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Genetic Analysis of the ϕX174 DNA Binding Protein

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The ϕX174 J protein is 37 amino acids in length and contains 12 basic residues. There are no acidic amino acids in the protein. The basic residues are concentrated in two clusters in the N-terminus which are separated by a proline-rich region. To investigate the morphogenetic functions of the J protein and possible mechanisms by which it may bind DNA, a genetic analysis was conducted. Lysine → leucine and arginine → leucine substitutions were generated within the basic amino acid clusters. At least three substitutions were required to eliminate viability *in vivo.* Lethal mutants with three or four substitutions exhibit dominant lethal phenotypes, indicating that the mutant proteins retain enough function to interfere with productive assembly. In cells infected with a dominant lethal mutant, noninfectious packaged particles were produced. Infectivity can be restored by second-site suppressors in the viral coat protein which disrupt polar interactions atop the threefold axis of symmetry in the capsid. The viability of strains containing compensating frameshift mutations within the proline-rich region suggests that only the proline residues in this segment are critical for efficient function.

INTRODUCTION

Bacteriophage ϕX174 is a simple T = 1 icosahedral virion of known genome sequence (Air *et al.,* 1978; Sanger *et al.,* 1978). *In vivo* studies have identified at least five assembly intermediates (Hayashi *et al.,* 1988). The atomic structure of the virion has been determined (McKenna *et al.,* 1992, 1994), which allows genetic and biochemical data to be interpreted within a structural context. The ϕX174 morphogenetic pathway is illustrated in Fig. 1. The first capsid intermediates are the 9S and 6S particles, pentamers of the coat and major spike proteins, respectively (Tonegawa and Hayashi, 1970). In a reaction dependent on the internal scaffolding protein, gpB, a 9S particle and 6S particle are joined, creating the 12S particle (Siden and Hayashi, 1974). Twelve 12S particles then associate with 240 molecules of the external scaffolding protein, gpD, to form the procapsid (Tonegawa and Hayashi, 1970; Fujisawa and Hayashi 1977a). After the procapsid is assembled, the single-stranded viral genome is synthesized and packaged along with the DNA binding protein, gpJ (Fujisawa and Hayashi, 1976). After the initiation of DNA packaging, gpB dissociates from the procapsid. The next intermediate, the 132S particle, still contains the external scaffolding protein, gpD, which then dissociates completing the maturation process (Fujisawa and Hayashi, 1977b).

The ϕX174 J protein is 37 amino acids in length and contains 12 basic residues. No acidic residues are present. The basic residues are concentrated in two clusters in the N-terminus of the protein, separated by a proline-rich region. The hydrophobic carboxyl terminus is conserved among all Microviridae viruses (Godson *et al.,* 1978; Kodaira *et al.,* 1992; Lau and Spencer, 1985; Sanger *et al.,* 1978) and has been shown to participate in DNA packaging (Aoyama *et al.,* 1983; Hamatake *et al.,* 1985, 1988; Kodaira and Taketo, 1984). The amino acid sequence of the wild-type J protein is presented in Fig. 2. In the mature virion, the J protein is present in 60 copies and forms an S-shaped polypeptide chain devoid of secondary structure (McKenna *et al.,* 1992, 1994). The lack of secondary structure argues against DNA binding mechanisms which rely on secondary structure, such as leucine zippers or helix-turn-helix motifs (Landshulz *et al.,* 1988; Pabo and Sauer, 1984). In addition, the amino acid sequence is inconsistent with the formation of a zinc finger (Miller *et al.,* 1985). These structural considerations suggest that simple charge interactions between basic amino acid residues and the negatively charged DNA phosphate background may be involved in nucleic acid binding. This mechanism has been observed in the DNA binding, basic amino acid terminal extensions of plant virus capsid proteins (Abad-Zapatero *et al.,* 1980; Liljas *et al.,* 1982), the L2 protein of human papilloma virus (Zhou *et al.,* 1994), and the bacterial histone-like proteins (Lammi *et al.,* 1984; Tanaka *et al.,* 1984).

Although a strict an analogy between the Microviridae J proteins and the histone-like proteins of bacteria may not be warranted, these two groups of proteins do share some similarities. Both groups of proteins are small and highly basic. As with the histone-like proteins (Holck and Kleppe, 1985; Zentgraf *et al.,* 1977), the ϕX174 J protein binds single-stranded DNA, double-stranded DNA, and RNA (Dalphin, 1989). Moreover, both the histone-like pro-

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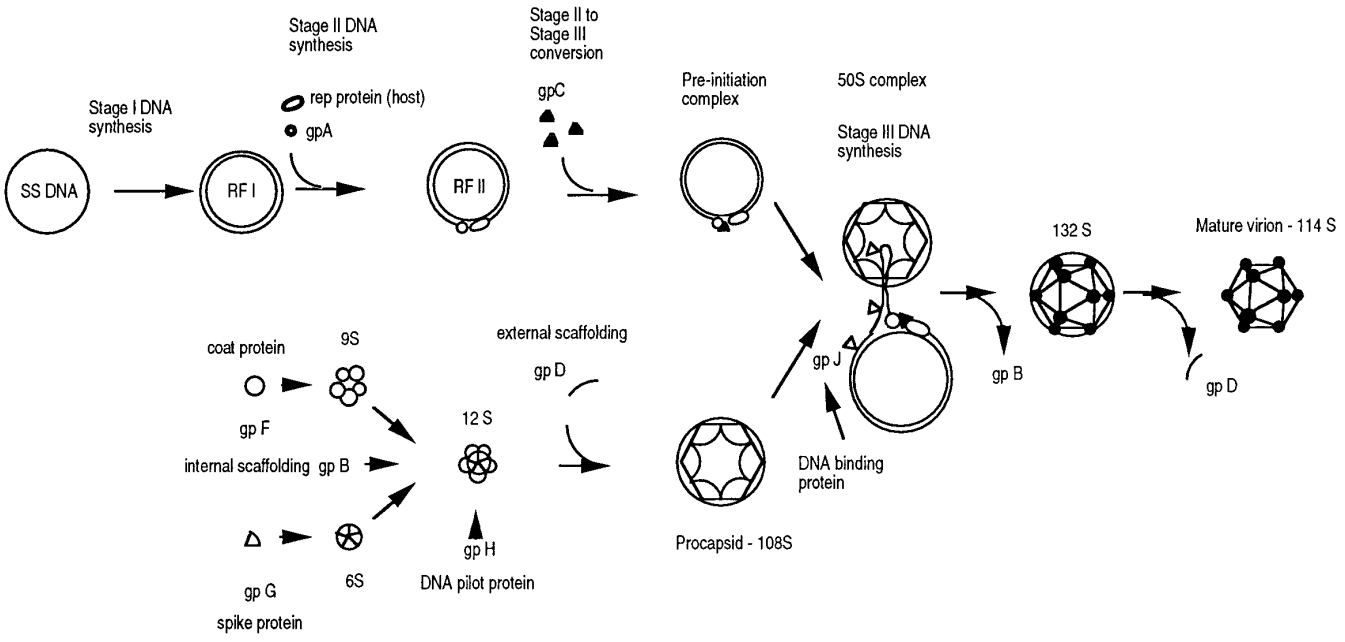


FIG. 1. The ϕX174 morphogenetic pathway.

teins and gpJ show little or no sequence specificity in DNA binding (Aoyama and Hayashi, 1985; Dalphin, 1989; Hamatake *et al.,* 1985). Crystallographic studies and the *in vitro* characterization of chemically modified histone-like proteins indicate that basic amino acid residues located within a small hydrophilic region of the proteins are critical to DNA binding activity (Lammi *et al.,* 1984; Zentgraf *et al.,* 1977).

In order to investigate the morphogenetic role of the J protein during assembly, and possible mechanisms by which it binds DNA, several site-directed J^- mutations have recently been isolated. These mutations confer substitutions at basic residues (lys/arg → leu) and in the proline-rich region. The phenotypes of the "charge-altered" J^- mutants and intragenic suppressors suggest that the mechanism of DNA binding is primarily mediated by charge–charge interactions. The phenotypes of viable compensating frameshift mutants in the proline-rich region indicate that only the proline residues in this segment of the polypeptide are critical for function. The *in vivo* assembly pathway was investigated in cells infected with one of the "charge-altered" mutants. Although packaged particles were produced in these infected cells, these particles were not infectious. Infectivity, however, can be restored by the introduction of extragenic suppressors in the viral coat protein at the threefold axis of symmetry.

MATERIALS AND METHODS

Bacterial strains, plasmids, phage plating, stock preparation, DNA isolation, DNA sequencing, media, and Burst experiments

The *Escherichia coli* C strains, protocols, and the plasmid pϕXJ have been previously described (Fane and Hayashi, 1991; Fane *et al.,* 1992; Hamatake *et al.,* 1988). C122 is the wild-type (*sup*°) host. BAF5 contains a *supE* mutation. BAF30 is a *recA* derivative of C122. The expression of the cloned gene is under temperature induction.

Site-directed mutagenesis in gene J

The *in vitro* DNA synthesis and transformation protocols have been previously described (Fane *et al.,* 1993). The phenotypes, genotypes, and generation of all of the J^- mutants are depicted in Fig. 2. Mutagenic oligonucleotides were purchased from Genosys Biotechnologies Inc. For the generation of the *J⁻3K → L I* and *J⁻3K → L II* mutants, the mutagenic oligonucleotides were designed to introduce three lysine → leucine substitutions into wild-type DNA. BAF30 pϕXJ cells were transfected with the *in vitro* DNA synthesis products, mixed with 2.0×10^8 BAF30 pϕXJ plating cells, and incubated at 42° until plaques appeared. Plaques were then stabbed into two indicator lawns, one seeded with BAF30 pϕXJ and one with C122. Putative mutants were identified as those which failed to grow, or grew very poorly, on the indicator plates seeded with C122. The genotypes of all mutants were verified by a direct DNA sequence analysis.

The *J⁻3K → L I* mutant retained a low level of viability, forming small plaques on C122. DNA isolated from this mutant was used as the template for the generation of additional "charge-altered" mutants. The *J⁻3K&R → L* mutant was generated with a mutagenic oligonucleotide designed to anneal to *J⁻3K → L I* DNA and introduce an additional R → L mutation at amino acid 6. The new mutant was identified by its inability to propagate on C122. The *J⁻K → L ALL* (all lysines changed to leucines) and *J⁻8BR* (eight basic residues removed) mutants were

also generated from the *J⁻3K → L I* parent. The mutagenic oligonucleotides were designed to mutate basic residues downstream from the mutations in *J⁻3K → L I.* Again, the new mutants were identified by their inability to propagate on C122 and verified by a direct sequence analysis.

The frameshift mutation, *FS(J⁻)40,* was recovered from a site-directed mutagenesis with a "doped" oligonucleotide designed to statistically introduce one substitution per genome in the region encoding residues 11 through 16 in gene J. It contains a deletion of cytosine at nucleotide 40. Substitutions for proline residues were also generated by the use of a doped oligonucleotide designed to statistically introduce one substitution per genome only in the proline codons. Mutants were generated as described above with the addition of a C122 indicator plate incubated at 24° to screen for *cs* (cold-sensitive) phenotypes.

Reversion analyses

Second-site, same-site, and compensating frameshift revertants were selected by plating 10^6–10^7 phage on C122 at 33°.

Site-directed mutagenesis in gene F

To verify the identity of the extragenic second-site suppressors of *J⁻3K → L II* located in gene F, oligonucleotide rescue experiments were performed (Fane *et al.,* 1993). A mutagenic oligonucleotide was designed to introduce the *su(J)-F S1F* mutation into the *J⁻3K → L II* background. As a control, a nonmutagenic primer was used. BAF 30 pϕXJ cells were transformed as described above. One hundred plaques were then stabbed into two indicator lawns, one seeded with C122 and incubated at 33° and one seeded with BAF30 pϕXJ and incubated at 42°. Rescue was defined as the ability to propagate on the C122 lawn.

Two rounds of oligonucleotide mediated mutagenesis were performed to place the *su*(*J*) − *FS1F* mutation in the *J⁻3K&R → L, J⁻K → L ALL,* and *cs*(*J*)*P16R* backgrounds. In the first round, an oligonucleotide designed to place an amber mutation, *am*(*F*)S1, at the site of the suppressor mutation was used. BAF5 (*supE*) pϕXJ cells were transfected with the mutagenized DNA and incubated at 42°. Progeny were stabbed into three indicator lawns, BAF30 pϕXJ, BAF5 pϕXJ, and BAF5. The *J⁻/am*(*F*)*S1* double mutants were identified as those progeny which only propagated on BAF5 pϕXJ. The *su(J)-F S1F* mutation was then introduced in the second round of oligonucleotide mediated mutagenesis. The *J⁻/su*(*J*)*-F S1F* mutants were recovered by selecting against the *am* phenotype on BAF30 (*sup*°) pϕXJ. The genotypes of all new mutants were verified by a direct DNA sequence analysis.

Preparation of radioactive lysates and sucrose gradient centrifugation

The protocols for the generation of the [^{3}H]leucine and [^{3}H]thymidine lysates and centrifugation are identical to those previously described (Ekechukwu *et al.,* 1995).

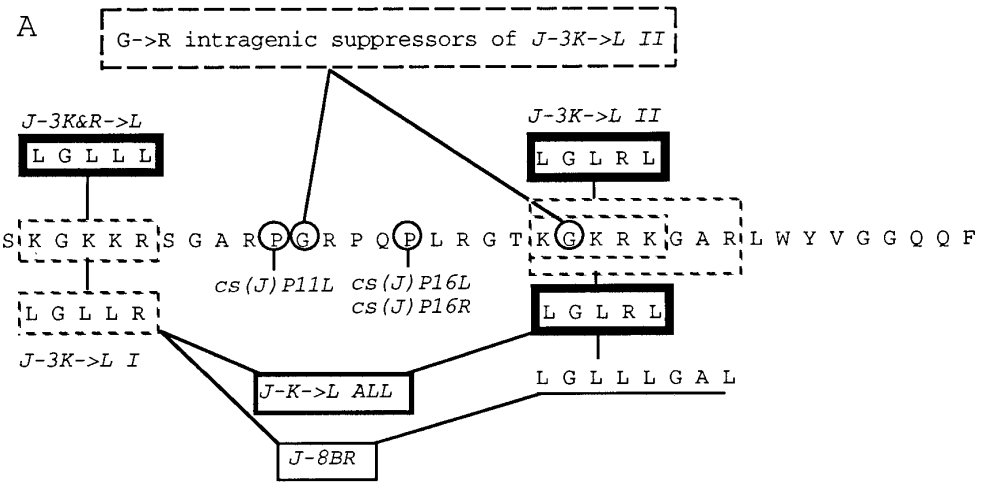
RESULTS

Phenotypes of *J⁻* mutants with substitutions at basic residues

The mutants described here are illustrated in Fig. 2. The most severely modified mutant which retains viability is the *J⁻3K → L I* mutant, in which the three lysine residues 2, 4, and 5 have been changed to leucine residues. This mutant, however, does not have a wild-type phenotype. Its plaque morphology is considerably smaller than wild-type, and burst size is considerably reduced *in vivo.* When a fourth mutation is added, Arg → Leu (*J⁻3K&R → L*), at position six, an absolute lethal phenotype results. In the *J⁻3K → L II* mutant, the three lysine residues at positions 21, 23, and 25 have been changed to leucine residues. Unlike the *J3 → K I* mutant, this mutant is not viable. And finally a fourth and a fifth mutant were generated, *J⁻K → L All,* in which all of the lysine residues have been changed to leucine residues, and *J⁻8BR* (eight basic residues removed). Both the *J⁻K → L ALL and J⁻8BR* mutants exhibit absolute lethal phenotypes.

The lethal *J⁻* mutants, with the exception of *J⁻8BR,* can only be efficiently propagated in cells harboring a maximally induced cloned *J* gene at 42° since expression is under heat induction. Intermediate levels of induction (37°) fail to efficiently complement these mutants, although the N-terminal amber mutant *am*(*J*)*S7* does propagate well under these conditions. This observation suggested that some of the mutant J proteins may retain some level of function. To test this hypothesis, the *J⁻* mutants were assayed for dominant phenotypes in co-infections where the wild-type:mutant J protein ratio would be near 1:1. The results of these experiments are presented in Table 1. All of the lethal *J⁻* mutants with amino acid substitutions at basic residues, with the exception of *J⁻8BR,* exhibit some degree of dominance. The amber mutant *am*(*J*)*S7* and the highly altered *J⁻8BR* mutant, on the other hand, are clearly recessive. These observations suggest that some of the mutant J proteins retain enough function to enter some intermediate, multimeric assembly complex, perhaps containing both protein and DNA, and inactivate it. This basis of dominance has been observed in other assembly systems, most notably VSV (Youngner *et al.,* 1986).

The burst sizes produced in the co-infections may reflect the extent to which the various J proteins can nonproductively interact with other phage components. In this model, the level of residual activity would be proportional to the severity of the dominant phenotype. For ex-



B

Wild type:

AAA CGT TCT GGC GCT CGC CCT GGT **C**GT CCG CAG CCG
K R S G A R P G R P Q P

Viable compensating frameshifts:

AAA ACG TTC TGG CGC TCG CCC TGG TGT CCG CAG CCG
K T F W R S P W C P Q P

ACG TTC TGG CGC TTC CGC CCT GGT CGT CCG CAG CCG
T F W R F R P G R P Q P

FIG. 2. Phenotypes and genotypes of the J$^-$ mutants. (A) The primary sequence of the wild-type J protein is contained on the central line. The basic residues are concentrated in two clusters, basic region I (amino acids 1–10) and basic region II (amino acids 17–28) in the N-terminus of the protein, separated by a proline-rich region (amino acids 11–16). The altered amino acids and the names of the mutants are depicted both above and below the wild-type J protein sequence. Viable mutants are indicated with hatched boxes. Nonviable substitutions are indicated with solid boxes. The thickness of the borders indicates the severity of observed dominant phenotypes in co-infections with wild-type ϕX174. (B) The nucleotide and amino acid sequences conferred by viable frameshift mutations are depicted below the wild-type nucleotide and amino acid sequences. The first nucleotide depicted in the wild-type sequence is at position 16 in the gene. The nucleotide position is determined from the start codon. The amino acid position, however, is determined from the first amino acid found in the mature virion as elucidated in the atomic structure. The deleted base in the parental mutant *FS(J)40* is indicated by bold text.

ample, the burst produced by cells co-infected with wild-type and *J$^-$K → L ALL* was 2.1, compared to the bursts of 0.8 and 0.1 for the co-infections involving the *J$^-$3K&R → L* and *J$^-$3K → L II* mutants, respectively. The 0.8 and 0.1 burst sizes reflect titers which were equal to the titer of unabsorbed virions determined 9.0 min postinfection. The 2.1 burst size, on the other hand, reflects a titer which was 6.0 times greater than the titer of unabsorbed virions, suggesting the actual production of progeny. The more extensive substitutions in the *J$^-$K → L ALL* may result in a less active J protein which, in turn, may lessen the severity of the dominant phenotype. The recessive phenotype of the *J$^-$8BR* mutant is consistent with this hypothesis.

In vivo characterization of "charge-altered" mutant morphogenetic pathways

The observed dominant phenotypes of the "charge-altered" mutants suggest that the mutant J proteins retain enough function to enter the morphogenetic pathway. In an effort to determine where and if assembly is affected, radioactive protein and DNA lysates were generated and analyzed by sucrose gradient sedimentation. The sedimentation profiles from the labeled protein experiments are presented in Fig. 3. In the lysates of wild-type infected cells (●), counts were detected to sediment at both 132S and 114S, the positions of the provirion and virion, respectively. The material from these peaks was infectious. Similarly, counts were also detected to sediment at 132S and 114S in lysates prepared from *J$^-$3K → L II*-infected cells (○). But unlike the material from the wild-type lysates, there was no infectivity associated with these peaks. Furthermore, counts sedimenting at 108S and 90S were also detected in the lysates prepared from *J$^-$3K → L II*-infected cells. The viral procapsid (108S) is the assembly intermediate into which the genome is packaged. The uninfectious 90S particle was observed in a series of experiments conducted by Aoyama and Hayashi (1985) in which genomes larger than unit length (100–

TABLE 1

Burst Sizes of J⁻ Mutants in Single Infections and Co-Infections with Wild-Type φX174

Infection	No. of basic residues in gpJ	Burst (ø/cell) 33°	23°
Wild-type	+12	31	25
J⁻3K → L II	+9	0.1	
J⁻3K & R → L	+8	0.3	
J⁻K → L ALL	+6	0.5	
J⁻8BR	+4	<0.1	
am(J)S7	Fragment	<0.1	<0.1
Wild-type X *J⁻3K → L II*	12 and 9	0.1	
Wild-type X *J⁻3K & R → L*	12 and 8	0.8	
Wild-type X *J⁻K → L ALL*	12 and 6	2.1	
Wild-type X *J⁻8BR*	12 and 4	11.5	
Wild-type X *am(J)S7*	12 and fragment	18.2	13.5
cs(J)P16R		41	1.2
cs(J)P16L		26	7.0
Wild-type X *cs(J)P16R*		17.7	1.6
Wild-type X *cs(J)P16L*		64	6.2

101%) were packaged. The possible connection between the experiments of Aoyama and Hayashi, using larger genomes as packaging substrates, and the experiments presented here, using defective J proteins, is analyzed in more detail under Discussion. Only partially packaged and empty capsids (70S) were observed in the sedimentation profiles generated from extracts of *J⁻K → L ALL* (▲)- and *J⁻8BR* (△)-infected cells.

Phenotypes conferred by mutations in the proline-rich region

To examine the role of the proline-rich region, we initially tried to recover mutants by oligonucleotide-mediated mutagenesis with a "doped" oligonucleotide designed to statistically introduce one substitution per genome in the region encoding residues 11 through 16. BAF30 pφXJ cells were transfected with the mutagenized DNA and 680 progeny were examined by stabbing plaques into two indicator lawns, one seeded with BAF30 pφXJ, the other with C122. Of the progeny examined, 10 displayed an altered plaque morphology, and 12 displayed absolute lethal phenotypes. The 12 displaying absolute lethal phenotypes were plated on BAF5 (*supE*), to assay for *am* phenotypes. Only 1 of these mutants exhibited an *am* phenotype. DNA was isolated from the remaining 11 mutants and sequenced in gene J. All 11 mutants contained a frameshift mutation, a deletion of nucleotide 40.

This frameshift mutation, designated *FS(J)40,* was then used in a reversion analysis. Compensating second frameshift mutations could dramatically alter the amino acid residues encoded between the two mutations. The genotypes of these viable revertants could reveal the important amino acid residues in this region. Revertants of *FS(J)40* were selected by plating on C122 at 33° and appeared at a relatively high frequency (5.0×10^{-3}). The DNA from six revertants was sequenced. Two genetically distinct pseudo-revertants were recovered. The amino acid sequences conferred by the compensation frameshift mutations are given in Fig. 2. Five of the revertants sequenced contained a simple addition of one A after nucleotide 18 to compensate for the deletion of the C at nucleotide 40. The sixth revertant, however, represented a more complex rearrangement. Although the deleted nucleotide at position 40 was restored, nucleotides 17 and 18 were deleted. To compensate for the two deleted nucleotides, an additional T and C were inserted after nucleotides 30 and 31, respectively. The latter mutant displayed a slight *cs* phenotype at 24° (eop = 0.1). Although the amino acid sequence between residues 7 and 14 was dramatically altered in these revertant strains, the proline residues were always conserved. While this may suggest that selecting for viability coselects for the conservation of the proline residues, the redundancy of the genetic code cannot be disregarded. In either case, the viable phenotypes of the compensating frameshift mutations indicate that the amino acid residues in this region, with the exception of the proline residues, are not critical for J protein function.

To directly examine the effects of substitutions for pro-

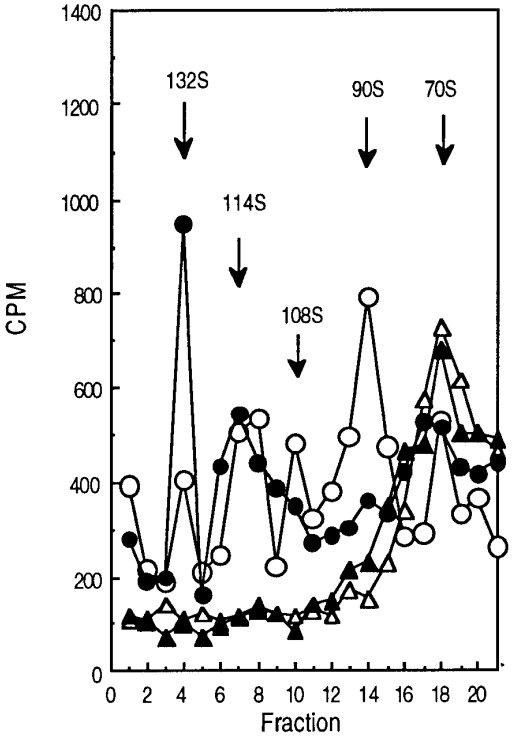


FIG. 3. Sedimentation profiles of viral particles from extracts of J⁻ mutant and wild-type infected cells. Symbols: (●) wild-type infected cells; (○) *J⁻3K → L II*-infected cells; (▲) *J⁻K → L ALL* infected cells; (△) *J⁻8BR*-infected cells.

line residues, a mutagenic oligonucleotide which would dope only the proline codons was used. BAF30 pϕXJ cells were transfected as described above. Progeny were then stabbed into three indicator lawns, one seeded with BAF30 pϕXJ incubated at 42°, one seeded with C122 incubated at 42°, and one seeded with C122 incubated at 24°. Of the 477 progeny examined, 5 lethal and 18 *cs* mutants were recovered. DNA was isolated and sequenced from the 5 lethal mutants and 5 *cs* mutants. Once again, all of the lethal mutants contained frameshift mutations, insertions or deletions between nucleotide positions 31 and 36. The amino acid sequences of the *cs* substitutions are given in Fig. 2. Two *cs* mutants, *cs*(J)*P16L* and *cs*(J)P16R, were assayed for dominant phenotypes in co-infections with wild-type ϕX174. Again, these mutations exhibit a dominant phenotype (Table 1).

Of the 34 mutants recovered and characterized in an effort to obtain lethal missense substitutions in the proline-rich region, none were recovered. All absolute lethals carried frameshift mutations. Although gene J is obviously a hotspot for frameshift mutations, this, in itself, does not explain the inability to recover lethal missense point mutations. Eighteen other mutations based on plaque morphology or cold-sensitive or amber phenotypes were isolated. This, taken with the genotypes of the compensating frameshift mutations, suggest that lethal missense point mutations in this region are very rare.

Reversion analyses

To further test the hypothesis that the ability of the J protein to bind DNA may involve simple charge–charge interactions, a second-site reversion analysis was conducted with the *J⁻3K → L II,* the *J⁻3K&R → L,* and the *J⁻K → L ALL* mutants. Revertants were selected by plating the mutants on the C122 host. Of the six revertants of *J⁻3K&R → L* characterized by a direct sequence analysis, all represented same-site events, either restoring the arginine codon or converting it to a phenylalanine codon. Since none of the altered codons in the *J⁻3K → L II* mutant could revert to encode a basic residue via one mutational step, it was reasoned that this mutant may be more useful for the isolation of second-site suppressors.

The results of the second-site reversion analysis conducted with this mutant are presented in Table 2. Both intragenic and extragenic suppressor were recovered. The intragenic suppressors conferred additional Gly → Arg substitutions at amino acid residues 13 and 23. These substitutions could theoretically elevate the charge of the protein from +9 to +10. The viable phenotypes of these intragenic suppressors, the "charge-altered" and compensating frameshift mutants with at least 10 basic residues suggest that 10 basic residues may be the minimal number required for viability. Two second-site suppressors were extragenic, both conferring substitutions in the first amino acid of the viral coat protein (see below). The mechanism by which these substitutions may suppress is addressed under Discussion. An attempt to isolate second-site suppressors of the *J⁻K → ALL* mutant was also made. No suppressors or revertants were isolated. If these mutants exist they occur at a frequency $<3.0 \times 10^{-8}$. Revertants of proline-altered mutants were also isolated. A DNA sequence analysis, however, indicated that all of the isolated revertants arose via same site revertant events, restoring the wild-type proline codon.

The extragenic suppressors of *J⁻3K → L II* conferred no secondary phenotypes (*ts* or *cs*). Therefore, they could not be localized by genetic complementation. They were initially identified by a sequence analysis of the genes which encode other viral structural proteins. To verify the identity of extragenic second-site suppressors of *J⁻3K → L II,* an oligonucleotide rescue experiment was conducted. The mutagenic oligonucleotide was designed to introduce the *su*(J)*-F S1F* mutation into the *J⁻3K → L II* background. A nonmutagenic oligonucleotide was used as a control. BAF30 pϕXJ cells were transfected with the *in vitro* DNA synthesis reactions and incubated at 42°. At least 100 plaques from each transfection were stabbed onto two indicator lawns, one seeded with BAF30 pϕXJ incubated at 42° and one seeded with C122 incubated at 33°. Rescue was defined as the ability to propagate on the C122 host. The frequency of rescue with the mutagenic primer was 12%. No rescue was detected by the nonmutagenic primer.

The inability to isolate second-site suppressors of the *J⁻3K&R → L* mutant may have been partly due to the high level of same site reversion events. Therefore, the extragenic suppressor *su*(J)*-F S1F* was placed directly into the *J⁻3K&R → L* background. The extragenic suppressor was also placed in the *cs*(J)*P16R* and *J⁻K → L ALL* background as well. Two rounds of mutagenesis were conducted as described under Materials and Methods. In the first round, an amber mutation was placed at the site of the extragenic suppressor. In the second round of mutagenesis, the oligonucleotide was designed to create the suppressor mutation. Selection against the amber mutation was employed. The eop's of these strains are also given in Table 2. The extragenic suppressor was able to restore viability to the parental *J⁻3K&R → L* mutant, demonstrating that the mechanism of extragenic suppression is not allele specific. The extragenic suppressor, however, was unable to restore viability to the *J⁻K → L ALL* or *cs*(J)*P16R* mutants.

DISCUSSION

Mechanism of J protein function and DNA binding

Three lines of evidence suggest that J protein–DNA interactions are mediated by the protein's highly basic primary sequence. (1) The protein lacks secondary structure; (2) there is a genetic redundancy of function, as

TABLE 2

Second-Site Suppressors of *J⁻3K → LII*

Mutant	Location			EOP[a] on C122
	Protein	Residue	Change	
J⁻3K → L II				9×10^{-5}
su(J)-J G13R/J⁻3K → L II	gpJ	13	Gly → Arg	5.0×10^{-2}
su(J)-J G23R/J⁻3K → L II	gpJ	23	Gly → Arg	0.5
su(J)-F S1F/J⁻3K → L II	gpF	1	Ser → Phe	1.0
su(J)-F S1P/J⁻3K → L II	gpF	1	Ser → Pro	1.0
J⁻3K & R → L				1.0×10^{-4}
su(J)-F S1F/J⁻3K & R → L	gpF	1	Ser → Phe	1.3
J⁻K → L ALL				$<3.0 \times 10^{-8}$
su(J)-F S1F/J⁻K → L ALL	gpF	1	Ser → Phe	1.8×10^{-4}
cs(J)P16R				1.0×10^{-5}
su(J)-F S1F/cs(J)P16R			Ser → Phe	1.0×10^{-7}

[a] Titer on C122 at 33°/titer on BAF30 pϕXJ at 42° for mutants with lethal phenotypes. Titer on C122 at 24°/titer on C122 at 33° for mutants with *cs* phenotypes.

demonstrated by the viability of mutants with ⩾9 basic residues; and (3) the severity of dominant lethal phenotypes correlates with the number of basic residues in the mutant proteins. Although the overall charge of the J protein is critical, other factors, such as spatial orientation, are most likely involved. This is evinced by the phenotypes of the two intragenic suppressors of the *J⁻3K → L II* mutant. The amino acid changes found in the parental strain are all contained within the second basic region of the protein. While Gly → Arg suppressors were found in both basic regions, the one located within basic region II is much more efficient at restoring viability than the one located in basic region I. The phenotypes of the mutants within the proline-rich region, furthermore, indicate that spatial considerations are involved. While the viability of the compensating frameshift mutants strongly argues that the amino acids in this region, with the exception of the proline residues, are not essential for efficient J protein function, single substitutions for the proline residues can confer cold-sensitive dominant phenotypes. Perhaps the proline residues function to optimize protein–DNA contacts between the two basic regions and the viral genome.

Mechanism of extragenic suppression

Radioactive DNA and protein extracts prepared from *J⁻3K → L II*-infected cells and analyzed by sucrose gradient sedimentation indicate that DNA packaging did occur. Particles sedimented at both 114S and 132S, the positions of the mature virion and the provirion, respectively. This material, however, was not infectious, suggesting that the mutant J protein somehow prevents the extrusion of DNA from the progeny capsids. Similar results were obtained by Aoyama and Hayashi (1985). In their experiments, ϕX174 was tested for the ability to package genome larger than unit length. The results of their experiments indicated that the capsid can package genomes as large as 101% and maintain infectivity. Above this value, genomes were still packaged but the resulting particles were not infectious. Interestingly, as the size of the genome increased, the recovery of the defective 90S particle increased as well. The results of the experiments presented here may be fundamentally related. What may be critical is the ratio of the total volume occupied by the viral genome to the total internal volume of the capsid. If this ratio exceeds a certain value, infectivity is lost. In the experiments of Aoyama and Hayashi (1985), there was an excess of packaged genome. In the experiments presented here, the genome may not have been sufficiently condensed by the mutant J proteins. Alternatively, the act of "genome gluttony" may produce "corpulent capsids" with perturbations on the coat protein surface which inhibit early stages of the viral life cycle.

The extragenic suppressors confer Ser → Phe or Ser → Pro substitutions at amino acid 1 of the viral coat protein. In a wild-type virion, this serine participates, via the γO, in three-way polar interactions directly atop the threefold axis of symmetry, interacting with serines 1 and 426 of neighboring proteins. The suppressing substitutions probably eliminate this interaction; the substituted sides contain no atoms capable of generating dipole moments. If a critical ratio between the total volume occupied by the viral genome to the total internal volume of the capsid must be maintained. The extragenic suppressors, through the elimination of three-way polar interactions, might compensate for a less condensed viral genome by expanding the capsid. Alternatively, if "overstuffing" the capsid leads to detrimental structural alterations on the surface of the viral coat, the elimination of the threefold interaction might restore the wild-type surface conformation. The atomic structure of the bacteriophage G4

capsid has recently been solved (McKenna *et al.,* 1996). Although the G4 genome is larger than the ϕX174 genome, its J protein contains fewer basic residues. Interestingly, the close range threefold interaction found in the ϕX174 structure is not present in the G4 capsid. To further investigate these phenomena, we are currently devising means to measure the packaging capacity of the defective J and extragenic suppressor mutants.

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