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Regulation of Filamentous Bacteriophage Length by Modification of Electrostatic Interactions Between Coat Protein and DNA

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Bacteriophage fd gene VIII, which encodes the major capsid protein, was mutated to convert the serine residue at position 47 to a lysine residue (S47K), thereby increasing the number of positively charged residues in the C-terminal region of the protein from four to five. The S47K coat protein underwent correct membrane insertion and processing but could not encapsidate the viral DNA, nor was it incorporated detectably with wild-type coat proteins into hybrid bacteriophage particles. However, hybrid virions could be constructed from the S47K coat protein and a second mutant coat protein, K48Q, the latter containing only three lysine residues in its C-terminal region. K48Q phage particles are approximately 35% longer than wild-type. Introducing the S47K protein shortened these particles, the S47K/K48Q hybrids exhibiting a range of lengths between those of K48Q and wild-type. These results indicate that filamentous bacteriophage length (and the DNA packaging underlying it) are regulated by unusually flexible electrostatic interactions between the C-terminal domain of the coat protein and the DNA. They strongly suggest that wild-type bacteriophage fd makes optimal use of the minimum number of coat protein subunits to package the DNA compactly.

The class I filamentous bacteriophage fd which infects *Escherichia coli* (others are M13 and f1) is a flexible particle about 890 nm long and 7 nm in diameter, composed of a circular single-stranded DNA genome (6408 nucleotides) enclosed within a tubular protein sheath (for reviews, see Marvin, 1978; Webster & Lopez, 1985; Model & Russel, 1988). There are five types of protein subunit in the capsid, four of which are minor coat proteins, the products of viral genes III, VI, VII and IX. Only a few copies of each of the minor coat proteins are present and these are arranged with the gene III and VI proteins at one end of the phage particle and the gene VII and IX proteins at the other (Grant *et al.*, 1981; Simons *et al.*, 1981; Webster *et al.*, 1981). The major coat protein is the product of gene VIII and approximately 2700 copies form a cylindrical structure around the DNA. The inner diameter of the protein tube is about 3·4 nm and the viral DNA is contained within this space, extending throughout the length of the particle (Makowski, 1984; Day *et al.*, 1988).

The major coat protein of fd, which is largely α-helical and contains 50 amino acid residues, is synthesized as a procoat molecule containing a leader-peptide of 23 residues (Fig. 1). Positively charged residues in the leader-peptide and in the C-terminal region of the coat protein are involved in inserting the procoat into the inner membrane of the host cell (Kuhn *et al.*, 1986; Wickner, 1988; Greenwood & Perham, 1989). After insertion, the leader-peptide is removed by leader-peptidase, leaving the mature protein spanning the membrane ready for bacteriophage assembly. The mature coat protein contains three domains, a negatively charged N-terminal domain, a hydrophobic central domain and a positively charged C-terminal domain. In addition to being involved in the membrane insertion, the C-terminal domain points towards the viral DNA when the coat protein is

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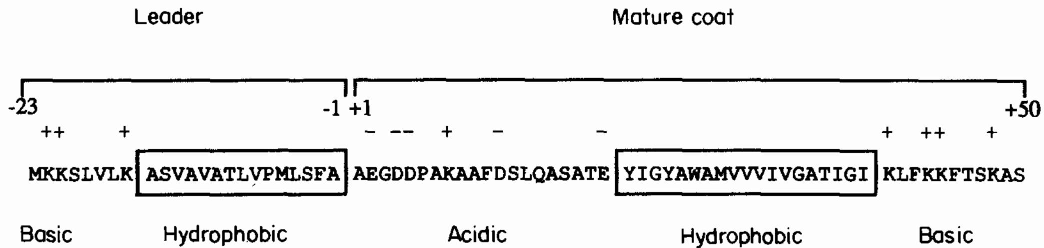

Figure 1. Primary structure of the procoat from bacteriophage fd. The amino acid sequence is inferred from the gene VIII nucleotide sequence for fd (Beck *et al.*, 1978). The hydrophobic domains that span the membrane are indicated by a box. Positively and negatively charged side-chains are indicated by + and −, respectively.

assembled into a mature phage particle, suggesting that its positive charges are involved in neutralizing the negatively charged phosphodiester links of the DNA (Marvin, 1978; Boeke *et al.*, 1980; Armstrong *et al.*, 1983).

Site-directed mutagenesis has been used to acquire important information about the mechanism of DNA packaging. The positive charge on lysine 48 in the coat protein is not essential for bacteriophage assembly, but its loss leads to an elongated particle in which commensurately fewer nucleotides are packaged per protein subunit (Hunter *et al.*, 1987). Conversion of lysine 48 to a negatively charged glutamate residue (K48E) does not permit viral assembly, but hybrid phage particles can be generated that contain both wild-type and K48E mutant coat proteins (Rowitch *et al.*, 1988). These viruses exhibit various lengths ranging up to twice that of the wild-type, strongly suggesting that as the average positive charge contributed by the C-terminal region of the coat protein falls, the DNA is forced to become essentially fully extended. These experiments all point to unusual flexibility in the DNA–protein interaction in these phages, an interaction dominated by electrostatic forces (Hunter *et al.*, 1987; Rowitch *et al.*, 1988).

An obvious question to ask is whether one or more additional positive charges in the C-terminal region of the fd coat protein would lead to more DNA packaged per unit length of viral filament and thus to commensurately shorter virions. An attempt to convert serine 47 to a lysine residue (S47K) proved unsuccessful, the mutation of the viral DNA not leading to a viable phage particle (G. J. Hunter, D. H. Rowitch & R. N. Perham, unpublished work). Serine 47 was chosen because of its proximity to lysine 48, already the subject of several successful mutations (Hunter *et al.*, 1987). By generating the S47K mutation in a different vector and expressing the mutant gene VIII from a plasmid, we have now been able to investigate the effects of the mutation without the need for accompanying phage viability.

The oligodeoxyribonucleotides S47K (d(TTGCTTTCTTGGTGAATTTCT)) and SELP (d(AATAAAGCCACAGAGCATA)) were synthesized by the method described by Mathes *et al.* (1984). Mutagenesis was performed in the vector pEf8P′, as described by Rowitch *et al.* (1988). The construct containing the mutated gene was designated pES47K. The mutant fd coat protein gene was cloned into the *E. coli* expression vector pKK223-3 by reconstituting it from two fragments (Rowitch *et al.*, 1988). The 5′ end of the gene was derived from the wild-type expression vector, pKf8P, and the 3′ end of the gene was excised from pES47K (Fig. 2). The construct was designated pKS47K.

E. coli TG1 cells transformed with pKS47K plasmid DNA were grown to an A_{600} value of 0·3 and were then induced with isopropyl-1-thio-β-D-galactoside (IPTG) (1 mM), as described by Rowitch *et al.* (1988). After various time intervals, samples were removed for analysis by means of urea/SDS/polyacrylamide gel electrophoresis (Simons *et al.*, 1979). A protein with mobility similar to that of the wild-type protein was observed after induction, and the levels of this protein increased with time. The identity of the plasmid-encoded product was further confirmed by means of an immunoblot employing antibody raised against bacteriophage fd (Rowitch *et al.*, 1988). Thus, it is clear that the S47K replacement in the C-terminal domain of the coat protein did not prevent membrane insertion or correct processing of the procoat molecule.

The ability of plasmid-encoded coat protein to participate in virus assembly was investigated by measuring the plaque-forming ability of bacteriophage R252 on uninduced and IPTG-induced cultures of *E. coli* HB2151 transformed with pKS47K or with the related plasmid pKf8P. The latter expresses the wild-type procoat (Rowitch *et al.* 1988). Bacteriophage R252 (Moses & Horiuchi, 1982) is an engineered class I (f1) bacteriophage containing the *am8H1* amber mutation in gene VIII in the codon encoding the amino acid at position 2 of the mature coat protein (originally derived from bacteriophage M13). It grows on the permissive *E. coli* strain K37 (*supI*) but not on non-suppressor strains, e.g. *E. coli* HB2151 (*su*⁻). The plaque-forming ability of bacteriophage R252 was raised by six orders of magnitude on induction of pKf8P-transformed cells, as noted by Rowitch *et al.* (1988), but the growth of bacteriophage R252 on *E. coli* HB2151 cells transformed with pKS47K was unaffected. The mutated coat protein is therefore unable to provide for the assembly of a viable phage particle.

Bacteriophage K48Q is a mutant of bacterio-

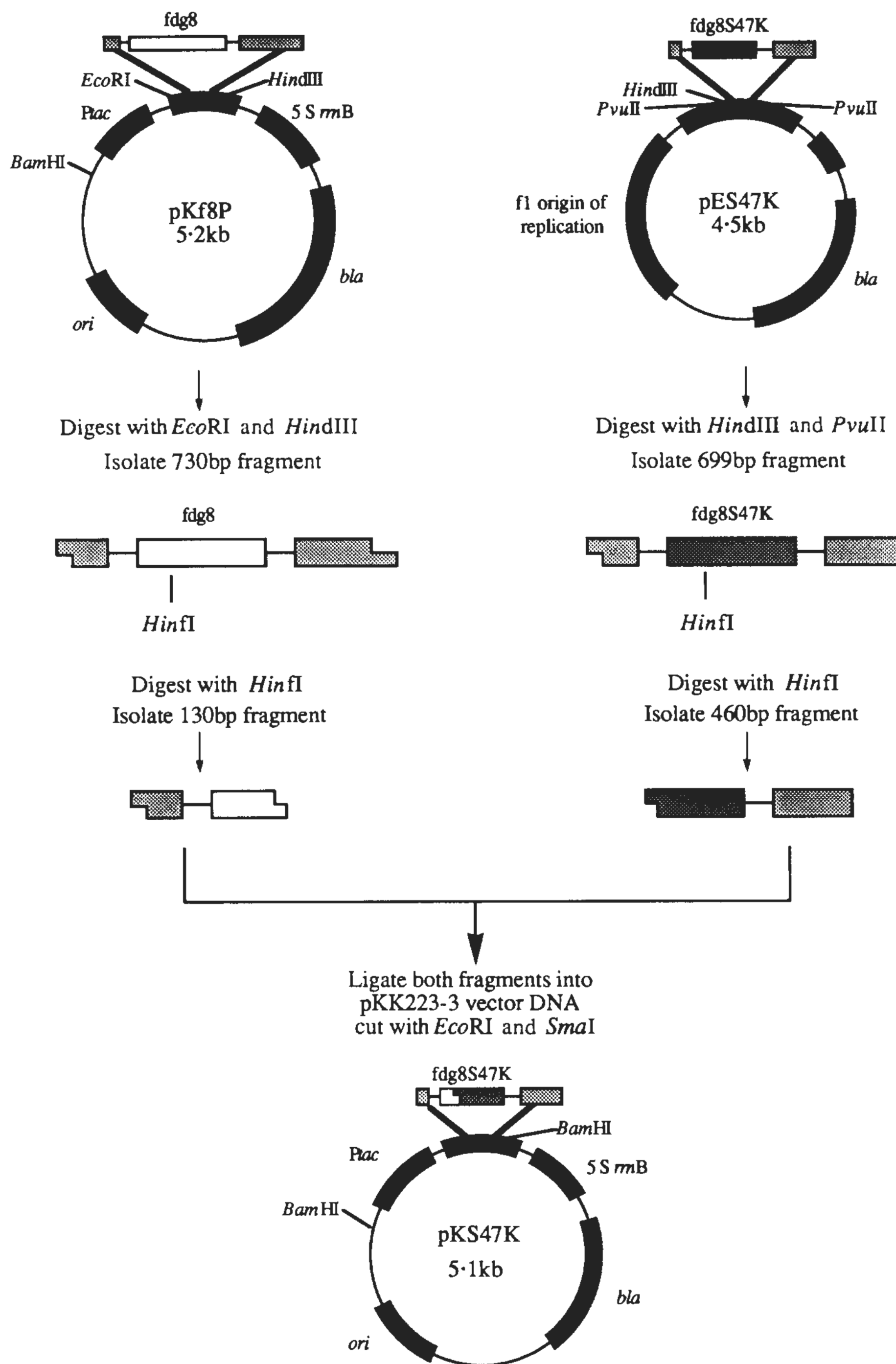

Figure 2. Construction of the plasmid pKS47K. DNA was digested with the enzymes indicated, fragments were isolated after polyacrylamide electrophoresis and ligated, as described by Rowitch *et al.* (1988). kb, 10^3 base-pairs; bp, base-pairs.

phage fd with only three positively charged residues in the C-terminal region of the coat protein and a corresponding increase (35%) in particle length. It also migrates more slowly on electrophoresis in 2% (w/v) agarose gels than does the wild-type virus (Hunter *et al.*, 1987). By growing wild-type bacteriophage fd on cells simultaneously expressing the K48Q protein, some mutant coat protein can be incorporated into the viral capsids and hybrid virions created. The hybrids have electrophoretic mobilities that vary between those of the pure K48Q mutant and wild-type fd (Rowitch *et al.*, 1988). When bacteriophage fd was grown on cells expressing the S47K protein from plasmid pKS47K, the progeny virions appeared to exhibit a slight decrease in electrophoretic mobility compared with wild-type fd, but the effect was small, suggesting little or no incorporation of the S47K protein. Similarly, peptide maps prepared from chymotryptic digests of the coat protein from the putative hybrid

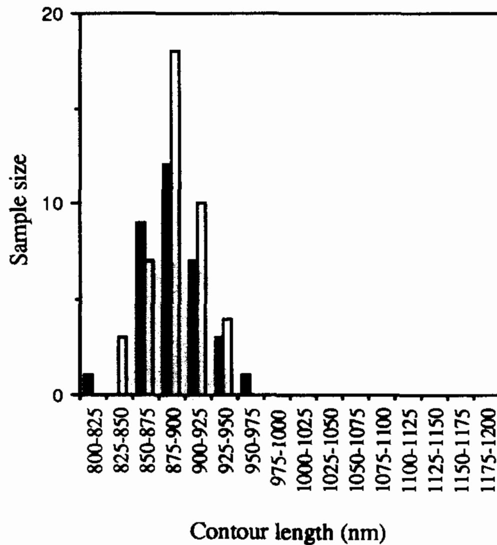

Figure 3. Particle contour lengths of fd/pKS47K hybrid phages compared with wild-type bacteriophage fd. Phages were examined by electron microscopy and the contour lengths (in nm) were measured directly from photographic negatives using a Graf/Bar sonic digitizer (Hunter *et al.*, 1987). Phage lengths were divided into categories within a range of 25 nm, e.g. all lengths from 900 to 925 nm formed one category. Data are displayed on histograms. Blocks of phage lengths: (■) fd; (□) fd/pKS47K.

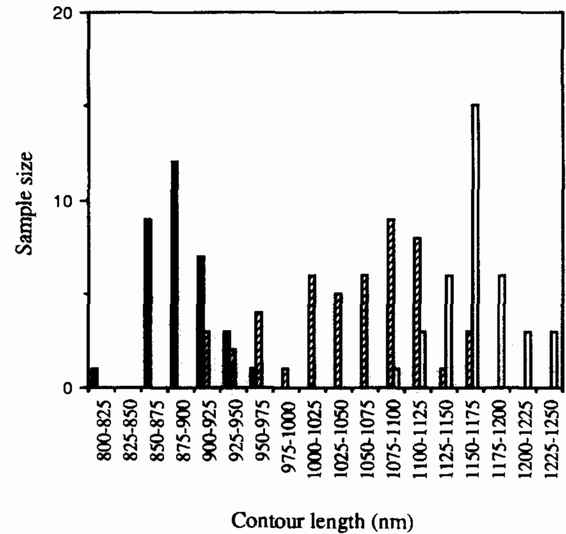

Figure 4. Particle contour lengths of K48Q/pKS47K hybrid phages (▨) compared with wild-type bacteriophage fd (■) and phage K48Q (□). Phages were examined by electron microscopy and the contour lengths (in nm) were measured and analysed as described in the legend to Fig. 3.

virions (Armstrong *et al.*, 1983; Rowitch *et al.*, 1988) revealed little or none of the peptide TKKAS characteristic of the S47K protein. A further test came from the examination of the virions in the electron microscope (Rowitch *et al.*, 1988). No shortening was detected (Fig. 3), and no other structural changes were noted.

In bacteriophage K48Q, the DNA is thought to be stretched out to follow a path about 35% longer than it does in wild-type fd. If the effect of the S47K coat protein were to compress the DNA back towards the wild-type length, it was argued that a hybrid phage capsid might be made which incorporated the K48Q protein with three lysine residues and the S47K protein with five. The above attempts to generate hybrid bacteriophages were therefore repeated with bacteriophage K48Q replacing bacteriophage fd. The progeny bacteriophage particles were prepared and analysed for changes in composition and structure. When subjected to agarose gel electrophoresis, the putative K48Q/S47K hybrid phages ran as a smear, with mobilities ranging from that of K48Q to approach that of wild-type fd (data not shown). This was a strong indication that hybrid bacteriophage particles had been produced. Further, peptide maps of chymotryptic digests of the coat proteins from the putative hybrids revealed two peptides, one (C3Q) identified (by amino acid analysis) as having the sequence TSQAS (derived from protein K48Q) and the other (C3K) as TKKAS (derived from protein S47K).

A sample of the putative K48Q/S47K hybrid bacteriophages was examined by electron microscopy. A wide range of contour lengths was observed (Fig. 4), from that of bacteriophage K48Q (1170 nm) down almost to that of wild-type fd (886 nm). The average length was 1046 nm, which is 18% longer than wild-type fd. Given that the pure K48Q bacteriophage particle is 35% longer than wild-type fd, the increase in length had been reduced by 44%. On the simple assumption of a direct relationship between the lengths of the phage particles and the number of positively charged residues in the C-terminal region of the coat protein, it can be calculated that, on average, the hybrids contained 22% S47K coat protein. This ratio of S47K to K48Q coat protein is consistent with the relative intensities of the spots derived from peptides C3K and C3Q on the peptide maps. The incorporation of the S47K protein clearly causes a decrease in length of the bacteriophage particle.

The S47K mutant protein is evidently capable of engaging at least in the elongation phase of filamentous bacteriophage assembly, with its associated protein–protein and protein–DNA interactions, but cannot sustain assembly on its own. There is a plausible explanation for these results. The filamentous bacteriophages are flexible hollow tubes, lined with positive charge, in which the single-stranded DNA is packaged without sequence specificity save that required in the helical hairpin loop that initiates assembly at the inner cell membrane (Ikoku & Hearst, 1981; Webster *et al.*, 1981; Dotto *et al.*, 1981; Dotto & Zinder, 1981; Russel & Model, 1989). Within this tube the negatively charged DNA must find its own spatial arrangement in accord with the positive-charge density created by the C-terminal regions of the coat protein subunits (Hunter *et al.*, 1987; Rowitch *et al.*, 1988; Day *et al.*, 1988). It is reasonable to suppose, therefore, that

the S47K mutant coat protein can be incorporated into hybrid particles with the K48Q mutant of bacteriophage fd because the higher positive-charge density of its C-terminal region offsets the lower positive-charge density of the C-terminal region of the K48Q protein, the length of the particle (and of the encapsidated DNA) reverting towards that of wild-type fd.

The nominal inner radius of the protein tube is thought to be about 1·7 nm, with the DNA necessarily filling a cylinder more than 1·4 nm in radius and extending in the virus to about 1·7 nm radius (Makowski, 1984; Day *et al.*, 1988). By analogy with the K48Q mutation, which causes a 35% increase in virion length (Hunter *et al.*, 1987), the additional positive-charge in the C-terminal region of the S47K coat protein might be expected to cause a significant shortening of the particle. If it were simply proportional to the number of positively charged side-chains in the C-terminal region of the coat protein, and there is no evidence to support or deny this (Hunter *et al.*, 1987; Rowitch *et al.*, 1988), a shortening of about 20% would be predicted. It is unlikely that the cylindrical hole in the virion could physically accommodate two antiparallel but non-base-paired strands of DNA packed significantly more densely than they are in wild-type bacteriophage fd. We infer that the S47K mutant coat protein may not be incorporated at detectable levels into hybrid phage particles with wild-type bacteriophage fd, because its presence would force too tight a packaging of the viral DNA.

In conclusion, it is worth noting the evolutionary implications of our results. In the interests of economy, it is to be expected that the DNA of the virus will be packaged with the fewest possible coat protein molecules. The protein–protein interactions define the helical symmetry of the coat, as for example in the generation of class I and class II virions (Marvin, 1978; Makowski, 1984). Lowering the number of positive charges provided by the C-terminal region of the fd coat protein requires more coat protein subunits to package the DNA, manifested in an elongation of the virion. As we have now shown, an increase in the number of positive charges is unacceptable. The wild-type fd virion thus appears to be the shortest particle possible, requiring the smallest number of coat protein subunits to package the DNA compatible with the helical symmetry adopted by the coat. It is reasonable to suppose that this is a result of evolutionary optimization of the DNA–protein interaction.

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References

Armstrong, J., Hewitt, J. A. & Perham, R. N. (1983). *EMBO J.* **2**, 1641–1646.

Beck, E., Sommer, R., Auerswald, E .A., Kurz, C., Zink, B., Osterburg, G., Schaller, H., Sugimoto, K., Sugisaki, H., Okamoto, T. & Takanami, M. (1978). *Nucl. Acids Res.* **5**, 4495–4503.

Boeke, J. D., Russel, M. & Model, P. (1980). *J. Mol. Biol.* **144**, 103–116.

Day, L. A., Marzec, C. J., Reisberg, S. A. & Casadevall, A. (1988). *Annu. Rev. Biophys. Biophys. Chem.* **17**, 509–539.

Dotto, G. P. & Zinder, N. D. (1983). *Virology*, **130**, 252–256.

Dotto, G. P., Enea, V. & Zinder, N. D. (1981). *Virology*, **114**, 463–473.

Grant, R. A., Lin, T.-C., Konigsberg, W. H. & Webster, R. E. (1981). *J. Biol. Chem.* **256**, 539–546.

Greenwood, J. & Perham, R. N. (1989). *Virology*, **171**, 444–452.

Hunter, G. J., Rowitch, D. H. & Perham, R. N. (1987). *Nature (London)*, **327**, 252–254.

Ikoku, A. S. & Hearst, J. E. (1981). *J. Mol. Biol.* **151**, 245–259.

Kuhn, A., Wickner, W. & Kreil, G. (1986). *Nature (London)*, **322**, 335–339.

Makowski, L. (1984). In *Biological Macromolecules and Assemblies. Virus Structures* (Jurnak, F. A. & McPherson, A., eds), vol. 1, pp. 203–253, Wiley, New York.

Marvin, D. A. (1978). In *The Single-Stranded DNA Phages* (Denhardt, D. T., Dressler, D. & Ray, D. S., eds), pp. 583–603, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Mathes, H. W. D., Zenke, W. M., Grunström, T., Staub, A., Wintzerith, M. & Chambon, P. (1984). *EMBO J.* **3**, 801–805.

Model, P. & Russel, M. (1988). In *The Bacteriophages* (Calendar, R., ed.), vol. 2, pp. 375–456, Plenum Publ. Corp., New York.

Moses, P. B. & Horiuchi, K. (1982). *Virology*, **119**, 231–244.

Rowitch, D. H., Hunter, G. J. & Perham, R. N. (1988). *J. Mol. Biol.* **204**, 663–674.

Russel, M. & Model, P. (1989). *J. Virol.* **63**, 3284–3295.

Simons, G. F. M., Konings, R. N. H. & Schoenmakers, J. G. G. (1979). *FEBS Letters*, **106**, 8–12.

Simons, G. F. M., Konings, R. N. H. & Schoenmakers, J. G. G. (1981). *Proc. Nat. Acad. Sci., U.S.A.* **78**, 4194–4198.

Webster, R. E. & Lopez, J. (1985). In *Virus Structure and Assembly* (Casjens, S., ed.), pp. 235–267, Jones & Bartlett Publ. Inc., Boston.

Webster, R. E., Grant, R. A. & Hamilton, L. A. W. (1981). *J. Mol. Biol.* **152**, 357–374.

Wickner, W. (1988). *Biochemistry*, **27**, 1081–1086.

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