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SAMase Gene of Bacteriophage T3 Is Responsible for Overcoming Host Restriction

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Deletion and point mutants of T3 have been isolated and used to show that the early region of T3 DNA is organized in the same way as that of T7 DNA. Homologous early RNAs and proteins of the two phages have been identified by electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate. Both phages have five early mRNA's, numbered *0.3*, *0.7*, *1*, *1.1*, and *1.3* from left to right, although no T3 protein that corresponds to the *1.1* protein of T7 has yet been identified. In general, corresponding early RNAs and proteins of the two phages migrate differently on gels, indicating that they differ in molecular weight and/or conformation. In both T7 and T3, gene *0.3* is responsible for overcoming the DNA restriction system of the host, gene *0.7* specifies a protein kinase, gene *1* specifies a phage- specific RNA polymerase, and gene *1.3* specifies a polynucleotide ligase. The *0.3* protein of T3 is responsible for the S-adenosylmethionine cleaving activity (SAMase) induced after T3 (but not T7) infection. However, cleaving of S-adenosylmethionine does not appear to be the primary mechanism by which T3 overcomes host restriction, since at least one mutant of T3 has lost the SAMase activity without losing the ability to overcome host restriction.

T3 and T7 are related bacteriophages that have similar genetic maps and similar programs of development after infection (2, 5, 12, 15). Both T3 and T7 can grow interchangeably on *Escherichia coli* B, K, and C strains (24; this work), apparently unaffected by the DNA restriction systems known to be present in the B and K strains (1, 16). Recently it has been found that the gene *0.3* protein of T7 acts after infection to overcome the DNA restriction-modification systems of B and K (24). Gene *0.3* is the leftmost gene identified in T7 and the first to be expressed after infection (21, 23).

The leftmost gene identified in T3, which is also one of the first to be expressed after infection, specifies the S-adenosylmethionine cleaving enzyme (SAMase) (2, 9, 11, 13). This enzyme would seem to be a logical one for overcoming host restriction after T3 infection, since S-adenosylmethionine is a cofactor required for the action of the restriction endonucleases of both *E. coli* B and K (1, 16). However, T7 produces no detectable SAMase activity (9), and Hausmann has isolated T3 mutants that have no detectable SAMase activity but which grow normally on B (11). By analogy with T7 (24), the ability to grow on B indicates that these SAMase mutants retain the ability to overcome host restriction.

To determine whether the T3 SAMase gene is responsible for overcoming host restriction, we isolated deletion and point mutants of T3 that are unable to overcome restriction. Analysis of these mutants showed that the SAMase protein is indeed responsible for overcoming host restriction, although we also confirmed that SAMase activity can be lost without losing the ability to overcome restriction. Further analysis showed that the entire early region of T3 is organized in the same way as that of T7.

MATERIALS AND METHODS

Bacteriophages. Wild-type T7, together with procedures for growth and plating of lysates, have been described (20). Wild-type T3 was obtained from J. Dunn, who obtained it from R. Hausmann (12). T3 strain 3356, a SAMase$^-$ mutant (11), was obtained from R. Hausmann.

***E. coli* strains.** The following *E. coli* strains are described in reference 24: B *(hspB)*, BBw/1 *(hspB sup)*, B707 *(hspB)*, B834 *(hspO)*, 011' *(hspK supE)*, C600 *(hspK supE)*, C600rm$^-$ *(hspO supE)*, C *(hspO)*, and C1757 *(hspO supD)*. The genotype *hspB* indicates that the strain both restricts and modifies with B specificity, *hspK* indicates K specificity, and *hspO* indicates that the strain neither restricts nor modifies; the presence of amber suppressors is indi-

cated by *sup*. B707 and B834 are an isogenic pair, as are C600 and C600rm$^-$. BL2 is a ligase-deficient derivative of B, and BR3 is a derivative of B that does not plate *0.7* mutants of T7 (22).

Isolation of T3 mutants. The techniques previously used to isolate point and deletion mutants of T7 (20, 22) were applied to T3. Point mutants were isolated from a lysate that had been grown in the presence of 20 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml. Conditional lethal amber mutants were identified by ability to grow on BBw/1 but not B. One of six tested (*am*2) was found to be in gene *1*, as judged by effect on protein synhesis (26). Point mutants that are unable to overcome host restriction were identified by ability to grow normally on B834 but not B. Unfortunately, such mutants produce some clearing in a spot test for growth on B, which makes them quite difficult to find. However, one amber mutant and one temperature-sensitive mutant were found. The amber mutant HR2 overcomes restriction in hosts that carry an amber suppressor but not in hosts that do not (Table 1); the temperature-sensitive mutant HR1 overcomes restriction when plated at room temperature (22 to 23 C) but is partially restricted when plated at 37 C (Table 1).

Deletion mutants of T3 were isolated from survivors of heat inactivation, as previously described for T7 (22). Lysates were diluted 100-fold in tryptone broth containing 10 mM EDTA and heated at 60 C (rather than 50 or 55 C for T7) to achieve appreciable rates of inactivation. To screen for mutants that are unable to overcome host restriction, phages were grown and plated on B834, and individual plaques were spot tested for ability to grow on B, BL2, and BR3, all three of which have an active restriction system. By testing on these three hosts we hoped to be able to find mutants in T3 ligase and protein kinase as well: ligase mutants of T7 (gene *1.3*) grow on B but not BL2 or BR3 (22), and mutants that lack only the T7 protein kinase (gene *0.7*) (17, 23) grow on B and BL2 but not on BR3 (22). We assumed that corresponding mutants of T3 would plate the same way.

Eight lysates, each grown on B834 from a different single plaque of T3, were heat inactivated for 4 h at 60 C, again grown on B834, and again heat inactivated for 4 h at 60 C. Survivors of the second heat inactivation were plated on B834, and approximately 80 plaques from each of the eight lysates (a total of 660 plaques) were spot tested on the three indicator host strains. Seventeen mutants, coming from six different lysates, were unable to overcome host restriction, and one strain appeared to be a ligase mutant. No strain that lacked only the protein kinase was identified. These 18 strains are numbered R1-R18. Patterns of early RNA and protein synthesis distinguished eight types of deletion, including the ligase deletion. Nine of the eighteen strains, coming from five different lysates, appeared to be identical to R1. The R1 type of deletion apparently arises relatively frequently in B834, and is the only type that was found in more than one of the eight independent lysates.

A set of ligase deletion mutants was also obtained, from a stock of T3 that had been grown on 011′ and then heat inactivated for 2 or 4 h at 60 C. Survivors were plated on B, and individual plaques were tested for growth on BL2 and BR3. After heating for 2 h, one ligase mutant was found from 585 plaques tested, but after heating for 4 h, 22 ligase mutants were isolated from 306 plaques tested. A

TABLE 1. *Plating efficiencies of T3 strain on different hosts at 37 C*[a]

T3 strain				Relative plating efficiency							
Designation	Genetic composition			on C *hspO*	on B *hspB*	on BBW/1 *hspB sup*	on B707 *hspB*	on B834 *hspO*	on 011′ *hspK supE*	on C600 *hspK supE*	on C600rm$^-$ *hspO supE*
	Initiator RNAs	Proteins *0.3*	Proteins *0.7*								
Wild	+	+	+	0.9	1.0	1.1	1.0	1.1	1.1	1.1	0.7
K7	+	+	0	1.0	0.8	1.1	0.8	1.2	0.8	1.1	0.8
3356	+	Point	+	0.4	1.0	1.1	1.0	1.0	0.5	0.6	0.7
HR1	+	*ts*	+	1.0	0.8×10^{-2}	11×10^{-2}	4.4×10^{-2}	1.0	1.2×10^{-2}	7.4×10^{-2}	0.8
HR2	+	*am*	+	1.2	2×10^{-5}	1.0	8×10^{-5}	1.0	0.8	1.1	1.0
R1	0	0	+	1.1	1×10^{-5}	2×10^{-5}	3×10^{-5}	0.9	2×10^{-4}	5×10^{-4}	0.4
R4	+	0	0	1.0	0.7×10^{-5}	3×10^{-5}	3×10^{-5}	1.0	3×10^{-4}	2×10^{-4}	0.4
R7	0	0	+	1.0	20×10^{-5}	9×10^{-5}	30×10^{-5}	1.0	17×10^{-4}	25×10^{-4}	0.5
R12	+	0	+	1.0	80×10^{-5}	25×10^{-5}	80×10^{-5}	1.0	60×10^{-4}	21×10^{-4}	0.8
R13	+	0	0	0.9	1.4×10^{-5}	2×10^{-5}	3×10^{-5}	1.1	2×10^{-4}	3×10^{-4}	0.6
R16	+	0	0	1.0	0.4×10^{-5}		2×10^{-5}	1.4	3×10^{-4}	3×10^{-4}	0.6
R17	0	0	0	0.9	0.7×10^{-5}	1×10^{-5}	3×10^{-5}	1.1	3×10^{-4}	4×10^{-4}	0.2
R14	Ligase deletion			1.0	0.8		0.9	1.4	1.1	0.9	0.4

[a] The T3 strains were grown in B834. Their genetic composition is summarized, but see text and Table 2 for details. The restriction specificity of the host strains and the presence of amber suppressors is noted below each host. At low plating efficiencies (less than 0.2 in the table), plaques are distinctly different from wild-type plaques, being smaller, sometimes more turbid, and taking longer to develop.

number of these mutants appeared to be identical, but at least five different types of deletion could be distinguished by analysis of RNA patterns. The 23 ligase mutants of this set are numbered LG101 to LG123. Again, no mutants that plated like the protein kinase mutants of T7 were detected.

Although no mutants that plated like protein kinase mutants of T7 were found in the above two sets of deletion mutants, T3 does induce a protein kinase activity after infection (see Fig. 4), and some of the deletions that are unable to overcome host restriction also lack the protein kinase activity (see Table 2). Therefore, a further attempt was made to isolate strains of T3 that lack only the kinase. Eight single plaques of wild-type T3 grown on B were cycled three times through growth on B followed by inactivation by heating for 4 h at 60 C. Two different mutants that delete the kinase but retain the ability to overcome restriction were found. As with T7 kinase mutants, the T3 kinase mutants do not grow on BR3. However, restriction of growth in BR3 is less severe for kinase mutants of T3 than for those of T7, so it is difficult to identify kinase mutants of T3 by spot tests on BR3. The two T3 kinase deletion mutants are designated K1 and K7. A T3 point mutant that lacks kinase activity was also found, and is designated K6.

Gel electrophoresis. Techniques for labeling and analyzing T7 RNAs and proteins (23) have been applied to T3. Cultures of *E. coli* C grown at 30 C in low phosphate B2 medium (25) to a concentration of about 5×10^8/ml were used for labeling. They were infected with about 10 phage particles per cell, using phage stocks that had been purified by CsCl banding. Details of labeling and electrophoresis on slab gels are given in the legends to the figures. After electrophoresis, the gels were dried for autoradiography.

Assay for SAMase activity. SAMase activity was assayed by the procedure of Herrlich and Schweiger (14). Cultures of *E. coli* growing at 30 C in tryptone broth at a concentration of 4×10^8 cells/ml were infected with purified stocks of phage at a concentration of 10 phage particles per cell. Virtually all of the cells were infected, with less than 0.1% retaining the ability to form a colony. At 10 min after infection, the cultures were centrifuged, and the cell pellets were suspended in 0.1 volume of lysis buffer (10 mM Tris-hydrochloride, pH 8, 22 mM NH_4Cl, 1 mM dithiothreitol, 4 mM EDTA, 5% glycerol, and 150 μg of egg white lysozyme per ml). The suspensions were frozen and thawed three times. To assay for SAMase, 5 μl of extract was mixed with 20 μl of S-adenosylmethionine (325 μg/ml in 50 mM Tris-hydrochloride, pH 8) containing 2 μCi of $^{14}CH_3$-labeled S-adenosylmethionine per ml. After incubation for 1 h at 30 C, 15 μl of reaction mixture was spotted on a cellulose thin-layer sheet (Eastman chromogram sheet 13255) and chromatographed in 65% ethanol. Unhydrolyzed S-adenosylmethionine remains at the origin, and the thiomethyladenosine produced by SAMase action (9) runs near the front. Results are readily apparent under a UV lamp or by autoradiography of the chromatogram.

RESULTS

SAMase and host restriction. Wild-type T3 plated with approximately equal efficiency on several different C, B, or K strains of *E. coli* (Table 1), indicating that, like T7, T3 is unaffected by the B and K host restriction systems. As outlined above, we isolated a set of seven deletion mutants of T3 that are unable to overcome host restriction, one mutant (HR2) that is amber in ability to overcome restriction, and one mutant (HR1) that is partially restricted at 37 C but not at room temperature (22 to 23 C). Plating efficiencies of these T3 strains on different hosts are given in Table 1. Deletion mutants that affect only the protein kinase (K7 in Table 1) or the ligase (R14 in Table 1) are not subject to host restriction, and strain 3356, a SAMase mutant of Hausmann (11), is not restricted either.

All of the T3 strains of Table 1 were tested for the ability to induce SAMase after infection. Figure 1 shows representative results obtained after infecting B834, which has no amber suppressor, and BBw/1, which carries an amber suppressor. As expected, wild-type T3 induces high levels of SAMase in both hosts, but neither T7 nor strain 3356 (the Hausmann SAMase mutant) produces detectable SAMase activity in either host. Mutants that affect only protein kinase or ligase produce normal levels of SAMase (data not shown), but deletion mutants that are unable to overcome restriction (represented by R12 in Fig. 1) produce no detectable SAMase activity. HR2, which is amber in ability to overcome restriction, is also amber in SAMase, producing high levels after infecting BBw/1 but little if any activity in B834. HR1, which is partially restricted at 37 C but not at room temperature, produces a low but detectable level of SAMase activity in both hosts. The activity induced by HR1 seems to be somewhat temperature sensitive but does not approach the wild-type level even when infection and assay are done at room temperature.

These results clearly show that a single T3 protein is responsible both for overcoming host restriction and for producing SAMase activity. However, strain 3356 has lost SAMase activity without losing the ability to overcome host restriction. This mutant, as all of Hausmann's SAMase mutants, was isolated in *E. coli* B (11), and T3 strains that have lost ability to overcome restriction (like similar T7 strains [24]) grow poorly in B even if they have previously been grown in B. Therefore, isolation of SAMase mutants in B should favor strains that retain the ability to overcome restriction, and it

seems likely that all of Hausmann's SAMase mutants are of this type. Of course T7 also overcomes restriction without inducing detectable SAMase activity.

It might be argued that wild-type T7 and strain 3356 of T3 both produce a SAMase that is active in vivo but not in extracts. However, the finding of Gefter et al. (9) that methylation of DNA occurs during infection by T7 (but not T3) suggests that S-adenosylmethionine must be available after T7 infection, an indication that T7 produces no SAMase activity in vivo. Equivalent information is not available for strain 3356.

Identification of T3 early proteins. T3 mutants have been used to identify and map the early RNAs and proteins of T3, using the techniques previously applied to T7 (19, 23). This analysis indicates that the early region of T3 is organized in the same way as that of T7, so the T7 numbering system has been adopted for the early genes of T3: gene *0.3* is SAMase, *0.7* is protein kinase, *1* is RNA polymerase, and *1.3* is ligase. For convenience, a map of the early region of T7 DNA is given in Fig. 2. The effects of different deletions on the T3 early RNAs and proteins are summarized in Table 2.

The time course of protein synthesis after infection of *E. coli* C by T7, T3, or by mutants affecting the T3 early proteins is shown in Fig. 3. The pattern of proteins made after T3 infection is similar to but distinctly different from that observed after T7 infection, as previously found by Hyman et al. (15). Positions of the four T3 early proteins that have been identified are indicated in Fig. 3, along with positions of the homologous T7 proteins.

Gene *1* specifies the phage RNA polymerase

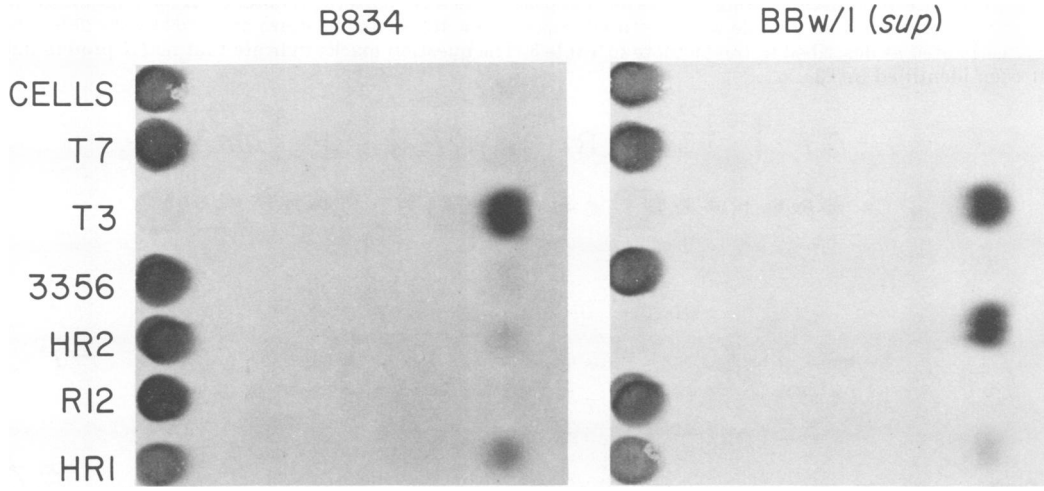

FIG. 1. *Assays for SAMase activity in extracts of cells infected with different phage strains. Autoradiograms of two sets of assays are given, the origin of the chromatogram being to the left in both sets. The host is given above each set, and the phage strain is identified to the left.*

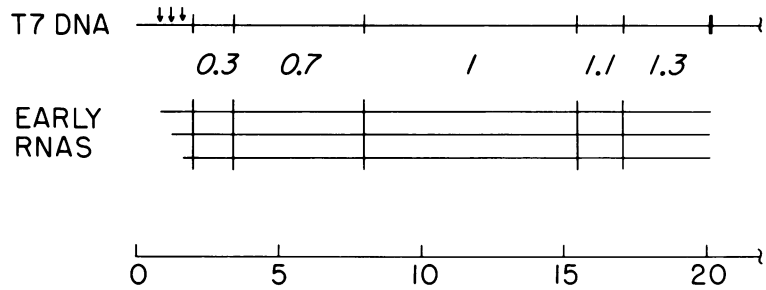

FIG. 2. *Map of the early region of T7 DNA (7, 19). Early RNAs are synthesized from left to right by E. coli RNA polymerase, beginning at three separate initiation sites (arrows) and ending to the right of gene 1.3 (thick line). The primary transcripts are cut by E. coli RNase III at five specific sites (thin vertical lines). Gene numbers are given above the RNAs; functions of the early genes and sizes of the early RNAs and proteins are summarized in Table 3. The scale is in units of percentage of the length of T7 DNA, beginning at the left end. The early region of T3 DNA is organized in the same way (see text).*

TABLE 2. *Properties of T3 deletions*[a]

Deletion location	T3 strain	RNAs								Proteins				
		Initiator RNAs	*0.3*	*0.7*	*1*	*1.1*	*1.3*	Terminator	Length of new RNA	*0.3*	*0.7*	*1.1*	*1.3*	Length of new peptide
Left of gene *1*	R1	0	0	0	+				4.8,5.5 (5.2,6.1)	0	+			
	R4	+	0	0	+				1.2	0	0			12,500
	R7	0	0	0	+				3.8	0	+			
	R12	+	0	0	+				3.8	0	+			
	R13	+	0	0	+				2.7	0	0			36,000
	R16	+	0	0	0				8.8	0	0			
	R17	0	0	0	+				1.2	0	0			
	K1	+	+	0	+				2.2	+	0			4,000
	K7	+	+	0	+				1.4	+	0			
Right of gene *1*	R14				0	0	0	0	~17			0	0	
	LG102				+	0	0	0	9.4			?	0	
	LG114				+	+	0	0	6.5			?	0	

[a] +, Presence of an RNA or protein in its normal position in a gel pattern; 0, absence. If no length is given for a new peptide, no new peptide was identified. Sizes of new RNAs and peptides produced by the deletions were estimated as described in the footnote to Table 3. The question marks indicate that no *1.1* protein has yet been identified in T3.

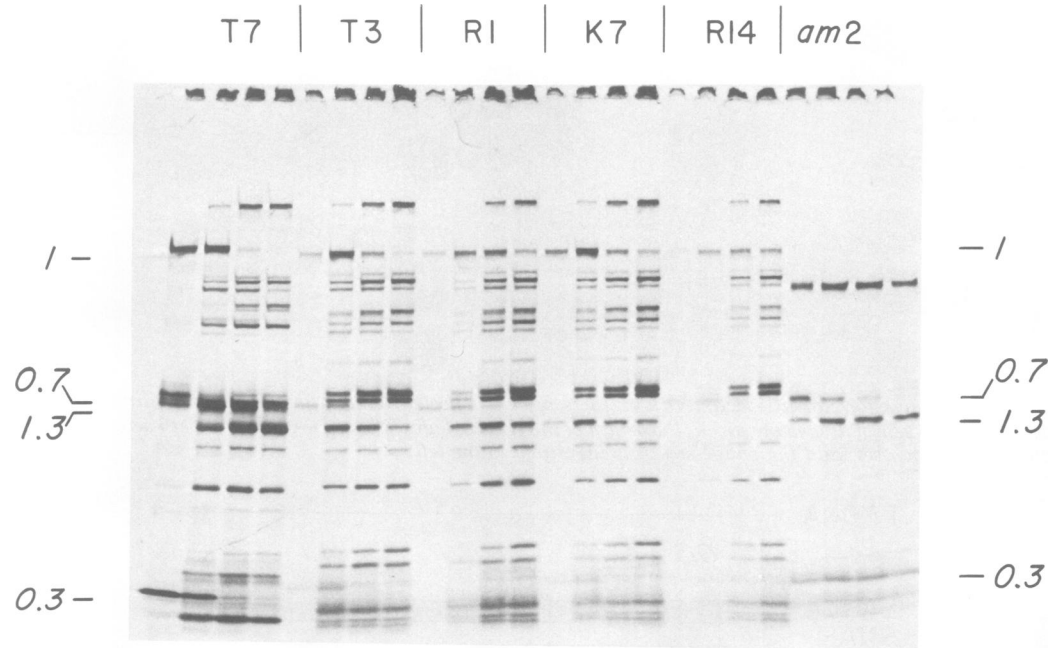

FIG. 3. *Time course of protein synthesis after infection by different phage strains. Samples were prepared and analyzed essentially as described in reference 23. The host cells were UV irradiated before infection to suppress synthesis of host proteins. Phage proteins were labeled with [^{35}S]methionine (40 μCi/ml, 400 μCi/μg) in 4-min pulses at different times after infection. Samples equivalent to 0.05 ml of culture were subjected to electrophoresis for 3 h at 150 V on a slab gel containing a 10 to 25% gradient of acrylamide plus a 5% stacking gel, using a discontinuous Tris-glycine-sodium dodecyl sulfate buffer system. The origin of electrophoresis is at the top of the autoradiogram and migration is downward. The phage strain is indicated above each set of four tracks; pulse times were 4 to 8, 8 to 12, 12 to 16, and 16 to 20 min after infection (left to right) in each set. R1, K7 and R14 are T3 deletion mutants described further in Table 2; am2 is a gene 1 amber mutant of T3. The positions of T7 early proteins are indicated to the left of the patterns; T3 early proteins are indicated to the right.*

(3, 6), which is needed for producing normal amounts of late protein (26). Early proteins are the only ones made in normal amounts after infection by *am2* in Fig. 3, so this amber mutation must be in gene *1*. The gene *1* protein is the only early protein affected, and a relatively long amber peptide is produced. As noted previously (15), the normal T3 RNA polymerase migrates slightly faster than T7 RNA polymerase during electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate.

The SAMase protein of T3 (gene *0.3*) migrates somewhat more slowly than the *0.3* protein of T7 and is made in smaller amounts (Fig. 3). The SAMase protein can be identified because the amber mutant HR2 does not produce it unless the host strain carries an amber suppressor, nor is it produced after infection by any of the SAMase deletions (see R1 in Fig. 3). HR2 does not seem to affect any other T3 protein, but some of the SAMase deletions also eliminate the kinase protein (Table 2). The SAMase point mutations of strains 3356 and HR1 do not appear to cause chain termination, since neither affects the migration of the SAMase protein on gels (not shown). As expected, none of the point mutations affects the gel patterns of the early RNAs (not shown).

T3, like T7 (17), induces a protein kinase activity after infection (Fig. 4). The specificity of the two kinase activities, as indicated by the spectrum of phosphorylated proteins produced after infection (Fig. 4), seems to be similar but perhaps not identical. The T3 protein identified as *0.7* in Fig. 3 seems to be responsible for the kinase activity, since only those deletion mutants that lack this protein are deficient in kinase activity (K7 in Fig. 3 and 4). The *0.7* protein of T7 usually migrates as a double or triple band (21, 23), but the *0.7* protein of T3 seems to migrate as a single band, slightly faster than the major band of *0.7* protein from T7. The ability of single T3 deletions to eliminate both the *0.3* and *0.7* proteins (Table 2) indicates that, as in T7, these two genes lie near each other in the DNA.

Ligase deletion mutants of T3 were identified by their inability to grow on ligase-deficient hosts. These mutants all lack a single early protein that migrates somewhat faster than the T7 ligase (see R14 in Fig. 3), and this protein should be the T3 ligase (gene *1.3*). Analysis of the RNAs produced after infection by ligase deletion mutants (see below) indicates that some of these deletions also eliminate the *1.1* RNA of T3, but no protein corresponding to the *1.1* protein of T7 has yet been identified in T3.

All four early proteins identified in T3 migrate on gels with mobilities different from their T7 counterparts, indicating a difference in molecular weight (18). Approximate molecular weights (calculated from relative mobilities) for corresponding early proteins of the two phages are given in Table 3.

Identification and mapping of T3 early RNAs. Analysis of the early RNAs produced after infection by different T3 deletion mutants demonstrates that the early region of T3 DNA is organized in the same way as the early region of T7 DNA. Most types of deletion already described for T7 (19, 23) have also been found in T3. Gel patterns of the early RNAs produced after infection by T7, T3, or representative T3 deletion mutants are given in Fig. 5. Most of the homologous early RNAs of T3 and T7 have different mobilities, and the relative sizes, as judged by migration, are summarized in Table 3. Identification and mapping of the T3 early RNAs is described below. The map of T7 early RNAs in Fig. 2 provides a useful reference.

The gene *1* RNA can be identified because it is the only T3 early RNA large enough to code for the gene *1* protein. The *0.7* RNA can be identified using deletions that eliminate only the *0.7* protein; deletions such as K1 and K7 affect only the *0.7* RNA (Table 2). All of the SAMase deletions affect both the *0.3* and *0.7* RNAs, but some do not affect the *0.7* protein. By analogy with T7 we assume that the *0.3* RNA is the messenger for the *0.3* protein, but our results do not rigorously exclude the possibility that the *0.3* protein could be specified by the left end of the *0.7* RNA. Thus, all of the SAMase deletions enter the *0.7* RNA, and the piece of *0.7* RNA left by the deletions that fall entirely within the *0.7* RNA (K1 and K7, Table 2) is still large enough to code for the *0.3* protein. However, this possibility seems very unlikely.

The SAMase deletions R1, R7, and R17 eliminate a set of smaller RNAs (I_1, I_2, and I_3 in Fig. 5), which are presumably the initiator RNAs of T3 (7, 24). The R1 deletion produces multiple fusion RNAs (Fig. 5), suggesting that it leaves most or all of the promoters for *E. coli* RNA polymerase still functional. R7 and R17 produce single fusion RNAs (Table 2), suggesting that these deletions eliminate all but the leftmost promoter. Apparently T3 DNA has at least three promoters to the left of gene *0.3*, but the exact number and location of the start sites has yet to be determined.

The following information is sufficient to order the T3 early RNAs.

Some deletions (R4, R12, and R13) (Table 2 and Fig. 5) affect only the *0.3* and *0.7* RNAs; therefore these two RNAs must come from adjacent regions of the T3 DNA. The R1 and R7

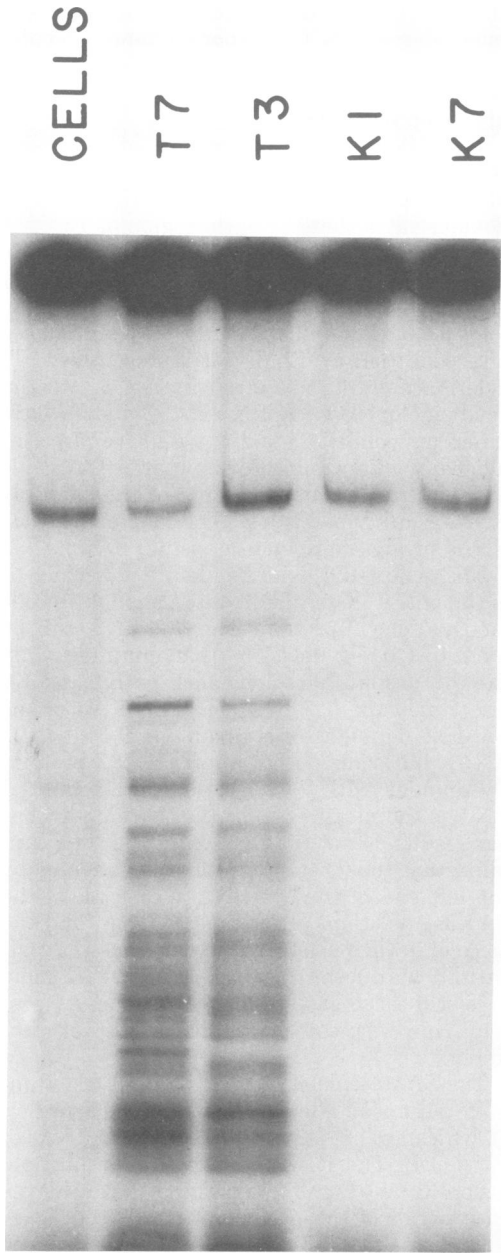

FIG. 4. *Protein kinase assays of different phage strains. Samples were prepared and analyzed essentially as described in reference 23. Host cells were not UV irradiated, 25 μCi of $^{32}PO_4$ per ml was added at the time of infection, and labeling was stopped after 10 min by adding a cyanide-phosphate mixture. Each sample, after heating in buffer containing 1% sodium dodecyl sulfate, was treated with 20 μg of pancreatic RNase per ml for 30 min at room temperature to eliminate RNA bands from the patterns. Amounts equivalent to 0.1 ml of culture were subjected to electrophoresis for 2.5 h at 150 V on a slab gel containing a 3 to 20% gradient of acrylamide, using a discontinuous Tris-glycine-sodium dodecyl sulfate buffer system. The origin of electrophoresis is at the top of the autoradiogram, and migration is downward. The phage strain is indicated above each track: K1 and K7 are T3 deletions that fall entirely within the 0.7 RNA (Table 2). The label at the origin and in the band closest to the origin is probably in DNA. The bands present only in T7- and T3-infected cells are phosphorylated proteins and are eliminated if the samples are treated with Pronase before electrophoresis (data not shown).*

deletions eliminate the initiator RNAs but not the *0.7* protein (Table 2); therefore the *0.3* RNA must lie between the initiator RNAs and the *0.7* RNA. The R16 deletion eliminates the *0.7* and *0.3* RNAs, producing a slightly larger gene *1* RNA and no other new RNA fragment (Fig. 5 and Table 2); therefore a portion of one of these RNAs must be fused to the gene *1* RNA, eliminating the other entirely. Since the *0.3* RNA has been placed between the initiator and *0.7* RNAs, the *0.7* RNA must lie next to the gene *1* RNA. Combining all of these observations establishes the order: initiators, *0.3, 0.7, 1*. Hausmann and Härle (13) have genetically mapped SAMase to the left of gene *1*, establishing the orientation relative to the genetic map.

Some ligase deletions (R14 in Fig. 5 and Table 2) also affect the gene *1* RNA, so the ligase RNA must lie to the right of the gene *1* RNA. As in

TABLE 3. *Relative sizes of T7 and T3 early RNAs and proteins*[a]

Gene	RNA		Protein		Function
	T7	T3	T7	T3	
0.3	1.6	1.6	8,700	11,500	Overcomes host restriction
0.7	4.6	4.3	42,000	40,000	Protein kinase
1	7.5	8.5	100,000	97,000	RNA polymerase
1.1	1.6	1.6	8,000	?	?
1.3	3.1	3.2	40,000	37,000	Ligase

[a] Sizes of RNAs and proteins were estimated from relative migration in gel electrophoresis, using T7 RNAs (19, 23) and proteins (21, 23) as standards. RNAs were analyzed on 2 and 2.25% gels, essentially as described in the legend to Fig. 5, and proteins were analyzed on gradient gels essentially as described in the legend to Fig. 3. The sizes should be regarded primarily as an indication of relative migration on these gels and may not be an accurate representation of the true sizes, since relative migration can change in other gel and buffer systems. RNA sizes are given in units of percentage of the length of T7 DNA, 1% being equivalent to 378 bases or a molecular weight of 130,000 (19). Protein sizes are given as molecular weights, 13,900 being equivalent to approximately 1% the length of T7 DNA (19).

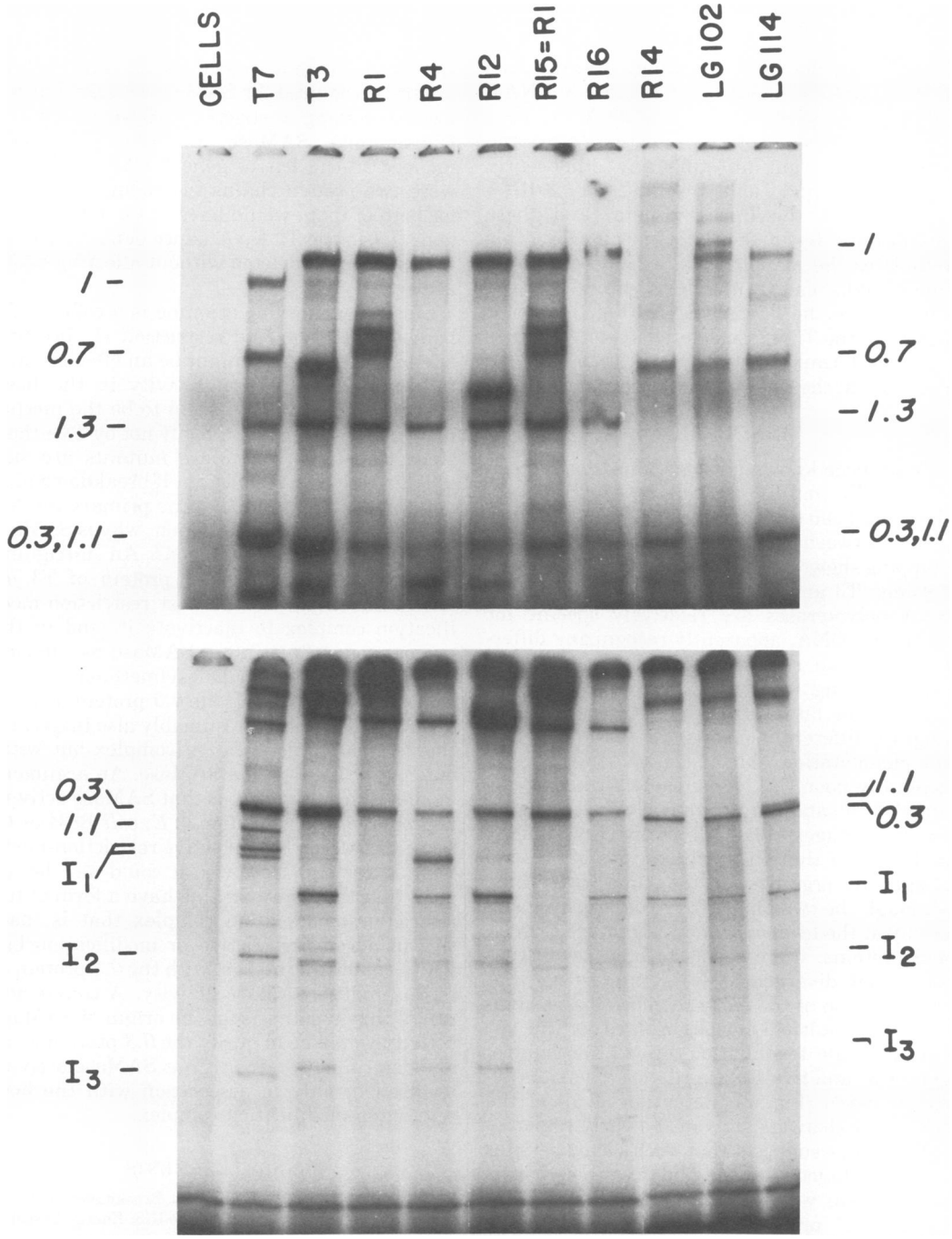

FIG. 5. *Early RNAs of T3 deletion mutants. Samples were prepared and analyzed essentially as described in reference 23. The host cells were UV irradiated before infection to suppress synthesis of host RNAs, and infection was in the presence of 360 μg of chloramphenicol per ml so that only early RNAs would be transcribed. The RNAs were labeled with 25 μCi of $^{32}PO_4$ per ml added at the time of infection, and labeling was stopped 10 min after infection by adding a cyanide-phosphate mixture. Samples equivalent to 0.05 ml of culture were subjected electrophoresis on two different slab gels: 2.5 h at 90 V on a gel containing 2.25% acrylamide plus 0.5% agarose, using a 0.05 M phosphate buffer-sodium dodecyl sulfate system, and 2.5 h at 150 V on a 3 to 20% gradient gel, as described in the legend to Fig. 4. The origin of electrophoresis is at the top of the autoradiograms; migration is downward. The phage strain is indicated above each track (see Table 2 for details). T7 early RNAs are identified to the left of the patterns; T3 early RNAs are identified to the right.*

T7 (19, 23), some ligase deletions enter the gene *1* RNA and eliminate the *1.1* and *1.3* RNAs, others affect the *1.1* and *1.3* RNAs without affecting the gene *1* RNA, and others affect the *1.3* RNA without affecting any other early RNA (Fig. 5 and Table 2). Thus, the *1.3* RNA must specify the ligase protein, and these RNAs must lie in the order: *1, 1.1, 1.3*. This completes the mapping of the T3 early RNAs and establishes that they are arranged in the same way as the T7 early RNAs (Fig. 2). Most if not all of the T3 ligase deletions seem to eliminate the termination site for *E. coli* RNA polymerase at the right end of the early region.

DISCUSSION

It has been known for some time that bacteriophages T3 and T7 are related (5). However, there has been considerable evolutionary divergence between the two phages: heteroduplex mapping shows only partial sequence homology between T3 and T7 DNAs (4); the T3 and T7 RNA polymerases are relatively specific for their own DNA, apparently recognizing different initiation sequences (6, 10); most of the corresponding early RNAs and proteins and many of the late proteins from the two phages migrate differently on gels (15, this work); and complementation during mixed infection is generally poor (13). Nevertheless, the genetic maps (2, 20) and the program of development of the two phages (12, 15) remain very similar, and we find that the early regions of the two phages are organized in the same way. Thus, although the two phages have diverged considerably at the level of primary structure of DNA and proteins, there seems to have been little functional divergence. It is interesting that transcription of the early region remains similar, with multiple initiation sites at the left end, a single termination site to the right of gene *1.3*, and five RNase III cleavage sites between genes (Fig. 2). Retention of all of the RNase III cleavage sites in the early region of both phages suggests that such cleavage sites are advantageous to the phage, although it is not yet clear why (8).

The first protein made after infection by either T7 or T3, the *0.3* protein, is responsible for overcoming host restriction. It is clear that the *0.3* protein is also required for the SAMase activity observed after T3 infection, but the Hausmann strain 3356 has lost SAMase activity without losing the ability to overcome restriction. We assume that strain 3356 carries a mutation in the *0.3* protein, but this has not been demonstrated directly. Our data do not exclude the possibility that the SAMase mutation of 3356 is in a second protein chain, which might be required for SAMase activity but not for overcoming restriction. However, one of Hausmann's SAMase mutants has been mapped to the left of gene *1* (13), so if there were two protein chains the coding sequences for both of them would have to lie to the left of gene *0.7* in the T3 DNA (since gene *0.7* can be almost entirely deleted without affecting SAMase activity).

Since S-adenosylmethionine is a cofactor for both modification and restriction (1, 16), production of a SAMase might be an effective way to eliminate restriction activity in the host. However, this does not seem to be the mechanism used by T7, and probably not by T3 either, since Hausmann's SAMase mutants are still able to overcome restriction. If breakdown of S-adenosylmethionine is not the primary mechanism for overcoming restriction, why is SAMase produced after T3 infection? An intriguing possibility is that the *0.3* protein of T3 interacts directly with the host restriction-modification complex to inactivate it, and in the process converts it into a SAMase by altering its normal use of S-adenosylmethionine as a cofactor. In this model, the *0.3* protein of T7 or of strain 3356 would presumably also inactivate the restriction-modification complex but without converting it into a SAMase. An argument against this hypothesis is that SAMase activity is produced after infection of *E. coli* B834 or C, neither of which has an active restriction-modification system. However, it could also be argued that these host strains have a form of the restriction-modification complex that is inactive in normal restriction or modification but which could still interact with the *0.3* protein of T3 to produce SAMase activity. A convincing test of this hypothesis for the origin of SAMase activity would be to purify the *0.3* protein of T3 and determine whether it has SAMase activity by itself or only in association with the host restriction-modification complex.

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