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Isolation of Recombinants Between T7 and T3 Bacteriophages and Their Use in In Vitro Transcriptional Mapping

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A variety of T3 × T7 recombinants were isolated from crosses between T3 and T7 parental phages carrying amber markers in various genes (gene 1 to gene 19). The genetic constitution of these recombinants was determined by reference to the selected markers and also directly by analysis of the proteins translated from the T3 × T7 recombinants in vivo. Although T3 and T7 phages are closely related, most T3 and T7 proteins differ slightly in size, and hence the genetic origin of a gene can be determined by protein analysis. The major transcripts read by T3 and T7 RNA polymerases from T3 × T7 recombinant phage DNAs vary, depending on which regions of the T3 or T7 chromosome are present. T7 RNA polymerase is unable to utilize major promoter sites employed by T3 polymerase at an appreciable rate, and the converse is also true. Hence the transcriptional pattern for a recombinant phage DNA obtained with the T3 or T7 polymerase allows a determination of the identity of the different promoter sites on the genome. The transcriptional analysis of T3 × T7 recombinant DNAs together with earlier observations has been used to map the promoter sites for five out of seven major T3 and T7 RNA species on the genetic maps of T3 and T7. The promoter sites for the T7 and T3 RNA species IIIa, IV, and V originate at the beginning of genes 7, 9, and 10, respectively; the promoter sites for the T7 and T3 RNA species I and II are located to the left of gene 11 and gene 13, respectively. No T3 × T7 recombinants were found for which the specificity of the phage RNA polymerase was not correlated with the corresponding promoter sites for species IIIa and I (the transcription of which covers 60% of the genome). That means that the RNA polymerase specified by the recombinant genome is able to read all of the information encoded in sequences read normally from major promoters by the enzyme on the wild-type phage genome. This suggests that the in vitro specificity for promoter site selection by the phage polymerases is also maintained in vivo.

RNA polymerases specified by T7 and T3 phages transcribe their homologous DNA templates with a high degree of selectivity in vitro. The enzymatically synthesized RNA is complementary to only one of the two DNA strands (*r* strand) (4, 7), and a major portion of this RNA is found in six discrete size classes of RNA, suggesting that there is accurate reading of both promoter and terminator regions on DNA by the phage polymerases (11). To locate the positions of these promoter and terminator regions, use has been made of both genetic and biochemical deletions (10) that give rise to truncated or deleted RNA species when transcribed in vitro. This has led to transcription maps that locate seven major promoters read on T7 DNA by T7 polymerase and on T3 DNA by T3 polymerase (10, 12). In addition, translation of certain in vitro T7 transcripts has established their genetic content and hence their map position (22). Another approach to transcriptional mapping employs recombinant bacteriophages that contain portions of both T7 and T3 genomes (2, 10). Neither of the two phage RNA polymerases is able to utilize the promoter regions specific for the other at an appreciable rate. (Although there is a promoter site on T3 DNA read by T7 RNA polymerase, this is not used by the T3 enzyme.) Hence analysis of the transcription products formed in separate reactions by T3 and T7 RNA polymerases with any given recombinant DNA as template establishes the presence or absence of specific promoter regions for T3 and T7 RNA polymerases, respectively.

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If the genetic composition of the recombinant is known, this can provide a powerful method for positioning of promoter sites.

MATERIALS AND METHODS

Phage and bacteria. Double amber mutants of T3 and T7 were obtained from H. Beier and R. Hausmann. The phage stocks used have been described previously (15). The phages T7 and T3 are from the collection of F. W. Studier and R. Hausmann. *Escherichia coli* strains Bs-1 and B/5 were used as the nonpermissive hosts and *E. coli* BBw/1 was used as the permissive host (15).

Preparation and purification of phage lysates. Nutrient broth (1%) was generally used as a liquid medium. Lysates were prepared by adding phage at a multiplicity of ~10 particles per cell to exponentially growing bacteria at 37°C. Concentration of phages was done by the procedure described by Yamamoto et al. (31). Concentrated phages were purified in a CsCl density gradient.

Chemicals and isotopes. ^{3}H-labeled UTP was purchased from Schwarz/Mann, and rifampin was obtained from Mann Research Laboratory. Other biochemical techniques have been described in previous publications (10, 11).

Isolation of recombinants between T3 and T7. Since the recombination frequency in crosses between T3 and T7 is very low (H. Beier, Ph.D. thesis, University of Freiburg, Freiburg, West Germany, 1973; 2, 14), the progeny of these crosses was plated on the nonpermissive host at a high concentration (ca. 10^7 phage particles per plate). To avoid am^+ revertants, crosses between T3 and T7 were performed with parental types carrying two amber mutants. In addition, the progeny of such crosses were plated on a mixed indicator (*E. coli* B/5 and B/5/3) and color agar plates, which allowed the distinction of T3 and T7 am^+ revertants and T3 × T7 recombinants. Plaques of T3 am^+ were turbid and showed a blue margin, and plaques of T7 am^+ were clear and produced a yellow margin, whereas plaques from a T3 × T7 recombinant, for example, would appear clear with a blue margin (H. Beier, Ph.D. thesis; 14). A sample of an exponentially growing culture (2 × 10^8 cells/ml) of permissive host bacteria (*E. coli* BBw/1) was added to an equal volume of a mixture of two different double amber mutants (multiplicity of infection was ten T3 particles and five T7 particles, respectively). The phages were allowed to adsorb for 5 min, and then the samples were centrifuged and resuspended in 1% nutrient broth. The cells were allowed to lyse, diluted 100-fold, and plated on indicator plates as mentioned above. Plaques which differed in size, margin, and host range from those of the two parental wild types were picked, and lysates were prepared as described above.

Enzyme assays. As a source of phage-coded enzymes cell-free extracts were prepared as previously described (16).

S-adenosylmethionine-cleaving enzyme (SAMase) was assayed by the method of Gefter et al. (9), and RNA polymerase was assayed as described by Chamberlin et al. (4). The specificity towards T3 and T7 antiserum, which is determined by the product of gene 17 (serum blocking protein [SBP]), was measured by mixing recombinant phages with diluted anti-T7 serum or anti-T3 serum. The mixture was incubated at 37°C for 20 min and then titrated for surviving PFU (20).

Labeling of phage proteins. Exponentially growing B_{s-1} cells in minimal medium (M9) were UV irradiated to inhibit host protein synthesis. To 0.4 ml of culture, 5 μl of ^{14}C-labeled hydrolysate (100 μCi/ml) and 10 μl of purified phages (ca. 10^{11} particles/ml) were added. The mixture was incubated at 37°C for 13 min (T3 and T7 am^+ phages) or for 20 min (T3/T7 recombinants). The sample was centrifuged, and the pellet was resuspended in 20 μl of sample buffer and heated for 90 s at 100°C before it was applied to the gel (27, 28).

SDS-polyacrylamide gel electrophoresis. A Tris-sodium dodecyl sulfate (SDS)-polyacrylamide gel system was used for preparing slab gels by the method described by Studier (27, 28). Instead of thick plastic strips between the two glass plates, 1-mm strips of silicon rubber were used. These were found to be more suitable for avoiding leaks while pouring the gel (O. G. Issinger, personal communication). The gel slab was loaded with 12 samples such that the volume of each sample did not exceed 10 μl.

Autoradiography. Gel slabs were dried according to Maizel (21). Autoradiography was performed by exposing the dried gel slab to Kodak no-screen X-ray film (NS-54T) for a period of 2 to 7 days.

In vitro synthesis and analysis of RNAs. T7 RNA polymerase and T3 RNA polymerase were purified by the methods of Chamberlin et al. (4) and Golomb and Chamberlin (12), respectively. Procedure for the in vitro synthesis of RNAs and the analysis of RNAs by acrylamide-agarose gel electrophoresis have been described elsewhere (10, 11).

Nomenclature. The nomenclature used for T3 and T7 transcripts and the promoter and terminator sites that govern their synthesis is described in the accompanying paper (12).

RESULTS

Isolation, selection, and characterization of recombinants between T3 and T7. The genetic maps of the related bacteriophages T3 and T7 are very similar (1). Yet the recombination rate in heterologous crosses is very low (H. Beier, Ph.D. thesis; 14). Upon simultaneous infection, about 5% of the mixed infected cells produce both parental phages, the other cells generally yield either T3 or T7 (14). Mutual exclusion between T3 and T7 is probably one of the reasons for the low recombination rates between these phages. Another reason is the partial homology and nonhomology of base sequences between T3 and T7 DNAs (6). Because of the low recombination rates (about 1,000 times less than in corresponding crosses with homologous phage), parental phages carrying two amber markers were used in crosses be-

tween T3 and T7 to avoid am^+ revertants. The use of double amber mutants also facilitates the genetic analysis of the T3 × T7 recombinants, since one can assume that multiple crossover events will occur at a reduced rate.

Several approaches were utilized to ascertain the genetic constitution of T3 × T7 recombinants in addition to the identity of selected markers. We routinely analyzed the recombinants with regard to the activity and template specificity of the phage RNA polymerase which is coded by gene 1 (4). In addition, we tested the specificity towards T3 and T7 antiserum, which is determined by the product of gene 17 (20).

It is noteworthy that one T3 × T7 recombinant (R39) was inactivated by T3 and T7 antiserum to the same extent. This seems to indicate that the recombinant phage displays both serum specificities, which could be explained by crossover within gene 17 (20). In some cases we checked the ability of the recombinants to code for an active *S*-adenosylmethionine-cleaving enzyme (SAMase) that depends on the T3-specific sam^+ gene and is absent in T7-infected cells (9, 13). The sam^+ gene is probably the leftmost gene on T3 DNA (16) and has recently been shown to be homologous to the 0.3 gene of T7 (29). Thus, we could analyze the T3 × T7 recombinants with respect to two nonselected markers at the "left" end of the genetic maps (SAMase and RNA polymerase) and one marker at the right end (SBP). For all of these markers, specific differences between T3 and T7 have been described.

At the beginning of our studies, we selected only recombinants that had the RNA polymerase specificity from one parental phage and the antiserum specificity from the other. However, later we found this selection method unsatisfactory in some respects. Although the recombinational origin of the phage stocks could be ascertained, the characterization of the genetic constitution still remained incomplete. Besides, we were discarding some of our most interesting recombinants by this kind of selection, in particular those which had originated from crossovers between T3 and T7 in the middle region of the genome (between 20 and 70%). Hence, we had to look for other approaches to characterize the genetic constitution of the isolated T3 × T7 recombinants. For this purpose, the analysis of the phage-induced proteins by means of the gel electrophoresis autoradiography method (27, 28) seemed promising. It had been shown recently that almost all T3 and T7 proteins are of different size in the two phages (O. G. Issinger and H. Falk, Arch. Virol., in press; 19, 29). Some of the T3 and T7 proteins differ only slightly with regard to their molecular weights, whereas the size of other proteins, such as the T3 RNA polymerase (gene 1 product) and the T7 major head protein (gene 10 product), were found to be about 5 and 10% lower, respectively, than those of the corresponding heterologous proteins (Issinger and Falk, in press; 25). We analyzed the phage-coded intracellular proteins on 10 and 12.5% SDS-polyacrylamide gels. Figure 1 shows an example of the protein pattern of a T3 × T7 recombinant (R60). The T3- and T7-coded proteins are shown for comparison. It seems clear that genes 1 and 4 are derived from T3 and that genes 10 and 12 are derived from T7 (Fig. 1A and B). Three bands are present in the region of the gene 6 protein in the R60 slot, whereas T3 and T7 each show only two bands. The origin of the first band from the top is unknown. However, the second protein migrates in the same position as the T3 gene 6 product, which suggests that gene 6 is derived from T3. To confirm our assumption that the gene 10 product had the molecular weight of the corresponding T7 protein, we analyzed the coat proteins of the R60 particle as well (Fig. 1C). The gene 11 product of R60 seems to have a molecular weight intermediate to the T3 and T7 gene 11 protein.

In Fig. 2 we present all the information available about the genetic constitution of the T3 × T7 recombinants. The origin of the genes from either T3 and T7 was determined – except for the terminal redundancy region – through the analysis of the protein pattern in a SDS-polyacrylamide gel, through direct enzymatic assays for SAMase (gene 0.3) and phage RNA polymerase (gene 1), and by measurement of SBP (gene 17).

The purpose of our present studies was to locate the promoter and terminator sites for the major RNA species IIIa, IV, and V and to confirm the exact location of the promoter sites for RNA species I and II. Preliminary experiments with extensively shortened T7 DNA and with DNAs containing deletions in the "left" region of the genome have revealed that the unmapped T7 transcripts must originate from promoter sites to the right of 30% (10). As it seemed very likely that these transcripts contained sequences only for the region from 40 to 50%, we were mostly interested in selecting genetic markers that would limit the crossover event(s) to the region between 40 and 60%, corresponding to the location of genes 6 and 12 (18). Thus, we chose markers that would select for a crossover between gene 5 and gene 6 (R55) or between gene 6 and various genes like gene 7 (R67 and R68), gene 9 (R71), and gene 13 (R84). The genetic markers we used are listed in Table 1.

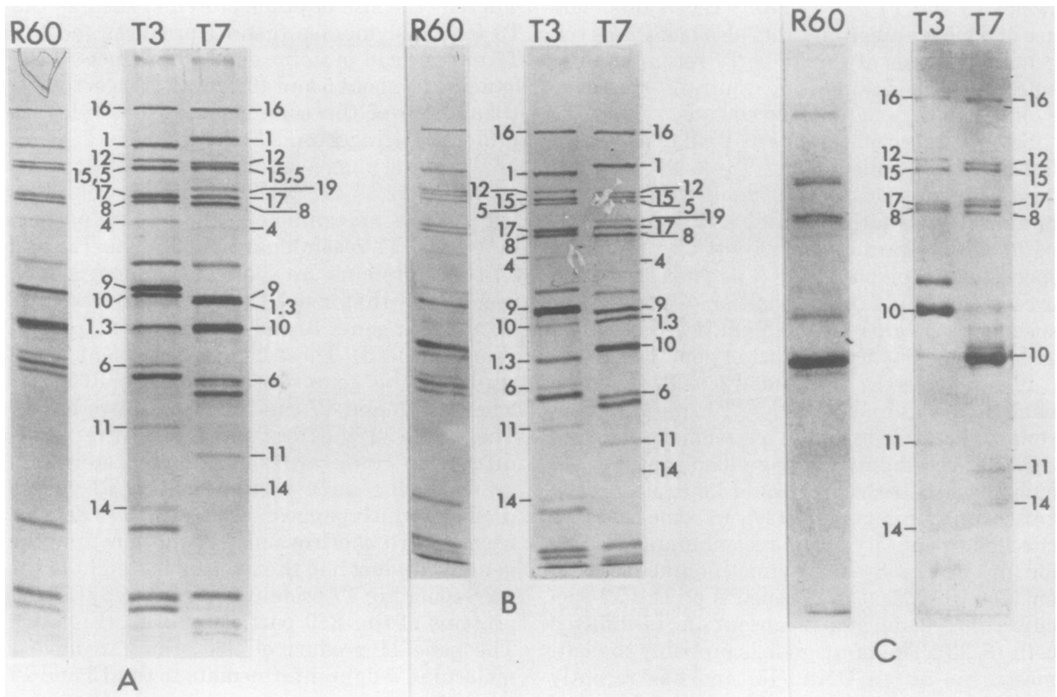

FIG. 1. *Protein patterns of T3 and T7 bacteriophages compared with those of a T3 × T7 recombinant (R60) shown by gel electrophoresis of labeled phage proteins (A, B) and coat proteins of phage particles (C). For labeling proteins, phages (ca. 10 particles/cell) and a mixture of* ^{14}C*-labeled amino acids were added to exponentially growing E. coli BS-1 cells that had been UV irradiated before infection. The cultures were centrifuged after 13 min (T3, T7) or 20 min (R60) of incubation at 37°C and resuspended in sample buffer (0.05 M Tris-hydrochloride, pH 6.8, containing 1% SDS, 1% mercaptoethanol, and 10% glycerol). After being heated for 90 s in a boiling-water bath, the samples were applied on slabs of 12% (A) or 10% (B) polyacrylamide gel as described by Studier (27, 28). The three slots of (A) and (B), respectively, are from the same gel. The number of the T3 and T7 genes that specify each band is given at the left or right of the patterns, respectively. When coat proteins were analyzed (C), a portion of purified phages (ca.* 10^{12} *phages/ml) was diluted with sample buffer (1:2) and heated for 90 s in a boiling-water bath.*

The most striking feature of almost all isolated T3 × T7 recombinants was revealed only after the careful characterization of their genetic constitution. Instead of originating from only a single crossover, all of them are derived from either two or four crossover events (Fig. 2). One of the reasons for the accumulation of crossovers and the invariance of an even number of events might be that T3 × T7 recombinants with heterologous terminal redundance regions on any single molecule cannot replicate effectively (18).

Our current studies of the transcription pattern of these recombinants, taken with the identification of the phage-specific RNA polymerases, revealed another possible reason for the multiple crossover events. It appears (see below) that the promoter sites for the major RNA species I and IIIa as well as the RNA polymerase gene must all derive from one of the two parental phages to give a viable recombinant genome.

Major RNA species transcribed from genetically modified templates (T3 × T7 recombinants) by T3 and T7 RNA polymerases. Transcription of T7 DNA by T7 RNA polymerase in vitro gives rise to six major size classes of RNAs comprising seven T7 RNA species (11). The transcription pattern of T3 RNA polymerase on T3 DNA resembles that of T7, although the homologous RNAs are of slightly different size (12). The finding that T7 RNA polymerase recognizes only the promoter site for RNA species IIIb on T3 DNA and the fact that T3 RNA polymerase reads all T7 major RNA species with only a low efficiency (10, 12) suggested that transcription of recombinant phage DNAs containing both T3 and T7 DNA sequences should be useful for locating the promoter sites of the unmapped major RNA species IIIa, IV,

	t_l	.3	.7	1.0	1.3	2	3	3.5	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	t_r
R8	○	●		⬤	●				●		●	●	●	●	●	●	●		●	◯		○		◯	○
R12	○	●		◐	○		◯		○		○	○	○	○	○	○	○		○	○		○		◯	○
R17	●	●		⬤	●		◯		○		◯	●	●	●	●	●	●		●	◐		○		●	●
R30	●	●		⬤	○		◯		○		●	●	●	●	●	●	●		●	●		○		◯	●
R39	●	●		◐	○		◯		○		○	○	○	○	○	○	○		○	○		◑		●	●
R55	○			◑	●				●	⬤	◯	○	○	○	○	○	○		○	○		○		◯	●
R60	●			⬤	●				●		●	●	●	⬤	○	◐	○		◯	◑		●		●	●
R67	○			○	○		◯		○		◯	◑	○	○	○	○	○		○	○		●		⬤	○
R68	○			○	○		◯		○		◯	◑	○	○	○	○	○		○	○		●		⬤	○
R71	●			●	●		◯		○		◑	●	●	⬤	●	●	●		●	●		●		⬤	●
R82	○			○	○				○		◯	○	○	○	○	○	○		○	◯		⬤		⬤	○
R84	●			○	○		◯		○		◯	○	○	○	○	○	○	⬤	●	●		●		⬤	●

FIG. 2. *Schematic representation of the genetic constitution of T3 × T7 recombinants by protein analysis. Genes were assigned to T3 or T7 mainly on the basis of protein size (T3, •; T7, ○) and selected genetic markers (T3, ●; T7, ◯). The antigenic specificity (gene 17) and the RNA polymerase specificity (gene 1) were checked for all recombinants, and the ability to code for an active S-adenosylmethionine-cleaving enzyme (T3 gene 0.3) was determined for some recombinants. Intermediate molecular weights or intermediate enzyme activities are shown as semiclosed circles. A semiclosed circle for gene 1 (e.g., R12, R39, and R55) implies that a crossover event occurred in the region of gene 1. The gene 1 product of the three recombinants has the molecular weight of T3 RNA polymerase and the template specificity of T7 RNA polymerase (e.g., R12 and R39) or an unstable enzyme with no measurable in vitro activity (e.g., R55). The assignment of the right-end terminal redundancy (t_r) was based on the presence or absence of RNA species VI; the assignment of the left-end terminal redundancy (t_l) was done tentatively assuming an identity of t_l and t_r. Large circles are selected markers.*

and V. The presence or absence of the six major RNA species in the in vitro transcription pattern of any of the recombinant phage DNAs would indicate directly which of the T3 or T7 promoter sites were contained in the recombinant phage DNA. Table 2 summarizes the transcription patterns of the twelve recombinant phages for which detailed information about the genetic constitution is known (Fig. 2).

The autoradiogram of RNAs transcribed from one representative recombinant DNA (R60) by T3 and T7 RNA polymerases is shown in Fig. 3e and f. This recombinant is of special interest because species V is not transcribed by either T3 or T7 RNA polymerase. The RNA species I, III, IV, and VI are transcribed by T3 RNA polymerase (Fig. 3e), and the RNA species II and III are transcribed by T7 RNA polymerase (Fig. 3f). As species IIIb is not transcribed by T3 RNA polymerase on either T3 or T7 DNA (12), the species III read by T3 RNA polymerase on R60 DNA should be in fact RNA species IIIa. For comparison, the transcription patterns of T3 and T7 RNA polymerase on T3 and T7 DNAs are also shown (Fig. 3a–d).

An RNA pattern equally unexpected was obtained on transcription of R67 and R68 DNAs. Neither species IV nor species V was read by either of the two RNA polymerases (Table 2). The transcription patterns of the remaining recombinants showed no unusual features. These recombinants showed either all of the T7 major RNA species (e.g., R12 and R82) or all of the T3 major RNA species (e.g., R17, R30, and R71) or all of the major RNA species but species VI from either T7 or T3 (e.g., R39, R55, R84, and R8, respectively).

Mapping of the major in vitro transcripts formed by T3 and T7 RNA polymerases on T3 and T7 DNA. As mentioned above, the presence or absence of one of the six major RNA species after the transcription of a recombinant phage DNA by T7 or T3 RNA polymerase should indicate directly which of the T3 or T7 promoter

TABLE 1. *Selection of T3 × T7 recombinants*[a]

Recombinant	Genetic selection	
R8	T7 *am*H13:*am*H280 (double gene 1⁻)	× T3 *am*A1:*am*H1 (gene 15⁻, gene 19⁻)
R12	T7 *am*H13:*am*H280 (double gene 1⁻)	× T3 *am*H283:*am*H1 (gene 3⁻, gene 19⁻)
R17	T7 *am*H13:*am*H280 (double gene 1⁻)	× T3 *am*H283:*am*A47 (gene 3⁻, gene 6⁻)
R30	T7 *am*H13:*am*H280 (double gene 1⁻)	× T3 *am*H283:*am*H1 (gene 3⁻, gene 19⁻)
R39	T7 *am*H13:*am*H280 (double gene 1⁻)	× T3 *am*H222:*am*H283 (gene 1⁻, gene 3⁻)
R55	T7 *am*H280:*am*H30 (gene 1⁻, gene 5⁻)	× T3 *am*A47:*am*H1 (gene 6⁻, gene 19⁻)
R60	T7 *am*H280:*am*H104 (gene 1⁻, gene 9⁻)	× T3 *am*H44:*am*A1 (gene 14⁻, gene 15⁻)
R67	T3 *am*H283:*am*A47 (gene 3⁻, gene 6⁻)	× T7 *am*N72:*am*A21 (gene 7⁻, gene 19⁻)
R68	T3 *am*H283:*am*A47 (gene 3⁻, gene 6⁻)	× T7 *am*N72:*am*A21 (gene 17⁻, gene 19⁻)
R71	T3 *am*H283:*am*A47 (gene 3⁻, gene 6⁻)	× T7 *am*H104:*am*A21 (gene 9⁻, gene 19⁻)
R82	T3 *am*A47:*am*A1 (gene 6⁻, gene 15⁻)	× T7 *am*H279:*am*A21 (gene 17, gene 19⁻)
R84	T3 *am*H293:*am*A47 (gene 3⁻, gene 6⁻)	× T7 *am*H338:*am*A21 (gene 13⁻, gene 19⁻)

[a] The recombinants were selected as wild-type recombinants from crosses between parental types each carrying two amber markers. The assignment of the T3 and T7 amber mutants to the corresponding genes were made according to Beier and Hausmann (1) and Hausmann (unpublished data) for T3 and T7 amber mutants, respectively. R67 and R68 are independent isolates.

sites were contained on the recombinant DNA. The combination of the data of the genetic constitution (Fig. 2) and the transcription patterns (Table 2) of the T3 × T7 recombinants should then provide sufficient information to locate the promoter sites for RNA species III, IV, and V.

The recombinant R60 is derived from four crossover events between the parental phages T3 and T7 in the region from gene 9 to gene 15 on the genetic map (Fig. 2). A comparison of the molecular weights of the R60-induced proteins with those of T3 and T7 (Fig. 1) reveals that the gene 10 and gene 12 products of R60 are derived from T7, whereas the gene 11 product is derived from T3. As species I is transcribed by T3 RNA polymerase and species II is read by T7 RNA polymerase (Table 2), it is very likely that the promoter site for species I originates to the left of gene 11 rather than at the beginning of gene 12 because the gene 12 product is entirely from T7. However, we cannot rule out the possibility that the promoter site for species I is in fact located to the right of gene 11, in which case a crossover might have occurred in the very left region of gene 12, causing no visible change in the molecular weight of the gene 12 product.

From the genetic constitution and the transcription pattern of R84, it can be concluded that the promoter site of species II is located to the left of gene 13 because the entire region of the right end of the R84 genome (from gene 13 on) is derived from T3 (Fig. 2), but most RNA species, including species II, are read by T7 RNA polymerase. If species I, in fact, originates to the left of gene 11, the promoter site for species II has to be located to the right of gene 12 because the two RNA species differ in their molecular weight to such an extent (ca. 10^6)

TABLE 2. *Ananysis of RNAs of T3 × T7 recombinants transcribed by T3 and T7 RNA polymerases*[a]

Recombinant	I		II		III		IV		V		VI	
	T3	T7	T3	T7	T3	T7	T3	T7	T3	T7	T3	T7
R8	+	−	+	−	+	+	+	−	+	−	−	+
R12		+		+		+		+		+		+
R17	+	−	+	−	+	+	+	−	+	−	+	−
R30		−		−(+)		+		−		−		−
R39		+		+		+		+		+		−
R55	−	+	−(+)	+	−	+	−	+	−	+	+	−
R60	+	−	−	+	+	+	+	−	−	−	+	−
R67	−	+	−	+	−	+	−	−	−	−	−	+
R68	−	+	−	+	−	+	−	(+)	−	(+)	−	+
R71	+	−	+	−	+	+	+	−	+	−	+	−
R82	−	+	−	+	−	+	−	+	−	+	−	+
R84	−	+	−	+	−	+	−	+	−	+	+	−

[a] Transcription patterns were obtained by inspection of autoradiograms as shown in Fig. 3. +, Presence of an RNA species; −, absence of an RNA species.

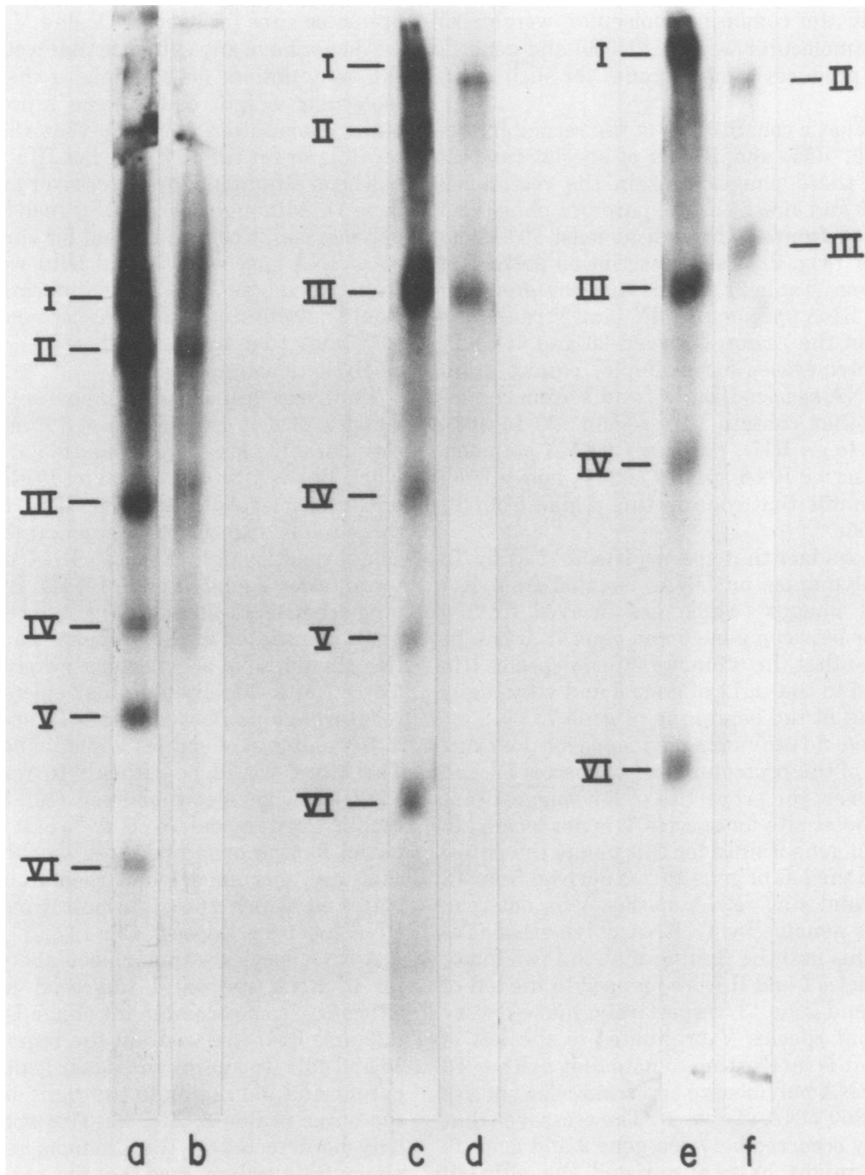

FIG. 3. *Autoradiogram of RNAs transcribed from T3 DNA, T7 DNA, and R60 DNA by T3 and T7 RNA polymerases. RNAs were synthesized in vitro in a 10-min synthetic reaction at 37°C with ³²P-labeled ATP as substrate; portions of the reactions were mixed with 0.25% SDS and placed directly on a 1.75% (a, b) or a 2% (c, d, e, f) acrylamide-0.5% agarose gel for analysis. After electrophoresis gels were analyzed by autoradiography. (a) RNAs transcribed from T3 DNA by T3 RNA polymerase; (b) RNAs transcribed from T7 DNA by T3 RNA polymerase; (c) RNAs transcribed from T7 DNA by T7 RNA polymerase; (d) RNAs transcribed from T3 DNA by T7 RNA polymerase; (e) RNAs transcribed from R60 DNA by T3 RNA polymerase; (f) RNAs transcribed from R60 DNA by T7 RNA polymerase. Track (b) demonstrates that T3 RNA polymerase utilizes all six major promoters on T7 DNA only poorly (12). Track (d) shows that T7 RNA polymerase transcribes only a single major RNA species (e.g., species III) with T3 DNA as template (10). With R60 DNA as template, T3 RNA polymerase reads RNA species I, III, IV, and VI efficiently, whereas T7 RNA polymerase reads species II and III.*

that only the combined molecular weights of gene 11 (molecular weight, 21,000) and gene 12 (86,000) products would account for such a difference.

The genetic constitution of the recombinants R17, R30, R55, and R71 is of special interest because these phages contain the region between 20 and 40% from one parental phage and the region from 40 through at least 70% from the other (Fig. 2). The transcription pattern of all of them (Table 2) reveals that the promoter sites for RNA species IIIa, IV, and V cannot be located in the region between 20 and 40%. T7 RNA polymerase, for example, cannot transcribe RNA species IIIa, IV, and V from recombinants that contain this region (20 to 40%) from T7 (e.g., R17), nor can T3 RNA polymerase transcribe RNA species III, IV, and V from recombinants that contain this region from T3 (e.g., R55).

From the fact that species IIIa is read by T3 RNA polymerase on DNAs isolated from R17 and R71 phages (which are derived from a crossover between gene 6 and gene 7), it can be concluded that the promoter site for species IIIa is located to the right of gene 6 and very likely originates at the beginning of gene 7.

We have no convincing evidence for the exact location of the promoter sites of species IV and V. However, the properties of R60 suggest that the promoter site for species V is not located to the left of gene 9 since for this phage the entire region to the left of gene 10 has derived from T3 (Fig. 2) and since RNA species V is not read from this genome by T3 RNA polymerase (Table 2). This and the finding that the two major RNA species I and II were mapped to the left of gene 11 and gene 13, respectively, make it very likely that species V originates to the left of gene 10. It is interesting to note that neither T3 nor T7 RNA polymerase can transcribe species V from R60 DNA (Table 2). The crossover that obviously occurred between gene 9 and gene 10 (Fig. 2) might have eliminated the efficient promoter site for both RNA polymerases.

On the assumption that RNA species IV and V have a common terminator, species IV has to originate to the left of gene 9 rather than to the left of gene 8 because the difference in the molecular weight of these two species (ca. 0.4×10^6) does not account for a protein with a molecular weight >40,000. However, the sum of the molecular weights of the gene 9 (40,000) and gene 8 (65,000) products is about 105,000.

The transcription patterns of R67 and R68 are difficult to interpret. Neither species IV nor species V is read by T3 or T7 RNA polymerase (Table 2). A double crossover to the left and to the right of gene 9 could have eliminated the promoter sites for species IV and V. However, we do not have any evidence that would support this assumption, for example, a change of the molecular weight of the gene 9 product. Another explanation could be that the common terminator for the RNA species IIIa, IV, and V has been eliminated by a crossover to the left of gene 11. Although species III is read by T7 RNA polymerase, it cannot be said for sure whether both RNA species (IIIa and IIIb) were in fact transcribed. To get more information, one would have to study the transcription pattern of R60 DNA that had been shortened by exonucleolytic cleavage (10).

Two large transcripts of apparent molecular weights of 8×10^6 and 6.5×10^6 are made in considerably less than equimolar amounts when T7 DNA is transcribed by T7 RNA polymerase (species VIII and IX). It is likely that these minor transcripts are generated by occasional readthrough of the T7 RNA polymerase termination signal at 56% (10, 12). By the mapping scheme presented in Fig. 4, a readthrough product initiated at the promoter for T7 species IIIa should have a molecular weight of $(2.0 + 5.5 = 7.5) \times 10^6$. By a similar calculation, the readthrough product of species IV should be 6.3×10^6, and that of species V should be 5.9×10^6. The latter would be difficult to resolve from species I by gel electrophoresis (10). We favor a readthrough model over a "weak promoter" model for the origin of these two RNAs since they are shortened when read from T7 templates in which the rightmost portion of the DNA has been excised (12).

A RNA species with a molecular weight similar to RNA species II was read with a low efficiency from recombinant phage DNAs (e.g., R30 and R55) that contain the region between 15 and 40% (including the ligase gene) from one parent and the region to the right of 40% from the other (Table 2, Fig. 2). This species could only be detected in the products read by the phage RNA polymerase that did not utilize the major promoters on the recombinant DNA. We interpret this RNA species as a transcript originating from a weak promoter site located in the region between 15 and 40% for which no in vitro RNA species have yet been clearly identified. Such a minor transcript would not normally be detected, since it would be obscured by the major transcripts I and II.

Does the specificity of the in vitro transcription duplicate that of site selection in vivo? Our studies of transcription of templates derived from T3 × T7 recombinants imply that "late" promoters on recombinant DNAs are used at an appreciable rate only by the homologous phage RNA polymerase (Table 2). Given

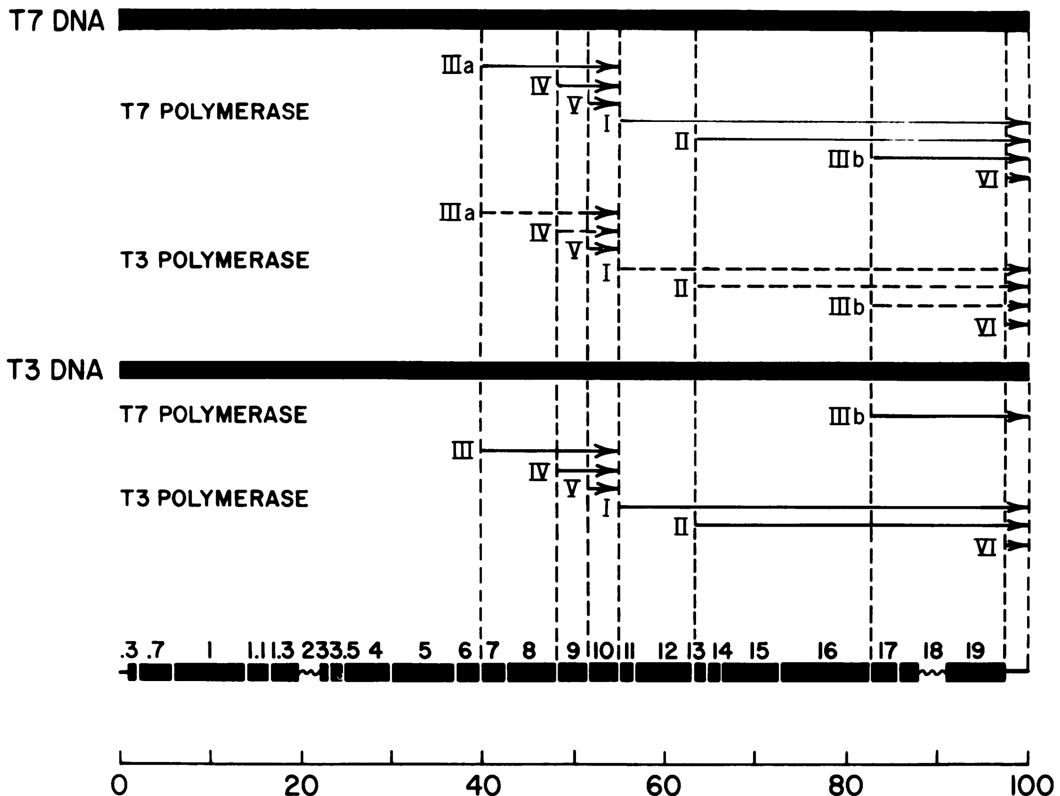

FIG. 4. *Representation of the major in vitro transcripts read by T7 and T3 RNA polymerase on T7 and T3 DNAs. The genetic map and sizes of RNAs and cistrons are those of T7 phage. Distances indicated by heavy lines on the genetic map show the approximate size of the numbered T7 genes as calculated from the sizes of their known protein products (27). The scale is calibrated as a percentage of total genome length. Positions of the T7 genes on the chromosome are assigned on the basis of data presented by Simon and Studier (26) and Hyman et al. (18). The positions of promoter and terminator sites for the major transcripts synthesized by T7 RNA polymerase on T7 DNA and by T3 RNA polymerase on T3 DNA were estimated from their molecular weights (11) (from transcription patterns of DNA templates shortened at both ends by exonucleolytic cleavage [10, 12]) and from the analysis of the transcription pattern of T3 × T7 recombinant phages. The T3 transcripts are not drawn to correct length. T7 RNA transcripts I to VI are read by T3 RNA polymerase with low efficiency (12).*

the high specificity of the two phage RNA polymerases, one might expect that recombinants with the gene for T3 RNA polymerase and the promoter sites for the major T7 RNA species would not be viable. The genetic constitution and the transcription patterns of most of the T3 × T7 recombinants were completely consistent with this expectation. However, for several recombinants (e.g., R12, R55, and R39), the gene 1 product had the molecular weight of the T3 RNA polymerase (data not shown), but transcriptional analysis showed that the genome contained the T7 promoters for all major RNA species but species VI (Table 2). To try to clarify the in vivo situation, we analyzed all T3 × T7 recombinants with regard to the activity and template specificity of the phage RNA polymerase. Figure 5 shows an analysis of the phage RNA polymerase activity in cell-free extracts of phage-infected cells assayed with T3 or T7 template DNA. The recombinants R60 and R71 (as well as R8, R17, and R30) had a preference for T3 DNA, the recombinants R84 (and R12, R39, R67, R68, R82) showed a preference for T7 DNA template, and the RNA polymerase of R55 had no measurable in vitro activity. Thus, the RNA polymerase of recombinants R12 and R39 displayed the specificity of T7 RNA polymerase, although having the molecular weight of T3 RNA polymerase. This in vitro specificity correlates exactly with that of the specific promoter sites for the major RNA species on these genomes. In the case of R55, we cannot say anything about the in vivo template specificity of

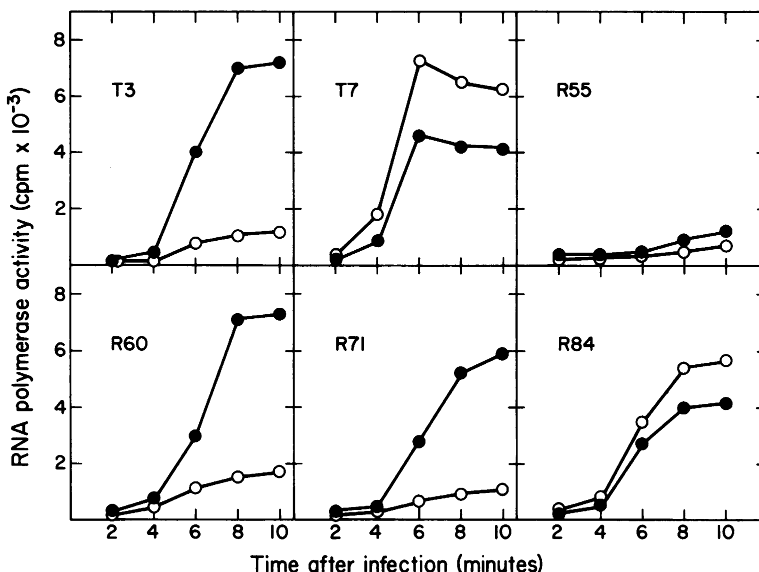

FIG. 5. *RNA polymerase activity in cell-free extracts of phage-infected host bacteria. Exponentially growing cells of E. coli BS-1 were infected with T3, T7, or various T3 × T7 recombinants (multiplicity, 10 particles per cell). At intervals, 10-ml samples of infected bacteria were pipetted into centrifuge tubes containing ice, chloramphenicol (300 μg/ml), and neutralized KCN (0.01 M). The samples were centrifuged without delay. The pellets were resuspended in 0.5 ml of buffer (0.05 M Tris-hydrochloride, pH 8, 0.01 M 2-mercaptoethanol, and 2 mg of bovine serum albumin per ml), sonically treated, and recentrifuged. The supernatant fluid was assayed at 37°C for rifampin-resistant RNA polymerase activity with minor modifications as described by Chamberlin et al. (4). The template DNA used was either T3 DNA (●) or T7 DNA (○). The RNA polymerase activity is here given as counts per minute of ^{3}H-labeled UTP incorporated into acid-precipitable material per assay.*

the RNA polymerase; however, we assume that this RNA polymerase may well also have the T7 promoter specificity.

The arrangement for the major RNA species III, IV, and V, as well as I, II, IIIb, and VI, is that of overlapping transcription units having a shared terminator near 56 and 99%, respectively (Fig. 4). If the RNA polymerase of a recombinant phage were able to recognize the specific promoter sites for species IIIa and I on the recombinant DNA, this would still result in the complete transcription of the entire region between 40 and 99%. Transcription of R60 by T3 RNA polymerase shows most of the major T3 RNA species but lacks species II and V. Thus, recombinants can apparently be viable even in the absence of some of the specific promoter sites for the major RNA species, as long as all regions of the recombinant genome are transcribed from a major promoter. It is noteworthy that the yield of progeny phages after infection with R60 is reduced and that the lysis of infected cells is delayed.

DISCUSSION

By studying the transcription patterns of a collection of T3 × T7 recombinant phages from which the genetic constitution was known, we have been able to map most of the promoter sites of the major RNA species transcribed by T3 and T7 RNA polymerases on T3 and T7 DNA. The resulting transcriptional maps are shown diagrammatically in Fig. 4 on a map of the T7 genome (note that for representational purposes T7 and T3 proteins and RNAs are shown as identical in size, although there are significant differences as discussed above). RNA species I and II originate at the beginning of genes 11 and 13, respectively. These results are in close agreement with our earlier suggestions, which were based on the use of biochemical deletions (10, 12). When artificially shortened T7 DNA templates were transcribed by T7 RNA polymerase, four (I, II, IIIb, and VI) of the seven major T7 RNA species were found to be truncated or deleted. This indicated that all are terminated at a common terminator site at or near the right end of the T7 DNA molecule. The RNA species IIIa, IV, and V originate at the beginning of genes 7, 9, and 10, respectively (Fig. 4), and share a common terminator near 55% (to the right of gene 10). Hence, there are at least two regions of overlapping transcription units on T3 and T7 DNA.

Translational mapping of T7 RNAs synthesized in vitro (22) has also been used to position promoters for two of the T7 RNA transcripts. Thus, T7 RNA species IV and V stimulate the synthesis of the T7 genes 9 and 10 proteins and of the gene 10 protein, respectively. These results, which demonstrate the existence of promoters for T7 RNA polymerase between genes 8 and 9 and between genes 9 and 10, are consistent with our mapping of the promoter sites for species IV and V. Since Niles and Condit (22) could not observe the synthesis of the genes 7 and 8 proteins from T7 species III and since T7 proteins other than those specified by genes 9 and 10 were synthesized (because of the presence of at least two different species, T7 species IIIa and IIIb), they were not able to locate the promoter site for species IIIa. The transcription patterns of recombinant DNAs that contain the region encoding genes 3 through 7 from one parental phage and the region encoding genes 7 through 15 from the other (Table 2, Fig. 3) gave convincing evidence that species IIIa is not transcribed from the DNA region coding for the class II proteins (20 to 40%).

Three of the RNAs (III, IV, and V) transcribed in vitro from a T7 DNA template by T7 RNA polymerase appear to comigrate in a polyacrylamide gel with in vivo late T7 RNAs, but there are no apparent in vivo counterparts for RNAs I, II, and VI (23). Dunn and Studier (8) have suggested that the in vivo RNAs that are synthesized by T7 RNA polymerase may arise from the post-transcriptional cleavage of larger precursor RNAs. Pachl and Young (24) have fractionated T7 RNAs synthesized in vivo on preparative polyacrylamide gels and have translated the RNAs in a cell-free system. Among the class III transcripts three overlapping RNAs (F, G, and H) are detected that are similar in size and in respect of their map position to the in vitro species IIIa, IV, and V.

The majority, or perhaps all, of the major in vitro transcripts are derived from the class III region (40 to 99%) of T3 and T7 DNA (Fig. 4). The class II region (20 to 40%) does not give rise to any major transcripts in vitro. However, when unfractionated late T7 RNAs (synthesized in vitro) were translated, almost all late T7 proteins (class II and III proteins) were present (22). The apparent lower level of transcription from the class II region might be due to "weak" promoters. If this region were transcribed as a unit with an initiation site at 17% and a termination signal at 40%, it would have a message with a molecular weight of 2.9×10^6; if it shared a termination signal with species IIIa, IV, and V (near 55%), its message would be 4.75×10^6 in molecular weight. RNAs of these sizes have not been resolved on gels (e.g., when T7 DNA was transcribed by T7 RNA polymerase), but if their molar yields were less than 10% of that of the major species, they might not be distinguishable from the background of major RNAs or unfinished chains in this region. However, such minor transcripts have been detected after transcription of recombinant DNA templates (e.g., R30 and R55). The genetic constitution of R55, for example, is thus that the region encoding the gene 1.3 through gene 5 is derived from T3 and the region encoding gene 6 through gene 19 is derived from T7 (Fig. 3) so that all major RNA species (besides VI) are transcribed by T7 RNA polymerase. The transcription of R55 DNA by T3 RNA polymerase results in a minor RNA transcript with a molecular weight similar to that of RNA species II (Table 2), and we suppose that this minor species becomes visible because of the absence of the major RNA species and/or because of a higher efficiency for "weak" promoters in the absence of specific "strong" promoters.

Given the overall pattern of the T3 and T7 transcriptional maps, what can one conclude about the functional aspects of the transcriptional pattern? The motif of overlapping transcription units is not clear. Formally speaking, it provides numerous start signals and "conserves" stop signals. There are several presumptive advantages to having many promoter sites. One obvious consequence is that all "late" genes can be transcribed nearly simultaneously. This is in fact the case in a normal T3 and T7 infection, where the appearance of class II proteins (e.g., DNA polymerase) coincides with that of class III proteins (e.g., major head protein) (Issinger and Falk, in press; 27). It is somewhat more difficult to produce justifications for the infrequency of stop signals on the T7 chromosome. Perhaps it is more efficient for an RNA polymerase to continue elongating the same RNA chain than to terminate and restart at another site.

If the specificity of T3 and T7 RNA polymerases observed in vitro (10, 12) is also expressed in vivo, then recombinants with genetic origin alternative from T3 and T7 in the region between 40 and 65% (where five of the seven promoter sites for the major RNA species are located) should not be viable. In fact we did not isolate recombinants of this kind. We observed a close relation between the RNA polymerase specificity of a recombinant phage and its corresponding promoter sites for the major RNA species I to V in most cases (Table 2; Fig. 2). With regard to the occurrence of overlapping transcription units, however, one would expect that the absence of one or two specific promoters on

the recombinant DNA would not be lethal but eventually only reduce the yield of progeny phages. Such a recombinant phage (e.g., R60) has been isolated and characterized (Table 2, Fig. 2).

The isolated T3 × T7 recombinants have proven to be very useful in transcriptional mapping. They should ultimately be of value for studying a variety of other phage functions as well. In principle it should be possible to map the gene for any function that is specific for only one of the two parental phages (e.g., host specificity). "Hybrids" have been isolated by crosses between related bacteriophages other than T3 and T7; examples are λ × 434 (5), λ × P22 (17), and P2 × 186 (3). In all cases, extensive recombination has been reported, as well as extended stretches of complete base sequence homology in heteroduplex derived from the two phages. T3 and T7 differ somewhat from these phage pairs in that they are incapable of extensive recombination (H. Beier, Ph.D. thesis; 14) and their DNAs have extensive sequences of partial homology (6).

The terminal redundancy of T7 is not homologous to that of T3 (6). If the terminal redundancy of a DNA is necessary for the replication of linear molecules (30) and if two phages have nonhomologous redundancies, as do T3 and T7, then the DNA products of a single recombinational event would not be terminally redundant and therefore would not be viable (18). The isolated T3 × T7 recombinants show an even number of crossover events (Fig. 2), and their genetic constitution makes it likely that all recombinants have homologous terminal redundancies. Physical maps of recombinants can be obtained from heteroduplex mapping of recombinant DNAs with T3 and T7 DNA and could be used to complement the protein mapping data and to confirm the assumption that the T3 × T7 recombinants do possess homologous terminal redundancy regions.

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