Bacteriophage P22 Tail Protein Gene Expression

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We have found that mutations which block bacteriophage P22 head assembly at or before the DNA packaging stage (1^- , 2^- , 3^- , 5^- , and 8^-) cause up to a 20-fold increase in the amount of tail (gene 9) protein made during infection. This correlation seems strong enough to warrant consideration of a control mechanism in which the failure to package DNA per se causes a large increase in the synthesis of tail protein. Our results indicate that one of the repressors required for maintenance of lysogeny, the *mnt* gene product, may be partially responsible for this phenomenon.

It is thought that there is a single promoter for all 15 late genes in the bacteriophage P22 late operon and that these genes are transcribed under the positive control of gene 23 protein from a single large operon as a single polycistronic mRNA (5, 23, 27). The structure and expression of the region of the chromosome near the tail protein gene is quite complex (Fig. 1). Located between the penultimate and last morphogenetic genes in the operon, 16 and 9, respectively, are several genes which are not involved in the normal late functions of phage assembly and cell lysis. These are the *sieA*, *mnt*, *arc*, and *ant* genes, which are required for superinfection exclusion and control of lysogeny. This region has been called the immunity I (*imm* I) region (reviewed in reference 23). The *sieA* and *mnt* genes appear to be expressed in lysogens, since only *sieA*⁺ lysogens exclude certain superinfecting phages (7, 25), and the *mnt* gene codes a repressor which prevents the expression of the *ant* gene during lysogeny (19). The *ant* gene product, antirepressor, has the capacity to inactivate the *c2* gene-coded prophage repressor, and thus it may function in prophage induction (8, 22, 23). The *arc* gene product negatively regulates the level of antirepressor during lytic infection (21). So in this region of the chromosome, which lies inside the late operon, are two genes which are expressed in the lysogenic state and two which are expressed early during lytic growth.

Regulation of transcription in this region must, therefore, be complex and is not fully understood. There are at least two promoters, one for *mnt* expression and one for *ant* and *arc* expression (19). The *ant* promoter (*P_{ant}*) causes initiation of transcripts made in the same direction as late operon mRNA. Transcription which starts in this region apparently does not result in expression of gene 9, even though it does proceed in the correct direction, since 23^- infections produce antirepressor but not tail protein (1, 23). Conversely, although there is at least one transcription termination signal in the *imm* I region, *T_{ant}* (1), expression of the tail protein gene requires transcription initiation far upstream at the late operon promoter (*P_{late}*), since transcriptionally polar insertions in gene 20 exert their effect fully on the tail protein gene expression (27). Susskind and Botstein (23) and Berget et al. (1) proposed that transcription initiates at *P_{late}* and is anti-terminated by the gene 23 protein after synthesis of a "leader transcript" (17). Subsequent downstream transcription is then immune to termination at *T_{ant}*, but transcription

initiated in the *imm* I region (without 23 protein) must stop at *T_{ant}*.

In this report, we describe an additional feature of the regulation of the expression of genes in this portion of the P22 chromosome. We show that the tail protein is made in much larger amounts in infections by DNA packaging-defective mutants than in wild-type infections and present a model which explains this aspect of the regulation of tail gene expression.

MATERIALS AND METHODS

Phage and bacterial strains and growth. The bacterial strains used were the isogenic *sup*⁺ and *sup*⁰ strains DB7004 and DB7000, respectively, from the collection of D. Botstein (28). All phage strains carried the *c1-7* or *c2⁻am08* and *13⁻amH101* alleles to block lysogeny and cell lysis, respectively. Other alleles used were *mnt1* (23, 29), *mnt⁻am343* from M. Susskind, *23⁻amH316* (3), *c2-27* and *Vy* (4), *5⁻ts5.1*, *3⁻ts3.1*, *2⁻ts2.1*, *8⁻tsN102* (10), *7⁻amH1375*, and *4⁻amH1368* (16). All other *am⁻* alleles used were those described by Botstein et al. (3). Growth medium and conditions have been previously described (11).

Analysis of infected cell extracts. Preparation of labeled and unlabeled cell extracts, sodium dodecyl sulfate (SDS)-discontinuous slab gel electrophoresis, and quantitation of protein in these gels from autoradiography or Coomassie brilliant blue staining have been described previously (11). Specific strains, times, etc., are given in the text and figure legends.

RESULTS

We observed that amber mutations in any of several late genes cause the tail protein (gene 9 product) to be substantially overproduced when compared with wild-type infections. Figure 2 shows an SDS-polyacrylamide gel analysis of whole lysates of cells infected with several different late mutants. Mutations which cause phage assembly to fail at or before the DNA packaging step cause the synthesis of high levels of tail protein. A systematic study of mutants defective in all of the various late genes shows that only 1^- , 2^- , 3^- , 5^- , and 8^- phage infections synthesize high levels of tail protein. This set of mutations corresponds exactly with those which fail to package DNA (3). Figure 3 shows quantitations of the amounts of tail protein synthesized in the various amber mutant infections. 4^- and 7^- levels are not shown; however, they both accumulate essentially wild-type amounts of tail protein. Single amber mutants which are unable to package DNA accumulated ca. 10 times the

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FIG. 1. The *Imm 1*-gene 9 region of the phage P22 chromosome. The known genes and transcripts are represented by the rectangles and lower arrows, respectively. The dashed areas indicate genes or transcription termination sites whose location is not accurately known. The size of the *sieA* protein is not known. The known transcription signals in this region are shown above the scale (1-kilobase-pair units). The figure is derived from Rutla and Jackson (18), Sauer et al. (19, 20), and Berget et al. (1).

wild-type amounts of tail protein. In numerous other experiments, depending upon the exact growth conditions and time after infection, these mutants always synthesized between 4 and 20 times the wild-type amount of tail protein. Several double mutants ($5^{-}8^{-}$, $1^{-}8^{-}$, $3^{-}8^{-}$, $3^{-}2^{-}$, and $5^{-}2^{-}$), in parallel experiments, all accumulated amounts of tail protein similar to those of the individual single mutants (data shown only for $5^{-}8^{-}$). Since the effects of two mutations are not additive, it is likely that a single mechanism is operating. In addition, the mutations which cause the high level of synthesis are epistatic to those not causing a high level of synthesis (i.e., $8^{-}16^{-}$ and $1^{-}16^{-}20^{-}$ were found to synthesize high levels of tail protein [data not shown]). Also shown in Fig. 3 are the results of infection by a $5^{-}9^{-}$ double amber mutant phage. The fact that it synthesized no material in the tail protein region of the SDS-polyacrylamide gel shows that a second hypothetical overproduced protein is not fortuitously comigrating with tail protein when DNA packaging is blocked.

Previous experiments with wild-type P22-infected cells pulse- and chase-labeled at 36 min after infection (6) and our own similar experiments labeled at later times after infection (data not shown) showed no metabolic instability in the tail protein itself. Therefore, we conclude that the mutants which accumulate high levels of tail protein in fact do synthesize it at a higher rate than strains which produce low levels.

FIG. 2. SDS-polyacrylamide slab gel electrophoresis of whole extracts of cells infected with various mutant P22 phages. Nonpermissive cells (DB7000) were grown to 2×10^8 cells per ml at 37°C in LB broth, infected at a multiplicity of 7, and shaken at 37°C for 90 min. Infected cells were harvested, samples were prepared, and gels were run and stained with Coomassie brilliant blue as described previously (11). In addition to the mutations of primary interest, which are indicated above the individual lanes, each of the infecting phage carried the *c1-7* and *13⁻amH101* alleles.

Figure 4 shows quantitation of tail protein made after infection by phage carrying a temperature sensitivity mutation in gene 2 or gene 5. Control experiments showed that tail protein synthesis in wild-type and 2^{-} *am* infections is not greatly affected by changes in temperature. However, 2^{-} *ts* and 5^{-} *ts* infections synthesize tail protein at essentially 2^{-} *am* and 5^{-} *am* levels, respectively, at the nonpermissive temperature (42°C) and at much reduced levels at permissive temperature (29°C). 3^{-} *ts* and 8^{-} *ts* mutants behaved like the 2^{-} *ts* mutants (data not shown). It is likely that in these mutant infections DNA packaging is not completely normal at 29°C , resulting in the synthesis of somewhat more than wild-type amounts at the permissive temperature. In addition to confirming the results with amber mutations, this experiment makes extremely unlikely the possibility that all these *ts* mutants fortuitously carry a secondary mutation which causes the synthesis of high amounts of tail protein (since the second mutation would also have to be *ts*).

Figure 5 shows that the excess tail protein made after infection by a 5^{-} *am* phage is biologically active. Israel et al. (9) found that P22 gene 9 protein could bind to noninfective, tailless heads in vitro to form infective phage particles. Since

FIG. 3. P22 tail protein accumulation in cells infected with various mutant phage. Samples were prepared and SDS-polyacrylamide gels were run as described in the legend to Fig. 2. Stained band intensities were quantitated as described previously (11). The ordinate values are expressed in arbitrary units per infected cell. All strains carried the *c1-7* and *13⁻amH101* alleles, except *mnt⁻5⁻8⁻*, which carried the *c2⁻am08* allele instead of *c1-7*. Control experiments have shown that *c2⁻* and *c1⁻* strains, which are otherwise isogenic, produce similar amounts of tail protein during lytic growth.

more than one tail part per particle is required for infectivity and since they do not appear to add cooperatively (9), it is not possible to use the *in vitro* assembly reaction as a linear assay for tail protein. Thus, its biological activity was measured in the experiment shown in Fig. 5 by a dilution assay in which the relative amounts of tail protein in different samples were determined by the amount of dilution required to reach identical extents of reaction. In the experiment presented, the 5^- lysate contained about five times as much tailing activity as the 10^- lysate. A 10^- lysate was chosen for comparison because DNA is packaged, but at least 80% of the tail protein remains unassembled (12; unpublished data). SDS-polyacrylamide gels of the same two samples showed that five to six times as much tail protein was physically present in the 5^- lysate as was present in the 10^- lysate (data not shown). We conclude that the specific activity of the tail protein was approximately equal in the two lysates and therefore that the excess tail protein made under 5^- conditions is biologically active.

Figure 6 shows that the rate of tail protein synthesis, when tail protein is being made at the high level, increases relative to coat protein with increasing time after infection. At 20 min after infection, tail protein in ca. 10% of the amount of coat protein has accumulated, whereas at 120 min tail protein in ca. 60% of the amount of coat protein has accumulated. It is difficult to measure the wild-type or 10^- amounts of tail protein in gels of whole extracts with precision, since the amounts present are not far above the amounts of host bands in regions of the gel. Thus, from this data, it is not possible to tell if the rate of tail protein synthesis is constant relative

FIG. 4. Tail protein accumulation in cells infected with phages which are temperature sensitive in DNA packaging. Bacteria (DB7000) growing in LB broth at the temperature indicated were infected, samples were prepared after 90 min, gels were run, and the tail and coat proteins were quantitated as described previously (legend to Fig. 2 and reference 11). The amount of tail protein measured was, in this case, normalized to the amount of coat protein present; however, since these strains produce similar amounts of coat protein, similar results are obtained if the absolute amount of tail protein was used for comparison. Each of the strains carried the $c1-7$ and $13^-amH101$ alleles in addition to those indicated below the bar graph.

to coat protein synthesis under DNA packaging-competent conditions.

Since gene 23 product is required for initiation at P_{late} , and because of the complex transcription in the *imm 1*-gene 9 region, we tested the possibility that the high levels of tail protein made in our experiments might be made independently of gene 23 protein (2, 14). Nonpermissive cells were infected with $c1^-5^-23^-13^-$ or $c1^-23^-13^-$ grown for 120 min at 37°C in LB broth, and the whole cell lysates were analyzed in SDS-polyacrylamide gels. No tail protein was made in either case (data not shown), so gene 23 product, or some protein under its control, is required for the synthesis of tail protein, even under the conditions which cause the high level of synthesis.

FIG. 5. Biological activity of the tail protein made under conditions of high level synthesis. Various amounts of tail donor lysates were mixed with a diluted tail-defective (9^-) lysate which contained 2×10^9 heads per ml and were incubated at 37°C for 30 min (control experiments showed that these conditions allowed the reaction to proceed to completion). The reaction mixtures were plated on permissive bacteria (DB7004) to measure the infective phage formed. The open circles represent data with a 10^- (low level) tail donor lysate, and the closed circles represent a 5^- (high level) tail donor lysate.

FIG. 6. Time course of tail protein accumulation. Nonpermissive cells were infected and harvested at the times indicated, proteins were separated by SDS-polyacrylamide gel electrophoresis, and bands were stained and quantitated as previously described (legend to Fig. 2 and reference 11). Open circles, closed circles, and closed triangles represent coat protein accumulated in 3^- , 10^- , and wild-type phage-infected cells, respectively. Closed squares, open squares, and open triangles represent tail protein accumulated in 3^- , 10^- , and wild-type phage-infected cells, respectively. Each of the phage strains also carried the $c1-7$ and $13^-amH101$ alleles.

An attractive model for this regulation is that RNA polymerase frequently terminates between genes 16 and 9 during wild-type infections, but does so less often when DNA is not being packaged. Since the operator for the *mnt* repressor lies between genes 16 and 9, the additional DNA, which is available when packaging is blocked, might titrate the *mnt* repressor, thereby allowing late transcription to proceed through this region more frequently. We therefore tested *mnt*⁻ mutants for their ability to produce tail protein. Figure 3 shows the results of quantitation of tail protein accumulated in cells infected with *mnt*⁻ and $5-8^-$ *mnt*⁻ compared with *mnt*⁺ and $5-8^-$ *mnt*⁺, respectively. The *mnt*⁻ mutation does affect the synthesis of tail protein. When DNA packaging is normal, the *mnt*⁻ mutation causes a reproducible 2.5- to 3-fold increase in the amount of tail protein made. When DNA packaging is blocked, the *mnt*⁻ mutation causes a 30% increase in tail protein synthesis. The data shown are for the mutation *mnt*⁻1. Similar experiments using an amber mutant (*am*⁻343) in the *mnt* gene gave identical results (data not shown), implying that the intermediate level of tail protein synthesis seen under *mnt*⁻ DNA packaging-competent conditions is not due to an intermediate level of functional *mnt* protein in the infected cells. In addition, infections by mutants defective in the operator for the *mnt* repressor (*Vy*; see references 2 and 13) accumulated two to four times more tail protein than did wild-type infections (data not shown), implying that the binding of the *mnt* repressor to its operator is important in attaining the low wild-type level of tail protein synthesis.

DISCUSSION

Our results show a perfect correlation between those late gene mutations which cause the P22 tail protein to be synthesized at high levels and those which block phage assembly at or before the DNA packaging step. We feel that this correlation warrants consideration of a mechanism for tail protein synthesis in which the DNA packaging process per se affects the amount of tail protein made during infection. Our measurements of the functional distance of gene 9

from its promoter (5) and the genetic work of Weinstock et al. (27) indicate that transcription of gene 9 must begin at P_{late} and that there is no promoter for gene 9 expression in the *imm* I region. Since the inability to package DNA does not affect the levels of other late proteins, the modulation of the level of tail protein synthesis must be exerted after the initiation of mRNA synthesis at P_{late} by RNA polymerase. In a separate report, we have shown that the functional half-life of the gene 9 mRNA is unaffected by mutations which block DNA packaging (5). These experiments show that the coupling between the rate of gene 9 expression and DNA packaging is not mediated by changes in mRNA decay rates. The effect is therefore most likely exerted at the level of termination of transcription or rate of translation.

The most attractive possibility is the following. In a DNA packaging-competent infection, most RNA polymerases transcribing the late operon terminate between genes 16 and 9; whereas when DNA is not being packaged, RNA polymerases terminate in this region less often. The most obvious consequence of the failure to package DNA which could be used as a regulatory signal is an increased amount of metabolically available phage DNA in the cell. Thus, if fairly small amounts of the *mnt* repressor were normally present after infection, such increased levels of unpackaged DNA might effectively titrate the repressor, leaving copies of the chromosome without repressor. If bound *mnt* repressor, or some consequence of its binding (such as possible increased transcription of the *mnt-sieA* region; see reference 19), could stop a polymerase traversing the late region, increased synthesis of tail protein could result when DNA is not packaged. Convergent transcription is known to be able to cause premature termination by RNA polymerase (26). This type of regulation of gene expression by a general masking of the DNA (causing a failure to titrate the *mnt* repressor) of a gene has been suggested for DNA in higher organisms (15), but it would be unique in procaryotes. Phage L, a close relative of P22 which does not have a complete *imm* I region (24; M. Hayden and S. Casjens, unpublished data) but does have a late operon nearly identical to that of P22 in other respects, does not overproduce its tail protein when DNA packaging is blocked (M. Adams and S. Casjens, unpublished data). This suggests that this regulation is unlikely to be required for P22 growth.

This model does not simply explain that fact that *mnt*⁻ infections produce substantially less tail protein than DNA packaging-defective infections. Transcription is quite complex in this region of the P22 genome, and the *arc* gene product is also a repressor which binds an operator in the *imm* I region (21). Thus, it could be partly responsible for not allowing all late operon RNA polymerases to transcribe through this region. Because they have other effects on late protein synthesis (21), we did not analyze *arc* mutants for their ability to make tail protein.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM21975 from the National Institutes of Health and National Science Foundation grant PCM8017177.

We thank Peter Berget, David Botstein, Bill Earnshaw, Vance Israel, Ethel Jackson, Miriam Susskind, Myron Levine, George Weinstock, Ken Lew, and Jonathan King for strains and helpful discussions.

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