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Functional Relationship between the J Proteins of Bacteriophages ϕX174 and G4 during Phage Morphogenesis

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The functions of the small DNA-binding protein, gpJ, of bacteriophages ϕX174 and G4 were examined by in vivo cross-complementation and sucrose gradient sedimentation. The morphogenetic roles of the two proteins may differ. The ϕX174 J protein may be required for the formation or stabilization of the ϕX174 prohead.

Bacteriophages ϕX174 and G4 are small icosahedral, single-stranded DNA phage of the class *Microviridae*. Their genomes are of similar length and organization (6, 14). Many of their proteins are cross functional (3).

The mature virions contain four structural proteins: the major coat protein, gpF; the major spike protein, gpG; the minor spike protein, gpH; and the small DNA-binding protein, gpJ. The least investigated of these four proteins is gpJ, which has been shown to be essential for DNA packaging both in vitro and in vivo (1a, 10, 11).

The amino sequences of the G4 and ϕX174 J proteins have been determined (6, 14). They are 25 and 38 residues long, respectively. The additional amino acids found in the ϕX174 sequence are located in the middle of the protein. The amino termini contain many basic residues. An N-terminal proteolytic fragment of the ϕX174 J protein binds and condenses DNA in vitro (1). The last 13 amino acids are conserved in 11 places. The last seven residues of the ϕX174 J protein have also been shown to be essential for DNA packaging in vitro (1).

In vivo complementation of ϕX174 *J*(Am) and G4 *J*(Am) mutants. To further the in vivo analysis of J-protein function, we assayed the ability of cloned ϕX174 and G4 *J* genes to complement newly constructed *J*(Am) mutants. The construction of the expression vector carrying the ϕX174 *J* gene has been described by Hamatake et al. (11). The *J* gene from G4 was cloned into the same vector (4). The plasmids will be referred to hereafter as pϕXJ and pG4J. G4 and ϕX174 *J*(Am) mutations were generated at the eighth codons of the *J* genes by oligonucleotide-directed mutagenesis (13).

To assay the ability of the cloned J proteins to cross-complement, the burst sizes of the ϕX174 *J*(Am) and G4 *J*(Am) mutants were measured in cells carrying the plasmids. These results are shown in Table 1.

Wild-type (wt) ϕX174 and G4 phage produced large bursts under all conditions. The burst sizes of the ϕX174 *J*(Am) and G4 *J*(Am) mutants in cells which do not harbor a plasmid were 3.4 and 0.6 phage per cell, respectively. In the presence of either pϕXJ or pG4J, the G4 *J*(Am) mutant produced bursts which were significantly higher. The ϕX174 *J*(Am) mutant, on the other hand, produced a burst only in the presence of pϕXJ. These results indicate that the G4 J protein does not productively contribute to ϕX174 development, making it unique among the G4 structural proteins. The other three structural proteins have been shown to function during the ϕX174 life cycle (3).

The hybrid G4 virions containing ϕX174 J protein generated in the above experiment were assayed for heat stability, UV and hydroxylamine sensitivity, and efficiency of adsorption. No differences were observed between the wt and hybrid G4 virions (data not shown).

Sedimentation profiles of extracts prepared from ϕX174- and G4 *J*(Am)-infected cells. To further investigate the roles the ϕX174 and G4 J proteins may play in phage development, phage DNA and proteins were radioactively labeled in ϕX174 *J*(Am) and G4 *J*(Am) infections under nonpermissive conditions. The phage used in these infections all had an additional *E*(Am) mutation which prevents the lysis of infected cells (12). HF4704 cells (2) at a concentration of 2.0×10^9 cells per ml were treated with mitomycin c and infected at a multiplicity of infection of 5.0 as described by Fujisawa and Hayashi (7), except that the leucine and lysine concentrations in the medium were 2.0 μg/ml. At $t = 11$ min, the infections were pulsed with 200 μCi of [*methyl*-^{3}H]thymidine and 300 μCi of either [U-^{14}C]lysine or [U-^{14}C]leucine and chased at $t = 40$ min by adding nonradioactive thymidine and amino acid to a final concentration of 20 and 10 mg/ml, respectively. The infections were stopped at $t = 60$ min, and lysates were prepared for centrifugation as described by Siden and Hayashi (15). To examine larger particles, the 5 to 30% sucrose gradients were spun at 45,000 rpm in an SW 50.1 rotor at 4°C for 80 min; to examine smaller particles, they were spun at 34,000 rpm for 16 h. Gradients were fractionated onto filter paper and incorporated label was precipitated with cold 6% trichloroacetic acid and counted. The results of these experiments are presented in Fig. 1 and 2.

The sedimentation profiles of larger particles in the extracts of cells infected with wt ϕX174 or wt G4 are shown in Fig. 1A and B, respectively; [^{3}H]DNA and ^{14}C-labeled protein sediments at 114S, the position of the mature virion. In the wt G4 extract, both isotopes also sediment at 140S. The sedimenting material is infectious (data not shown) and analogous to the 132S particle of ϕX174 (8, 17), the penultimate species in the assembly pathway.

In the extracts of cells infected with ϕX174 *J*(Am) or G4 *J*(Am) mutants (Fig. 1C and D, respectively), no [^{3}H]DNA sediments at 114S, 132S, or 140S. In the extract of the G4 *J*(Am)-infected cells, however, ^{14}C-labeled protein sediments at approximately 108S. By analogy to ϕX174 development, the sedimenting protein is most likely the G4 prohead (9). The fact that no prohead is present in the extract of the ϕX174 *J*(Am)-infected cells is a bit surprising, because the J protein is not a constituent of this particle (9).

Figure 2 contains the sedimentation profiles of ^{14}C-labeled

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TABLE 1. In vivo complementation of φX174 and G4 *J*(Am) mutants

Phage	Burst size (no. of phage/cell)[a]		
	BAF30(pφXJ)	BAF30(pG4J)	BAF30
φX174 *J*(Am)	26	4.2	3.4
G4 *J*(Am)	110	92	0.6
wt φX174	82	38	40
wt G4	420	450	860

[a] The BAF30 strain is a *recA56 srl*::Tn*10* derivative of BTCC122 (16). The protocol for these experiments is as given by Fane and Hayashi (5). TK (1.0% tryptone, 0.5% KCl) plates and liquid culture medium were supplemented with ampicillin (50 μg/ml) for strains bearing plasmids.

protein of smaller particles. In the extracts of the *J*(Am)-infected cells (open symbols), there is much more ^{14}C-labeled protein sedimenting at the 12S position than in the extracts of wt-infected cells (filled symbols). The 12S particle, an early assembly intermediate composed of the major coat and spike proteins, is known to accumulate when prohead production is inhibited (7, 15). ^{14}C-labeled protein sedimenting at the 18S position appears in all the extracts but that of the φX174 *J*(Am) mutant. The exact role of the 18S particle in φX174 assembly, however, remains obscure. It is not known whether it is an assembly intermediate or a degradation product of a larger particle.

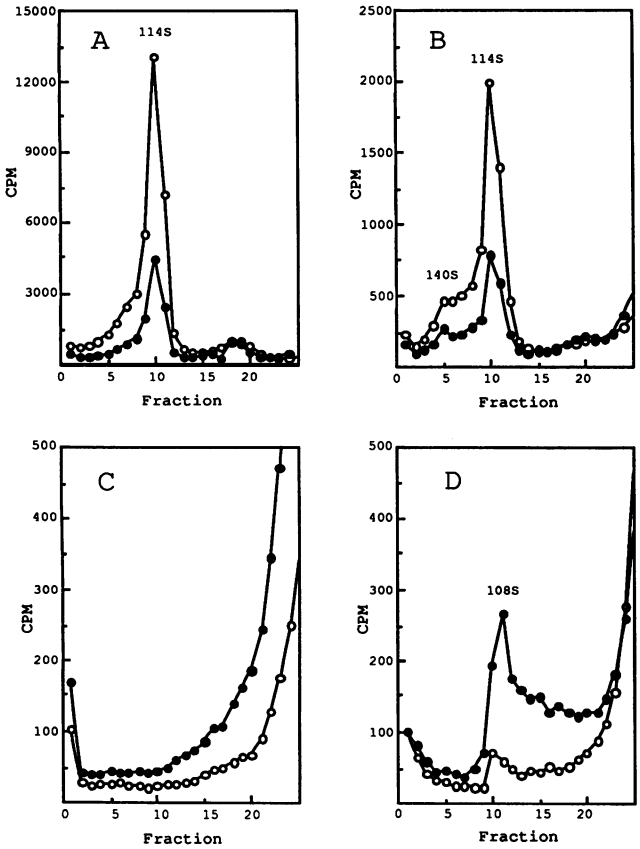

FIG. 1. Sedimentation profiles of larger particles from extracts of *J*(Am) mutant-infected cells. (A), wt φX174, (B) wt G4, (C) φX174 *J*(Am), (D) G4 *J*(Am). Symbols: ○, incorporated [^{3}H]thymidine; ●, incorporated [^{14}C]leucine.

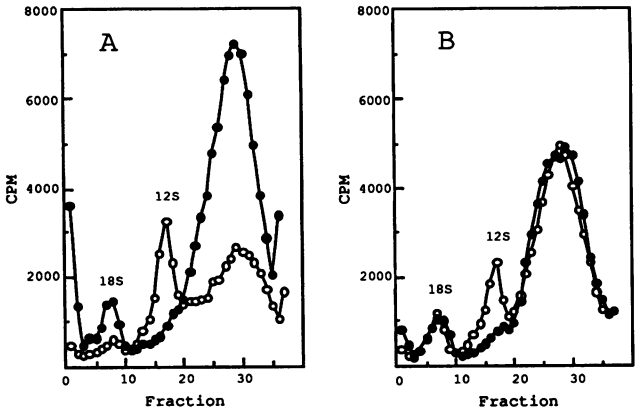

FIG. 2. Sedimentation profiles of smaller particles from extracts of *J*(Am) mutant-infected cells. (A) φX174 wt- and *J*(Am)-infected cells; B, G4 wt- and *J*(Am)-infected cells. Symbols: ■, incorporated [^{14}C]leucine in wt-infected cells; ○, incorporated [^{14}C]leucine in *J*(Am)-infected cells.

The accumulation of the 12S particle and the absence of the prohead species in the φX174 *J*(Am) extracts is consistent with an hypothesis that the J protein plays some role in φX174 prohead morphogenesis or stabilization. It should also be noted that there is preponderance of 12S material in extracts of G4 *J*(Am)-infected cells. The incorporation of ^{14}C-labeled protein into the 108S material, furthermore, was only one-third of that observed incorporated into the infectious particles of the wt G4 extracts. While the presence of the G4 J protein may not be required for the formation or stabilization of the G4 prohead, it may occur more efficiently in its presence.

Sedimentation profiles of extracts prepared from HF4704 pG4J cells infected by *J*(Am) mutants. To determine whether the basis for the inability of the G4 J protein to function during the φX174 life cycle also involved prohead morphogenesis, the particles synthesized in HF4704 pG4J-infected cells were analyzed by sucrose gradient sedimentation as described above. The sedimentation profiles of the larger particles are depicted in Fig. 3.

As can be seen in the extracts of the G4 *J*(Am)-infected

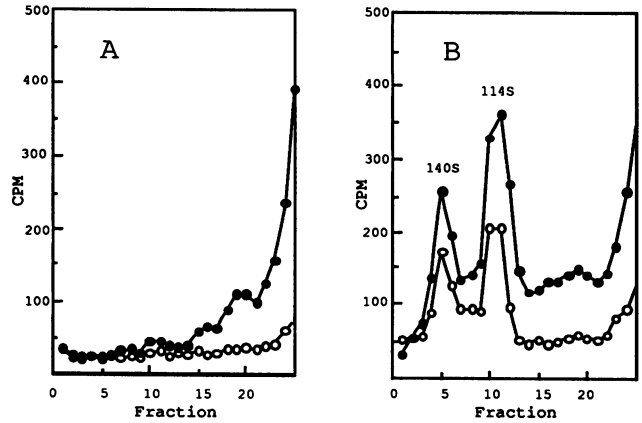

FIG. 3. Sedimentation profiles of larger particles from extracts of infected cells harboring pG4J. (A) φX174 *J*(Am), B, G4 *J*(Am). Symbols: ○, incorporated [^{3}H]thymidine; ●, incorporated [^{14}C] lysine.

cells (Fig. 3B), [^{3}H]DNA and ^{14}C-labeled protein sediment at the positions of the mature virion and the 140S particle. The righthand shoulder of the ^{14}C peak at the 114S position extends farther out than the corresponding shoulder of the ^{3}H peak, specifically fraction 12. The specific infectivity of fractions 10, 11, and 12 were 2.2×10^6, 1.5×10^6, and 2.2×10^5 PFU per ^{14}C cpm, respectively. This suggests that the ^{14}C-labeled protein in fraction 12 is not associated with infectious material and indicates the presence of the G4 prohead. In the extracts of the φX174 *J*(Am)-infected cells (Fig. 3A), on the other hand, there is no [^{3}H]DNA or ^{14}C-labeled protein sedimenting at either 132S or 114S. The presence of the G4 J protein does not significantly alter the structures synthesized by the φX174 *J*(Am) mutant. This observation suggests that the information needed for φX174 prohead formation or stabilization may not be found in the G4 J protein. The possible requirement of the φX174 J protein for φX174 prohead morphogenesis or stabilization may be the morphogenetic distinction between the two phages which underlies the failure of the G4 J protein to function in the φX174 life cycle.

The behavior of the φX174 *J*(Am) mutant also appears to differ from the previously characterized *insJ* mutant which contains a frameshift at codon 20 (11). In cells infected with this mutant, phage synthesis proceeds through the formation of the DNA packaging complex or 50S particle of which the φX174 prohead is an integral component (1, 10). This suggests that the information needed for the formation or stabilization of the prohead is found in the first half of the protein, perhaps between amino acids 9 and 20 which are not present in the G4 sequence.

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