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BACTERIOPHAGE T4 TAIL LENGTH IS CONTROLLED BY ITS BASEPLATE

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SUMMARY

Purified T4 baseplate, when treated with high concentrations of pancreatic RNase, are inactive in in vitro complementation assays. Their ability to initiate tail tube assembly is not altered; but the most probably length of the tube-baseplate formed is only 800A, compared to 1000A, the normal tube length, when untreated baseplates are used. Thus, baseplates help to regulate tube length, possibly by a template mechanism. Several minor baseplate proteins which may be involved in determining the length, including gp54, are missing from RNase - treated baseplates. These effects may be due to an unidentified protease contaminant of the RNase, since they are inhibited by phenylmethane sulfonyl fluoride.

INTRODUCTION

Bacteriophage T4 has a tail of well-defined length, as do the other tailed phages. No investigator has reported observing a T4 virion with a tail deviating significantly from the normal length of almost exactly 1000 Å (1,2). The mechanism(s) by which bacteriophage tail length is so stringently regulated is unknown (3). Perhaps the most obvious hypothesis is that some macromolecule, distinct from the tail protein subunits, serves as a template along which the subunits polymerize. However, direct evidence for the existence of templates (e.g. visualization by electron microscopy, or variation in tail length caused by mutation of a gene whose product is a baseplate protein) has eluded all investigators to date.

We have previously reported methodology for studying the in vitro assembly of the T4 tail tube (4). Baseplates and gp19 may be separately purified, and when incubated together will react to yield tube-baseplate complexes in which the most probable length is the same as the tail length in normal T4 phage.

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Since the putative template is probably either a protein or a nucleic acid (5), the strategy we have adopted to demonstrate its existence is to treat baseplates with various proteases and nucleases, with the hope that conditions which selectively cleave the template may be found. We then look for changes in the distribution of tube lengths when baseplates so treated are mixed with gp19. In this communication we report some encouraging preliminary results obtained when T4 baseplates are treated with pancreatic RNase.

MATERIALS AND METHODS

Phage parts: The procedures used for preparing T4 baseplates, tube protein subunits and extracts for in vitro complementation assays have been described elsewhere (4).

Enzymes: RNase was from Worthington (Code RAF). Two different preparations were used; both gave similar results in the experiments described. Trypsin and chymotrypsin were obtained from Sigma, and elastase from Worthington. Phenylmethylsulfonyl fluoride (PMSF)[≠] came from Sigma.

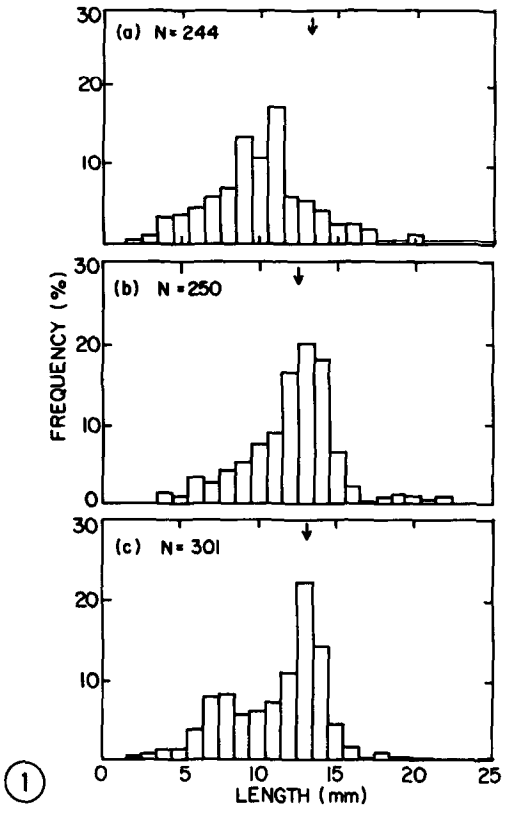
Treatment of baseplates with proteases and RNase. To an aliquot of purified baseplates (50-150 µl) was added to 10-25 µl of enzyme; the mixture was incubated at 37°C for 1 hr. Both baseplates and enzymes were prepared in a buffer consisting of 0.01 M Tris, 0.02 M $MgSO_4$, 0.01 M mercaptoethanol, 0.0001 M EDTA (pH 7.4). The baseplate-enzyme mixture was then either frozen for later analysis by SDS electrophoresis (4) or mixed with an aliquot of gp19 and further incubated for 1 hr at 30°C. The concentrations of baseplates, gp19 and enzyme used are described in the text. The baseplate-enzyme-gp19 mixture was also frozen for later examination by electron microscopy as described elsewhere (4).

RESULTS

When purified baseplates and gp19 are incubated together at 30°C for 1 hr the lengths of the resulting tube-baseplates may be measured from electron micrographs of the reaction mixture. A typical histogram of tube lengths is shown in Fig. 1b. The most probable length is found to be equal within experimental error to the length of a normal phage tail, 1000 Å. While shorter than normal tube-baseplates are quite common, longer than normal structures are rare. We have shown elsewhere that distributions of this type are obtained over a wide range of initial gp19: baseplate concentration ratios (4).

It had been suggested that if the T4 baseplate possessed a template mole-

[≠]Abbreviations: PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate.



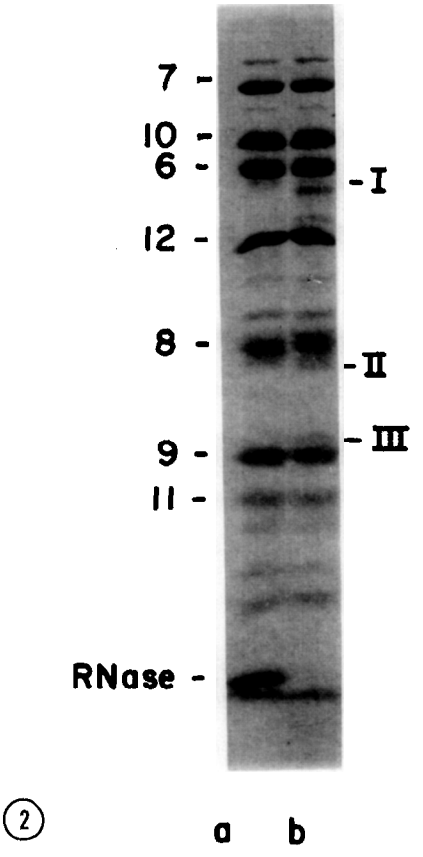


Figure 1. Effect of RNase treatment of baseplates on core-baseplate length.
a) 0.15 ml of baseplates (0.3 mg/ml) were preincubated in the presence of RNase (1.7 mg/ml) for 60 minutes at 37°C. 0.1 ml of gp19 (0.8 mg/ml) was then added and the mixture incubated for 60 minutes at 30°C. The reaction was stopped by immersing the samples in ice water and immediately freezing.
b) Same as (a) except that the RNase was replaced by buffer.
c) Same as (a) except that RNase was added after the first 60 minutes incubation along with the gp19.

Figure 2. Effect of RNase on protein composition of baseplates as determined by SDS gel electrophoresis. Baseplates and RNase were mixed and incubated as described in Materials and Methods. Final concentrations of baseplates and RNase were ~0.4 mg/ml and 1 mg/ml respectively. The number of the major gene products are given at the left and the bands which are absent from the RNase treated baseplates are indicated at the right. A. Baseplates treated with RNase. B. Baseplates treated with buffer.

cule, it might be a double-stranded RNA (5). We therefore treated baseplates with pancreatic RNase, which is known to degrade double-stranded RNA in low-salt buffers (6,7). We then determined the distribution of tube-baseplate lengths

when the treated baseplates were mixed with gp19. If very high concentrations of RNase were used we did indeed observe a change in the distribution of tube lengths. This is illustrated in Fig. 1a, which was obtained from an experiment in which baseplates were incubated for 1 hr at 37° with RNase at 1.7 mg/ml. The most probable tube length in Fig. 1a is only 800 Å, compared to 1000 Å for untreated baseplates. Fig. 1c shows the length distribution obtained when baseplates, gp19, and RNase were incubated together simultaneously (i.e., baseplates were not preincubated with RNase). The distribution is similar to that obtained with untreated baseplates, although the subsidiary maximum at 500 Å may indicate some effect of RNase on baseplates which had not yet begun to serve as initiation sites for tube polymerization. These histograms show that RNase does not cause a shortening of tube lengths by action on gp19, and that the T4 baseplate is involved in controlling the length of the phage tail.

Electron microscopic comparison of populations of RNase-treated and untreated baseplates which had been incubated with gp19, showed that RNase treatment had no effect on the fraction of baseplates that served as initiators of tube polymerization.

Since such high concentrations of RNase are required to upset the ability of the baseplate to regulate tail length, it is doubtful that the activity usually attributed to RNase is responsible. One possibility is that the RNase is contaminated with a protease. To check for this we compared the protein composition of baseplates treated with RNase to that of untreated baseplates by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. We observed that three minor protein bands were absent from electrophoretograms of baseplates treated with RNase (Figs. 2a, b). These have been labeled I, II and III in order of decreasing molecular weight. The major protein bands have been labelled with their corresponding gene number by comparing the banding pattern to published patterns (8,9). We believe that band I corresponds to contaminating sheath protein in our baseplate preparation because the mobility matches that reported for sheath protein, the intensity of the band varies from one prepara-

tion of baseplates to another, and we have observed contracted sheaths as a contaminant of baseplates by electron microscopy. Band II, which has a mobility slightly greater than gp8, is most likely either gp5 or gp48. Sometimes band II is not resolved from the other bands of similar mobility. Band III, which has a slightly lower mobility than gp9, is interesting because the only baseplate protein found by other workers (9,10) in this area of the electrophoretogram is gp54. The gene 54-polypeptide has been shown to be required to initiate core polymerization and has been suggested as a possible length determining protein as well (5). In the discussion which follows we shall concentrate on band III because it is reproducibly well-resolved from the gp9 band and because we are confident that its identification as gp54 is correct. The simplest interpretation of these results is that a protease contaminant of the RNase selectively degrades the proteins corresponding to bands I, II and III in Fig. 2.

As further evidence for a protease contaminant, we found that when the RNase is treated with the serine protease inhibitor PMSF, it is not able to degrade the proteins corresponding to bands I and III (band II was either absent or not resolved in this experiment).

PMSF was also effective in inhibiting the ability of RNase to inactive baseplates in in vitro complementation assays. Baseplates which were treated with RNase had only 27% of the activity of untreated baseplates. The activity of baseplates treated with PMSF + RNase rose to 63% of the control. The reason full activity was not observed was that the solvent used for the inhibitor itself inhibited the complementation assays by 30-40%.

If a serine protease contaminant is indeed responsible for the effects of RNase on baseplates, it should be possible to reproduce the results of Figs. 1 and 2 using a protease instead of RNase. Since both the identity of the protease and the concentration required to do this are unknown, we have first tried to duplicate the SDS gel electrophoresis experiments using the pancreatic serine proteases trypsin, chymotrypsin and elastase. All three of these enzymes are effective in selectively removing bands I and III from SDS gel patterns.

The effects which these enzymes may have on tube assembly, especially the length, have not yet been determined.

DISCUSSION

We have shown that when bacteriophage T4 baseplates are incubated with high concentrations (>1 mg/ml) of RNase and are then mixed with tube protein subunits, the resulting tube-baseplates are shorter than normal. Thus the baseplate helps to regulate phage tail length. A plausible explanation of these results is that the RNase (or, more likely, some contaminating activity in it) modifies a baseplate component whose function is to serve as a template for tube polymerization. When the template is cleaved, the fragment which remains attached to the baseplate is still capable of initiating tube assembly, but growth of the tube is prematurely terminated. This model is consistent with the electron microscopic observations that the fraction of baseplates that react with gp19 is independent of RNase treatment.

We have tentatively identified one of the protein bands (band III, Fig. 2), found to be absent from SDS gel electrophoretograms of RNase-treated baseplates, as the product of gene 54. The function of this gene product during phage assembly is to convert the baseplate into an initiator of tube assembly. It has also been suggested that gene 54 protein may function as a template for regulating tube length (5). It is tempting to postulate that the effect of the protease contaminant on tube length is due to cleavage of the gp54 template. It should then be possible to identify the fragment of gp54 that remains attached to the baseplate after cleavage. We have not observed any degradation products of band III by SDS gel electrophoresis after RNase treatment of baseplates. This might be due either to overlapping of the fragment with other protein bands, or because several fragments of different molecular weights are produced, none of which is present in quantity sufficient to be detected. Such difficulties might be circumvented in future experiments by using baseplates selectively radiolabelled in gp54. These could be prepared by incubating unlabelled 54$^-$ baseplates with a labelled gp54 donating extract.

The effect of proteolytic enzymes on baseplates is still somewhat uncertain. We found that band III was removed from baseplates which were treated with trypsin, chymotrypsin, or elastase. Kikuchi and King (personal communication) observed that T4 baseplates and tube-baseplates were inactivated by trypsin, papain, and carboxypeptidase A, but that collagenase inactivated only baseplates. However, they "were unable to detect substantial cleavage of any baseplate proteins by SDS gel electrophoresis." The reason for this disagreement is unclear.

It is important to note that gp54 is long enough to be the length-determining template for the 1000 Å tail. The molecular weight of gp54, determined by SDS gel electrophoresis (9), is 36,000 daltons, corresponding to about 327 amino acids of average residue weight 110. The extended length of a polypeptide chain is about 3.6 Å/amino acid residue, so the gp54 polypeptide chain would stretch 1180 Å when fully extended. In this conformation, of course, it would be extremely sensitive to proteolytic attack.

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REFERENCES

1. King, J. (1968). J. Mol. Biol. 32, 231-262.
2. Smith, P. R., Aebi, U., Josephs, R. and Kessel, M. (1976). J. Mol. Biol. 106, 243-275.
3. Casjens, S. and King, J. (1975). Ann. Rev. Biochem. 44, 555-611.
4. Wagenknecht, T. and Bloomfield, V. A. (1977). J. Mol. Biol. 116, 347-359.
5. King, J. (1971). J. Mol. Biol. 58, 693-709.
6. Bishop, J. M. and Koch, G. (1967). J. Biol. Chem. 242, 1736-1743.
7. Edy, V. G., Szekely, M., Loving, T. and Dreyer, C. (1976). Eur. J. Biochem. 61, 563-572.
8. King, J. and Laemmli, U. (1973). J. Mol. Biol. 75, 315-337.
9. Kikuchi, Y. and King, J. (1975). J. Mol. Biol. 99, 673-694.
10. Berget, P. B. and Warner, H. R. (1975). J. Virol. 16, 1669-1671.