group to achieve close-packing within both facet and edge. This contrasts with the continuously curved capsid model of Caspar and Klug (5), in which the polypeptide is usually assumed to be the structure unit. Here, the number of quasiequivalently related bonding configurations required for the structure unit tends to infinity as the capsid increases in size.

Our results for adenovirus suggest that larger units than the polypeptide always should be considered as possible structure units when evaluating viral architecture. Large, weakly-interacting building blocks permit accurate assembly into a highly restricted set of related locations. Stability is later conferred by the addition of a different structural component. This new approach casts light, for example, on the controversial demonstration of an all-pentameric polyoma capsid (6), and on the dissociation of the small plant viruses (7). In polyoma, one class of 60 pentamers bonds identically to give an icosahedral shell, with another class of 12 pentamers completing the capsid. In the small plant viruses, one set of intersubunit interactions within the hexameric clusters is almost identical to that of the pentamers. In addition, the clusters move apart as units when calcium is removed.

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REFERENCES

1. Burnett, R. M. 1984. Structural investigations on hexon, the major coat protein of adenovirus. *In* Biological Macromolecules and Assemblies. A. McPherson and F. A. Jurnak, editors. Virus Structures. John Wiley & Sons, Inc., New York. 1:337–385.
2. Burnett, R. M., M. G. Grutter, and J. L. White. 1985. The structure of the adenovirus capsid. I. An envelope model of hexon at 6 Angstrom resolution. *J. Mol. Biol.* In press.
3. Burnett, R. M. 1985. The structure of the adenovirus capsid. II. The packing symmetry of hexon and its implications for viral architecture. *J. Mol. Biol.* In press.
4. van Oostrum, J., and R. M. Burnett. 1984. Architecture of the adenovirus capsid. *Ann. NY Acad. Sci.* 435:578–581.
5. Caspar, D. L. D., and A. Klug. 1962. Physical principles in the construction of regular viruses. *Cold Spring Harbor Symp. Quant. Biol.* 27:1–24.
6. Rayment, I., T. S. Baker, D. L. D. Caspar, and W. T. Murakami. 1982. Polyoma virus capsid structure at 22.5 Angstrom resolution. *Nature* (*Lond.*). 295:110–115.
7. Robinson, I. K., and S. C. Harrison. 1982. Structure of the expanded state of tomato bushy stunt virus. *Nature* (*Lond.*). 297:563–568.

DNA INJECTION APPARATUS OF PHAGE P22

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During the assembly of double stranded DNA phages, DNA molecules are translocated from the cytoplasm into preformed procapsid shells, and condensed into a tightly wound state (Earnshaw and Casjens, 1980). At a later stage in the life cycle the process reverses, and the chromosome is released from the capsid and injected into the cytoplasm of a new host cell. At the heart of these processes is the unique vertex that morphologically forms the head/tail junction (Murialdo and Becker, 1978). This vertex is assembled as the very first stage of capsid assembly, serving first as the initation complex for procapsid assembly (Black and Showe, 1983).

We will refer to this vertex as the DNA translocating or portal vertex. A feature of these vertices in phages T4, λ, and ϕ29 is a special "portal protein" species, which forms a 12-fold ring at the vertex (Bazinet and King, 1985).

In the mature phage the portal vertex is located at a junction marked by symmetry mismatch. The tails of all known double-stranded DNA phages have sixfold rotational symmetry, while the capsid vertices have fivefold symmetry. Hendrix (1978) has proposed that this mismatch is a fundamental aspect of the enzymatic machinery of DNA packaging, and reflects the need for a rotation of some structure within the specialized vertex during DNA translocation.

In the Hendrix model the symmetry mismatch between pentameric and hexameric structures provides the metastable states permitting rotation. If there is rotation at this vertex, the portal proteins probably play a central role (Bazinet and King, 1985).

P22 is a double-stranded DNA phage infecting *Salmonella typhimurium*, whose cell attachment and DNA injection apparatus is simple in comparison with phages T4 and λ. It has been the subject of extensive genetic and biochemical investigation, and its structure and assembly pathway is understood in considerable detail (King and Casjens, 1974; Thomas et al., 1982; Fuller and King, 1982). The procapsid of P22 is assembled through the copolymerization of two major proteins, the coat and scaffolding subunits, and four minor proteins (Fuller and King, 1982). After shell completion, the portal vertex of the procapsid interacts with a complex of DNA packaging proteins and replicating DNA (Earnshaw and Casjens, 1980). After initation of DNA packaging, but before its completion, all of the scaffolding subunits exit from the procapsid, and the coat protein lattice expands and stabilizes forming the mature capsid (King and Casjens, 1974). At the termination of packaging a headful of DNA is cut from the replicating concatemeric DNA, releasing the packaging proteins.

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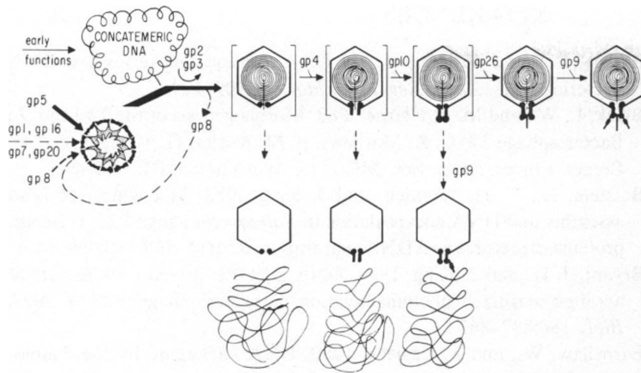

FIGURE 1 P22 DNA packaging and capsid maturation. Concatemeric DNA is packaged into procapsids through the portal vertex and cut into genome-size lengths by the products of genes 2 and 3. This packaging step is accompanied by a 12% expansion in the radius of the capsid and the exit of the scaffolding subunits, resulting in a metastable DNA-filled capsid. The subsequent proteins assemble at the portal vertex to stabilize this intermediate and form the DNA injection and cell attachment apparatus (Strauss and King, 1984). In their absence, the filled capsids are unstable and lose their DNA.

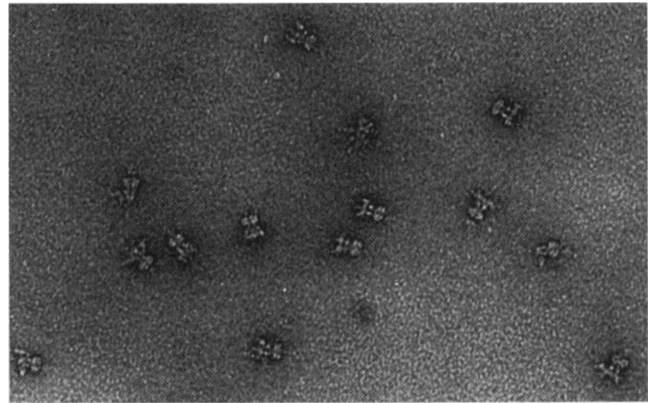

FIGURE 2 Isolated DNA injection and cell attachment apparatus. These structures are from a sucrose gradient fraction and have been negatively stained with uranyl acetate. The channel through which DNA passes during injection is visible in a number of cases. Compare with Fig. 3 for identification of the features.

The newly packaged DNA is metastable until three additional proteins add (Strauss and King, 1984), forming the morphological neck that provides the valve and channel needed for DNA injection (Fig. 1). Finally the tail spikes associate, producing infectious particles.

Though the procapsid contains six protein species, only three of them—coat, scaffolding, and gene 1 protein—are required for the assembly of a procapsid competent to package DNA. The remaining three minor species function only later in the life cycle during the DNA injection process and are dispensable for DNA packaging and particle assembly (Botstein et al., 1973; Poteete and King, 1977; Bryant and King, 1984).

The 90,000 d product of gene 1 (gp1) is of particular interest. 10–15 molecules of gp1 are incorporated into the procapsid. In its absence procapsid shells assemble but these are unable to package DNA. These inactive procapsids cannot be rescued by subsequent addition of gene 1 protein. This suggested that the gene 1 protein was the P22 portal protein, providing part of the machinery for DNA packaging and ejection. To determine if gp1 was located at the portal vertex, we attempted to isolate the DNA injection apparatus from the mature phage.

RESULTS

The procedure developed to isolate the injection apparatus involved heating purified wild type P22 particles to 60°C in the presence of 0.1 M sodium EDTA and 0.1% triton X-100, in phosphate buffer. This physically disrupted the particles, probably due to the destabilization of the condensed DNA. Many of the disrupted capsids had lost their tail structures, which could be observed relatively intact in electron micrographs of the material. The intact injection structures were concentrated by differential centrifugation and purified by sucrose gradient centrifugation (80 min, 37,000 rpm, 20°C, in an SW50.1 swinging bucket rotor) and examined more closely in electron micrographs of negatively stained samples. The sedimentation coefficient was ~60*S*.

The morphology of the isolated tail structures is shown in Figs. 2 and 3. The structure can be thought of as a shaft, one end having six tail spikes attached, and the other end having a collar or ring. The collar end must have been associated with the phage capsid. The shaft corresponds to the neck structure that can be observed on phage particles lacking the tail spikes. This shaft is assembled after the

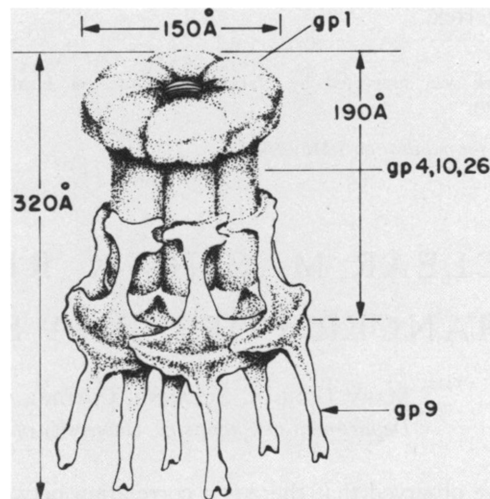

FIGURE 3 Schematic drawing of injection apparatus. The collar of gene 1 protein is assembled as part of the initiating vertex of the procapsid, and probably has rotational symmetry different from the fivefold capsid vertex. In the procapsid (prior to the assembly of the shaft) the collar probably sits between the outer coat protein shell and inner scaffolding shell. Though the overall structure is "closed" in the mature virus, attachment to the phage receptors must trigger the opening of a channel allowing the DNA to exit the capsid.

DNA is packaged into the procapsid, and requires the products of genes 4, 10, and 26, as shown in Fig. 1.

The protein composition of the fractions containing the isolated injection apparatus revealed five bands, whose mobilities corresponded to that of the gene 1 protein, gp9 tail spike polypeptide, the polypeptides specified by genes 4, 10, and 26, and the gene 5 major coat protein. Because we know that the shaft can only be assembled after DNA packaging, the collar must correspond to the structure within the capsid on which the shaft is assembled. In particles that have lost their chromosome but maintain the portal vertex, the collar can be observed within the capsid shell. This collar must be composed of the gene 1 product.

DISCUSSION

Though the mechanism of DNA transport into and out of the capsid remains obscure, features of the assembly of the machinery are becoming clearer. The procapsid of P22 is initiated in the cytoplasm without the participation of a membrane site (Lenk et al., 1975). Initiation must require interactions of coat, scaffolding, and gene 1 portal subunits, as well as the DNA injection proteins. We think the primary organizer is a complex of scaffolding protein, because certain mutants of the scaffolding protein exist that do not block shell assembly, but prevent incorporation of gp1 and the other minor proteins (unpublished results). However, some additional mechanism is needed to limit these interactions to one vertex. The scaffolding protein autoregulates its own synthesis, at the level of translation (Wyckoff and Casjens, 1984). Such a messenger RNA/scaffolding complex is an excellent candidate for an organizing center for assembling six different proteins into the portal vertex.

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REFERENCES

Bazinet, C., and J. King, 1985. The DNA translocating vertex of dsDNA bacteriophages. *Annu. Rev. Microbiol.* 39:109–129.

Black, L. W., and M. K. Showe. 1983. Morphogenesis of the T4 head. *In* Bacteriophage T4. C. K. Mathews, E. M. Kutter, G. Mosig, and P. B. Berget, editors. Amer. Soc. Microbiol. Washington, DC. 219–245.

Botstein, D., C. H. 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.

Bryant, J. L., and J. King. 1984. DNA injection proteins are targets of acridine-sensitized photoinactivation of bacteriophage P22. *J. Mol. Biol.* 180:837–863.

Earnshaw, W., and S. Casjens. 1980. DNA packaging by the double-stranded DNA bacteriophages. *Cell.* 21:319.

Earnshaw, W., and J. King. 1978. Structure of phage 22 coat protein aggregates formed in the absence of the scaffolding protein. *J. Mol. Biol.* 126:721–747.

Fuller, M. T., and J. King. 1982. Assembly in vitro of bacteriophage P22 procapsids from purified coat and scaffolding subunits. *J. Mol. Biol.* 156:633–665.

Hendrix, R. 1978. Symmetry mismatch and DNA packaging in large bacteriophages. *Proc. Natl. Acad. Sci. USA.* 75:4779–4783.

King, J., and S. Casjens. 1974. Catalytic head assembly protein in virus morphogenesis. *Nature (Lond.).* 251:112–119.

King, J., E. V. Lenk, and D. Botstein. 1973. Mechanism of head assembly and DNA encapsulation in *Salmonella* phage 22. II. Morphogenetic pathway. *J. Mol. Biol.* 80:697–731.

Lenk, E., S. Casjens, J. Weeks, and J. King. 1975. Intracellular visualization of precursor capsids in phage P22 mutant infected cells. *Virology.* 68:182–199.

Murialdo, H., and A. Becker. 1978. A genetic analysis of bacteriophage λ prohead assembly in vitro. *J. Mol. Biol.* 125:57–74.

Poteete, A. R., and J. King. 1977. Functions of two new genes in *Salmonella* phage P22 assembly. *Virology.* 76:725–739.

Poteete, A. R., J. Jarvik, and D. Botstein. 1979. Encapsulation of phage P22 DNA in vitro. *Virology.* 95:550–564.

Strauss, H., and J. King. 1984. Steps in the stabilization of newly packaged DNA during phage P22 morphogenesis. *J. Mol. Biol.* 172:523–543.

Thomas, G. J., Y. Li, M. T. Fuller, and J. King. 1982. Structural studies of P22 phage, precursor particles, and proteins by laser Raman spectroscopy. *Biochemistry.* 16:3866–3878.

Wyckoff, E., and S. Casjens. 1984. Autoregulation of the bacteriophage P22 scaffolding gene. *J. Virol.* 53:192–197.

NUCLEAR MAGNETIC RESONANCE STUDIES OF AN SV40 ENHANCER CORE DNA SEQUENCE

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We have observed that there is a correlation between the GTG/CAC sequence centered on the position corresponding to the sixth base of the messenger RNA of the *lac* operon with the observed biological properties of the *lac* operon DNA sequence (1) and the minimum imino proton spin lattice relaxation time in the region where the *lac* repressor binds. Position six also corresponds to the single base pair substitution leading to an operator mutation with the highest basal level of *lac* operon enzymes, i.e., the weakest repressor binding. The galactose operon, another inducible sugar utilization system, has two operators, an external operator with symmetrically located GTG/CAC sequences and an internal operator with one GTG/CAC sequence located homologously. Mutations have been found that affect the GTG/CAC sequences in the external operator. A number of interesting DNA sequences containing GTG/CAC where protein is expected to interact specifically are shown in Fig. 1. The selection of sequences

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