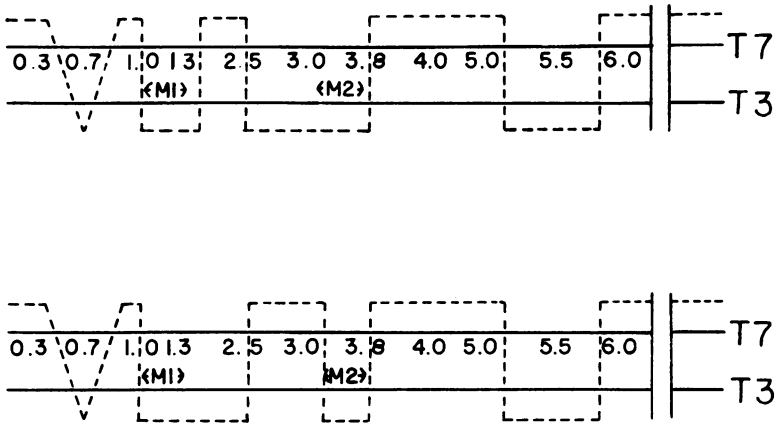

FIG. 5. Schematic diagram of the two most likely patterns of crossovers between T7 and T3 that led to the formation of BO1. BO2 is similar, but no longer has the deletion of gene 0.7. The corresponding genes of T7 and T3 are aligned, but the diagram is not drawn to scale. M1 and M2 refer to those regions of T3 identified genetically as necessary for growth of the recombinant phage in male strains of *E. coli*.

can be detected. These data show that one region of T3 DNA necessary for growth on male cells (designated M1 in Fig. 5) lies between the 1-193 mutation and the left end of the ligase deletion mutant LG26. Despite the apparent tight linkage between the ligase gene and the region required for growth on males, we cannot conclude that the two markers are in fact physically extremely close to each other. This is because recombination between the selected markers is presumably confined to regions of homology between the two phages. In this experiment exact homology is probably limited to the left half of gene 1, since the right half of this gene and DNA ligase itself appear to be predominately of T3 origin. This caveat is supported by the fact that the recombination frequency between selected markers is reproducibly 10-fold lower with 1-193, BO2 and ligase mutants than when using the same mutations in normal T7 strains.

Unfortunately, most of the reciprocal crosses could not be performed. We have been unable to introduce by recombination a T7 ligase mutation (deletion or the amber mutation HA13) into BO2. Although both 2-64 and 2-139 amber mutations have been successfully recombined into BO2, they were both too leaky (in the hybrid phage background) to be used in this analysis, which initially selects for am^+ recombinants. This problem does not arise in the 1-193, BO2 × 2-64 cross, since a single recombination event gives rise to am^+ phage that can grow on males. Multiple crossovers are required to segregate the 2-64 mutation with the T3 sequences necessary for growth of the hybrid phage on male strains. Therefore, the frequency of occurrence of recombinants with the genotype 2-64, BO2 would be so low as to be undetectable in these experiments.

None of the crosses involving 3-29, BO2 resulted in a substantial number of the wild-type recombinants being able to grow on male cells (Table 4). Since these crosses involved markers both to the left and to the right of gene 3, this is indicative that a second locus or region (the first being gene 1 to gene 1.3) that is necessary for growth on males lies to the right of the 3-29 mutation. This second region (designated M2 in Fig. 5) also lies to the left of the 4-20 mutation, since a high proportion of the wild-type recombinants in the 4-20, BO2 × 3-29 cross grow on males, whereas few do so in the 4-20, BO2 × 5-28 cross. This conclusion is reinforced by the 5-28, BO2 × 4-20 cross, where 92% of the wild-type recombinants grow on males, whereas only 74% do so in the 5-28, BO2 × 3-29 cross. In this last experiment, a proportion of the recombinants can result from a crossover between the 3-29 mutation and the presumed T3 insertion; thus, the percentage of am^+ progeny that grow on males is reduced.

In the experiments of Table 4, with amber mutant derivatives of BO2 and T7 and where selection (to am^+ recombinants) was made for a crossover between the 3-29 and 5-28 mutations. recombination frequencies were always greater than 30% and sometimes equal to the T7 controls. However, since there is only little homology between T7 and T3 genes 3 and 3.5 and only partial homology in the gene 4 region (11, 17), the size of the M2 region, which is presumably of T3 origin, is likely to be rather small. In fact the essential function encoded by the M2 region

appears to lie within the 1,010 bp that separate the 3-29 mutation from the *Mbo*I recognition site in T7 gene 3.8 (14).

A summary of the conclusions of this genetic data is shown in Fig. 5, which also shows the two most likely patterns of crossovers between T7 and T3 that occurred in the formation of BO1. It is possible that additional crossovers occurred and have not been detected by any of the above analyses. The two regions that are necessary for productive growth on male strains of *E. coli* are designated M1 and M2 (for class I and class II regions, respectively, essential for growth on males.

DISCUSSION

Recombinants between T7 and T3 have proven useful, in particular in the characterization of the pronounced template specificity of the parental phage RNA polymerase (3, 16). In the absence of T7 mutants that could overcome F-mediated restriction (and since we were also unable to isolate them), we thus embarked on a search among T7-T3 recombinants for a phage that was predominately T7, but which preserved the T3 functions necessary for growth on males. This search among the rare T7-T3 recombinants was successful in that we have isolated a phage that appears to contain the class III (structural) genes together with some of the class I and II genes of T7. As determined by restriction enzyme analysis, approximately 75% of the recombinant phage DNA is of pure T7 origin, with most of the remaining fragments being of neither parent. Only two *Hpa*I fragments appear to be uniquely T3; these fragments encompass the right end of the gene 1 protein (RNA polymerase) through gene 1.3 (DNA ligase).

T7 and T3 RNA polymerases show marked template specificity for the homologous phage DNA, but a number of hybrid phage RNA polymerases have been described in which this specificity is largely lost (16). It is possible that the RNA polymerase of BO2 is a similar hybrid, since it is probable that the phage also contains T3 promoters between gene 1 and gene 1.3, yet it is unlikely that promoters throughout the whole genome are all of the T3 sequence.

It is not clear why the original T7-T3 recombinant, BO1, has a deletion of the gene 0.7 region. Although BO1 was the only recombinant of the third class of original hybrid phages that was analyzed in detail, all of the members of this class that we originally isolated gave the same fragments after *Mbo*I digestion, and all were restricted by *E. coli* BR3. Both parental phages were phenotypically 0.7^+, but we cannot exclude the formal possibility that a minor population of phage with deletions of gene 0.7 recombined to give BO1. Alternatively, a region of homology between T7 and T3 may exist in the early region, but at separate physical and genetic sites, thus leading to deletion formation. The deletion of gene 0.7 in BO1 does explain the difficulty experienced in introducing into this phage a mutation in gene 1 by recombination. The hybrid phage BO1 lacks homology with T7 1-193 on both sides of the amber mutation, and the right-hand nonhomology is the region required for growth on males.

Only five of all of the standard T7 mutations tested failed to be recombined into the 0.7^+ derivative of BO1, i.e., BO2. Four of these primarily affect gene 1.3, DNA ligase. This is understandable, since by protein analysis this gene appears to be of T3 origin, and by heteroduplex mapping T7 and T3 DNA ligases show only partial homology (11, 17). The fifth T7 mutant strain, which could not be recombined intact into BO2 was T7 3-29, 6-147. Either single amber mutation, but never both, was found among recombinant progeny phage. Since one of the two regions found to be essential for growth on males genetically maps between genes 3 and 4, simultaneous acquisition of amber mutations in genes 3 and 6 would therefore require a double crossover and might therefore be expected to be undetectable in the absence of selection.

Assuming that the deletion of gene 0.7 is the result of recombination, at least eight crossovers between T7 and T3 occurred in the formation of BO1 and all other phages of this hybrid class. These must occur in pairs, since the terminal redundancy must be from the same phage (3). Three of these double events have been detected by physical means, and the fourth is suggested by the genetic mapping data. It is not clear why at least four separate regions of T3 recombined into T7 to generate a T7-like phage that, unlike its parent, productively infects male cells. The first of these lies in the 0.7 region and has already been discussed. Genetic analysis of the recombinant phage indicated that only two regions are essential for growth. These regions, designated M1 and M2, lie between the gene 1 mutation, *am*193 and gene 1.3, and between the gene 3 mutation, *am*29 and gene 4. M1 is part of a T3 insertion that may be physically larger than the genetic data suggests, but it is not known by how much. The *Mbo*I-G fragment of T7 is missing in BO2, and these sequences encode part of gene 1.7 (14). Therefore, gene 1.7, if present, must be at least partly of T3 origin in the hybrid phage. The product of gene 2.5 (single-stranded DNA-binding protein) appears, by protein gel analysis, to be a hybrid. We do not know which end of the protein is from T3 and which end is from T7. Either the insertion, which includes M1, extends through to gene 2.5, thereby encod-

ing a protein whose N terminus is of T3 origin and whose C terminus is of T7 origin, or it terminates before gene 2.5, thus leading to a protein with an N terminus of T7 origin and a C terminus of T3 origin (Fig. 5). The last of the T3 insertions in BO2 lies between genes 5 and 6. This insertion does not appear to be necessary for growth of the hybrid phage on male strains and has recently been recombined to yield a phage, e.g., BO4, that still grows productively on male strains of *E. coli*. It is noteworthy that three of the original insertions affect elements of DNA replication: gene 5 (DNA polymerase), gene 2.5 (single-stranded DNA-binding protein) and the T7 replication origin (26). The latter is replaced by one of the regions of T3 that encodes a function (M1) that is essential for the hybrid phages to grow on males. Since, in the original T7 × T3 cross we only picked large plaque-forming phage, it is possible that we also selected for proteins (e.g., gp5 or gp5.5 [or both] and gp2.5) that could efficiently interact with a T3 origin sequence as well as other T7 proteins.

A more detailed discussion of the specific content of the regions M1 and M2 is presented in the accompanying paper (29). It is notable that in 1971 Morrison and Malamy (24) proposed a translational control hypothesis to explain the abortive infection of T7 in male cells. They suggested that T7 encoded two translation initiation factors, one that was necessary for class II protein synthesis and one that was necessary for class III protein synthesis. They favored the idea that both of these factors were themselves class II proteins, while acknowledging that one of them may in fact be a class I gene product. We have no evidence for a translational control mechanism per se, but the fact that there are two regions of T3 DNA (one in the class I region and the second in the class II region) that must be present in a predominately T7-like phage to productively infect male cells suggests that this model may justify further consideration.

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