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The Virion Proteins Encoded by Bacteriophage φK and Its Host-Range Mutant φKhT: Host-Range Determination and DNA Binding Properties[1]

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The microvirid phage φK, specific for *Escherichia coli* K12, contains a circular single-stranded (SS) DNA in the icosahedral virion, which comprises four phage gene products, F (capsid), G (major spike), H (minor spike), and J (core). φKhT, a host-range mutant of φK, can grow on *E. coli* C and B, besides K12, and is more thermosensitive than the parental phage φK. Sequencing analysis revealed that the genome of φK and φKhT consists of 6,089 nucleotides (nt), and codes for eleven genes, whose sequences are similar to those of α3, φX174, and G4 infective to strain C. In φKhT, two nt had changed: one is in the gene G, resulting in replacement of the 75th codon Ala with Ser, and the other is at 67th codon of the gene H: Val to Ala. Chemically synthesized gene J protein composed of 23 amino acids (aa) binds to φK SS DNA more tightly than and preferentially over the host *E. coli* SS-DNA-binding protein (SSB). These results indicate that the two spike proteins G and H are involved in the determination of φK host-range, and support a model in which the gene J protein functions in packaging the viral SS DNA into the virion vesicle.

Key words: bacteriophage, DNA-binding protein, host-range, φK, virion proteins.

Various strains of microvirid (isometric) phages of *Escherichia coli* have been extensively used as probes for elucidation of various biochemical and genetic problems, including gene structure, DNA replication, transcription, and host-parasite relationship (*1, 2*). These phages contain a circular SS DNA in the icosahedral virion, which is composed of four phage gene products, F (capsid), G (major spike), H (minor spike), and J (core), and are classified into three major groups: (i) the first group consists of φX174, S13, and G6, (ii) the second contains G4, φC, and U3, and (iii) the third includes α3, φK, and St-1 (*2, 3*). φX174 and G4 can grow only on *E. coli* C, and α3 infects *E. coli* B as well as *E. coli* C, whereas φK and St-1 propagate on *E. coli* K12 (*3*). Total genome sequencing has been accomplished with three species φX174 (*4*), G4 (*5*), and α3 (*6*). Comparative studies have indicated that the three phages closely resemble each other, and code for the same series of eleven genes A, A*, B, C, K, D, E, J, F, G, and H. However, the details of the mechanism of the host-range determination are still unknown (*2*): no host-range mutants have so far been isolated from the phages φX174, G4, and α3 (*2*), and it is unclear which of virional protein(s) F, G, H, and/or J is determinant for the host-range (or adsorption).

As in φX174, G4, and α3 (*2*), the life cycle of φK consists of three successive stages (*3, 7-9*): (i) synthesis of parental double-stranded (RF) DNA (stage I), (ii) replication of progeny RF DNA by a rolling circle (RC) mechanism (stage II), and (iii) RC-type synthesis of progeny viral SS DNA driven by packaging into a virional vesicle composed of φK gene products F, G, and H (stage III or morphogenesis). The stages I and II depend upon host *E. coli* SSB (*7, 10*), whereas the stage III is thought to rely on φK gene J protein, instead of SSB (*11, 12*). However, the function of the gene J protein remains to be clarified in detail: *e.g.*, whether or not the gene J protein binds to SS DNA more preferentially over SSB.

In order to obtain further insight into role of the virion-forming proteins F, G, H, and J in determination of host-range and interaction with DNA, total genomes of φK and its host-range mutant φKhT were sequenced, and a 23-aa φK gene J protein was chemically synthesized. The results obtained in this study clearly indicate that the φK host-range is determined by the major (G) and minor (H) spike proteins, and that the synthetic J proteins bind to the viral SS DNA more tightly than and preferentially over SSB *in vitro*.

MATERIALS AND METHODS

Phages and Bacterial Strains—Bacteriophage φK and *E. coli* K12 W3110, B, C, and their lipopolysaccharide-defective (LPS) mutants (Table I) were from our laboratory stock (*3*). φK was multiplied in *E. coli* K12 W3110 (*7*). φKhT, which infects *E. coli* C and B as well as K12 (see below), is a host-range mutant spontaneously derived from φK, and was propagated on *E. coli* C or K12 W3110. Other phages, *E. coli* strains, and plasmids used in this study are

[1] Nucleotide sequence data have been registered with EMBL Data Library (accession number: X60323).

TABLE I. **Strains of *E. coli*, phages, and plasmids.**

Strain	Properties	Source/ Reference
E. coli bacteria		
K12 W3110	Delivative of K12	Lab. stock
C	Wild-type	Lab. stock
C61	LPS mutant of C	Lab. stock
C71	LPS mutant of C	Lab. stock
C23	LPS mutant of C	Lab. stock
BB	Wild-type	Lab. stock
BB1	LPS mutant of BB	Lab. stock
BB5	LPS mutant of BB	Lab. stock
XL1-blue	*supE44, hsdR17, recA1, endA1, gyrA46, thi, relA1, lac⁻, F'[proAB⁺, lacI^q, lacZΔM15, Tn10(tet^r)]*	(*19*)
JM109	*recA1, supE44, endA1, hsdR17, gyrA96, relA1, thi, Δ(lac-proAB), F'[traD36, proAB⁺, lacI^q, lacZΔM15]*	(*20*)
Phage		
φK	Wild-type	Lab. stock
φKhT	Host-range mutant of φK	This study
	Wild-type	Lab. stock
M13mp18	M13-derived vector for sequencing	(*21*)
M13mp19	M13-derived vector for sequencing	(*21*)
M13KO7	M13-derived helper phage for sequencing	(*22*)
Plasmid		
pUC18	2.7-kb, *E. coli* vector, Ap^r, *lacZ*	(*21*)
pUC118	3.2-kb, *E. coli* vector, Ap^r, *lacZ*	(*21*)
pUC19	2.7-kb, *E. coli* vector, Ap^r, *lacZ*	(*21*)
pUC119	3.2-kb, *E. coli* vector, Ap^r, *lacZ*	(*21*)

LPS, lipopolysaccharide.

summarized in Table I.

Enzymes and Biochemicals—Restriction enzymes, phage T4 DNA ligase, *E. coli* DNA polymerase I (Klenow fragment), and reagents used for dideoxy sequencing were purchased from CalBiochem. (USA), New England Biolabs (USA), Takara Shuzo (Kyoto), and Nippon Gene (Toyama). Buffers for the enzymes were as recommended by the manufacturers. [α-^{35}S]dCTP was from NEN (USA). All other materials used in this study were prepared as described previously (*6, 13, 14*).

DNA Sequencing Analysis—Cloning and sequencing of φK DNA was carried out essentially as described by Kodaira *et al.* (*6*). Restriction enzyme fragments of the φK RF DNA were cloned into sequencing vectors (M13mp18, M13mp19, pUC118, or pUC119) using *E. coli* strain XL1-blue or JM109 (Table I). SS and RF DNAs as a direct template for sequencing were prepared from these clones by standard methods (*15*). Sequencing was performed by the chain termination method (*16*) with universal and reverse vector primers.

Biological Assay—Biological properties of phages were examined as reported previously (*13*). For determination of heat stability, φKhT (or φK) propagated on *E. coli* C (or W3110) were incubated at 60°C in dilution fluid (*3*), and the surviving fractions were periodically titrated, using C or W3110 as the indicator. For eclipse assay, cells of *E. coli* C (or W3110) exponentially growing at 37°C in LB broth (A_{660}: 0.25) were mixed with chloramphenicol (50 μg/ml). After 10 min, phage was added (input multiplicity: 0.1) with $CaCl_2$ (final concentration: 5 mM). At intervals, an aliquot was removed, then treated with chloroform, and the remaining free phage was titrated on host strain C or W3110.

Chemical Synthesis of Protein—Gene J protein of φK was chemically synthesized using an Applied Biosystems Peptide Synthesizer (Model 430A) with a standard solid-phase peptide technique, and then purified by high-performance liquid chromatography. The 23-aa alignment synthesized in this study was KKARRSPSRRKGARLWYVGGSQF (Fig. 3A). The synthetic protein is soluble in H_2O or Tris-HCl buffer (pH 7.3), and can be stored stably at 4°C.

RESULTS AND DISCUSSION

Properties of the Host-Range Mutant φKhT—φK infects *E. coli* K12 derivatives but not strain C, differing in host-range from the related phages φX174, G4, and α3. Intact particles of φK were unable to be adsorbed onto cells of *E. coli* C, but the DNAs (SS and RF) efficiently transfect this strain upon Ca^{2+}-treatment or electroporation (data not shown). A host-range mutant φKhT was spontaneously obtained from φK at approximate frequency of 10^{-8} on *E. coli* C. Unlike the parental strain, φKhT can infect *E. coli* B besides C and K12 at almost the same efficiency; the rate of eclipse of φKhT on K12 W3110 was identical to that of φK (Fig. 1a). As to host-range, φKhT resembles α3 (*3*), and can be adsorbed onto LPS mutants of *E. coli* C (*e.g.*, C61 and C23) and B (BB1 and BB5). The plating efficiencies of φKhT on these LPS strains are shown in Table II, compared with those of φK and α3.

φKhT is somewhat unstable; the phage particles were more sensitive to heat treatment at 60°C in dilution fluid (*3*) than those of φK (Fig. 1b). The virion particles of φKhT are composed of four major proteins F, G, H, and J. By SDS polyacrylamide gel electrophoresis (PAGE), their molecular weights (MW) were estimated to be approximately 52.3, 35.8, 21.8, and 2.8 kDa, respectively. These values are identical to those of the parent φK (*14*).

Genome Structures of φK and φKhT—To identify structural genes of the four virion-forming proteins (F to J), the total genomes of φK and φKhT were sequenced. After establishment of restriction enzyme cleavage maps (data not shown), appropriate restriction fragments of φK (φKhT) were cloned into the sequencing vector using *E. coli* strain XL1-blue or JM109 (Table I). SS and RF DNAs were prepared from the resulting recombinant clones, and their sequences were determined from both strands of the viral and complementary DNA by the chain termination method (*16*).

The circular SS DNA of φK (φKhT) consists of 6,089 nt (EMBL Data Library accession number X60323), and is 2, 53, and 703 nt longer than the genome of α3 (*6*), G4 (*5*), and φX174 (*4*), respectively. As in the three phages, φK has eleven homologous genes (A, A*, B, C, K, D, E, J, F, G, and H), each of which is encoded in the viral strand DNA, preceded by a possible ribosome-binding sequence (*17*). The gene organization of φK thus established is illustrated in Fig. 2, compared with that of α3, φX174, and G4. As a whole, φK shows an extensive sequence homology (89.3%) with α3, and moderately resembles φX174 and G4 (approximately 50% homologies). In Fig. 3, the deduced aa alignments of φK virion-forming proteins, F (capsid), G (major spike), H (minor spike), and J (core), are shown in comparison with those of α3, φX174, and G4.

Properties of the Virional Proteins F, G, H, and J—Like

α3 (*6*), ϕK gene F codes for a 431-aa protein, which is 4 residues longer than that of ϕX174 (*4*) and G4 (*5*). The capsid (F) protein is highly conserved among the four microvirid species (Fig. 3B): the degree of aa similarity is 91.2% between ϕK and α3, 70.8% between ϕK and ϕX174, and 63.1% between ϕK and G4 (66.0% between ϕX174 and G4). Despite the similarity, the three phages α3, ϕX174, and G4 can not substitute ϕK gene F protein for their own protein *in vivo* (*6*).

Among the four virion-forming proteins, the major spike protein (G) is most divergent (Fig. 3C). The ϕK gene G protein consists of 187 aa, like that of α3 (*6*), and is 12 and 10 residues longer than that of ϕX174 (*4*) and G4 (*5*), respectively. Concerning aa arrangement, ϕK is closely related to α3 with 88.2% similarity, and is relatively remote from ϕX174 and G4 with 31.6 and 28.3% coincidence, respectively (42.0% between ϕX174 and G4). The ϕK gene G product functions in α3 *in vivo*, but not in ϕX174 or G4 (*6*).

ϕK codes for a 332-aa gene H protein, which is 2 and 4 residues longer than that of α3 (*6*) and ϕX174 (*4*), respectively, and 5 residues shorter than that of G4 (*5*). The aa conservation among the four minor spike proteins is as follows (Fig. 3D): 97.3% between ϕK and α3, 77.6% between ϕK and ϕX174, and 71.7% between ϕK and G4 (65.5% between ϕX174 and G4). Complementation of the gene H proteins has not been detected between ϕK (α3) and ϕX174 (G4)(*6*).

In order to elucidate the molecular basis of host-range determination, the total genome sequence of ϕKhT was compared with that of ϕK; ϕKhT has two nt changes (Fig. 4). One is from G (ϕK) to T (ϕKhT), located at the 223rd nt in the gene G. Consequently, the 75th aa of the ϕKhT

TABLE II. **Plating efficiency of ϕKhT.** Cells of *E. coli* were infected with ϕKhT, ϕK, or α3, and plated at 37°C. *E. coli* strains used were as indicated in Table I. Relative plaque yield is presented.

Strain	Phage		
	ϕK	ϕKhT	α3
E. coli K12			
W3110	1.0	8.2×10^{-1}	$<1.0\times10^{-5}$
E. coli C			
C	6.3×10^{-5}	1.0	1.0
C61	$<1.0\times10^{-5}$	1.0	5.8×10^{-1}
C71	$<1.0\times10^{-5}$	$<1.0\times10^{-5}$	$<1.0\times10^{-5}$
C23	1.1×10^{-5}	6.7×10^{-1}	7.6×10^{-1}
E. coli B			
BB	4.3×10^{-5}	2.3×10^{-1}	8.9×10^{-3}
BB1	1.1×10^{-7}	2.4×10^{-1}	$<1.0\times10^{-5}$
BB5	$<1.0\times10^{-5}$	3.0×10^{-1}	1.1×10^{-2}

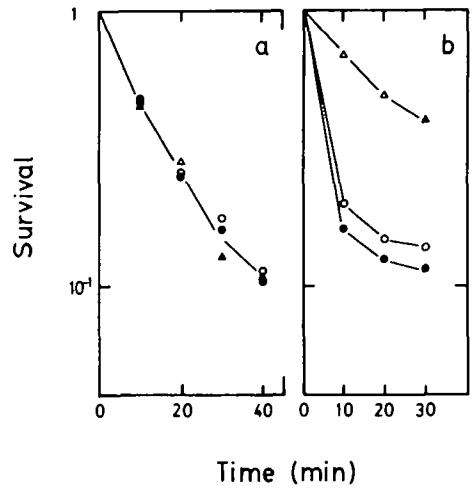

Fig. 1. **Eclipse (a) and heat stability (B) of ϕKhT mutant.** (a) *E. coli* C (or W3110) cells exponentially growing at 37°C in LB broth were mixed with chloramphenicol (50 μg/ml). After 10 min, phage was added at a multiplicity of infection of 0.1, together with $CaCl_2$ (final concentration: 5 mM). At the indicated time, an aliquot was removed and treated with chloroform, and the remaining free phage was titrated on C (or W3110). ○, ϕKhT on C; ●, ϕKhT on W3110; △, wt on W3110. (b) phage particles propagated on *E. coli* C were incubated at 60°C in dilution fluid and the surviving fractions were periodically determined, using C and K12 W3110 as the indicator. ○, ϕKhT on C; ●, ϕKhT on W3110; △, wt on W3110.

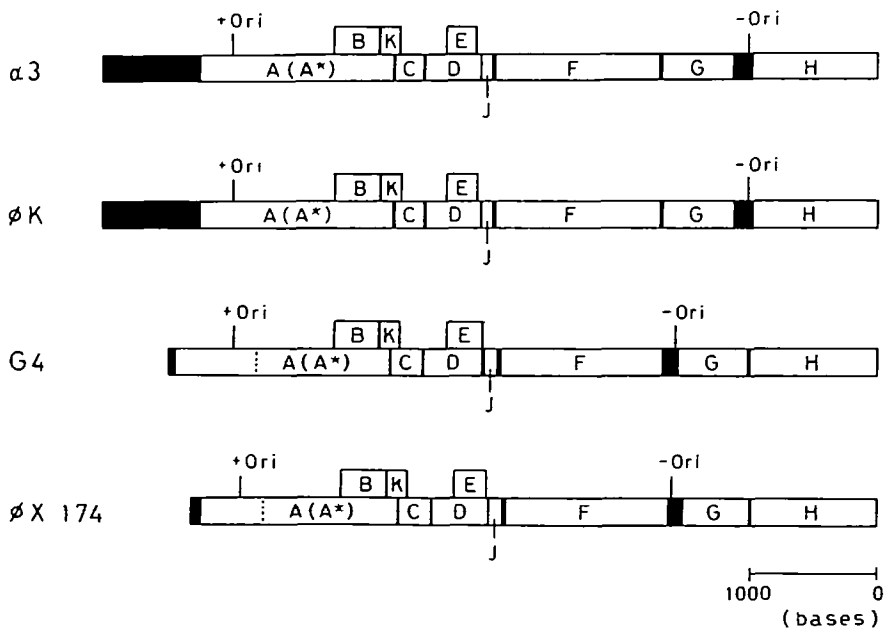

Fig. 2. **Genetic maps of the genomes of α3, ϕK, G4, and ϕX174.** The genetic maps of α3, ϕK, ϕX174, and G4 are drown to scale from their nucleotide sequences: α3 from Kodaira *et al.* (6), ϕK from this study, G4 from Godson *et al.* (*5*), and ϕX174 from Sanger *et al.* (*4*). The two replication origins for viral and complementary strand are shown as $+ori$ and $-ori$, respectively (6). The dotted lines in gene A of G4 (*5*) and ϕX174 (*4*) indicate the putative starting positions for gene A*.

Fig. 3 (Continued on next page)

minor spike protein is altered to Ser from Ala in φK; the other one, from T (φK) to C (φKhT), is at the 200th nt in the gene H: the aa replacement of minor spike protein (H) is from Val (φK) to Cys (φKhT). In the capsid (F) and core (J) proteins, φKhT has no such aa changes. These sequencing results clearly demonstrate that φKhT is a double mutant bearing two missense mutations in genes G and H, suggesting that, at least in φK, the two spike proteins G and H are involved in the host-range determination. In α3 as well (*13*), comparable results have been obtained; three missense strains of gene H (from Ile to Val at 56th aa, from Gly to Cys at 69th aa, and from Leu to Phe at 71st aa) showed altered heat sensitivities and eclipse rates. These facts suggest that the N-terminal domain of the gene H protein (see Fig. 3D) is important for functional and/or structural interaction among virional proteins F, G, H, and J (see below). Certain host-range mutants of φX174 also have alterations in genes G and H (*18*).

In order to discern the contributions of gene G and H to the altered host range and heat stability, phage strains having single missense mutations were constructed by exchanging a *Ppu*M1 restriction fragment of 876 bp (from nucleotide 4059 to 4934, containing the mutated region of gene G), between φKhT and wild-type φK. Thus, each RFI DNA was cut into two fragments and purified by repeated agarose gel electrophoresis. Ligation of the wild-type large fragment with the φKhT G segment resulted in an RFI molecule having a single missense G mutation. Conversely, ligation of the φKhT large fragment with the wild-type G segment yielded an RF molecule with a single missense H

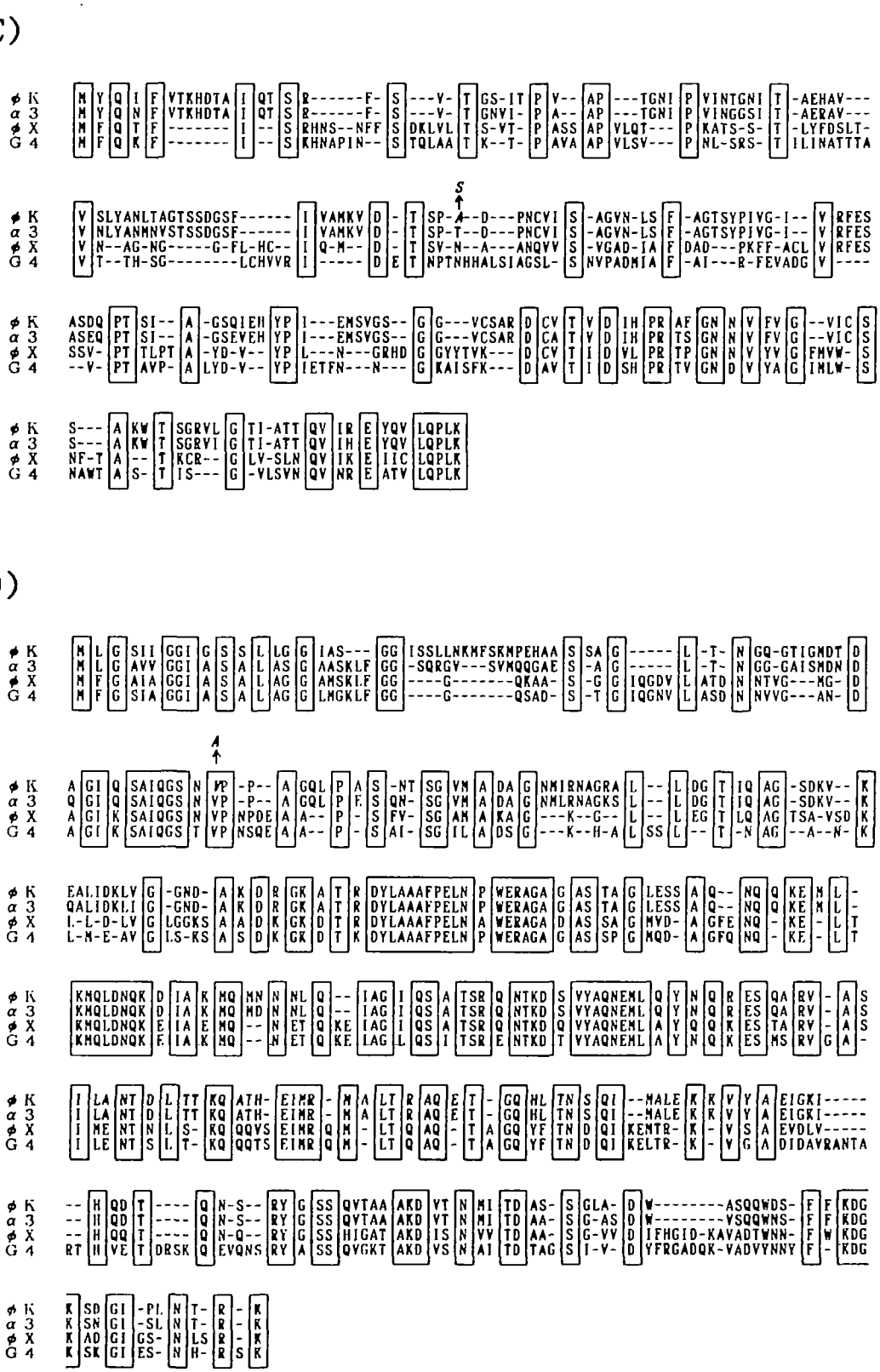

Fig. 3. **Comparisons of the ϕK virional proteins J, F, G, and H with those of α3, ϕX174, and G4.** Amino acid sequences of ϕK, α3, ϕX174, and G4 are from this study, Kodaira *et al.* (*6*), Sanger *et al.* (*4*), and Godson *et al.* (*5*), respectively: A, core (J) protein; B, capsid (F) protein; C, major spike (G) protein; D, minor spike (H) protein. Dashes represent gaps inserted to optimize the protein alignment. Conserved aa residues through the four phage species are boxed. The aa alignment of synthetic gene J protein (J-chem, see text) is shown in (A). Amino acid changes found in the G and H proteins of ϕKhT are indicated by ↑.

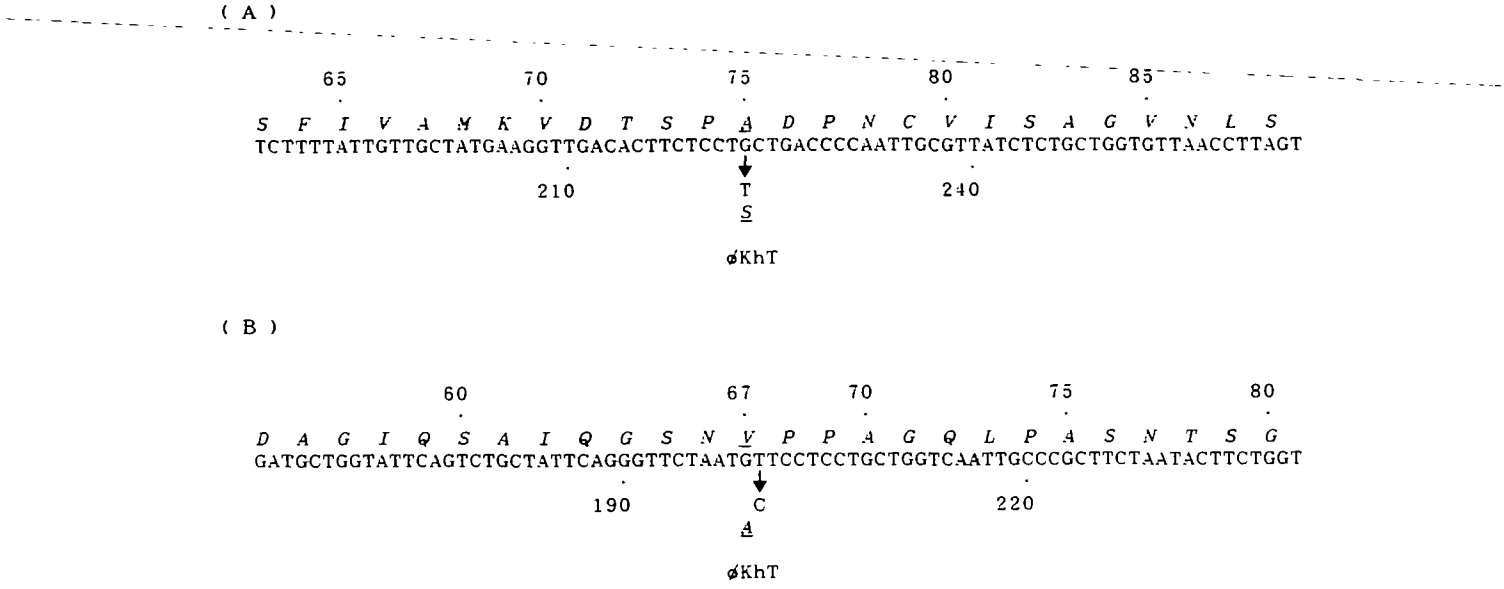

Fig. 4. **Nucleotide sequences of the genes G and H of φK and φKhT.** Relevant parts of nucleotide and amino acid sequences are presented. The nucleotide numbers of the genes G (A) and H (B) are from their starting codons, ATG. The sites of the changed nucleotides and amino acids in φKhT are indicated by the arrows (↓).

mutation. After Ca^{2+}-dependent transfection and single plaque isolation, the properties of the missense G (msG) strain and the missense H (msH) were tested.

In *E. coli* C and B, plating efficiency of the msG strain was, like that of the wild-type φK, negligibly low, whereas the msH mutant infected *E. coli* C at the relative efficiency of 10^{-3} of K12 W3110, and easily reached the level of φKhT by further propagation on C cells. As to heat stability at 60°C, the msG strain was indistinguishable from wild-type φK. The heat inactivation profile of the msH mutant was complex: an initial slow decline was followed by a rapid loss of infectivity, suggesting that the msH phage preparation is somewhat heterogenous. When the msH mutant was repeatedly propagated on *E. coli* C, the progeny exhibited a heat inactivation profile similar to that of φKhT. [As can be seen in Fig. 1b, the φKhT preparation contained a minor fraction relatively resistant to the heat treatment. The ratio of this fraction increased during storage at 4°C, accompanied with gradual loss of bulk infectivity. The nature of this change (confomational?) is presently unknown.]

These results suggest that plaques of φK initially formed on *E. coli* C were relatively heat-stable msH type with lower plating efficiency on this host, and φKhT, which is the *msH msG double mutant thermosensitive* and highly infective to C, was derived from the msH strain during propagation on C. At any rate, cells of *E. coli* C and B were rather insensitive to the single mutants of msH or msG type.

Like the three phages (α3, φX174, and G4), the φK gene J codes for a small and highly basic core protein of 24-aa identical to that of α3 (*11*), which is shorter than that of φX174 (*4*) composed of 38 aa and G4 of 25 aa (*5*). The four gene J proteins are structurally similar (Fig. 3D): (i) a basic N-terminal half rich in Lys and Arg (albeit diverged in aa sequence), (ii) a conspicuously conserved C-terminal half, whose identical decapeptide KGARLWYVGG is thought to be a DNA-binding domain (R. McKenna *et al.*, personal communication), and (iii) a C-terminal aromatic residue (Phe in φK, α3, and φX174; Tyr in G4). As reported previously (*6*), α3 can use the φK gene J product *in vivo*, but not that of G4 or φX174, and *vice versa*.

These results on the four virional proteins (F to J) indicate that φK and α3 are functionally related, and remote from φX174 and G4, whereas φX174 and G4 are evolutionally closer.

DNA-Binding Properties of the Gene J Protein—The detailed structure and function of the gene J protein are still unknown, because of difficulty in preparation of the gene J protein from phage particles in sufficient amounts for analysis *in vitro*.

To investigate DNA-binding properties, the gene J protein of φK was chemically synthesized as described in "MATERIALS AND METHODS." The synthetic protein (termed J-chem) is 23 aa long, with the sequence shown in Fig. 3A; in the present synthesis, the N-terminal residue Met was omitted, according to a previous report (*4*) that the gene J protein isolated from φX174 particle was devoid of the N-terminal residue. The J-chem protein with a calculated MW of 2.7 kDa is soluble in H_2O as well in Tris-based buffers (*e.g.*, 50 mM Tris-HCl, pH 7.3), and shows a random structural circular dichroism profile with no significant bands. Its electrophoretic mobility on a 17% SDS-polyacrylamide gel is the same as that of the natural gene J protein isolated from φK particle (data not shown).

Binding of the J-chem protein to φK circular SS DNA was examined by gel retardation analysis. SS DNA (0.2 μg) was mixed with various amounts of J-chem ranging from 0 to 0.2 μg in a reaction buffer (50 mM Tris-HCl, pH 7.3), incubated at 25° C for 30 min, and then electrophoresed on a 1% agarose gel. As shown in Fig. 5 (lanes 1-4), SS DNA incubated with J-chem moved in front of the naked SS DNA, faster than expected from the mass of the complex, and its electrophoretic mobility was accelerated with

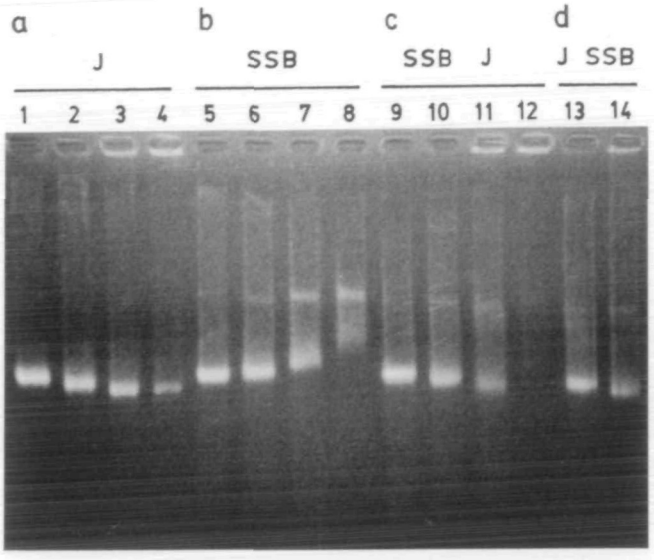

Fig 5 **Characterization of *in vitro* interaction between chemically synthesized gene J protein (J-chem) and ϕK DNA.** a: ϕK SS DNA (0.2 μg) was mixed with J-chem (lane 1, 0 μg, lane 2, 0.05 μg; lane 3, 0.1 μg; lane 4, 0.2 μg), incubated at 25°C for 30 min, and each mixture was subsequently electrophoresed on a 1% agarose gel. b: ϕK SS DNA (0.2 μg) was mixed with SSB (lane 5, 0 μg; lane 6, 0.1 μg; lane 7, 0.2 μg, lane 8, 0.3 μg) and analyzed as in (a). c: ϕK SS DNA (0.2 μg) was incubated with SSB (0.1 μg) for 30 min at 25°C, and then J-chem (lane 9, 0 μg; lane 10, 0.05 μg; lane 11, 0.1 μg; lane 12, 0.2 μg) was added. After further incubation for 30 min, each mixture was analyzed as in (a). d: ϕK SS DNA (0.2 μg) was incubated with J-chem proteins (lane 13, 0.05 μg, lane 14, 0.1 μg) for 30 min at 25°C, and then SSB (0.1 μg) was added. After further incubation for 30 min, each mixture was analyzed as in (a).

increasing J-chem concentration. Preliminary studies with fluorescence spectroscopy have shown that an SS DNA molecule of ϕK seems to be saturated by the J-chem proteins at a weight ratio (J-chem/SS DNA) of 1.0, that is, approximately 600 molecules of J-chem cover one SS DNA. In addition, J-chem bound to ϕK RF DNA tightly, but the complex moved more slowly than the naked RF DNA, as in *E. coli* SSB (data not shown). These results clearly indicate that the gene J proteins bind to both of SS and RF DNAs, and selectively condense SS DNA, but not RF DNA, into a compact form. Hamatake *et al.* (*12*) reported that gene J proteins purified from ϕX174 particles have an ability to bind to RF as well as to SS DNA.

Binding of SSB to ϕK SS DNA was examined under the same conditions as used for J-chem. SS DNA (0.2 μg) in the presence of SSB (ranging from 0 to 0.3 μg) moved more slowly than the naked SS DNA, and its mobility decreased with increasing SSB (Fig. 5, lanes 5-8), although two complexes appeared: their conformations are presently unknown. These results suggest that SSB binds tightly to SS DNA, but the complex is not so compact as to move faster than the naked SS DNA on the gel. To clarify whether SSB is replaced by gene J protein in morphogenesis, competition experiments between J-chem and SSB were performed. ϕK SS DNA (0.2 μg) was incubated with SSB (0.1 μg) in the reaction buffer at 25°C for 30 min, and then J-chem (from 0.05 to 0.2 μg) was added. After further incubation for 30 min, the mixtures were electrophoresed on a 1% agarose gel. As presented in Fig. 5 (lanes 9-11), SSB associated with SS DNA is substantially replaced by J-chem.

TABLE III **Infectivity of ϕK DNA treated with J-chem or SSB.** Single- or double-stranded DNA of ϕK was mixed with synthetic gene J protein (J-chem) or SSB in 50 mM Tris-HCl (pH 7.3), incubated at 25°C for 30 min, and transfected to Ca^{2+}-treated *E. coli* K12 W3110 (*7*).

Protein	(μg)	Relative titer	
		SS-DNA (0.2 μg)	DS-DNA (0.4 μg)
None		1.0	1.0
J-chem	0.1	2.4×10^{-2}	1.0
	0.2	5.0×10^{-3}	1.0
	0.3	$<1.0\times10^{-3}$	1.0
	0.4	—	1.0
	0.5	—	1.0
SSB	0.1	5.5×10^{-1}	1.0
	0.2	1.7×10^{-1}	1.0
	0.3	7.0×10^{-2}	1.0
	0.4	$<3.0\times10^{-3}$	1.0
	0.5	—	1.0

Infectivity of SS or RF DNA complexed with J-chem was investigated by Ca^{2+}-dependent transfection assay (*7*). As shown in Table III, infectivity of ϕK SS DNA was markedly reduced in the presence of J-chem as well as SSB, although the latter was less inhibitory than J-chem, whereas transfecting activity of ϕK RF DNA was not prevented by J-chem or SSB.

The present *in vivo* and *in vitro* observations thus strongly indicate that ϕK nascent SS DNA preferentially binds to gene J (core) protein, replacing host SSB, and hence is packaged into a virional vesicle composed of three gene proteins F (capsid), G (major spike), and H (minor spike). It is also evident that two spike proteins G and H determine the host-range of ϕK, through still unidentified protein-protein and protein-LPS interaction(s). Taking advantage of the ϕK host-range mutant and synthetic gene J protein, further studies on structural and functional interactions among the virion-forming proteins are in progress.

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