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Assembly-Controlled Autogenous Modulation of Bacteriophage P22 Scaffolding Protein Gene Expression

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In the assembly of bacteriophage P22, precursor particles containing two major proteins, the gene 5 coat protein and the gene 8 scaffolding protein, package the DNA molecule. During the encapsidation reaction all of the scaffolding protein molecules are released intact and subsequently participate in further rounds of DNA encapsidation. We have previously shown that even though it lies in the center of the late region of the genetic map, the scaffolding protein gene is not always expressed coordinately with the remainder of the late proteins and that some feature of the phage assembly process affects its expression. We present here in vivo experiments which show that there is an inverse correlation between the amount of unassembled scaffolding protein and the rate of scaffolding protein synthesis and that long amber fragments of the scaffolding protein can turn down the synthesis of intact scaffolding protein in *trans*. These results support a model for scaffolding protein regulation in which the feature of the assembly process which modulates the rate of scaffolding protein synthesis is the amount of unassembled scaffolding protein itself.

The notion that proteins involved in the assembly of macromolecular structures can turn off their own synthesis when not assembled is attractive, since excess unassembled protein subunits can simply eliminate the production of additional subunits when the structure (or a sufficient number of structures) is completed, and production remains off until assembly begins again. In this report, we present evidence that a bacteriophage P22 assembly protein, the scaffolding protein, can depress its own synthesis in vivo when unassembled.

The genetics and molecular biology of bacteriophage P22 have been intensively studied (reviewed in reference 27). The switch to late protein synthesis is controlled by the gene 23 product. This regulation is thought to occur by an RNA polymerase antitermination mechanism in which the gene 23 protein causes readthrough at a termination site(s) a short distance downstream from the late promoter, P_{late} (2, 18, 24, 25, 27). The existence of a single late promoter in P22 has not been proven directly, but studies with polar insertion elements (28) and measurements of the temporal length of the lag before exponential decay of individual gene mRNAs after the addition of rifampin (4) strongly support this idea. There are 15 known genes in the late operon; 2 are required for lysis of the infected cell, and 13 are required for DNA packaging and assembly of progeny phage particles.

The pathway by which the protein products of the 13 morphogenetic late genes assemble into infectious phage has also been extensively investigated (reviewed in reference 10). As is the case with many other double-stranded DNA bacteriophages, a protein particle called a prohead is assembled first, which is subsequently filled with DNA (15, 22). The prohead contains ca. 420 molecules of coat protein (gp5 [gp*X* refers to the protein product of gene *X*]) on the exterior, 200 to 300 molecules of scaffolding protein (gp8) in the interior, and 10 to 30 molecules each of gp1, gp7, gp16, and gp20 (3, 7, 15, 23). The prohead encapsidates DNA in a complex reaction with the following features: one phage length of DNA (43,500 base pairs) is cut from the replicating concatameric DNA (3), the products of genes 2 and 3 must act but are not present in the final phage particle (3, 5, 21), and all of the molecules of scaffolding protein (gp8) exit intact from the structure. These scaffolding protein molecules can then recycle by reacting with newly synthesized coat protein, gp1, gp7, gp16, and gp20 to form new proheads (13). Thus, the scaffolding protein in effect catalyzes the assembly of proheads and the encapsidation of DNA, even though 200 to 300 molecules are required for a single catalytic event.

Although it lies in a nearly central position in the late operon and is not synthesized in the absence of the gene 23 product, the scaffolding protein is not always synthesized coordinately with the other late proteins (14). Mutants which accumulate proheads (lacking gene 1, 2, or 3 function) synthesize scaffolding protein at a higher rate relative to the other late proteins than wild-type phage or phage defective in any of the other late functions, and gene dosage experiments have shown that this regulation results in the maintenance of coat and scaffolding protein subunits in the proper ratio for prohead assembly. This regulatory circuit couples scaffolding protein synthesis to the process of phage morphogenesis.

In this report we present in vivo experiments which support the view that the feature of the assembly process which is important in this modulation is the state of assembly of the scaffolding protein itself, and that unassembled it negatively regulates the expression of its own gene.

MATERIALS AND METHODS

Phage and bacterial strains. The bacterial strains used were the su^+ strain DB7004 and su^- strains DB21 and DB7000 from the collection of D. Botstein (22, 29). All phage strains carried the 13^-amH101 mutation to prevent lysis of infected cells and the *c*1-7 mutation to ensure that only lytic infections occurred. The other nonsense alleles used were 3^-amN6, 5^-amN114, 8^-amH202, and 8^-amN123 (3). The 8^-tsN102

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and *cs*RN26D mutations were described by Jarvik and Botstein (12).

Analysis of infected cell extracts. The detailed procedures used in the preparation of labeled and unlabeled cell extracts, sodium dodecyl sulfate (SDS)-discontinuous slab gel electrophoresis, and quantitation of proteins in these gels have been described previously (14). Specific strains, times, etc., are given in the text.

Measurement of amounts of assembled and unassembled scaffolding protein. Nonpermissive cells (DB7000) were infected at 2×10^8 cell per ml and grown in LB broth (6) at 30°C for the times indicated. The cells were chilled and concentrated 40-fold in LB broth by low-speed centrifugation, lysed by shaking with chloroform, and DNase I (Worthington Diagnostics) treated to reduce viscosity, and the cell debris was removed by centrifugation at 10,000 rpm for 5 min in a Sorvall SS-34 rotor. The supernatant was layered on a sucrose-cesium chloride step gradient as previously described (5), containing steps of 20% sucrose, a CsCl density of 1.4 g/cm^3, and a CsCl density of 1.6 g/cm^3, and centrifuged at 45,000 rpm for 45 min in a Spinco SW50.1 rotor. Unassembled proteins remain at the top of the gradient, proheads and empty heads collect in a sharp band on top of the CsCl step with a density of 1.4 g/cm^3, and phage collect on the more dense CsCl step. These three fractions were collected and dialyzed against SDS sample buffer (20). The three fractions were then diluted to equal total volumes with SDS sample buffer, and equal volumes were analyzed by SDS-polyacrylamide gel electrophoresis. Protein bands in the gels were quantitated by Coomassie brilliant blue staining as previously described (14). Samples taken from positions between the three fractions described above contained very little scaffolding protein.

RESULTS

Our previous results have shown that there is a correlation between those amber mutants which accumulate proheads and do not recycle scaffolding protein and those which synthesize high amounts of scaffolding protein (14). The most obvious possibility for the mechanism of the regulation of the synthesis of scaffolding protein is that unassembled (recycling) scaffolding protein turns down the rate of synthesis of additional scaffolding protein. We present here experiments designed to test this hypothesis in vivo.

Levels of unassembled scaffolding protein. The above model predicts that the amount of free, unassembled scaffolding protein present in the infected cell should be inversely related to the rate of synthesis of scaffolding protein. Wild-type P22- and 2$^-$-, 3$^-$-, and 5$^-$-infected cells were analyzed for amounts of assembled and unassembled scaffolding protein as described above. The results of such an experiment at 80 min after infection are shown in Table 1. There is an inverse relationship between the total amount of scaffolding protein made and the amount of scaffolding protein unassembled in cells infected by various mutant phage. We have previously shown that scaffolding protein is stable in these extracts, so the amount accumulated reflects the overall rate of synthesis (5, 14).

It is also informative to consider the synthesis of scaffolding protein with time in a wild-type infection. We have previously shown that in such an infection, scaffolding protein synthesis begins at a relatively high rate coordinately with the other late proteins, but its rate of synthesis slows down significantly before those of the other late proteins (14). Figure 1 shows the levels of assembled and unassembled scaffolding protein at various times after infection. The unassembled scaffolding protein concentration increases with time during infection. Since the only sedimentable form of scaffolding protein is the prohead structure (5), sedimentable scaffolding protein was used as a measure of the relative number of proheads in the infected cell. Thus, the rate of synthesis of scaffolding protein is constant or

TABLE 1. Amounts of unassembled gp8 in mutant-infected cells[a]

Mutant	gp8 unassembled	gp8 in proheads	Total gp8[b]
Wild type	1.5	3.8	6.0
5$^-$	3.9	<0.1	4.2
2$^-$	<0.5	20.5	23.2
3$^-$	<0.4	25.2	27.3

[a] The amounts of gp8 were measured at 80 min after infection as described in the text and are given in arbitrary units per infected cell.

[b] The total gp8 was determined by gel electrophoresis of the whole infecteed cell extract (14) in parallel with the analysis of the amounts of proheads and unassembled. In general, ca. 10% of the gp8 was lost in the cell debris and unlysed cells.

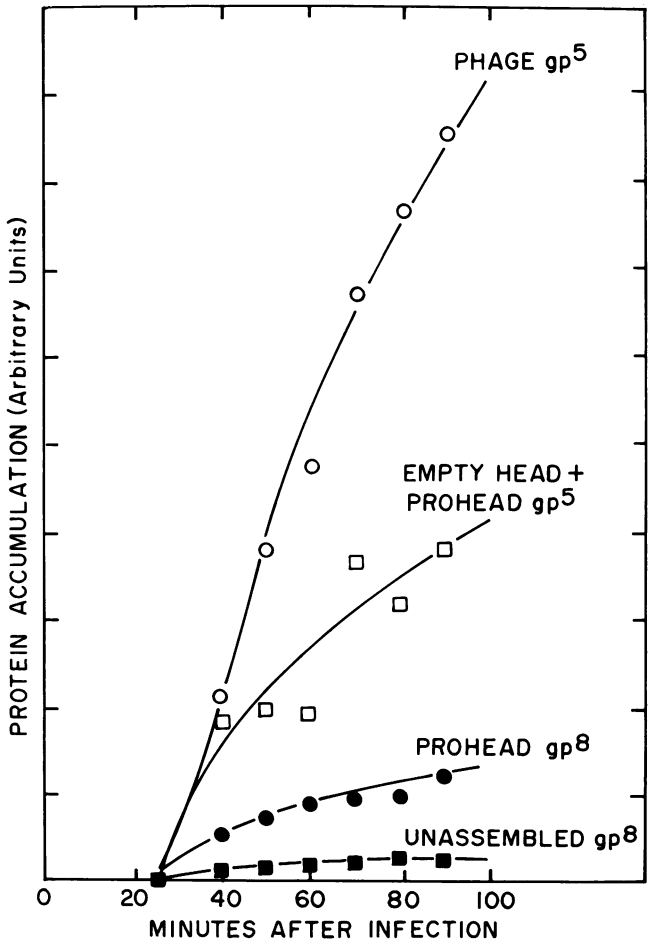

FIG. 1. Levels of assembled and unassembled scaffolding protein after infection. Cells were infected with P22 *c*1-7 13$^-$*am*H101, and at various times after infection, portions were removed and the amounts of assembled and unassembled scaffolding protein were measured as described in the text.

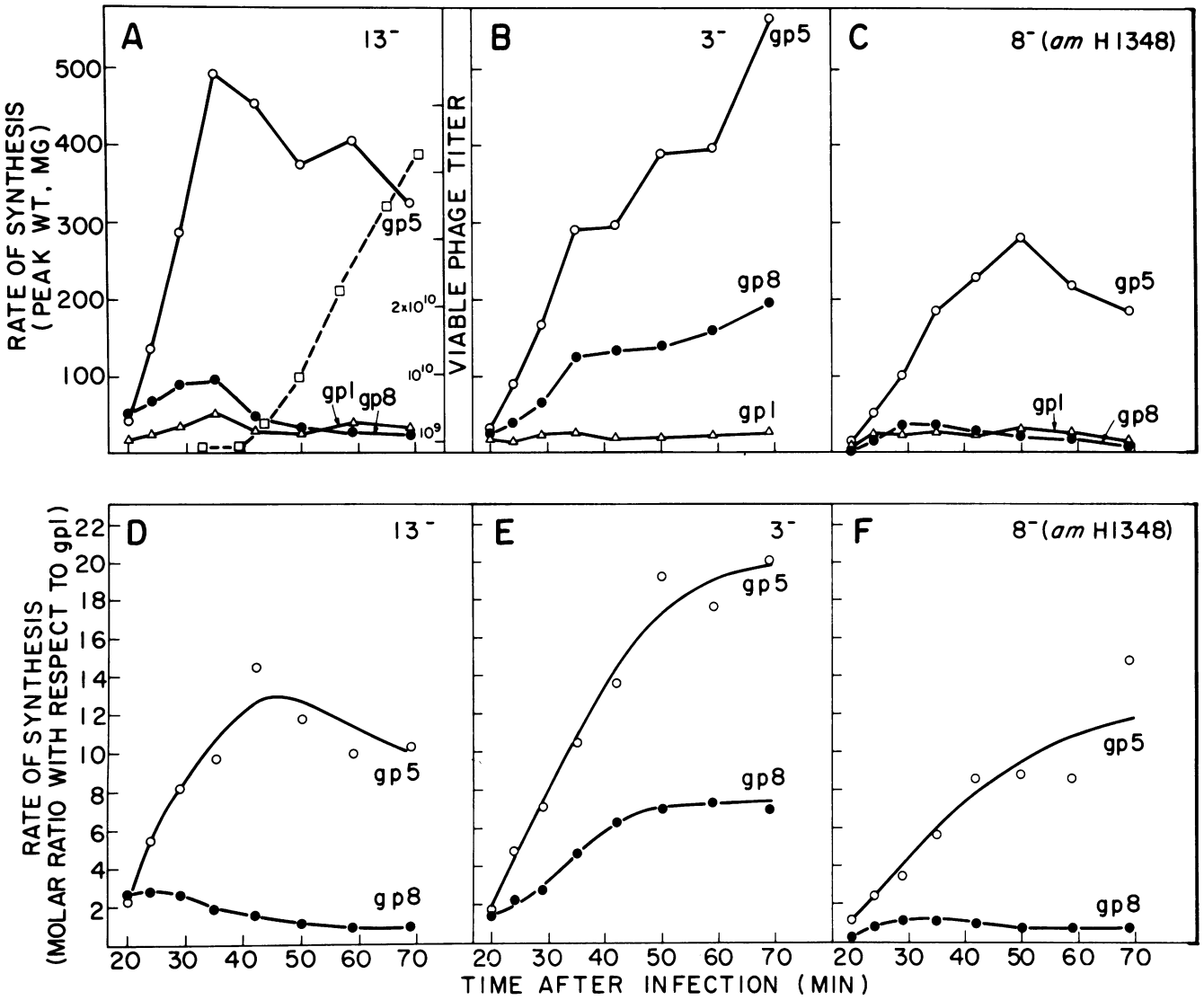

FIG. 2. Synthesis of a scaffolding protein amber fragment. DB21 su⁻ cells were infected at 30°C, pulse-labeled at 4 min with ^{14}C-labeled mixed amino acids, and harvested by freezing in a dry ice-acetone bath, and the proteins separated and quantitated as described previously (14). The top panels show the rates of incorporation at various times after infection, and the bottom panels show this data corrected to molar amounts and normalized to the amount of gp1, whose synthesis is not affected by the mutations being compared, Symbols: ○, coat protein or its amber fragment; ●, scaffolding protein or its amber fragment; △, gene 1 protein; □, viable phage. The mutations of primary interest are given in each panel; all phages also carried the *c*1-7 and 13⁻*am*H101 alleles.

decreasing between 40 and 90 min after infection and is inversely related to the amount of unassembled scaffolding protein, rather than proportional to the number of proheads in the infected cell (which might have been thought to turn on scaffolding protein synthesis).

Synthesis of scaffolding protein amber fragments. We previously reported that those amber fragments of scaffolding protein which can be visualized in SDS-polyacrylamide gels are not overproduced, as might be expected if scaffolding protein negatively regulates its own synthesis (14). Figure 2 shows that upon closer inspection, one of these amber fragments is in fact synthesized at a consistently lower rate in P22 8⁻*am*H1348-infected cells than the scaffolding protein is synthesized in 5⁻-infected cells, even though the amber fragment was shown to be stable (14). Although this effect is not large, it has been very reproducible. We have also found that other point mutations available in gene 8 do not cause overproduction of the scaffolding protein when compared with the wild-type. Double-mutant phage carrying 8⁻*ts*N102 or 8⁻*cs*RN26D (blocked at nonpermissive temperatures in DNA packaging and prohead assembly, respectively; data not shown) and an amber mutation in gene 5 (*am*N114) to block any structure formation accumulate scaffolding protein to the same level as the 5⁻8⁺ control at both permissive and nonpermissive temperatures (data not shown). These results can be explained in terms of the autoregulatory hypothesis if the assumption is made that the amber (N-terminal and unassembled) fragments have regulatory activity. If this regulatory activity were somewhat stronger than the wild-type protein, it would explain why the amber fragment is made at a lower level than that of unassembled wild-type protein in parallel infected cells. The *ts* and *cs* mutations tested apparently affect only the assembly function of the scaffolding protein.

Long scaffolding protein amber fragments have regulatory activity. To test more directly the hypothesis that the long amber fragments of scaffolding protein can modulate the level of expression of the scaffolding protein gene, we performed several mixed infections which demonstrate that the amber fragments can depress the expression of a functional scaffolding protein gene in *trans*. Because of interpretational difficulties when phage assembly and scaffolding protein recycling are taking place, we chose to perform the experiments either in a 3^- or 5^- mutational background. The first of these experiments is shown in Fig. 3 and 4. This experiment compares the effect of relatively long gp8 amber fragments with a short fragment on the expression of a functional gene 8. The two mutations used which generate long amber fragments, *am*H202 and *am*H1348, produce fragments with molecular weights of 21,000 and 12,000, respectively (14). The molecular weight of intact scaffolding protein is ca. 40,000 (3, 31). The mutation *am*N123 maps near the N terminus of the gene by deletion mapping (E. Wyckoff, personal communication), and no *am*N123 amber fragment has been seen even in high-percentage or gradient acrylamide gels (data not shown). Nonpermissive cells were infected at various ratios and constant multiplicity (20 or 30) with a phage carrying an intact (8^+) scaffolding protein gene and another phage carrying an amber mutation in the scaffolding protein gene. Both phage carried a 3^- mutation (*am*N6). The mutation in gene 3 blocks assembly at the DNA packaging step and assures that proheads accumulate and scaffolding protein is not recycling. Nearly all (>95%) of the scaffolding protein made in these infections is present in proheads of prohead-like structures (data not shown). Parallel cultures infected at different ratios of input parental phage were pulse-labeled with ^{14}C-amino acids to measure

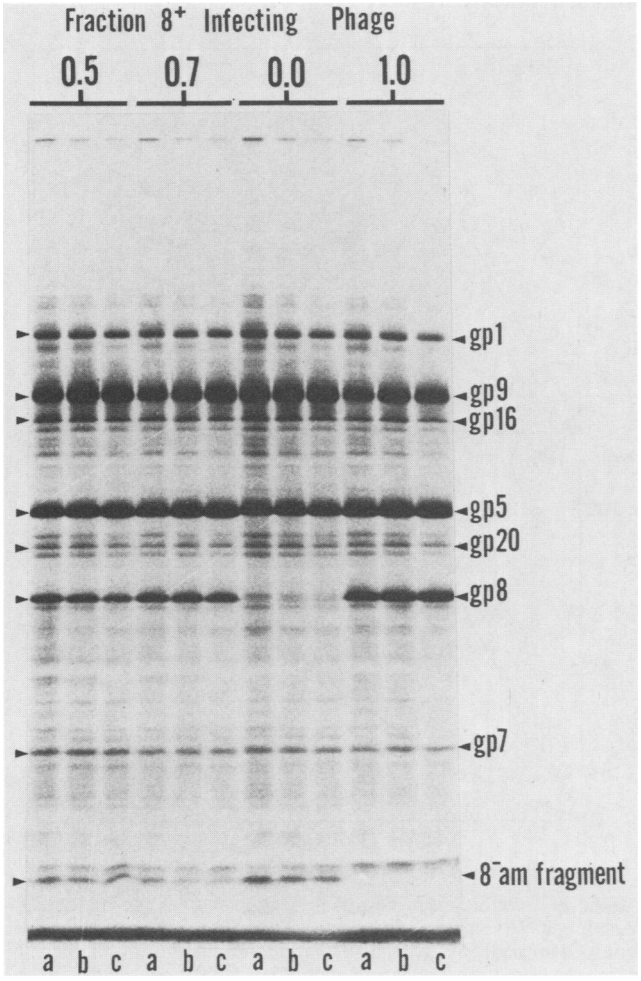

FIG. 3. Autoradiogram of a polycarylamide slab gel of extracts of 3^-8^+-3^-8^- mixedly infected cells. Cells were infected and pulse-labeled for 2 min with ^{14}C-amino acids. Cells were harvested at the times indicated, and the proteins were displayed in a 12 to 22% linear gradient polyacrylamide gel as described in the text. This autoradiogram is somewhat overexposed to make the protein bands more visible. The fraction of 3^-8^+ infecting phage is given above the lanes, and the labeling time after infection is indicated below (lanes a, 40 min; lanes b, 50 min; lanes c, 60 min).

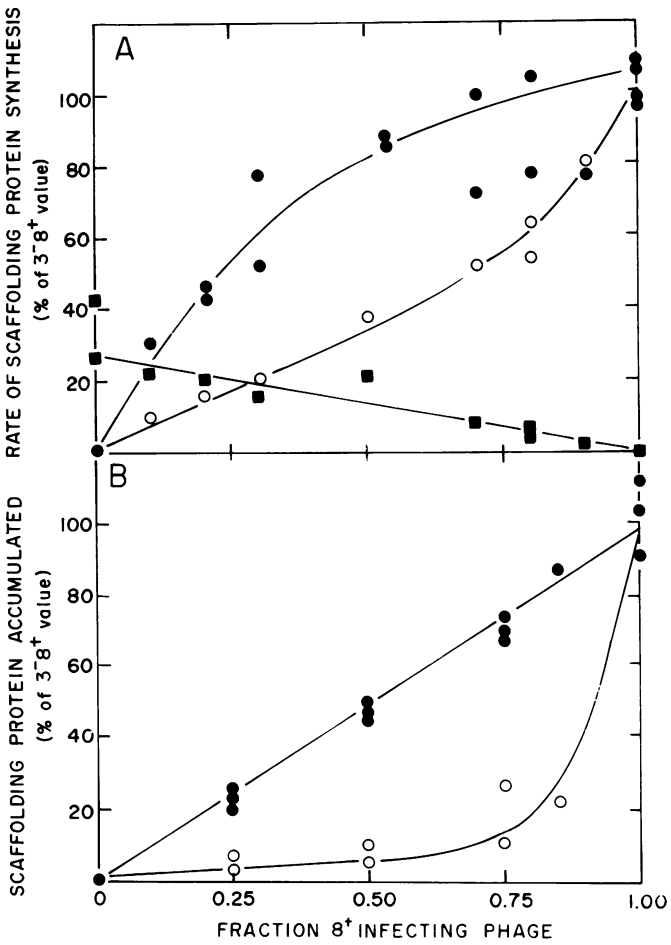

FIG. 4. Scaffolding protein synthesis in 3^-8^+-3^-8^- mixedly infected cells. Nonpermissive infected cells were pulse-labeled for 2 min with ^{14}C-amino acids in minimal medium (A) or grown in LB broth (B). The proteins were displayed in polyacrylamide slab gels and quantitated by autoradiography (A) or Coomassie brilliant blue staining (B) as described in the text. Each panel shows the combined results of several identical experiments. In (A) the total multiplicity of infection was 30, and samples were analyzed at 50 min after infection. In (B) the multiplicity of infection was 20, and samples were analyzed at 120 min after infection. In both cases, infecting parental phage carried the mutations *c*1-7, 13^-*am*H101, and 3^-*am*N6, and the fraction of 8^+ infecting phage is given on the abcissa. Symbols: ●, scaffolding protein synthesis in the 3^-8^+-3^-8^-*am*N123 mixedly infected cells; ○, scaffolding protein synthesis in the 8^+3^--3^-8^-*am*H1348 mixedly infected cells; ■, the rate of synthesis of the *am*H1348 amber fragment.

rates of synthesis. SDS-polyacrylamide gels were run to display the total proteins in each of the cultures. One such gel is shown in Fig. 3. Each of the protein bands of interest was quantitated, and the results are shown in Fig. 4A. The coninfecting gene 8 *am*H1348 fragment caused a depression of the rate of synthesis of complete scaffolding protein compared with that produced by the 8^-*am*N123 mutant. As the fraction of the *am*H1348 parent increases, there is a linear increase in the amber fragment synthesis rate. Qualitatively similar results were obtained when the total accumulated scaffolding protein was measured, even though the two types of experiments were done under very different growth conditions. The results of one such experiment are shown in Fig. 4B. An identical experiment, in which the 8^-*am*H202 allele replaced *am*H1348, gave very similar results.

To more firmly rule out other mechanisms, such as amber fragment poisoning of assembly which in turn modulates the synthesis of scaffolding protein, a similar mixed infection experiment was performed in which both of the parents carry an amber mutation in the coat protein gene. In this infection no scaffolding protein is in the assembled state, and the long amber fragment effect seen in the previous experiment should be present only if the fragment itself is directly responsible for the effect. The results of such an experiment are shown in Fig. 5. The total scaffolding protein accumulated by 80 or 125 min after infection shows that the mixed infection which produces a long amber fragment always synthesizes substantially less complete scaffolding protein than the infection in which one of the parents makes a short amber fragment. In this experiment we included as a control the 8^-*am*N123 8^-*am*H202 double mutant, which should produce only the short amber fragment, even though it carries both mutations. It behaves in this analysis indistinguishably from the *am*N123 phage. Since the only difference between the *am*N123 mixed infections and the *am*H1348 or *am*H202 mixed infections is the mutation in gene 8, we conclude that the long amber fragments must be directly responsible for depressing the synthesis of scaffolding protein. One feature of the results in Fig. 5 deserves further comment—the fact that the *am*H202 curve is approximately linear and the *am*N123 curve is bowed upwards. The *am*H202 curve is expected to be linear, since both the intact protein and the amber fragment are unassembled and have regulatory activity in this experiment. The upward bowing of the *am*N123 curve is explained if one notes that in the 5^-8^+ case, the scaffolding protein gene is fully "repressed." Since the short fragment has no regulatory activity, as the fraction of 8^+ parental phage decreases, the functional 8 genes present have the capacity to produce more scaffolding protein until a concentration sufficient to depress expression is reached.

DISCUSSION

The phage P22 scaffolding protein gene is under the positive transcriptional control of the 24 and 23 gene products, since it is not made when either protein is defective (3, 18, 27). However, we previously noted that the expression of the scaffolding protein gene does not always parallel the expression of the remainder of the late genes. More specifically, about three- to fivefold more scaffolding protein is made during infections mutationally blocked in a step which causes proheads to accumulate, whereas the amounts of the other late proteins made do not change (14). These studies indicated that the rate of synthesis of scaffolding protein is modulated in some way by the phage assembly process.

The experiments presented here show that (i) the amount of unassembled scaffolding protein in wild-type P22-infected cells at various times after infection or in various P22 mutant-infected cells is inversely related to the rate of synthesis of scaffolding protein, and (ii) long amber fragments of the scaffolding protein have the ability to depress synthesis of complete scaffolding protein, even in the absence of prohead assembly. In addition, in experiments reported elsewhere, we have shown that scaffolding protein is the only phage-coded protein required for this regulation to occur in vitro (30). We feel that these facts combine to provide a compelling argument for a control mechanism in which the feature of the assembly process which is critical in the modulation of the rate of scaffolding protein synthesis is the state of assembly of the scaffolding protein present. According to this model, the scaffolding protein itself depresses the synthesis of additional scaffolding protein when it is unassembled, but not when it is assembled into proheads. Thus, any excess of scaffolding protein over the available coat protein will depress additional scaffolding protein synthesis.

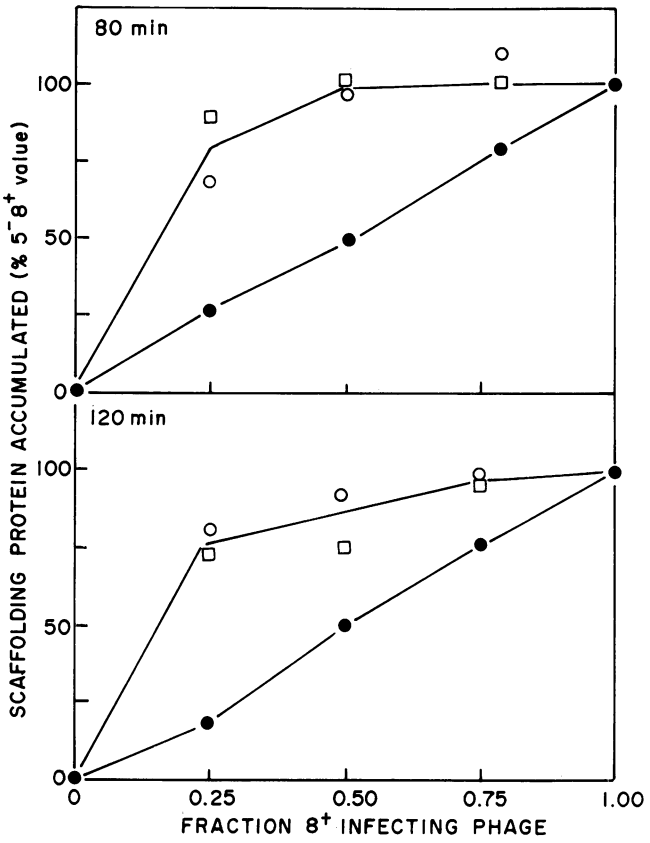

FIG. 5. Scaffolding protein synthesis in 5^-8^+-5^-8^- mixedly infected cells. Nonpermissive cells were infected, samples were removed for analysis at 80 and 120 min after infection, total proteins were separated in polyacrylamide slab gels and visualized by staining with Coomassie brilliant blue, and the proteins of interest were quantitated as described in the text. Both of the infecting parental phage carried the mutations *c*1-7, 13^-*am*H101, and 5^-*am*114. One of the parents was 8^+ and one was 8^-; the fraction of the 8^+ infection phage is given on the abcissa. Symbols: □, the scaffolding protein accumulated in 5^-8^+-5^-8^-*am*N123-infected cells; ●, the scaffolding protein accumulated in 5^-8^+-5^-8^-*am*H202-infected cells; ○, the scaffolding protein accumulated in 5^-8^+-5^-8^-*am*H202 8^-*am*N123-infected cells.

The synthesis of this catalytic morphogenetic protein is regulated by its own state of association with other proteins, and thus by the assembly process itself. Although the unassembled coat protein of the RNA bacteriophages acts as a translational repressor of the phage-coded RNA replicase (17), and the *Escherichia coli* RNA polymerase β and β′ subunit genes may be regulated in such a way that the holoenzyme and the subassembly $\alpha_2\beta$ depress synthesis (but free subunits do not [9, 11, 26]), P22 scaffolding protein and *E. coli* ribosomal proteins (19) represent the current, clearest examples of proteins which participate in a multicomponent assembly process, whose synthesis is directly regulated by the assembly process itself. In both these cases, the indicator is the state of assembly of the protein itself, and regulation is autogenous and negative. This mode of regulation is attractive and may be used in other more complex assembly systems and organisms to regulate the synthesis of structural proteins or catalytic proteins required in large amounts (1, 8, 16).

The fact that some amber fragments of the scaffolding protein have regulatory activity suggests that the N-terminal portion of the protein contains the regulatory activity, and since the active amber fragment of the 8$^-$*am*H1348 has an apparent molecular weight of only ca. 12,000, the regulatory site must reside in the N-terminal one-third of the 40,000-dalton scaffolding protein. Genetic mapping of these amber mutations strongly supports the idea that these fragments are in fact N terminal and not "restart" fragments (L. Wyckoff, unpublished data). This amber fragment does not appear to be able to assemble into proheads (L. Hall and J. King, unpublished data), suggesting that the assembly function of the protein requires the C-terminal portion of the scaffolding protein.

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LITERATURE CITED

1. **Ben-Ze'ev, A., S. Farmer, and S. Penman.** 1979. Mechanisms regulating tubulin synthesis in cultured mammalian cells. Cell **17**:319–325.
2. **Botstein, D., and I. Herskowitz.** 1974. Properties of hybrids between *Salmonella* phage P22 and coliphage lambda. Nature (London) **251**:584–589.
3. **Botstein, D., C. Waddell, and J. King.** 1973. Mechanism of head assembly and DNA encapsulation in *Salmonella* phage P22. I. Genes, proteins, structures and DNA maturation. J. Mol. Biol. **80**:669–695.
4. **Casjens, S., and M. Adams.** 1984. Posttranscriptional modulation of bacteriophage P22 scaffolding protein gene expression. J. Virol. **53**:185–191.
5. **Casjens, S., and J. King.** 1974. P22 morphogenesis. I. Catalytic scaffolding protein in capsid assembly. J. Supramol. Struct. **2**:202–224.
6. **Chan, R., and D. Botstein.** 1972. Genetics of bacteriophage P22. I. Isolation of prophage deletions which affect immunity to superinfection. Virology **49**:257–276.
7. **Ernshaw, W., S. Casjens, and S. Harrison.** 1976. Assembly of the head of bacteriophage P22: X-ray diffraction from heads, proheads and related structures. J. Mol. Biol. **104**:387–410.
8. **Elgin, S., and H. Weintraub.** 1975. Chromosomal proteins and chromatin structure. Annu. Rev. Biochem. **44**:725–774.
9. **Fukuda, R., M. Taketo, and A. Ishihama.** 1978. Autogenous regulation of RNA polymerase beta subunit synthesis *in vitro*. J. Biol. Chem. **253**:4501–4504.
10. **Georgopoulos, C., K. Tilly, and S. Casjens.** 1983. Lambdoid phage head assembly, p. 279–304. *In* R. Hendrix, J. Roberts, F. Stahl, and R. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Glass, R.** 1977. Identification of an amber fragment of the beta subunit of *Escherichia coli* RNA polymerase: a yardstick for measuring controls on RNA polymerase subunit synthesis. Mol. Gen. Genet. **155**:83–88.
12. **Jarvik, J., and D. Botstein.** 1975. Conditional-lethal mutations that supress genetic defects in morphogenesis by altering structural proteins. Proc. Natl. Acad. Sci. U.S.A. **72**:2738–2742.
13. **King, J., and S. Casjens.** 1974. Catalytic head assembling protein in virus morphogenesis. Nature (London) **251**:112–119.
14. **King, J., C. Hall, and S. Casjens.** 1978. Control of the synthesis of phage P22 scaffolding protein is coupled to capsid assembly. Cell **15**:551–560.
15. **King, J., E. Lenk, and D. Botstein.** 1973. Mechanism of head assembly and DNA encapsulation in *Salmonella* phage P22. II. Morphogenetic pathway. J. Mol. Biol. **80**:697–731.
16. **Komeda, Y., and T. Iino.** 1979. Regulation of expression of the flagellin gene (*hag*) in *Escherichia coli* K-12: analysis of *hag-lac* gene fusions. J. Bacteriol. **139**:721–729.
17. **Kozak, M., and D. Nathans.** 1972. Translation of the genome of a ribonucleic acid bacteriophage. Bacteriol. Rev. **36**:109–157.
18. **Lew, K., and S. Casjens.** 1975. Identification of early proteins coded by bacteriophage P22. Virology **68**:525–533.
19. **Nomura, M., S. Jinks-Robertson, and A. Miura.** 1982. Regulation of ribosome biosynthesis in *Escherichia coli*, p. 91–104. *In* M. Grunberg-Manago and B. Safer (ed.), Interaction of translation and transcriptional controls in the regulation of gene expression. Elsevier Science Publishing Co., Inc., New York.
20. **O'Farrell, P., L. Gold, and W. Huang.** 1973. The identification of prereplicative bacteriophage T4 proteins. J. Biol. Chem. **248**:5499–5501.
21. **Poteete, A., and D. Botstein.** 1979. Purification and properties of proteins essential to DNA encapsulation by phage P22. Virology **95**:565–573.
22. **Poteete, A., V. Jarvick, and D. Botstein.** 1979. Encapsulation of phage P22 DNA *in vitro*. Virology **95**:550–564.
23. **Poteete, A., and J. King.** 1977. Function of two new genes in *Salmonella* phage P22 assembly. Virology **76**:725–739.
24. **Roberts, J.** 1975. Transcription termination and late control in phage lambda. Proc. Natl. Acad. Sci. U.S.A. **72**:3300–3304.
25. **Roberts, J., C. Roberts, S. Hilliker, and D. Botstein.** 1976. Transcription termination and regulation in bacteriophages P22 and lambda, p. 707–718. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Scaife, J.** 1976. Bacterial RNA polymerases: the genetics and control of their synthesis, p. 207–225. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Suskind, M. M., and D. Botstein.** 1978. Molecular genetics of bacteriophage P22. Microbiol. Rev. **42**:385–413.
28. **Weinstock, G., P. Riggs, and D. Botstein.** 1980. Genetics of bacteriophage P22. III. The late operon. Virology **106**:82–91.
29. **Winston, F., D. Botstein, and J. H. Miller.** 1979. Characterization of amber and ochre suppressors in *Salmonella typhimurium*. J. Bacteriol. **137**:433–439.
30. **Wyckoff, E., and S. Casjens.** 1984. Autoregulation of the bacteriophage P22 scaffolding protein gene. J. Virol. **53**:192–197.
31. **Youderian, P., and M. Susskind.** 1980. Identification of the products of bacteriophage P22 genes, including a new late gene. Virology **107**:258–269.