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Bacteriophage T4 RNA ligase is gene 63 product, the protein that promotes tail fiber attachment to the baseplate

(multifunctional protein)

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ABSTRACT **RNA ligase and tail fiber attachment activities, normally induced following bacteriophage T4 infection of *Escherichia coli*, are not induced when gene 63 amber mutants of T4 infect nonpermissive host cells. Both activities are induced when these mutants infect permissive hosts, or when revertants of these mutants infect nonpermissive hosts. When one of these mutants infects a host that carries *supF*, both activities are more than normally heat labile. RNA ligase, purified to homogeneity, promotes the tail fiber attachment reaction *in vitro* with a specific activity similar to that of the most highly purified preparations of gene 63 product isolated on the basis of tail fiber attachment activity. We conclude that T4 RNA ligase is gene 63 product. The RNA ligase and tail fiber attachment reactions differ in requirements and in response to some inhibitors, suggesting that the two activities of the gene 63 product may be mechanistically unrelated.**

Bacteriophage T4 induces the formation of two ATP-dependent polynucleotide ligases: DNA ligase (reviewed in ref. 1) and RNA ligase (2). DNA ligase plays several key roles in DNA replication and repair, including the joining of Okazaki fragments during discontinuous DNA synthesis (3). The physiological role of RNA ligase is unknown. However, it has attracted considerable interest as a reagent for *in vitro* synthesis of nucleic acids. This versatile enzyme, which has been purified to homogeneity (4, (4, 5,§), catalyzes the ligation of single-stranded RNA and DNA (2, 4, 5, 6–12) and promotes the T4 DNA ligase-catalyzed joining of duplex DNAs with completely base-paired ends (13). The mechanism of the RNA ligase reaction involves adenylylated enzyme and adenylylated nucleic acid intermediates (9–12, 14,§). Thus far, RNA ligase activity has been detected reliably only after T-even phage infection (2). Its appearance within about 3 min after infection at 37° is blocked by chloramphenicol, suggesting that a phage protein is respoinsible for the activity (2).

We report here that RNA ligase is the product of gene 63 (is *gp* 63). This gene previously was shown to code for the tail fiber attachment (TFA) protein, which promotes noncovalent joining of tail fibers to the phage baseplate (15, 16, ¶). Tail fiber attachment normally is the last step in T4 morphogenesis. Although the reaction proceeds slowly in the absence of TFA protein, this factor is required for a normal burst of phage, and gene 63 amber (*am*) mutants form only minute plaques on nonsuppressing hosts. *In vitro*, the presence of TFA protein increases the rate of the TFA reaction by up to two orders of magnitude (15). Because *gp*63 apparently is not part of the virion (17), this protein belongs to the intriguing class of nonstructural proteins required for phage assembly (16, 18), and is the first member of this class to be purified to homogeneity.

MATERIALS AND METHODS

Strains of Bacteria and Phage. Derivatives of *Escherichia coli* strain B, nonpermissive for T4 *am* mutants and either permissive or nonpermissive for *rII* mutants, have been described previously (15, 16, 20). Strain CR63 carries *supD* and is permissive for both *am* and *rII* mutants; CR63(λ) is nonpermissive for *rII* mutants. KM 159 and KM 1593, isogenic K-12 *strA* strains that carry no amber suppressor and *supF*, respectively, were obtained from K. McEntee.

Phage mutants were derivatives of T4D from the California Institute of Technology collection. The three gene 63 mutants *am*M69 (15), *am*MN23 (17), and *am*MNc79 (17) make minute plaques at 42° or 37° and no plaques at 23°. The multiple mutant X379 carries *am*B17 (gene 23), *am*M69 (gene 63) and the deletion *rdf*41 (*rII*); X4E carries *am* mutations in tail fiber genes 34, 35, 37, and 38; and X1, used for preparation of TFA protein (15, ¶) carries *am* mutations in DNA synthesis genes 41, 42, 43, 44, and 45. T4 RNA ligase mutants were isolated from T4D phage that had been treated with 0.4 M hydroxylamine (19) in two stages for a total of 144 hr to a survival level of 6×10^{-9} (19 lethal hits per particle). Survivors that plated on strain CR63 at 30° were used to infect *E. coli* B at 43°. Midway through the latent period the infected cells were harvested; extracts were prepared by freezing and thawing (20) and assayed for RNA ligase activity.

Enzymes. RNA ligase and TFA protein were purified by methods to be described elsewhere.§ Unless indicated otherwise, the RNA ligase used was the hydroxylapatite II fraction, which is physically homogeneous, and the TFA protein used was fraction III, which is about 10% pure. A nearly homogeneous preparation of RNA ligase purified by a different procedure (5) was obtained from J. Last. Bacterial alkaline phosphatase type III S was obtained from Sigma Chemical Co.; pancreatic DNase I and RNase IA were obtained from the Worthington Co.

Assays. The standard RNA ligase assay measures the conversion of [5′-^{32}P]r$A_{\overline{20}}$ to a phosphatase-resistant form (ref 4;

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Abbreviations: *am*, amber (referring to suppressor-sensitive nonsense mutations); *gp*, gene product; Tes, 2-{[tris(hydroxymethyl)methyl]amino}ethanesulfonate; TFA, tail fiber attachment.

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§ N. P. Higgins, A. Geballe, T. J. Snopek, A. Sugino, and N. R. Cozzarelli, unpublished data.

¶ W. B. Wood, M. P. Conley, H. L. Lyle, and R. C. Dickson, unpublished data.

Table 1. Effects of gene 63 mutations on RNA ligase, TFA, and DNA polymerase activities

Phage	Activity, units/mg protein: RNA ligase	TFA	T4 DNA polymerase
T4(A5x3)*	12	5.1	10
A5x4	0.9	1.7	6.6
*am*MN23	<0.2	0.5	7.6
*am*MNc79	<0.2	<0.2	7.3
*am*M69	<0.2	<0.2	9.1
rev(*am*M69)†	11	4.2	12

Preparation of infected-cell extracts and assays for RNA ligase, TFA (assay A), and DNA polymerase activities were performed as described in *Materials and Methods*.

* Isogenic with A5x4 except for apparently normal ligase gene (isolated from final backcross of A5x4).

† Spontaneous revertant of *am*M69.

RNA cyclization reaction). The reaction mixture (20 μl) contained 50 mM Tris·HCl at pH 7.5, 10 mM $MgCl_2$, 20 mM dithiothreitol, 1 mM ATP, bovine serum albumin at 0.1 mg/ml, 10 μM[5′-^{32}P]r$A_{\overline{20}}$, and 0.005–0.05 unit of enzyme. After 30 min at 37°, the reaction mixture was treated with 1 unit of bacterial alkaline phosphatase at 65° for 20 min and radioactive material adsorbable by Norit (activated charcoal) was measured. One unit of RNA ligase catalyzes the formation of 1 nmol of product in 30 min.

The two assays used for TFA activity measure the conversion of tail-fiberless particles to infectious phage. Assay A employed the optimum conditions for tail fiber attachment (15, §). The 100-μl reaction mixture contained 10 mM 2-{[tris(hydroxymethyl)methyl]amino}ethanesulfonate (Tes) at pH 7.5, 1 mM EDTA, 2 mM dithiothreitol, 1 M $(NH_4)_2SO_4$, 3×10^6 tail-fiberless phage particles, and excess tail fibers (as determined experimentally), as well as TFA protein or RNA ligase. The tail fiber preparation was the supernatant fraction of a centrifuged (30,000 × *g* for 50 min) extract (20) of X379-infected Bb cells, and the particles were purified by differential centrifugation from a lysate of X4E-infected B/5 cells (20). In some experiments RNA ligase and tail fiber preparations were freed of small molecules by filtration through Sephadex G-50. For purification of ligase the column was equilibrated with 50 mM Tris·HCl, pH 7.5/14 mM 2-mercaptoethanol/10% (vol/vol) glycerol, and for fiber purification it was equilibrated with 10 mM Tris·HCl, pH 7.5/0.5 mM EDTA. Assay B was carried out using the standard RNA cyclization reaction mixture, except that RNA substrate was replaced by tail fibers and particles as in assay A, and Tris, which appears to inhibit the TFA reaction, sometimes was replaced by Tes. Both assay mixtures were incubated at 30° for 10 min, and the titer of infectious phage was measured by plating on CR63(λ). The increase in titer in these assays is not a linear function of either time or amount of TFA protein added (15). The kinetics of the TFA reaction have been interpreted in terms of a model of random tail fiber attachment with a pseudo-first order rate constant, *k*, which can be calculated from the increase in titer (15). Most of the data on TFA activity in this report are expressed in terms of *k* values. Duplicate determinations of *k* values by this method show a mean standard deviation of about 20%.

T4 DNA polymerase was assayed at 37° according to Goulian *et al.* (22).

Other Methods. Linkage between known *am* mutations and mutations that cause ligase defects was estimated by crossing an *rII* derivative of a ligase mutant with the *am* mutant, selecting for *rII*$^+$ *am*$^+$ recombinants, preparing infected-cell extracts with about 15 such recombinants, and assaying for ligase activity. Infected-cell extracts were prepared using B or B/5 bacteria as previously described (20). Most of the experiments reported in this paper have been performed in Chicago and in Pasadena with similar results.

RESULTS

Isolation of RNA Ligase Mutants. Mutants lacking RNA ligase were isolated by screening survivors of phage stocks mutagenized with hydroxylamine to a level of 19 lethal hits per particle (see *Materials and Methods*). From the genome size, the proportion of essential genes in T4 (18), and the molecular weight of RNA ligase (about 42,000) these phage can be estimated to have received about 0.25 hits per RNA ligase gene. Thus about 22% $[(1 - e^{-0.25}) \times 100]$ of such mutagenized phage would be expected to sustain a defect in the RNA ligase gene. In the experiment 38 survivors were tested, and 6 showed markedly reduced RNA ligase activity after infection. One of these mutants was backcrossed successively five times to standard-type T4D to remove extraneous mutations. The resulting phage, designated A5x4, induced little RNA ligase but normal levels of T4 DNA polymerase (Table 1). Nevertheless, A5x4 formed normal plaques on all *E. coli* strains tested including B and K-12, the set of 27 California Institute of Technology "hospital" strains (23), and K-12 strains that carried *recA*, *lex*, *uvrA*, *recB*, or *lig* mutations. One-step growth experiments showed that A5x4 had a normal latent period and burst size in strain B at temperatures ranging from 30°–43°. The other five RNA ligase mutants, although not as extensively backcrossed before testing, also made normal plaques on strain B.

Gene 63 Mutants Lack RNA Ligase. Preliminary mapping (see *Materials and Methods*) placed the A5x4 mutation in the region of gene 63. In the course of refining the genetic map, we discovered to our surprise that nonpermissive cells infected by any one of three gene 63 amber mutants lack RNA ligase activity (Table 1). All three mutants induced normal levels of T4 DNA polymerase. The RNA ligase defect in one of these mutants (*am*M69) was shown to be caused by the nonsense mutation, by the following tests. First, when *am*M69 was cross to *rII*A105 at a parental ratio of 1:10, 12 *rII*$^+$*am*$^+$ progeny that were assayed all induced normal RNA ligase activity, indicating that *am*M69 does not carry an unlinked second mutation that affects RNA ligase. Furthermore, when an *rII* derivative of A5x4 was crossed with *am*M69, none of 12*rII*$^+$*am*$^+$ progeny that were assayed induced RNA ligase, indicating close linkage of the ligase defect to *am*M69. Second, *am*M69-infected KM 1593 (*supF*) cells were found to contain appreciable RNA ligase activity, although at only about one third of the standard-type level. Third, 4 *am*$^+$ revertants of *am*M69 induced normal RNA ligase levels, as shown for one such revertant in Table 1. The frequency of these revertants was about 10^{-5}, in the range expected for a single mutational event. Backcrosses of these revertants to T4D did not segregate *am* progeny.

RNA Ligase Promotes Tail Fiber Attachment. The product of gene 63 is known to promote the attachment of tail fibers to the phage baseplate in the terminal step of T4 phage assembly (15). The TFA protein responsible for this activity has been partially purified and characterized (15, 16, §). TFA protein was concluded to be the product of gene 63 on the basis of the demonstration that *am*M69 produced abnormally heat-labile TFA activity upon infection of *supF* cells (15). As shown in Table 1, infection of nonpermissive host cells with any one of the gene 63 mutants *am*M69, *am*MN23, and *am*MNc79 produces little or no TFA activity. Moreover, the ligase-defective

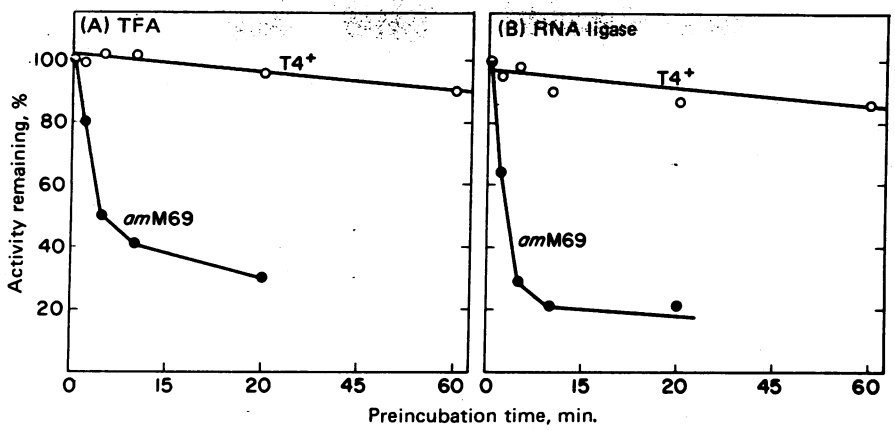

FIG. 1. Thermal inactivation of TFA and RNA ligase activities in extracts of *am*M69- and T4D$^+$-infected *supF* cells. Extracts were diluted to a protein concentration of 1.6 mg/ml in a buffer containing 0.01 M Tris·HCl at pH 7.5, 5 mM $MgSO_4$, and 17% glycerol. The diluted extracts were incubated at 41°, and at the times indicated samples were removed and assayed at 30° for TFA activity (assay A) and RNA ligase activity. At 0 min the *am*M69 extract exhibited about one third the activity of the T4$^+$ extract in both assays.

mutant A5x4 also produces subnormal levels of TFA activity (Table 1), suggesting that its defect also is a gene 63 mutation. The ability of A5x4 to form normal plaques is explained by this demonstration that its gene 63 defect is only partial compared to those of the *am* mutants.

The finding that gen·63 *am* mutations eliminate both TFA and RNA ligase activities suggests that both may reside in the protein *gp*63. This suggestion was proven correct in two ways. First, in *am*M69-infected *supF* host cells, both TFA and RNA ligase activities were shwon to be markedly more heat labile than the corresponding activities in standard-type T4D-infected *supF* cells (Fig. 1). This result indicates that *gp*63 is responsible for both activities. Second, physically homogeneous RNA ligase was shown to promote rapid tail fiber attachment (Table 2). The specific TFA activity of this preparation was similar to that found for a nearly homogeneous preparation of TFA protein purified on the basis of TFA activity.[§] Moreover, the ratio of TFA and RNA cyclization activities was roughly constant for several fractions that represented different stages in the independently developed purification procedures for TFA protein and RNA ligase (Table 2). When either pure RNA ligase or partially purified TFA protein preparations were fractionated by chromatography on Sephadex G-100 (in the presence of 20 mM Tris·HCl, pH 7.5/100 mM KCl/0.01 mM ATP/2 mM dithiothreitol/0.5 mM EDTA/10% glycerol), the TFA and RNA cyclization activities eluted together. Therefore, both genetic and biochemical evidence confirm that TFA and RNA ligase activities reside in one protein, which is the product of gene 63.

Table 2. Comparison of TFA and RNA cyclization activities in five enzyme preparations

Enzyme preparation	Activity, units/ml: RNA ligase	TFA	Ligase/TFA
1. Hydroxylapatite II	2600	500	5.2
2. DEAE-cellulose	110	29	3.8
3. Fraction III	1300	210	6.2
4. Crude extract	820	130	7.2
5. DEAE-cellulose II	85	12	7.1

Enzyme preparations 1 and 2 were purified in Chicago on the basis of RNA cyclization activity. Preparation 1 is physically homogeneous and preparation 2, from an intermediate purification step, is about 5% pure. Preparations 3 and 4 were made in Pasadena; preparation 3 is from an intermediate step in a purification based on TFA activity. Preparation 5 is a nearly homogeneous RNA ligase that was purified using a different procedure than that used for preparation 1 (ref. 5; supplied by J. Last).

Comparison of Requirements and Inhibitors of the RNA Cyclization and TFA Reactions. The conclusion from the preceding section suggests either that a reaction similar to nucleic acid ligation is involved in tail fiber attachment, or that *gp*63 has two functionally unrelated activities. We have compared requirements and responses to inhibitors of the two reactions *in vitro*. Previous studies showed no specific cofactor requirements for the TFA reaction except for a divalent or monovalent cation (15, 16). The same requirements were observed for the TFA reaction promoted by purified RNA ligase under conditions optimal for tail fiber attachment (Table 3). Under these conditions addition of EDTA, PP_i, or RNase, at concentrations that eliminate RNA cyclization under RNA ligase assay conditions, has no marked inhibitory effect on the

Table 3. Requirements for tail fiber attachment promoted by RNA ligase

Reaction mixture	Relative activity
1. Complete*	100
2. + 5 mM EDTA	60
3. + 1 mM $MgCl_2$ + 2 mM PP_i	100
4. − RNA ligase + heated (80°, 2 min) RNA ligase	<2
5. + RNase, 1.5 mg/ml	95
6. − Dithiothreitol†	93
7. − Dithiothreitol + 5 mM *N*-ethylmaleimide	<2
8. − Dithiothreitol + 0.2 mM PCMS	<2
9. − RNA ligase‡	2.5
10. − RNA ligase + 0.2 mM PCMS‡	2.1

PCMS, *p*-chloromercuribenzenesulfonate.

* Assay A for tail fiber attachment was carried out with 0.2 μg of RNA ligase; the tail fibers and RNA ligase were filtered through Sephadex G-50 prior to use. For reactions 1–8, incubation was for 10 min; the *k* value (see *Materials and Methods*) calculated for the complete reaction (0.15 min^{-1}) was set equal to 100.

† The activity observed under these conditions varied from the level shown to as low as 30.

‡ Reactions 9 and 10 were incubated for 180 min; the 10-min *k* value was computed from the titer as described previously (15) for comparison to that of the complete reaction.

Table 4. Comparison of requirements for TFA and RNA cyclization activities under RNA ligase assay conditions

Reaction mixture	Relative activity	
	TFA	RNA cyclization
Complete*	100	100
− ATP	83	1
− ATP + 1 mM PP_i	91	<1
− Mg^{2+} + 5 mM EDTA†	63	<1
+ 1 M $(NH_4)_2SO_4$	770	<1

* Assay B for TFA and the standard assay for RNA cyclization were carried out with 0.3 and 0.003 μg of RNA ligase, respectively. Both tail fibers and RNA ligase were filtered through Sephadex G-50 before use. Extents of TFA and RNA cyclization were measured after 0, 15, 30, 60, 120, and 180 min. The rates observed in the complete reactions (k value for TFA = 0.008 min^{-1}) were set equal to 100.

† The usual 10 mM $MgCl_2$ was omitted but the tail-fiberless particle preparation contributed 0.06 mM $MgCl_2$ to the TFA reaction mixture.

TFA reaction (Table 3). ATP addition stimulates the TFA reaction by up to 2-fold, but is not required as it is for the RNA cyclization reaction. The ATP effect on the TFA reaction is probably due to stabilization of the TFA protein. ATP significantly stabilized the TFA activities of both purified RNA ligase and partially purified TFA protein to heat inactivation (data not shown).

On the basis of the foregoing results, the TFA reaction exhibits requirements and responses to inhibitors that appear quite different from those of the RNA cyclization reaction. However, an objection to this conclusion is that the optimal assay conditions for the two activities are quite different; most notably the TFA assays in Table 3 were done in the presence of 1 M $(NH_4)_2SO_4$. The TFA reaction will proceed, although not as efficiently, under conditions quite similar to those of the standard RNA cyclization assay (15). When the two activities were compared under these conditions (Table 4), omission of ATP, addition of EDTA, or addition of PP_i abolished RNA cyclization but did not significantly inhibit tail fiber attachment. Addition of 1 M $(NH_4)_2SO_4$ inhibited RNA cyclization completely but greatly stimulated tail fiber attachment. In additional experiments, when RNA ligase was preincubated with ATP or PP_i under conditions known to charge and discharge the enzyme, respectively (14, ‡), there was no appreciable effect on its TFA activity under either set of assay conditions (data not shown). Thus there are clear differences between the RNA cyclization and TFA activities with respect to requirements and effects of certain inhibitors.

The two activities are similar in that both are inhibited variably by omission of dithiothreitol from the reaction mixture and strongly by addition of either of the sulfhydryl blocking reagents *p*-chloromercuribenzenesulfonate or *N*-ethylmaleimide (Table 3). These reagents have only slight effects on the attachment of tail fibers in the absence of TFA protein (Table 3) or on the infectivity of complete phage particles. Moreover, experiments in which TFA protein was preincubated with these reagents showed that their inhibitory effect can be accounted for almost entirely by elimination of the TFA activity (data not shown).

DISCUSSION

Earlier studies revealed two similarities between RNA ligase and TFA protein. First, both activities are unusual among phage-induced functions in that they are produced throughout the latent period; they appear a few minutes after infection and increase continuously to levels that represent about 1% of the total soluble protein in lysis-inhibited cells 2–3 hr after infection at 37° (15, ‡). Second, the molecular weight of RNA ligase (4, 5) is the same as that of the TFA protein subunit (16, §), about 42,000. An apparent dissimilarity is that in gel filtration experiments with crude extracts the TFA protein chromatographed as a presumed dimer with an apparent molecular weight of about 80,000 (15), whereas the active form of RNA ligase behaves as a monomer (5). However, under the conditions used in these studies the RNA ligase and TFA activities cochromatographed as monomers, and preliminary experiments showed that the gel filtration behavior of TFA protein can vary with the elution medium.

We have presented two lines of evidence that RNA ligase, TFA protein, and *gp*63 are identical. (*i*) Three independently isolated gene 63 *am* mutants lack both TFA and RNA cyclization activities. The two phenotypes have not been separable genetically. Reversion of the *am* mutation restores both activities. Growth of one *am* mutant in a *supF* host strain produced RNA ligase and TFA activities that both are substantially more heat labile than normal, indicating that gene 63 codes directly for both proteins. (*ii*) The ratio of RNA cyclization to TFA activity remains roughly constant throughout protein purifications based on assays for either activity. Physically homogeneous RNA ligase is active in both reactions. These findings provide compelling evidence that the two proteins are the same, and that they are coded by gene 63 of the phage.

The role of *gp*63 in promoting tail fiber attachment is well established, but its mechanism is unclear. The reaction does not absolutely require TFA protein. In its absence, tail fiber attachment proceeds at a slow rate both *in vivo*, to produce a burst of 10–15 phage per infected cell during the normal latent period, as well as *in vitro* (15). This property accounts for the leakiness of gene 63 *am* mutations and the finding that they can be partially suppressed by prolongation of the latent period or by growth at higher temperatures, which increase the rate of unpromoted tail fiber attachment (unpublished results). The presence of TFA protein increases the rate of tail fiber attachment up to 100-fold. However, it is unclear whether this effect represents true catalysis. The level of TFA protein present does not affect the end point of the *in vitro* reaction (15), and *gp*63 does not appear to be present in the completed virus (17). However, the molar amount of TFA protein added to the *in vitro* reaction to produce a measurable increase in the rate of tail fiber attachment under standard conditions is around an order of magnitude greater than the level of tail fibers present, and several orders of magnitude greater than the number of attachment events that normally are measured. A similar situation probably exists *in vivo*, where the numbers of both *gp*63 molecules and tail fibers (24) are estimated to be approximately 10^4 per cell by the end of the latent period.

A two-step mechanism whereby *gp*63 promotes conversion of either particles or tail fibers to an activated form for subsequent attachment is unlikely, because mixtures of tail-fiberless particles and tail fibers isolated from infected cells in which gene 63 was functional do not combine efficiently *in vitro* unless a source of TFA protein is present (15). Support for this view also comes from experiments (unpublished) in which particles and fibers were preincubated separately with purified RNA ligase and then assayed for rate of infectious phage production when mixed with the other component. No effect of preincubation was seen.

The finding of RNA ligase activity so far only in T-even-infected bacteria is consistent with the notion that the sole physiological role of *gp*63 is in tail fiber attachment. On the other hand, the protein's ability to catalyze ligation of nucleic acids with a very high turnover number and its appearance shortly after infection argue that it also may serve functions prior to the final step in morphogenesis. Superficially, *am* mutations in gene 63 appear to have no effect on early events in the latent period (15), but more careful physiological experiments are required to explore this point.

With regard to the mechanism of tail fiber attachment, the identity of the RNA ligase and TFA proteins suggests two alternatives: either *gp*63 carries two active sites whose functions are unrelated, or the phosphodiester-bond-forming activity of RNA ligase is involved in tail fiber attachment. The protein appears to have a sulfhydryl group essential to both activities, on the basis of stabilization of its activities by reducing agents and inactivation by sulfhydryl-blocking reagents. ATP also stabilizes both activities. Otherwise, the ligation and TFA reactions show marked differences. TFA proceeds in the absence of ATP or Mg^{2+}; ligation requires both these reagents. TFA proceeds in the presence of PP_i, EDTA, and 1 M $(NH_4)_2SO_4$; any one of these reagents blocks ligation. A remote possibility is that tail fiber attachment is related to the final step of the cyclization reaction. This step is the formation of a phosphodiester bond between a 5′-adenylylated donor and a 3′-hydroxyl-terminated acceptor molecule with release of AMP (9–12). RNA ligase catalyzes this reaction with many donors of the general form AppX, transferring the −pX group to the 3′-OH group of an oligonucleotide acceptor (T. England, R. I. Gumport, and O. C. Uhlenbeck, personal communication). This reaction does not require ATP, and could be involved in tail fiber attachment if either the tail fiber or a baseplate protein is isolated from infected cells in the activated donor form. Arguing against this possibility are (*i*) the evidence that the bond formed between the tail fiber and the baseplate is noncovalent, because it does not survive treatment with sodium dodecyl sulfate and sulfhydryl compounds (24) and (*ii*) the failure to find phosphorus associated with any of the proteins of the phage following dissociation by the same treatment (25). The lack of inhibition of the TFA reaction by ATP (which adenylylates RNA ligase to render it inactive in the transfer reaction) or by 1 M $(NH_4)_2SO_4$, RNase, and EDTA also discourages this view. Instead, the evidence so far favors the hypothesis that *gp*63 is a bifunctional protein which catalyzes covalent ligation of nucleic acids and promotes noncovalent attachment of tail fibers to the baseplate in reactions that are mechanistically quite distinct.

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1. Lehman, I. R. (1974) *Science* **186**, 790–797.
2. Silber, R., Malathi, V. G. & Hurwitz, J. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 3009–3013.
3. Okazaki, R., Okazaki, T., Sakabe, K., Sugimoto, K., Kainuma, R., Sugino, A. & Iwatsuki, N. (1968) *Cold Spring Harbor Symp. Quant. Biol.* **33**, 129–144.
4. Snopek, T. J., Sugino, A., Agarwal, K. L. & Cozzarelli, N. R. (1976) *Biochem. Biophys. Res. Commun.* **68**, 417–424.
5. Last, J. A. & Anderson, W. F. (1976) *Arch. Biochem. Biophys.* **174**, 167–176.
6. Kaufmann, G. & Littauer, U. Z. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3741–3745.
7. Kaufmann, G. & Kallenbach, N. R. (1975) *Nature* **254**, 452–454.
8. Walker, G. C., Uhlenbeck, O. C., Bedows, E. & Gumport, R. I. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 122–126.
9. Sugino, A., Snopek, T. J. & Cozzarelli, N. R. (1977) *J. Biol. Chem.* **252**, 732–738.
10. Ohtsuka, E., Nishikawa, S., Sugiura, M. & Ikehara, M. (1976) *Nucleic Acids Res.* **3**, 1613–1623.
11. Sninsky, J. J., Last, J. A. & Gilham, P. T. (1976) *Nucleic Acids Res.* **3**, 3157–3166.
12. Uhlenbeck, O. C. & Cameron, V. (1977) *Nucleic Acids Res.* **4**, 85–98.
13. Sugino, A., Goodman, H. M., Heyneker, H. L., Shine, J., Boyer, H. W. & Cozzarelli, N. R. (1977) *J. Biol. Chem.* **252**, 3987–3994.
14. Cranston, J. W., Silber, R., Malathi, V. G. & Hurwitz, J. (1974) *J. Biol. Chem.* **249**, 7447–7456.
15. Wood, W. B. & Henninger, M. (1969) *J. Mol. Biol.* **39**, 603–618.
16. Wood, W. B. & Bishop, R. J. (1973) in *Virus Research*, eds. Fox, C. F. & Robinson, W. F. (Academic Press, New York), pp. 303–324.
17. Vanderslice, R. W. & Yegian, C. D. (1974) *Virology* **60**, 265–275.
18. Wood, W. B. & Revel, H. R. (1976) *Bacteriol. Rev.* **40**, 847–868.
19. Tessman, I. (1968) *Virology* **35**, 330–333.
20. Edgar, R. S. & Wood, W. B. (1966) *Proc. Natl. Acad. Sci. USA* **55**, 498–505.
21. Depew, R. E., Snopek, T. J. & Cozzarelli, N. R. (1975) *Virology* **64**, 144–152.
22. Goulian, M., Lucas, Z. J. & Kornberg, A. (1968) *J. Biol. Chem.* **243**, 627–638.
23. Wilson, J. H. (1973) *J. Mol. Biol* **74**, 753–757.
24. Ward, S., Luftig, R. B., Wilson, J. H., Eddleman, H., Lyle, H. & Wood, W. B. (1970) *J. Mol. Biol.* **54**, 15–31.
25. Dickson, R. C. (1973) *J. Mol. Biol.* **79**, 633–647.