Correlation of Genetic Loci and Polypeptides Specified by Bacteriophage T1

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SUMMARY

The effects of amber mutations in the 24 known essential genes of phage T1 on phage-directed protein synthesis were examined by SDS–polyacrylamide gel electrophoresis of radiolabelled polypeptides from infected non-permissive cells. Mutations in nine genes (genes 1, 2, 3, 3-5, 5, 7-8, 10, 13-3 and 15) each resulted in the failure to synthesize a single polypeptide. Since the synthesis of each of these polypeptides was at least partially restored in permissive infections and wholly restored in amr revertant infections we conclude that the affected polypeptide is the primary gene product. Mutations in genes 13-7, 16 and 17, which are required for head formation, are all defective in the synthesis of a 68 000 mol. wt. non-structural polypeptide. Mutants in the head genes 13 and 14 fail to cleave P7p to P7, the major structural component of proheads. Gene 14 mutants also fail to make a 45 000 mol. wt. non-structural polypeptide which appears to be involved with the gene 13 product in the P7p cleavage reaction. The protein products of the DNA genes 1, 2 and 3-5 are, as expected, synthesized predominantly early in infection whereas those of the remainder, which determine head and tail formation, are made in greater amounts late in infection. Gene 3, a tail gene which has been mapped within the ‘early’ DNA gene cluster, codes for a 75 000 mol. wt. polypeptide synthesized predominantly late in infection. This observation suggests that the early genes 1, 2, 3-5 and 4 do not form an operon under the control of a single early promoter.

INTRODUCTION

Genetic analysis of phage T1 has identified 24 phage-coded gene products essential for replication (Michalke, 1967; Figurski & Christensen, 1974; Ritchie & Joicey, 1980). An additional three or four functions are known which are not identified by conditional lethal mutations and may not be essential. These include gene 2-5 which is involved in host shutdown and DNA degradation (Borchert & Drexler, 1980), host range (Drexler & Christensen, 1961), plaque colour mutations (Trautner, 1960), and the function responsible for inactivating exonuclease V coded for by the host recBC genes (J. C. Pugh & D. A. Ritchie, unpublished results). As in many medium-sized, DNA-containing phages, the T1 gene functions are clustered on the genetic map into groups of related functions as shown in Fig. 1 (Figurski & Christensen, 1974; Ritchie & Joicey, 1980; Ramsay, 1981).

Polyacrylamide gel electrophoresis of polypeptides synthesized during phage T1 infection has provided a minimum estimate of 30 to 35 different phage-coded polypeptides (Toni et al., 1976; Martin et al., 1976; Wagner et al., 1977). The estimated total mol. wt. of these polypeptides is 1-3 × 10^6 to 1-6 × 10^6, accounting for 80% to 100% of the coding capacity of the T1 genome and suggesting they represent most if not all T1 gene products. Between 12 and 15 of these proteins are structural components of the phage particle (Toni et al., 1976; Martin et al., 1976; Wagner et al., 1977).

The kinetics of T1 protein synthesis has been interpreted broadly in terms of three classes of polypeptides, early, late and continuous, according to the time of synthesis during infection.

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Table 1. Genetic map of phage T1 showing the relative locations of the known essential genes and the representative amber mutants used in this study. The mutant phenotypes were compiled from the data of Figurski & Christensen (1974), Ritchie & Joicey (1980) and Ramsay (1981). Phenotype symbols are: DO, defective in DNA synthesis; DA, premature arrest of DNA synthesis; rec, defective in recombination; T, defective in tail production; H, defective in head production.

Phage and bacteria. Phage T1 wild-type was obtained from Dr L. A. MacHattie and is the variant described as T1 Ds +÷ and more recently as Type 3 by Ramsay & Ritchie (1982). The T1 amber (am) mutants used in this study are shown with their map locations and functions in Fig. 1. Mutants numbered below 100 were isolated by Michalke (1967) and those numbered above 200 were isolated in this laboratory (Ritchie & Joicey, 1980). Escherichia coli strain B (sup °) was used as the non-permissive host for all growth experiments and KB-3 (sup +) was the permissive strain used to prepare phage stocks and for control infections. 35S-labelled T1 + phage were prepared in E. coli B growing in M9 medium (Anderson, 1946) at 37 °C. Thirty ml of exponential phase cells (3 x 108 cells/ml) were infected at 5 phage/cell and 5 min later 300 \( \mu \)Ci [35S]methionine (100 Ci/mmol; Amersham International) was added and the culture aerated until lysis, which was completed by the addition of chloroform. Purification by polyethylene glycol–dextran sulphate phase partition and centrifugation in CsCl was as described by Martin et al. (1976).

Preparation of labelled intracellular proteins. Bacteria were grown in M9 medium with aeration at 37 °C to 2 x 109 cells/ml. Ten ml volumes were irradiated with about 1000 J/m² of u.v. light to reduce host cell protein synthesis and 1 ml samples were immediately infected with phage (5 to 10 p.f.u./cell) and aerated at 37 °C. Proteins were labelled either early (1.5 to 4.5 min post-infection) or late (12.5 to 16.5 min post-infection) by the addition of 50 \( \mu \)Ci [35S]methionine (100 Ci/mmol; Amersham International) was added and the culture aerated until lysis, which was completed by the addition of chloroform. Purification by polyethylene glycol–dextran sulphate phase partition and centrifugation in CsCl was as described by Martin et al. (1976).

Gel electrophoresis. Cells were harvested by centrifugation, washed twice in 1 ml volumes of 50% (v/v) ethanol and suspended in 0.2 ml of a solution containing 5% (w/v) SDS, 20% (w/v) 2-mercaptoethanol and 10% (v/v) glycerol dissolved in the stacking gel buffer used for electrophoresis and the proteins solubilized and denatured by heating at 100 °C for 2 min.

Polypeptides were separated by electrophoresis in polyacrylamide gel slabs with a 5 to 16% linear gradient of acrylamide and a discontinuous SDS buffer system as described by Martin et al. (1976). Polypeptide bands were identified following autoradiography using Kodirex KD 54T X-ray film. Mol. wt. were estimated by reference to the T1 particle proteins using the values determined by Martin et al. (1976).
Fig. 2. Autoradiograms of electrophoresed polypeptides from non-permissive E. coli B cells infected with am mutants in the T1 early genes. (a) Late-labelled samples, (b) early-labelled samples. Polypeptide profiles for T1 phage particle proteins (T1φ) and for wild-type infected cells (+) are included as standards. The particle polypeptides are labelled P1 to P11 according to Martin et al. (1976). The track labelled U shows the uninfected cell pattern. Each mutant track is labelled with the am mutant number above the gene number. Filled arrows indicate the positions of missing polypeptide bands and open arrows show new bands presumed to be the corresponding truncated amber polypeptide. Migration is from top to bottom.

RESULTS

The basic experimental design was to infect non-permissive cells with am mutant phage, label the phage-specific polypeptides synthesized during a short pulse and then separate the polypeptide chains by SDS-polyacrylamide gel electrophoresis. Mutant and wild-type gel patterns were compared to identify band differences which might indicate which polypeptide was defective for a particular mutant. Because of the chain-terminating nature of am mutations the mutant polypeptide will be shorter than the corresponding gene product from wild-type infections or from mutant infections of permissive cells. This should lead to the absence of the polypeptide from its wild-type position in the gel and possibly to the appearance of a new band of lower mol. wt.

Genes 1 to 4

These genes mostly control T1 DNA replication. Genes 1 and 2 are required to initiate DNA synthesis and genes 3-5 and 4 are responsible for the maintenance of DNA synthesis during the later stages of infection, for concatemer formation and genetic recombination (Figurski & Christensen, 1974; Ritchie et al., 1980; Walling & Christensen, 1981). Gene 3 has been assigned a role in tail formation but is included in this group because of its map position: possibly this gene product has a more complex function.

The polypeptide patterns for these mutants are shown in Fig. 2. The band missing from am20 (gene 1) infections is a polypeptide of approx. 18000 mol. wt. previously identified by Martin et al. (1976) as an early gene product. Infections with the gene 2 mutants am5 and am221 both failed to synthesize the 65000 mol. wt. early polypeptide; however, only for am221 has a
truncated gene product (mol. wt. 48000) been identified. Gene 3 (am41) mutant infections were unable to make a prominent late band which runs a little slower than the gene 2 product. The mutant polypeptide is only marginally smaller than the 75000 mol. wt. wild-type gene 3 product. Another gene 3 mutant, am235, was missing the same band. Gene 3-5 (am201) infections did not synthesize a low mol. wt. (approx. 20000) band whereas gene 4 (am23) infections showed an apparently wild-type profile (data not shown). This difference substantiates the complementation data which indicate that genes 3-5 and 4 specify different polypeptides in spite of having indistinguishable phenotypes (Ritchie et al., 1980). The gel patterns in the summary diagram (Fig. 6) show clearly that genes 1, 2 and 3-5 specify polypeptides synthesized predominantly early in infection whereas the gene 3 product is made predominantly late.

**Genes 5 to 11-5**

These genes specify functions required for tail formation (Figurski & Christensen, 1974; N. Ramsay & D. A. Ritchie, unpublished results). Of the representative amber mutants examined, no differences from the wild-type polypeptide pattern were detected in extracts of non-permissive cells infected with aml8 (gene 6), am35 (gene 7), am32 (gene 8), am13 (gene 9), am29 (gene 11) and am304 (gene 11-5). The gel profiles for the remaining mutants, aml5 (gene 5), am208 (gene 7-8) and am19 (gene 10) are given in Fig. 3. The am15 profile reveals a complex pattern involving changes to several bands. As will be shown below (Fig. 5), these differences, with the exception of that affecting the particle protein P1 (mol. wt. 152000), were also found in the lysates from permissive infections and an amr+ revertant and must result from additional genetic differences not associated with the nonsense mutation in gene 5.

Am19 (gene 10) lysates are missing the minor structural protein P2 (mol. wt. 117000). This correlation was confirmed by showing that am204, another gene 10 mutation, also failed to synthesize P2. The major tail protein P10 (mol. wt. 26000) was absent from extracts of am208 (gene 7-8)-infected cells.
**Fig. 4.** Autoradiograms of electrophorosed polypeptides from non-permissive *E. coli* B cells infected with *am* mutants in the T1 head genes. All samples were labelled late in infection. Symbols are described in the legend to Fig. 2.
Fig. 5. Autoradiograms of electrophoresed polypeptides from permissive E. coli KB-3 cells infected with the em mutants illustrated in Fig. 2, 3 and 4. All samples were labelled late in infection. The particle polypeptides P1, P4/5, P10 and P11 are indicated on each gel track as reference points. All other symbols are described in the legend to Fig. 2.
Phage T1 protein synthesis

Genes 12 to 18

This third group of genes codes for the structural proteins of the T1 head and those which catalyse head assembly and DNA packaging (Figurski & Christensen, 1974; N. Ramsay & D. A. Ritchie, unpublished results). The polypeptide profiles for non-permissive infections for this group of mutants are given in Fig. 4, with the exception of am37 (gene 12), am246 (gene 14-5) and am30 (gene 18) whose patterns were indistinguishable from wild-type.

The am10 mutant (gene 13) failed to synthesize the particle protein P7 (mol. wt. 33000). This is a major head component accounting for about 50% of the mass of the head and is present at approximately 250 to 500 molecules per particle (Martin et al., 1976; Wagner et al., 1977). A second gene 13 mutant, am260, was also unable to synthesize P7 (data not shown); however, both am10 and am260 synthesized P7p, the higher mol. wt. precursor of P7. P7 was also missing from non-permissive cells infected with am45 (gene 14) and similarly the P7p precursor was made normally. However, am45 was also defective in the synthesis of a non-structural protein of mol. wt. of approximately 45000. Both bands missing from non-permissive am45 infections were partially restored in permissive infections (Fig. 5). The gene 13-3 mutant am283 was unable to make the second major head polypeptide, P11, a 16000 mol. wt. molecule present at an estimated 200 to 400 molecules per particle (Martin et al., 1976; Wagner et al., 1977). The particle protein P4 was identified as the product of gene 15 (aml1).

Non-permissive infections by am216 (gene 13.7), am4 (gene 16) and am7 (gene 17) were all defective in the synthesis of a polypeptide of about 68000 mol. wt. and in the case of am216 this band was replaced by a truncated polypeptide of 42000 mol. wt.

Polypeptides synthesized during permissive infections

As an essential control to our identification of genes and polypeptides, the polypeptide profile of each mutant was determined following infection of permissive cells (Fig. 5). It would be expected that suppression of the amber mutation would restore, at least partially, the gene product that had been identified as missing. This was indeed observed with all the mutants tested. In fact, in many cases the suppression was only partial and showed not only the synthesis of the wild-type gene product but also considerable amounts of the truncated amber polypeptide.

It is worth noting that in permissive infections with am45 both the affected proteins showed some restoration of synthesis, and also that am216 (gene 13.7), am4 (gene 16) and am7 (gene 17) which all appear to affect the same band showed restoration of synthesis of the same protein.

The am+ revertants of many mutants were also analysed and with the exception of am15, noted above, showed patterns identical to our standard wild-type following infection of either B (supo) or KB-3 (sup+) bacteria (data not shown).

DISCUSSION

This study has concentrated on correlating the 24 known T1 essential genes with the polypeptides they specify. Our data considerably extend the analysis of Wagner et al. (1977), which was based on 10 genes. Furthermore, our gene–polypeptide assignments have been clarified considerably by including in our analysis the polypeptide patterns of am mutants from infections of permissive cells and of am+ revertants. These additional controls have been particularly helpful in identifying the effects of the strain differences known to exist among the T1 parental stocks used to isolate am mutants (Ramsay, 1981; Ramsay & Ritchie, 1982; P. Ooi & J. R. Christensen, personal communication).

A summary of our results is presented in Fig. 6. A detailed comparison of our results with those of Wagner et al. (1977) and K. Hercules (personal communication) is difficult because of the use of different purification schemes and of gel systems with different degrees of resolution. However, the three sets of data show a large measure of agreement. To date, polypeptide products have been associated with 18 of the 24 known essential genes. Those for which nothing is yet known are the tail genes 6, 7, 8 and 11.5 and the head genes 12 and 18. Of the 18 positive assignments there is general agreement for the DNA genes 2 and 3-5, the tail gene 10, and the head genes 13, 13-3, 15 and 16. For the remaining genes there is either only partial agreement among the three studies or none at all.
Our analysis agrees with that of Wagner et al. (1977) in identifying gene 5 as coding for the particle protein P1 described by Martin et al. (1976). The former authors also report the appearance of a truncated mutant polypeptide of 125000 mol. wt. However, this is almost certainly the identification of a new polypeptide associated with the strain difference between the standard T1+ and the parent T1+ from which am15 was isolated. The 125000 mol. wt. polypeptide is present in our am+ revertants of am15 and we have shown that gene 5 lies in a region of strain variability (Ramsay & Ritchie, 1982).

The results for the gene 3 mutants am6 (Wagner et al., 1977) and am41 (this paper and K. Hercules, personal communication) deserve comment. All three studies report the absence of a band of about 75000 mol. wt. from non-permissive infections and the studies by Wagner and Hercules also indicate the absence of a band equivalent to the 57000 mol. wt. tail structural protein P4 (Martin et al., 1976; N. Ramsay & D. A. Ritchie, unpublished results). Our observation that synthesis of the 75000 mol. wt. polypeptide is partially restored in am41 infections of permissive cells would suggest that this is the primary product of gene 3. The absence of P4 may reflect a strain difference [P4 is missing from type 1 T1 strains (Ramsay, 1981; Ramsay & Ritchie, 1982)] or it may derive from the post-translational cleavage of the 75000 mol. wt. polypeptide. The location of gene 3 within the early cluster is somewhat anomalous since its gene product is a component of the phage tail. Moreover, it is synthesized predominantly late in infection whereas the polypeptides specified by the surrounding genes 1, 2 and 3-5 are expressed predominantly early (Fig. 2 and 6). This casts doubt on the possibility that the early gene cluster is coordinately expressed as a unit from an early promoter region. Alternatively, the map location of gene 3 may not be correct in spite of this being the agreed position from three independent studies (Michalke, 1967; Figurski & Christensen, 1974; Ramsay & Ritchie, 1982). Careful re-mapping of this region will be necessary to resolve this uncertainty.
Phage T1 protein synthesis

Mutants in two genes (genes 13 and 14) fail to make P7, the major constituent of T1 proheads (N. Ramsay & D. A. Ritchie, unpublished results). P7 is derived from the 40000 mol. wt. precursor P7p by loss of a 7000 mol. wt. fragment (Toni et al., 1976; Martin et al., 1976). Gene 13 and 14 mutant infections synthesize normal amounts of P7p, indicating that neither codes for this major head protein. The gene 14 mutant, in addition, fails to synthesize a polypeptide of 45000 mol. wt. One explanation for these observations is that the 45000 mol. wt. protein is the gene 14 product which together with the gene 13 protein is required for the formation of proheads, a process which would require the cleavage of P7p to P7.

The other unresolved complex result concerns the polypeptide of approximately 70000 mol. wt. which is absent from extracts of non-permissive cells infected with am216 (gene 13-7), am4 (gene 16) and am7 (gene 17). K. Hercules (personal communication) also reported the absence of this band from am4 and am7 infections. The only known common feature of these genes is that they all code for head functions.

Our recent studies of T1 particle assembly (N. Ramsay & D. A. Ritchie, unpublished results) based on the identification of particle polypeptides present in purified subcomponents of T1 phage particles lead to the conclusion that P4 and P10 are respectively minor and major tail components, P5 and P7 are respectively minor and major components of the prohead structure, and P5, P7 and P11 are present in both full and empty heads, with P11 forming the major outer shell protein. P2 and P12 are tentatively classed as tail components and P1, P3, P8 and P9 as head components. With the exception of P1 this classification agrees fully with the present results of gene–polypeptide assignments based on the polypeptide profiles from am mutant infections of non-permissive cells.

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