CMLS, Cell. Mol. Life Sci. 56 (1999) 580–603
1420-682X/99/080580-24 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Scaffolding proteins and their role in viral assembly

T. Dokland

Institute of Molecular Agrobiology, 1 Research Link, The National University of Singapore, Singapore 117604, Fax + 65 872 7007, e-mail: dokland@ima.org.sg

Received 17 May 1999; received after revision 5 July 1999; accepted 6 July 1999

Abstract. Scaffolding proteins are proteins that are required to catalyse, regulate or modulate some step in the assembly of a macromolecular complex. They associate specifically with the nascent protein complex during assembly, but are subsequently removed, and are absent from the mature structure. Scaffolding proteins have been described primarily from viral systems, in particular from the double-stranded DNA bacteriophages, but most likely play a more general role in macromolecular assembly, a fundamental process in all biological systems. Scaffolding proteins may act in a specific fashion, by actively encouraging the formation of correct protein-protein interactions, or more generally by nucleating and promoting assembly. They may also work to ensure the fidelity of the assembly process by preventing the formation of improper interactions, in many ways similar to the role of molecular chaperones in protein folding. In viruses, scaffolding proteins are found both in the form of internal cores and external bracing, and may form elaborate and complex structures. This review will focus on the viral scaffolding proteins, for which an increasing amount of structural and functional information has recently become available.

Key words. Virus; bacteriophage; capsid; assembly; morphogenesis; chaperones.

Introduction

Most proteins in the cell are part of larger macromolecular complexes in their active form, or as it was put in a recent series of reviews, the cell is a 'collection of molecular machines' [1]. A considerable amount of information has over time been accumulated on the structures of some of these machines, but far less is known about the fundamentally important process of macromolecular assembly, the process by which they are put together spatially and temporally.

The study of macromolecular assembly dates back to the studies on virus and bacteriophage assembly in the 1950s and 1960s [2–4], and most of what is known about macromolecular assembly today still arises from in vitro and in vivo studies of viral systems. In fact, with the advent of more powerful and accessible structural methods to complement the biochemical and genetic data, this field has experienced somewhat of a renaissance in recent years.

These systems exhibit self-assembly, in which the assembly process proceeds along ordered pathways that are regulated solely through the sequential generation of unique complementary interfaces and conformational switching events [5–7]. Although more complex cellular morphogenetic processes may be under additional temporal control at the level of transcription, self-assembly clearly still plays an important role. The energy required for the self-assembly process is typically intrinsic to the proteins themselves and stored as 'conformational energy' in the interacting components [8]. In the cell, of course, many processes, including assembly, are driven by nucleotide hydrolysis.

The concept of a scaffolding (or scaffold) protein arose from studies on the double-stranded (ds) DNA bacteriophages P22 [9] and T4 [10], where it was found that certain proteins were associated with immature capsid structures (assembly intermediates), termed procapsids or proheads, but were absent from the mature virions. In scaffolding protein mutants, no assembly would occur, or only aberrant capsid-related structures were formed. Subsequently, scaffolding proteins were identified in a number of other viral systems, and became well established as a common theme in the assembly of dsDNA bacteriophages [11, 12]. Scaffolding proteins have also been found in some other systems, both viral and nonviral, and are likely to play a far more important role in macromolecular assembly in general than is generally recognized. (Many of the proteins that bind and regulate the polymerisation of actin and tubulin, for example, may be considered de facto scaffolding proteins.)

Scaffolding proteins have been likened to a higher-order equivalent of molecular chaperones, operating at the level of quaternary structure in the same way that chaperones act on the secondary and tertiary structure of proteins. Chaperones usually act by inhibiting unproductive folding and preventing illicit interactions between unfolded or partially folded protein chains, but may in some cases also actively promote correct folding [13]. Like chaperones, scaffolding proteins may act by preventing the formation of improper assemblies, but in general tend to play a far more active role in determining the structure of the final assembly. Most traditional chaperones exhibit little specificity for their substrate; scaffolding proteins, on the other hand, are quite specific and not generally usable for more than one structure. In addition, scaffolding proteins are not necessarily catalytic per se, since they may be involved in only one round of assembly, and are sometimes degraded proteolytically after use. However, there is a close connection between folding and assembly, and the scaffolding proteins do affect both secondary and tertiary structure in the course of their action. Conversely, many of the typical chaperone proteins have functions in the assembly and disassembly of macromolecular complexes as well [13].

A note on terminology: Many proteins function as 'scaffolds' for subsequent assembly, for example in the way that a viral core particle functions as the scaffold for the outer shell layer, or titin may act as a scaffold for actin assembly. These proteins are often called scaffold(ing) proteins in common usage; however, the term 'scaffolding protein' as used in this review refers specifically to a protein which is required for some step in the correct assembly of a macromolecular structure and is subsequently removed from the complex. (Sometimes proteolytic fragments of the scaffolding protein remain with the complex after completion of assembly, but since its role in the assembly process has been played out, it can still be considered a scaffolding protein, rather than simply a structural component of the virion.) This distinction between scaffolding proteins and other structural proteins may be somewhat arbitrary, however, as there may be nothing really fundamentally different between the way they work.

Virus assembly and the role of scaffolding proteins in the assembly process have been reviewed numerous times before [7, 11, 12, 14–16]. In this review, I will focus on the scaffolding proteins themselves, their structures and biochemical properties, as there have been many recent developments in this area. I will concentrate on the 'classical' scaffolding proteins from the dsDNA bacteriophages and other viral systems, for which a fair amount of structural or mechanistic information is available. A discussion of the many cytoskeletal proteins that in principle could be considered scaffolding proteins is outside the scope of this review.

Virus structure and assembly

Since most of the discussion of scaffolding proteins relates to virus assembly, a few general points about viral structure and assembly are appropriate here.

Although viruses may be isometric, elongated, filamentous or irregularly shaped, here we will be concerned mainly with the so-called spherical or isometric viruses of icosahedral symmetry (and their prolate cousins). The icosahedron is a closed, isometric surface consisting of 20 triangular faces arranged with 60-fold symmetry. Although the isometric viruses are not necessarily icosahedrally shaped, they still conform to the icosahedral symmetry. The 60-fold symmetry implies that the structural proteins must be present in the capsid in multiples of 60. The exact multiple that is utilised is reflected in the triangulation number (T number), and this number can be thought of as a subdivision of the triangular faces into smaller triangles. The utilisation of more than 60 subunits implies that there will be T nonequivalent subunit types in the shell. This is reflected in the capsid proteins as conformational variation, which can range from slight to dramatic. Many viruses, however, including the dsDNA bacteriophages, are only quasi-icosahedral, as they have a unique fivefold vertex containing specialised structures such as the tail. Prolate capsids are derived from the icosahedral ones by elongation at the equator [17].

Some simple viruses may assemble through direct association of the genome and capsid proteins. More complex viruses, such as the dsDNA bacteriophages, however, assemble through far more elaborate assembly pathways [12, 14, 16]. Such an assembly pathway may

start from an initiator complex, often containing a 12-fold (or 13-fold) symmetric connector, onto which the precursor capsid, or procapsid is assembled, often with the aid of scaffolding proteins (fig. 1). The genome is subsequently packaged into this precursor capsid through a largely unknown mechanism, most likely involving the connector. During this process, large conformational changes in the shell may take place, together with the removal of the scaffolding proteins. Although less is known about the assembly of other large viruses, the herpes- and adenoviruses appear to assemble in a similar fashion.

Classification of scaffolding proteins

The viral scaffolding proteins known at this point can be roughly classified into three groups: (i) the icosahedrally ordered, external scaffolds of bacteriophage P4 and the ϕX174-related phages; (ii) the internal, corelike scaffolds, like those of P22, herpes and λ; and (iii) the prolate core of the T4-like phages (table 1, fig. 2). Internal scaffolds appear to be more common, but more structural information is available on the external scaffolds, which have icosahedrally ordered structures. The internal corelike scaffolds of spherical viruses appear to lack such order and are thus less amenable to structural analysis; nevertheless, from a functional point of view the internal scaffolds are better characterised than the external ones. The T4 prolate core is a far more complex structure consisting of several proteins, and will be considered separately.

Note that this classification scheme is relatively arbitrary at present, and much more information is needed on more different proteins before classification into groups based on functional or evolutionary relationships can be attempted. So far, only the internal corelike scaffolds appear to form a coherent group. A number of scaffolding proteins of other viruses, not to mention those present in nonviral systems, have not been characterised in sufficient detail to establish whether they share any structural, functional or evolutionary properties, and cannot be classified into a specific group at present.

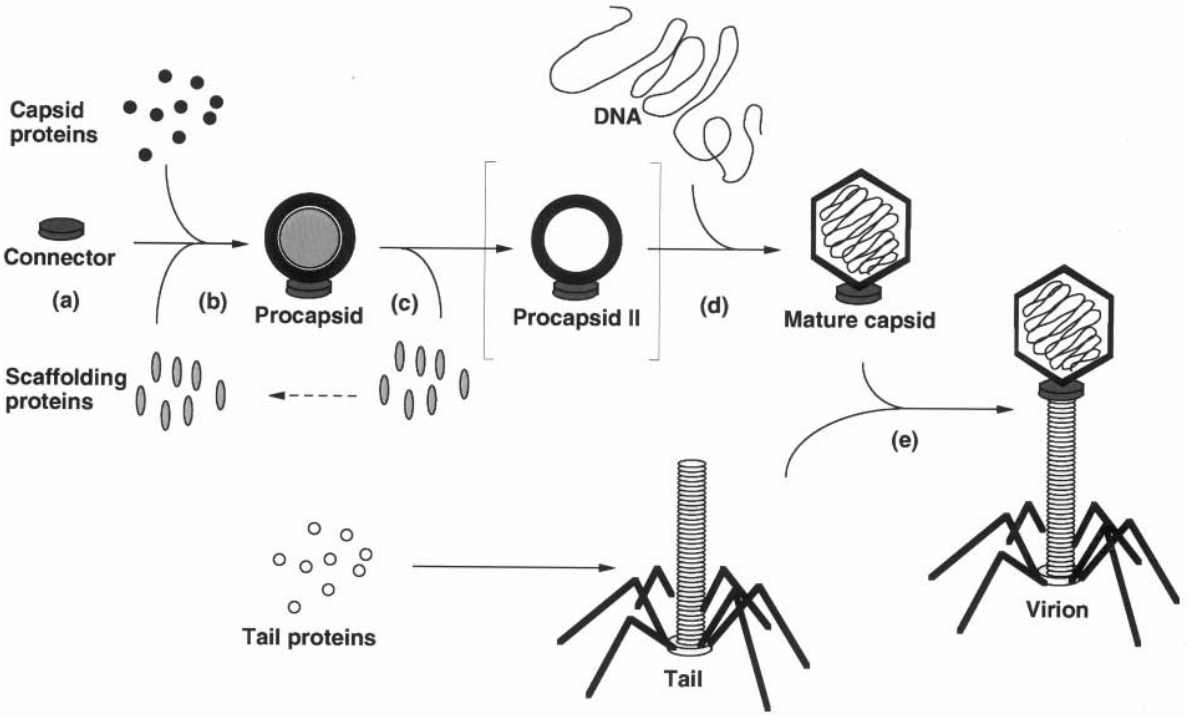

Figure 1. General assembly pathway for a dsDNA bacteriophage. (*a*) Initiation of assembly at the connector; (*b*) scaffolding-dependent procapsid assembly; (*c*) removal and possibly recycling of the scaffolding protein; (*d*) DNA packaging may occur after or simultaneous with scaffold removal, and is accompanied by major structural changes in the shell; (*e*) tail attachment and capsid finishing.

Table 1. An overview of viral scaffolding proteins.

Virus	Protein	Size (kDa)	Copy no.	Function/location	Properties	Reference
ϕX174	D	16.9	240	external scaffolding	forms asymmetrically organized outer shell; four conformations; α helical	[18, 20]
	B	13.8	60	internal	partially ordered	[18, 20]
P4	Sid	27.2	60/120	external, size-determining scaffold	elongated, α helical, forms 'cage' connected at 3f axes	[31, 40]
P2/P4	gpO	31.4	~70	internal scaffold	cleaved; fragment remains inside capsid	[143]
P22	gp8	34.0	~200 –300	internal core	elongated, α helical; forms dimers and tetramers; recycled	[52, 54]
λ	gpNu3	13.4	~200	internal scaffolding	not cleaved	[71]
T7	gp9	33.8	~156	internal scaffolding core	not cleaved	[74, 76, 77]
herpes	VP22a*	40.0	~600	internal core	elongated, cleaved	[98]
T4	gp22	29.9	~576	main internal core	elongated; pI = 4.3; 80% α helix. Cleaved; fragment remains inside capsid.	[7, 105, 121]
	gp67	13.0	~341	internal core		
	gp68	17.0	~240	internal core		
	IPI, IPII, IPIII	10, 11 21.7	~360 each	(dispensable) core proteins		
	gp21	23.2	~72	protease, centre of scaffold		
ϕ29	gp7	11.3	~180	internal scaffold	recycled	[137–139]

*Many related viruses for which the scaffolding proteins have different names: HSV, VP22a/UL26.5/ICP35; CMV, pAP/UL80a; VZV, UL33.5

External scaffolding proteins

ϕX174 protein D

The only scaffolding protein structures known to high resolution are those of the single-stranded (ss) DNA *Escherichia coli* bacteriophage ϕX174, for which the X-ray structure of a scaffolding-containing procapsid was recently determined to 3.5 Å resolution [18–20]. Prior to the determination of the crystal structure, cryo-electron microscopy (EM) had shown the procapsid at 25 Å resolution [21]. The ϕX174 procapsid contains 60 copies each of the major capsid protein F (48.4 kDa), the fivefold spike protein G (19.0 kDa) and the internal scaffolding protein B (13.8 kDa), whereas 240 copies of protein D (16.9 kDa) forms an external scaffold. Both the B and D scaffolding proteins are required for procapsid assembly [22–25]. The following discussion will concentrate on the external scaffolding protein D; protein B will be described later, together with the other internal scaffolding proteins.

Pentamers of the F and G are through to comprise the earliest assembly intermediates [22, 23, 26] (fig. 2). In cells infected with mutants in protein D, 12S particles, which may consist of a pentamer each of the F and G proteins, accumulate [26, 27]. These particles may represent an assembly intermediate, possibly forming a substrate for the D protein, in the presence of which functional 108S procapsids are produced [23, 24]. Either during or subsequent to DNA packaging the procapsid undergoes major structural rearrangements, and the internal scaffolding proteins are removed, generating a 132S particle [18, 21, 24]. Finally, the external scaffolding is removed, perhaps only upon lysis of the cell [28] (fig. 2).

In the ϕX174 procapsid, there are four copies of the external scaffolding protein D per asymmetric unit (a total of 240). The most striking features of the D scaffolding protein are its unusual arrangement on the surface of the particle and its great structural variability. The four symmetrically nonequivalent copies are arranged in an asymmetric fashion, where each subunit presents a different surface to the other capsid proteins and its environment. One subunit (D1) is located near the pentameric spikes where it interacts with the G protein; the other three subunits (D1–D3) are lined up along the twofold interface, across which subunits D2 and D4 make contact with twofold related subunits in the adjacent asymmetric unit (fig. 3A). D4 makes the primary contact with the underlying F protein. The external scaffolding provides the main, if not only, contact between the G protein spikes and the underlying F protein shell as well as the main contact between adjacent pentameric subunits [18, 21].

The four subunits are organised into two asymmetric dimers (D1:D2 and D3:D4) which can be mutually superimposed with an r.m.s. deviation of less than 2.5 Å for 67% of the residues [18] (fig. 3A, 4). This dimer could represent an intermediate in the assembly pathway. Biochemical studies, however, suggested that the

D protein existed as a tetramer in solution [29]. If so, this tetramer is most likely not represented in the scaffold itself [18]. At higher protein concentration, protein D forms a higher-order 20S complex, of which the nature is at present unknown [29], although it could suggest that the D protein may have the capability to form shells in the absence of other viral proteins. Under physiological conditions, however, scaffold assembly is likely to be dependent on the interaction between scaffolding and capsid proteins.

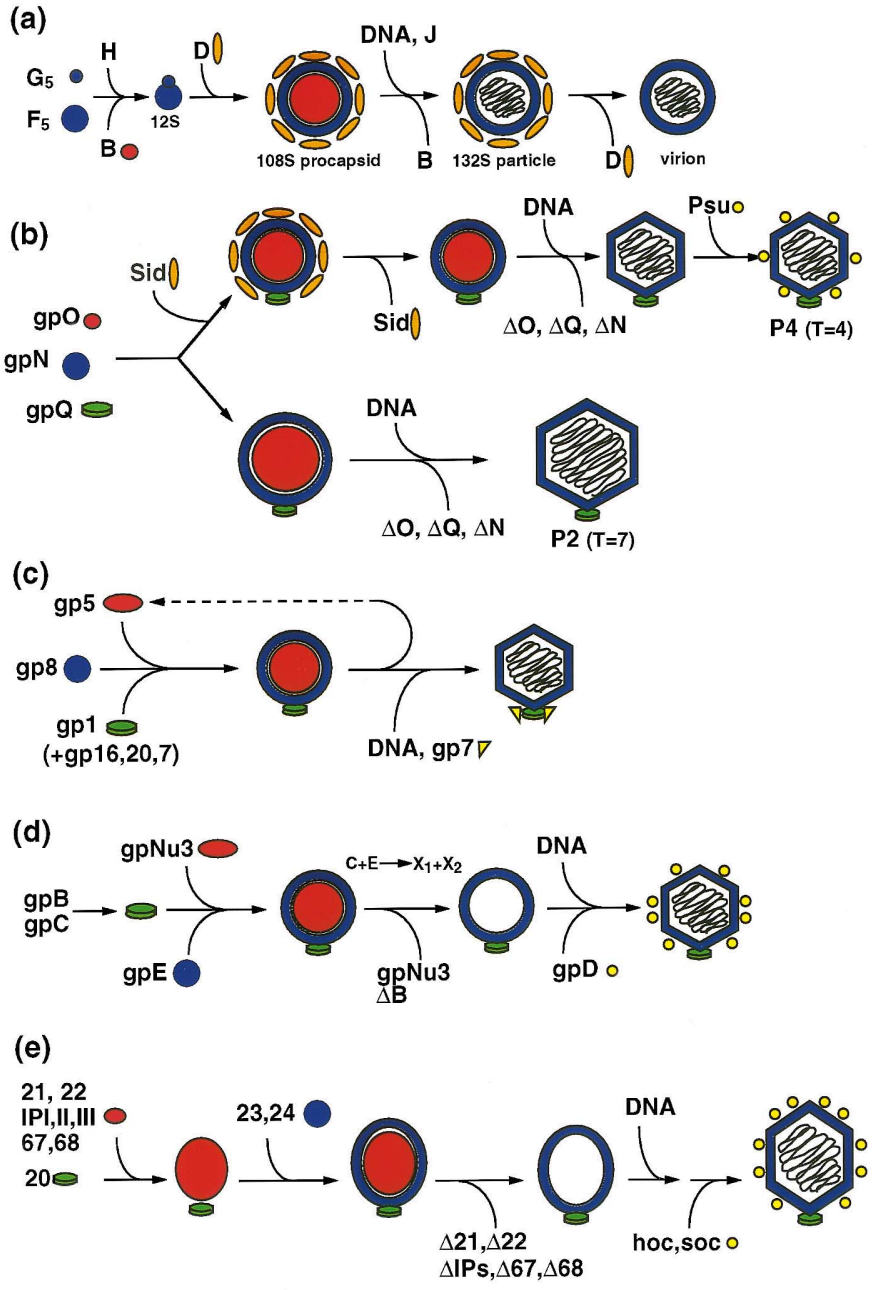

Figure 2. Comparison of assembly pathways for several of the viruses discussed in the text. Scaffolding proteins are shown in red (internal) or orange (external), whereas capsid proteins are coloured blue, connector protein green and decoration proteins yellow. The schematics have been somewhat simplified, and tail assembly has been omitted. Protein cleavage reactions are indicated as protein → protein* + Δprotein (e.g. N → N* + ΔN). (*a*) ϕX174 [18], (*b*) P2/P4 [31, 34], (*c*) P22 [43], (*d*) λ [15, 65], (*e*) T4 [7, 105].

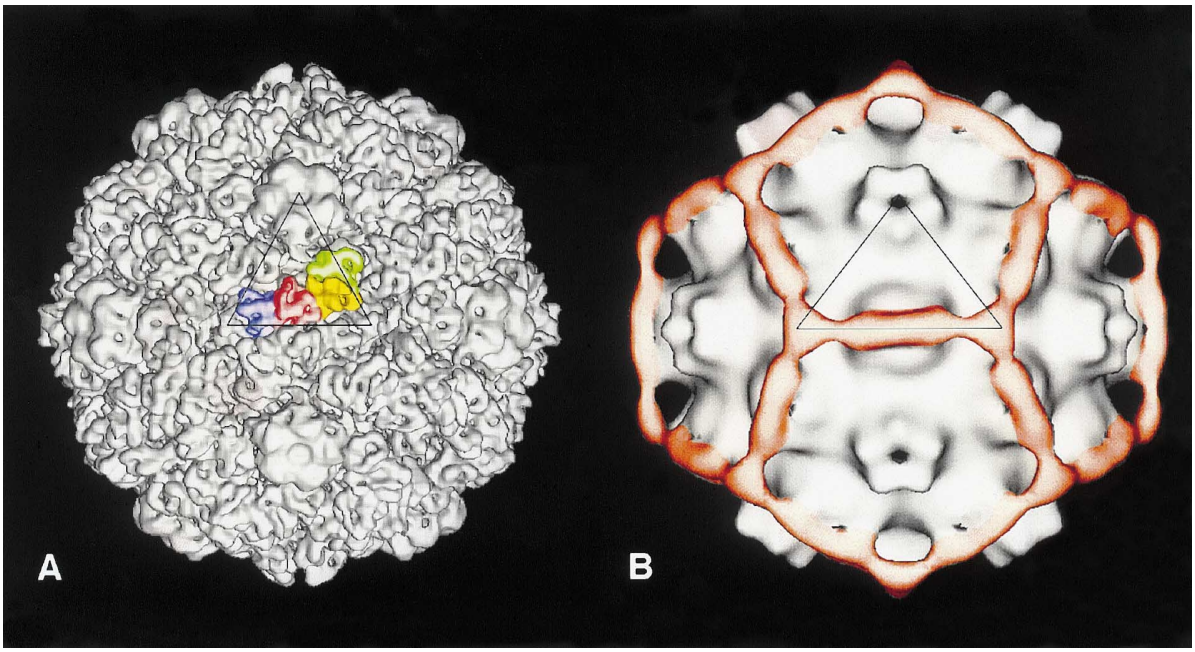

Figure 3. Structures of the external scaffolds of ϕX174 (*A*) and P4 (*B*). (*A*) Surface representation at 10 Å resolution of the ϕX174 procapsid, viewed down a twofold axis [18]. One asymmetric unit triangle is indicated. The four D scaffolding proteins within the asymmetric unit (triangle) are coloured: D1, green; D2, yellow; D3, red; D4, blue. The particle diameter is 330 Å. (*B*) Surface representation at about 30 Å resolution of the P4 procapsid electron density map [31]. The Sid scaffolding protein is shown in orange, and the asymmetric unit triangle is indicated. The P4 procapsid is approximately 400 Å in diameter. The view is the same as in (*A*), although the particles are not exactly to scale.

The D protein itself is largely α helical, consisting of seven main helical segments separated by short (< 10 residues) loop segments. One slightly longer loop (18 residues) forms some β structure (fig. 4). The four subunits share a structurally conserved core of about 75 residues, whereas in the remainder of the protein there is great conformational variation. In general, these variable regions represent domains of functional importance in the capsid assembly. Subunits D1 and D3 are almost identical, but the other subunits differ in several aspects: (i) the N-terminal helix 1 is rotated by $\sim 100°$ around residue Q22 in D4 relative to the other subunits; (ii) helix 3 is kinked at G61 in D1 and D3, but is straight in D2 and D4; (iii) loop 5 has variable secondary structure and forms the main twofold contact in the scaffold; (iv) the C-terminus is partly disordered in D1, D2 and D3, but forms a long α helix in D, which forms the most significant contact between the scaffold and the underlying F protein shell [18, 20].

The different conformations in the D protein suggest how the scaffold—and thus the whole shell—may be assembled through a series of conformational switching events. At least two conformational switches can be distinguished: (i) A switch from a kinked to a straight helix 3 in D2 and D4. This switch would be required to change the subunit into a nonsticky conformation once it has bound to its partner (D1 or D3). Otherwise, it would have the potential to continue adding more subunits infinitely [18, 30]. (ii) Switching of loop 5 in D4 into a conformation able to form interactions across the twofold interface. This may be related to and perhaps triggered by the formation of the long, ordered C-terminal helix 7 in D4 and its binding to the underlying F protein. The twofold interaction is the crucial step that ties together the precursor pentamers across the twofold axis; without it, the pentamers cannot assemble further, as apparently the F protein is not able to form these interactions unaided. The existence of mutations in protein F that can suppress conditionally lethal mutations in the N-terminal helix 1 of protein D suggests that there is a functional interaction between the D and F proteins in a region that is not seen to make contact in the procapsid X-ray structure. However, these regions may have been in contact at an earlier stage in the assembly process. This model is supported by analysis of the procapsid EM reconstruction, which probably represents a stage in the assembly process prior to maturational conformational changes in the shell [18,

20, 21]. Once the capsid proteins have been brought into proximity by the scaffold, additional switches in the F capsid protein, probably involving a set of α helices at the threefold axes, reinforces the shell, so that the scaffolding proteins can be removed [18, 20]. Likewise, the G protein spikes, which are not in contact with the capsid protein in the procapsid, must undergo conformational changes to allow the interactions between the F and G proteins to be established [20, 21].

The D protein is highly conserved between different members of the *Microviridae*, except in the N-terminal 20 amino acids and a shorter segments near the C-terminus. In spite of this conservation, the D proteins exhibit little cross-functionality between different species, suggesting that the species specificity is conferred by the less conserved N- and C-terminal regions [B. Fane, personal communication].

P4 Sid protein

Another scaffolding protein for which a three-dimensional structure is available, albeit at low resolution, is the external scaffold of *E. coli* dsDNA bacteriophage P4 [31], a satellite virus of dsDNA phage P2. P4 depends on P2 for most structural gene products, including the major capsid protein, gpN [32–34]. In the bacteriophage P2–P4 system, the choice of morphogenetic pathway is made through the presence or absence of a single scaffolding protein. P2 normally forms an icosahedral T = 7 capsid from 415 copies of the gpN-derived protein N* [35] (fig. 2). During a co-infection of P2 and P4, or on infection of a P2 lysogen with P4, a smaller T = 4 capsid is formed containing only 235 copies of gpN-derived protein [35]. This capsid can package the P4 genome, while effectively excluding the three times larger P2 genome from being packaged. The gene product responsible for this size determination is the 244-amino acid, 27-kDa P4-encoded protein Sid (for Size determination), which acts as a scaffolding protein during P4 assembly [36] (fig. 2). Mutations in the *sid* gene lead to a failure to produce small capsids [37]. In addition, mutations in *N*, called *sir* (*sid* responsiveness), are known that renders the gpN capsid protein resistant to the effect of Sid [38]. These *sir* mutations can be suppressed by second-site mutants in *sid*, so-called 'super-sid' mutants [K.-J. Kim et al., unpublished results].

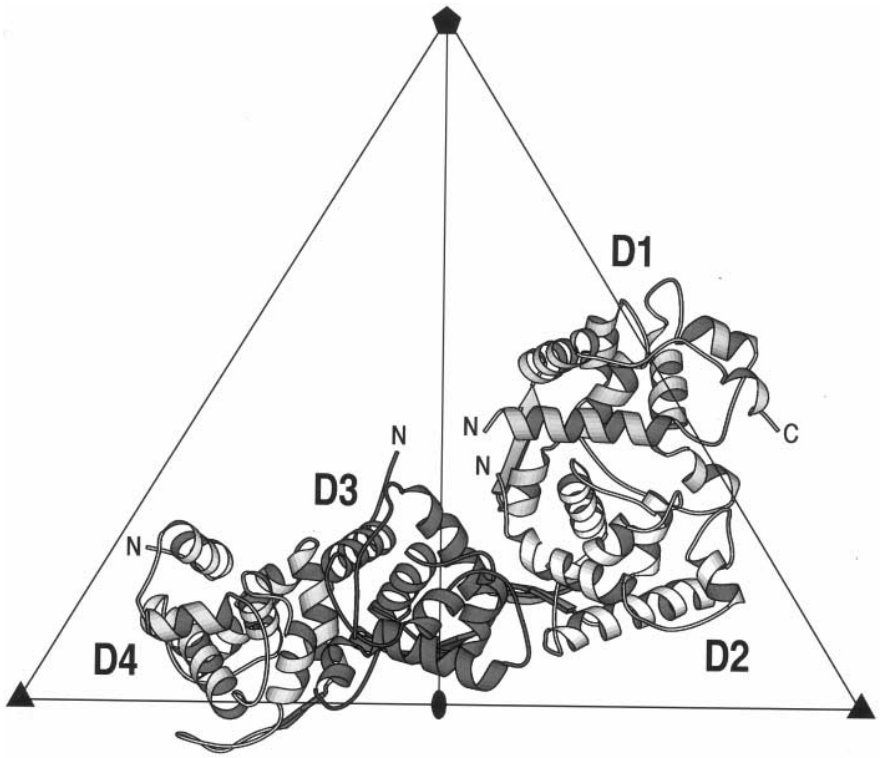

Figure 4. Ribbon representation of the four copies of D scaffolding protein within one asymmetric unit (triangle) of the ϕX174 procapsid [18], corresponding to the coloured portion in figure 3A. The two threefold, the twofold and the fivefold axes of symmetry are indicated, as are the N– and C–termini that are visible in this view.

This analysis thus identifies gpN as the most likely target for Sid. The *sir* mutations are clustered in the centre of gpN, and could affect either the flexibility (or 'intrinsic curvature') of the gpN protein, and thereby its ability to form small capsids, or the interaction of gpN with Sid [35].

True procapsids of either P2 or P4 have not been identified; however, expression of the gpN protein together with Sid protein leads to the production of a set of small Sid-containing $T = 4$ particles, which are assumed to be equivalent to procapsids. However, these particles lack the portal structure and hence cannot package DNA [39]. In three-dimensional (3D) reconstructions of these procapsids, the Sid protein is seen to form an embracing 'cage', which connects at the threefold axes and makes contact with the underlying shell at the twofold axes [31] (fig. 3B). Only two of the gpN subunits in the twofold hexamer appear to take part in this gpN-Sid interaction; the remaining four subunits are apparently untouched, as are the pentamers.

Volume measurements in the reconstruction favoured a number of 120 copies of the Sid protein in the scaffold, although a $T = 1$ model of 60 copies is still a possibility [31]. The shape of the Sid protein (consistent with both models) is an $\sim$100-Å-long tube with a constriction in the middle. This suggests a two-domain structure for Sid, where one domain is the trimer-forming domain and the other domain forms the Sid-gpN and the twofold Sid-Sid interactions (fig. 3B).

Such a two-domain structure is supported by genetic data, which showed a clustering of a number of the *sid* mutations in the C-terminal region [40]. (Other mutations were located near the N-terminus (N27Q, T34P), and in the centre of the sequence (G107V, T112K), but the significance of this is unclear.) Also the super-sid mutations are clustered in the C-terminal part of the Sid protein, and are not allele-specific with respect to the sir mutations. Taken together this suggests that the C-terminal region may be involved in Sid-Sid interactions, e.g. at the threefold axes, and that the super-sid mutations strengthen these interactions [K.-J. Kim et al. unpublished results]. Recent results from my laboratory, comparing the in vitro assembly properties of full-length and truncated Sid protein, also support this conclusion [unpublished].

Secondary structure predictions predict a predominantly α helical structure for Sid, with seven main α helical stretches [40], but a high degree of predicted disorder or flexibility is also seen over most of the sequence [unpublished results]. The 27-kDa Sid protein has an apparent mass of 31 kDa by SDS-polyacrylamide gel electrophoresis (PAGE), suggesting that the protein has an unusual shape. By EM the Sid protein also appears to be fibrous is nature. Attempts to crystallize the Sid protein have not been successful, probably due to the shape and flexibility of the protein, as well as a tendency of the protein to aggregate.

The structure of the external scaffold and its interaction with the capsid hexamers led to a model in which the Sid protein would act upon a hexamer subassembly [31]. More recently, Erik Jacobsen [University of Oslo, Norway, personal communication] proposed a simpler and thus perhaps more plausible model, in which the gpN trimer forms the basic building block. In either model, the curvature enforced upon the subassemblies by the Sid protein would force the generation of pentamers at the appropriate places. The advantage of the trimer model is that it assumes no separate subassembly of pentameric structures, whereas the pentamer/hexamer model assumes that there must be a control point for the production of pentamers vs. hexamers from the same gpN monomers. No gpN subassemblies of any kind have yet been observed, however.

Internal corelike scaffolds

P22 protein gp8

From a functional point of view, the gp8 protein of the *Salmonella typhimurium* dsDNA bacteriophage P22 is the best-described scaffolding protein. During P22 assembly, about 2–300 copies of gp8 associate with the 420 copies of gp5 required to make a $T = 7$ shell [9, 41–43] (fig. 2). During DNA packaging, the gp8 proteins are removed and used in subsequent cycles of assembly. The gp8 protein shows no propensity to assemble on its own in vitro [44–46], and the gp5 capsid protein assembles only a few unclosed shells and protein aggregates in an 8^- mutant infection [47]. This suggests that it is the interaction between coat and scaffolding proteins that leads to a switching from a nonassembling to an assembling form [46].

In the P22 procapsid, gp8 forms a spherical core of about 200 Å diameter [47, 48] (fig. 5). However, the core is not observed in 3D reconstructions of procapsids, suggesting that the scaffolding protein itself is not arranged with icosahedral symmetry [49]. Some increased density is visible in the central part of the core, and a small domain is found to form a specific interaction with gp5 at the inside of the capsid shell. During assembly, scaffolding protein copolymerises with coat protein and is added to the edge of the growing shell [44, 45], unlike e.g. bacteriophage T4, where initially a complete scaffolding core is formed, around which the capsid protein is assembled (see below). P22 assembly is compatible with a wide range of scaffolding:capsid protein ratios, from 0.2:1 to more than 1:1 [44, 45]. The interaction between gp8 and gp5 is relatively weak, as the gp8 protein can be removed from procapsid shells by a mild treatment in 0.5 M guanidine hydrochloride (GuHCl), which does not disrupt the shell itself.

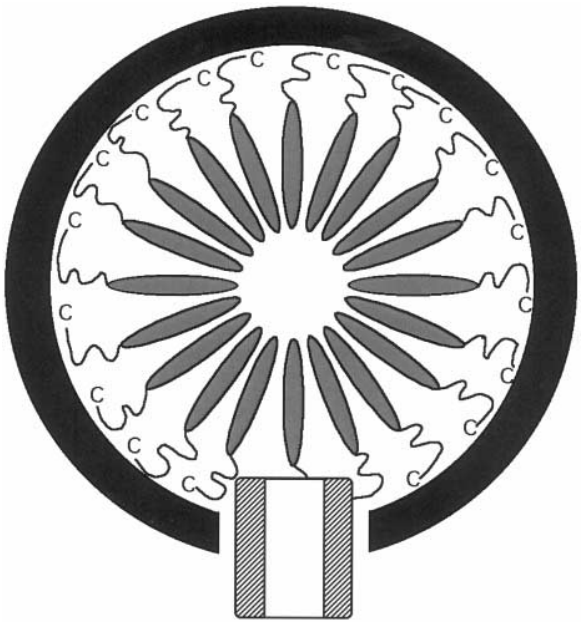

Figure 5. Schematic diagram of the P22 procapsid, which also serves as a model for the internal cores of λ and herpesvirus. The scaffolding proteins have an elongated shape, and the C-termini make contact with the capsid protein. There is a low-density region between the core itself and the outer shell. The connector (hatched) also forms interactions with the scaffolding proteins, which are required for connector integration.

In solution, the gp8 scaffolding protein exists in equilibrium between monomers, dimers and tetramers. The dimer seems to be the active species in promoting capsid assembly [50] (fig. 6). In fact, dimerisation of gp8 could provide the driving force for capsid assembly if binding of gp5 to gp8 favours dimer formation, which would bring the coat protein monomers together spatially [51]. The C-terminal half (residues 141–303) of the protein is required and sufficient both for dimer formation and to direct capsid assembly [52, 53]. The N-terminal part (residues 1–140) is required for tetramerisation, and plays a role in stabilising the scaffolding protein and ensuring the fidelity of the assembly process [53, 54]. The C-terminal 11 residues of gp8 form a 'coat protein recognition domain' which binds to gp5 (fig. 6). These residues form part of an amphipathic helix-loop-helix motif of about 30 amino acids length, which exposes a number of positively charged residues which probably form specific, electrostatic interactions with the coat protein [51, 53, 54]. Incidentally, this domain structure is shared between P22 and the herpesviruses (see below).

Overall, the gp8 protein is rich in prolines and charged amino acids. It has an elongated shape of 247 Å by 22 Å [50, 55, 56]. Spectroscopic studies have suggested an α helical content of about 40% and a highly flexible tertiary structure comprised of multiple α helical domains, which reaches its final conformation only upon binding to the coat proteins [57]. Significant secondary structure changes take place both within the scaffolding and coat proteins upon assembly as well as during maturation, which is associated with the release of scaffolding protein [57–59].

Although the P22 capsid protein is able to form both normal T = 7 and smaller T = 4 capsids in vivo in the absence of scaffolding protein, only the larger capsids are produced in the presence of scaffolding protein [47, 60, 61]. The elongated shape and size of the scaffolding proteins provides a simple steric mechanism for ensuring that the correct size capsid is made, since it would be too large to allow the formation of a T = 4 capsid (fig. 5).

There is also a specific interaction between scaffolding protein and the gp1 portal protein [62]. Scaffolding protein is required for incorporation of connectors into the shell, and the aberrant shells that are formed in the absence of scaffolding protein do not contain connectors [47]. The same dependence on scaffolding protein is found in both phage λ and $\phi 29$ (see below). Unlike $\phi 29$, however, the scaffolding protein of P22 is sufficient to ensure correct size determination of the shell, and perfectly shaped, albeit inactive, procapsids can be made from gp5 and gp8 alone in vitro [44].

λ protein gpNu3

The *E. coli* dsDNA phage λ has a similar T = 7 structure to P22, consisting of 405 copies of the major capsid protein gpE, the same number of an additional nonessential 'decoration protein' gpD and a specialised gpC-containing collar structure at the portal vertex [63, 64]. The whole assembly process of λ has been reproduced in vitro using mutant extracts [65] (fig. 2). The gpNu3 protein of phage λ plays a similar role in the assembly process to the P22 gp8 protein. It is absent from the mature capsid and accumulates in 160S procapsid-like particles produced in wild-type and certain mutant infections (mutants in gpC and groEL) [66–68]. However, these 160S procapsids are not active in DNA packaging [67]. The only procapsids that are active in DNA packaging sediment at 135S and do not contain gpNu3. Such active, gpNu3-less procapsids are found during wild-type and terminase (gpA) mutant infections [67]. Thus, the action of gpNu3 as a scaffolding protein must be only transient in nature. X-ray solution scattering analysis indicated that the core was unstable and was rapidly lost from gpNu3-containing procapsids [69]. The complete cores seen in some mutant infections could therefore be an artefact caused by a failure to release the scaffolding protein.

Several similarities exist between the P22 gp8 protein and gpNu3. In the absence of gpNu3, the major capsid protein gpE is primarily present as monomers or small oligomers and a few aberrant assemblies [66, 67, 70]. Conversely, gpNu3 never associates into larger aggregates in the absence of gpE [15, 68]. Furthermore, gpNu3 is also required for the assembly and/or integration of the connector structure in addition to its function as a scaffolding protein. *Nu3* mutants fail to assemble the gpB-containing 25S preconnector intermediate, and mutants in *B* fail to incorporate gpNu3, suggesting a direct interaction between the connector and the scaffold [65, 67, 68]. The gpNu3 sequence is identical with the C-terminal part of gpC, the collar protein. gpC forms interactions with the gpB connector at an early stage of assembly, and could perhaps serve as a nucleation site for further scaffolding protein assembly, if gpC-gpNu3 interactions were identical to gpNu3-gpNu3 scaffolding interactions. However, gpNu3-containing preheads accumulate in a C^- infection, which suggests that the role of gpC in assembling the core is more indirect.

The gpNu3 protein itself is a hydrophilic, 131-amino acid protein with a pI of 4.2. In solution, it probably exists primarily as a dimer or trimer, and has an unusual elongated shape of about 90 Å length and an axial ratio of about 13 [71]. These parameters are not unlike those of its gp8 counterpart in P22. Although gpNu3 is only about half the length of gp8, only the C-terminal half of gp8 was necessary for scaffolding action in the P22 system [53]. Based on this analogy gpNu3 would form interactions with the capsid protein at the C-terminal end and intrascaffolding interactions at the N-terminal end (fig. 5).

Low-angle X-ray solution scattering experiments indicated that the gpNu3 core is a 100–150 Å thick shell of lower density than the outer shell, separated from it by a low density 'connecting' region [69], consistent with its elongated shape [71]. Small-head (T = 4) mutants in the gpE capsid protein also contain gpNu3, demonstrating that under these conditions the capsid protein can direct the size of the scaffolding core [72]. Another difference from P22 is that gpNu3 is completely degraded after assembly is completed [66].

Bacteriophage T7 gp9

The capsid of the *E. coli* dsDNA bacteriophage T7 has T = 7 icosahedral symmetry, similar to the phages λ and P22, and contains a coaxial, internal connector-core structure at one vertex [73]. The prohead (procapsid) contains about 140 copies of the gp9 scaffolding

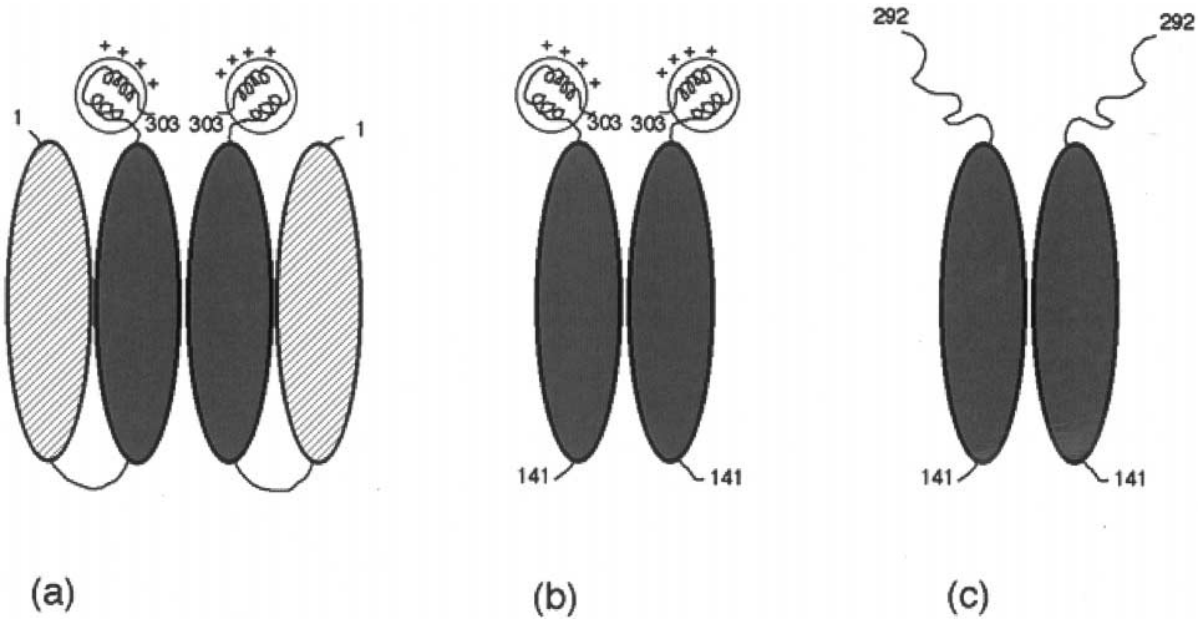

Figure 6. Model for the gp8 scaffolding protein of P22. (*a*) Full-length gp8 (303 amino acids), (*b*) N-terminally truncated gp8 (residues 141–303), (*c*) N– and C-terminally truncated protein (residues 141–292). The N-terminally truncated protein (*b*) is sufficient for promoting capsid assembly, whereas the N-terminal 140 residues ((*a*), hatched) are involved in tetramerization of uncertain function. Removal of the C-terminal 12 residues (292–303) destroys procapsid assembly activity (*c*). These residues form charged interactions with the capsid protein via an amphipathic α helix. Residues 141–292 are sufficient for dimer formation. Adapted from [54].

protein, along with the 415 copies of gp10 capsid protein that comprise the shell [73–75]. This ratio is similar to that found for the P22 procapsid. T7 in addition codes for three minor proteins, gp14, gp15 and gp16, which are associated with the permanent internal core [74]. Mutants of any of these minor core proteins or of the connector protein gp8 produce tubular structures called polycapsids, which lack any scaffolding protein [76, 77].

T7 assembly has been reproduced both in vivo and in vitro [77]. No capsid-related structures are seen during expression of the capsid protein alone, but prohead-like shells containing scaffolding and capsid proteins are produced when both proteins are expressed together. Similar results were obtained in vitro using purified components: scaffolding-containing, procapsid-like structures were found when gp9 and gp10 were coassembled. Efficient assembly in vitro requires the scaffolding protein to be present in large excess, without which the shells remain unclosed [77]. If only small amounts of scaffolding protein are made available, most of the capsid protein assembles into polycapsids. Coexpressions including the connector protein gp8 in addition to gp9 and gp10 result in the production of heads that look like mature (expanded), empty capsids and lack both scaffolding and connector proteins [77]. Apparently, the presence of connector protein favours the conversion of procapsid-like structures to expanded shells, even though the resulting shells contain neither scaffolding nor connector protein. Probably the minor core proteins, gp14, gp15 and gp16, are essential for the incorporation of connectors into the procapsids.

Interestingly, the T7 assembly data favours a model in which initially formed incomplete shells are subsequently closed by the insertion of connectors [77]. This model goes against the more commonly held view for dsDNA phage assembly: that the connectors act as the initiator for shell (and scaffold) assembly.

Based on its unusual mobility by SDS-PAGE, the gp9 scaffolding protein is likely to have an extended conformation in solution, similar to those of P22 and λ [77]. It is also highly sensitive to protease digestion, indicative of high structural flexibility. An elongated shape for gp9 was also suggested by EM studies, which showed a core having fibrous projections that made contact with the shell [76].

Herpesviridae scaffolding proteins

The herpesviridae family of large dsDNA animal viruses include herpes simplex virus (HSV), cytomegalovirus (CMV) and varicella-zoster virus (VZV). These viruses undergo an assembly process similar to that of the dsDNA bacteriophages, including the production of an empty capsid precursor which is subsequently filled with DNA, and the dependence of the assembly process on scaffolding proteins [78, 79]. (Unless otherwise noted, the following discussion refers to the most well-described member, HSV.)

The HSV virion contains a 1250 Å-diameter, T = 16 icosahedral nucleocapsid [80–82] comprising at least five different proteins, enclosed inside a complex lipid envelope (see [79]). The nucleocapsid contains 960 copies of VP5, the major capsid protein, arranged as hexamers and pentamers, and 320, 640 and 900 copies of the three proteins VP19c, VP23 and VP26, respectively. Three different nucleocapsid-related particles are seen in HSV infections: native, DNA-filled C capsids, scaffolding core-containing B capsids, presumably an assembly intermediate, and empty A capsids, probably an abortive byproduct.

The scaffolding cores found in the B capsids consist of three major proteins, VP22a, VP21 and VP24, which are all produced from the two overlapping reading frames UL26 and UL26.5. VP22a is the main component of the core, and is present in about 1000 copies [83]. VP24 is a maturational protease, which is initially expressed as a polyprotein containing the minor scaffolding protein VP21 at its C-terminus (fig. 5). VP21 in turn includes all of VP22a at its C-terminus. Maturation of the B capsids requires cleavage of the C-terminal 25 residues of VP22a and VP21 by the VP24 protease [84]. This is concomitant with a structural change in the core of the B capsids [85]. In addition to its scaffolding core function, VP22a also serves to transport the major capsid protein to the nucleus, which is the site of virus assembly [86–88].

The role of VP22a (and other scaffolding-related proteins) in HSV assembly has been elucidated both through the use of a baculovirus assembly system [89–91] and an in vitro system [92–94], as well as through traditional mutational analysis [88, 95].

Expression of all the HSV nucleocapsid structural proteins in the baculovirus system results in capsidlike structures indistinguishable from normal nucleocapsids. If any of the major mature capsid proteins (VP5, VP23 or VP19c) are left out, VP22a-containing particles of 400–600 Å diameter are produced [90], the same size as the cores seen inside HSV nucleocapsids [96]. Similar size particles are also produced when VP22a is extracted from B capsids with 2 M GuHCl and reassembled in vitro [97]. In the absence of both VP22a and VP21 scaffolding proteins, mostly aberrant structures are formed [90, 91], like those seen in null mutants of the UL26 open reading frame (which removes VP22a, VP21 and VP24) [95]. However, some closed capsidlike particles are produced in the absence of VP22a if VP21 is present. Apparently, the less abundant VP21 (whose C-terminal part is identical to VP22a) can to some extent substitute for VP22a; however, the resulting par-

ticles lack obvious cores [91]. In the absence of VP21/VP24, but in the presence of VP22a, particles similar to B capsids are produced [91].

More rounded scaffolding-containing particles appear in an in vitro system after about 1 h of assembly. These particles mature into angular particles looking like B capsids over a period of 8 h [93, 98] and probably represent procapsids, similar to those seen in the bacteriophages. Wedge-shaped intermediates, appearing to consist of a partial procapsid shell, are seen after about a minute of assembly, demonstrating that the scaffolding protein coassembles with the capsid proteins.

The C-terminal 12 residues of VP22a form specific interactions with the major capsid protein [99] (fig. 5). These residues are predicted to form an α helix, and contain an essential Phe residue. In CMV, the C-terminal 16–21 amino acids of the scaffolding protein, called assembly protein, or pAP, is required for this capsid protein interaction [87, 100]. Swapping this region between VP22a and pAP changes the capsid protein specificity [100, 101]. Likewise, HSV assembly was supported by the VZV scaffolding protein if the 23 C-terminal residues were swapped with those of VP22a [102]. Between the more homologous bovine and human HSV, the UL26 and UL26.5 open reading frames (encoding VP22a, VP21 and VP24) can be exchanged without loss of function [103].

The C-terminal region is not essential for forming intrascaffolding interactions, since mature VP22a, in which the 25 last residues have been cleaved off, can reassemble into corelike particles in vitro after particle disruption [97]. Thus, while the C-terminal part of the protein forms specific interactions with the capsid proteins, the N-terminal portion forms the interactions required to assemble a scaffolding core (fig. 5). In HSV-1 these interactions are formed between residues 165 and 234 [104]. In CMV, residues 34–52 of pAP were essential for forming the intrascaffolding interactions [87].

The core appears not to be icosahedrally ordered either in the procapsid or in the B capsid [80, 85, 98]. However, difference mapping between B capsids containing cleaved and uncleaved scaffolding protein revealed that a small part of the scaffolding protein forms rod-shaped densities near the VP19a and VP23 triplex proteins, which comprise the connections between the capsid protein hexamers and pentamers [85]. Radial density profiles suggest that the protein has an elongated shape of about 250 Å length and is composed of three domains separated by low-density linkers [98]. In particular, there is a low-density region between the capsid shell and the core, suggesting a rather tenuous connection between the scaffold and the capsid shell [80, 85, 98] (fig. 5).

Many of the structural as well as functional aspects of the herpesvirus scaffolding proteins are thus quite similar to those found in the dsDNA phages, and especially in P22 [53], suggesting, perhaps, an evolutionary relationship between herpesvirus and the dsDNA bacteriophages.

Prolate scaffolding core

The *E. coli* dsDNA bacteriophage T4 is not only one of the most complex of all viruses, but also one of the most well characterized of all assembly systems [7, 105]. T4 has a prolate capsid (1170 Å long and 860 Å wide) containing 960 copies of the major capsid protein, gp23* (a cleaved form of gp23), with a related protein, gp24*, comprising the fivefold vertices. A simple connector structure is found at the portal vertex where the very complex tail is attached.

During T4 assembly, an elongated scaffolding core can be seen inside the assembling capsids [10] (fig. 2). The T4 core is a complex structure consisting of at least seven proteins (gp21, gp22, gp67 and the nonessential gp68, IPI, IPII and IPIII) [7, 105–107]. The main component of the core is gp22, which comprises 38% of the core mass. Core-containing T4 procapsids, so called T particles, are unstable, but can be isolated from certain mutant infections, such as mutations in the processing protease, itself a part of the core [108, 109]. Naked cores are produced in mutants of the major capsid protein, gp23 [106, 110]. Both naked cores and procapsids can be produced in vitro from purified, disrupted procapsids [111, 112]. This contrasts with the previously described, simple, isometric viruses, where the scaffolding protein shows little propensity to assemble in the absence of capsid protein. Morphogenetically correct, albeit nonactive prohead shells can be produced using only the four proteins gp20 (connector), the gp23 capsid protein and the two scaffolding proteins gp22 and gp67 [105].

Polyheads—open-ended, tubular forms of the gp23 capsid protein—are formed in several T4 mutants. Mutants in gp20 (connector) or gp24 (fivefold vertex) form polyheads which contain a scaffolding core of gp22, while those formed in the *22*$^-$ mutants contain multiple layers of gp23, suggesting that gp23 assembly requires a template, which would normally comprise the scaffolding core [105, 113]. In vitro, gp23, isolated by disruption of polyheads reforms polyheads in the absence of any scaffolding proteins [114, 115]. Conversely, the core proteins can form 'polycores' in the absence of gp23 and gp20, the connector [116]. In the presence of connector protein, however, the core proteins can form either naked cores [111, 112] or star-shaped cores with connectors at each point of the star [7, 117]. Taken together, these studies suggest a defining role of the

gp20 connector in initiating core assembly as well as determining the size of the scaffolding core. The core in turn, nucleates the assembly of the shell [105].

Both point mutations in gp22 and mutants in the other major scaffolding proteins, gp67 and the nonessential gp68 produce aberrant viruslike particles of a variety of shapes and sizes [118–120]. Apparently gp22 alone is sufficient to cause the formation of closed shells, whereas gp67 is required to ensure the fidelity of the assembly. Size variants have also been found in shell protein mutants, but always of normal width, whereas gp67 mutants also generate width variants, suggesting that the core is the main determinant in specifying the width of the particle [120].

The main core protein, gp22, is a highly charged (pI = 4.3), predominantly α helical protein, and is predicted to form a three-stranded coiled-coil structure [121]. gp22 can self-assemble in vitro into filamentous ribbons [122, 123]. The core has been proposed to contain from 6 to 10 such ribbons arranged in a helical configuration [123–125]. Scanning transmission EM observations of the ribbons suggest that gp22 exhibits an elongated rodlike shape [122, 123]. The apparently odd mismatch between the sixfold symmetry of the core and the fivefold symmetric shell may function in a kind of Vernier mechanism for length determination of the head [118, 125]. (Incidentally, a 6-fold symmetry would also be consistent with the 12-fold symmetry of the connector [126].)

The three core proteins IPI, IPII and IPIII are present in large amounts, but some phage particles are assembled normally without them [105]. There seems to be a synergistic action with gp22, however, since double mutants in both gp22 and IPIIII form only aggregates of capsid protein rather than the normal multilayered polyheads seen in gp22 mutants [10, 127].

The gp21 protein forms a dense kernel at the centre of the core [109], where it acts as a core-associated protease, called the T4 PPase. T4 PPase is responsible for maturational cleavage of the capsid proteins as well as cleavage and consequent release of the core components [128, 129]. This cleavage is normally required for the maturation of the capsid, manifested as expansion of the shell. Without cleavage the assembly process is halted at the procapsid stage [108, 109]. Fragments of all the core proteins remain inside the capsid after expansion.

The T4 core is thus a complex structure, apparently very different from the simple cores of the isometric phages, like P22. Yet the main core protein, gp22, shares several similarities with the core proteins of the isometric phages, including an elongated shape, high α helical content and high charge. Perhaps these physical properties are especially advantageous for its function as a scaffolding core.

Miscellaneous viral scaffolding proteins

Bacteriophage ϕ29 gp7

The *Bacillus subtilis* dsDNA bacteriophage ϕ29 virion has a 540-Å long, 450-Å wide prolate capsid consisting of two T = 3 caps with a central cylindrical segment, and contains 235 copies of the gp8 capsid protein [130–132]. The virion also contains a 12-fold symmetric connector or portal made of gp10, the portal protein. ϕ29 codes for one scaffolding protein, gp7, which is required for procapsid assembly. The procapsid contains about 180 copies of gp7, which are recycled in subsequent rounds of assembly [131, 133–135]. No obvious scaffolding cores are seen inside the procapsid in cryo-electron micrographs or in 3D reconstructions [132], suggesting that the scaffold is only transient or unstable, like in phage λ.

The entire assembly pathway of ϕ29, including DNA packaging and formation of infectious virions, has been reproduced in vitro [136–140]. Although the ϕ29 capsid is prolate and thus superficially T4-like, the assembly process and the properties of the scaffolding core are more like those of the simple isometric phages, such as λ.

The gp7 scaffolding protein has a high α helical content and is expressed in *E. coli* as soluble monomers [137, 139]. Expression of the gp8 capsid protein alone primarily results in the formation of inclusion bodies, which after denaturation and renaturation form aberrant shells [139]. In the absence of gp10, the portal or connector protein, the capsid and scaffolding proteins copolymerise upon expression into capsidlike structures which vary in size and shape [139]. Coexpression of gp7 with gp10 leads to the formation of a soluble, but unstable complex of the two proteins. The same is found if the two proteins are mixed in vitro [139]. Thus, gp7 has affinity both for the gp8 capsid protein and the gp10 portal vertex protein [137]. In contrast, gp10 and gp8 do not form complexes upon coexpression, indicating that the scaffolding protein gp7 is required to mediate the contacts between connector and capsid proteins. If all three proteins gp7, gp8 and gp10 are expressed together, however, a homogeneous population of correctly shaped particles is produced, which can be matured into infectious virions in vitro [137–139]. Hence, although assembly can be initiated in the absence of gp10, the portal protein is required for correct size determination.

This size determination is apparently mediated through interactions between the scaffolding and portal proteins. Temperature-sensitive mutants in gp7 produce T = 3 isometric particles that also lack connectors [132]. Most likely, therefore, it is the connector and its associated internal core structure that specifies the elongated shape of the wild-type, prolate capsid. The gp7 mutants,

while able to promote shell assembly, are unable to incorporate the connector structure and, hence, form only isometric heads. In phage P22, scaffolding protein is also required to incorporate portal protein, although the scaffolding protein alone is sufficient for the formation of correctly sized shells. In this isometric case the size determination problem is simpler, as only one dimension (radius) needs to be specified. The likely events in the assembly of $\phi 29$ are therefore that the connector acts as the initiator, which organises the scaffolding protein, which then acts upon the capsid protein assembly [137–139].

P2 protein gpO

Bacteriophage P2, the helper virus of satellite phage P4, encodes a protein, gpO, which is required for shell assembly in vivo [141] and is believed to comprise an internal scaffold [142]. gpO is associated with a subset of empty procapsid-like particles produced during both P2 and P4 infections and is absent from mature capsids [143], although a cleavage product of gpO, O*, remains inside the mature capsid [144]. The presence of gpO leads to reduced formation of aberrant capsids in an in vivo expression system [39]. However, gpO is not found associated with the procapsid-like particles formed in these expressions [39]. Cleavage of gpN and gpO into its respective cleavage products is mutually dependent [141], but gpO itself does not appear to have protease activity.

gpO is also required for assembly of the P4 satellite phage [32]. Expression of both Sid and gpO together with gpN leads to increased fidelity of assembly, but results in a mixture of particles of P4-like and P2-like sizes [39]. This suggests that there is a competition between gpO and Sid in terms of size determination [39, 142]. Clearly, additional control points must exist for efficient assembly and size determination in vivo. Most likely, this involves the gpQ connector protein [144, 145].

The properties of gpO protein have not been characterized in sufficient detail to classify it in any particular group of scaffolding proteins. However, by analogy with other isometric dsDNA phages it is perhaps likely to share many properties with the internal scaffolding proteins of P22 and λ.

ϕX174 protein B

As mentioned previously, assembly of the *E. coli* ssDNA phage ϕX174 depends on an internal scaffolding protein, protein B, in addition to the already described external scaffolding protein D [22, 23]. In cells infected with mutants in the B protein, pentamers of the F and G proteins accumulate, and no capsids are produced [26, 27]. The role of the B protein in the assembly process is not well understood, and it seems to act both on these early intermediates and in the formation of twofold contacts later in assembly, as well as playing an important role in the conformational changes accompanying capsid maturation [20].

In the ϕX174 procapsid X-ray structure, the internal scaffolding protein B forms tight interactions on the internal surface of the F capsid protein through a number of polar contacts [18, 20]. The N-terminal half of protein B is not seen in the procapsid structure, indicating that this part of the protein is flexible and/or not icosahedrally ordered. The ordered C-terminal half of the protein forms one long, radially oriented α helix and two smaller helices. The last few residues, which make a small amphipathic helix, contain several aromatic as well as basic residues, and fit into a pocket in the F protein [20] (fig. 7).

The interaction site for protein B on protein F also forms the binding site for the DNA-associated protein J, which enters the capsid during DNA packaging. This suggests that a competition between these two proteins during capsid maturation may be important in releasing the B protein and possibly also in triggering the conformational change of the capsid [18, 20]. The B protein has to exit the capsid upon DNA packaging. The procapsid EM structure displays large holes at the threefold axes through which the B protein could escape and potentially also forms the port of entry for the genome [21]. The B protein must therefore be flexible enough to exit through these 20-Å holes.

The C-terminal region 20 amino acids or so is the most conserved part of protein B between ϕX174 and the related viruses $\alpha 3$ and G4. The rest of the protein is highly variable. Nevertheless, the ϕX174 B protein is able to complement the highly divergent $\alpha 3$ B protein, suggesting that only the more conserved C-terminus is required for interaction with the F protein [146]. The ϕX174 B protein, on the other hand, is not able to complement the more closely related G4 protein, although mutants in the G4 F protein have been isolated which enable G4 to utilise the ϕX174 B protein [146]. Furthermore, a chimeric B protein, having its N-terminal part from G4 and its C-terminal part from ϕX174 did support assembly of ϕX174, but not of G4. This shows that the B protein consists of two more or less independent functional domains: a species-specific C-terminal domain which makes contact with protein F, and an N-terminal domain which forms interactions with other B proteins. This division into two domains is reminiscent of that seen in the isometric dsDNA phages and in the herpesviruses, although the B protein of ϕX174 appears to exhibit more icosahedral order than these other scaffolding proteins.

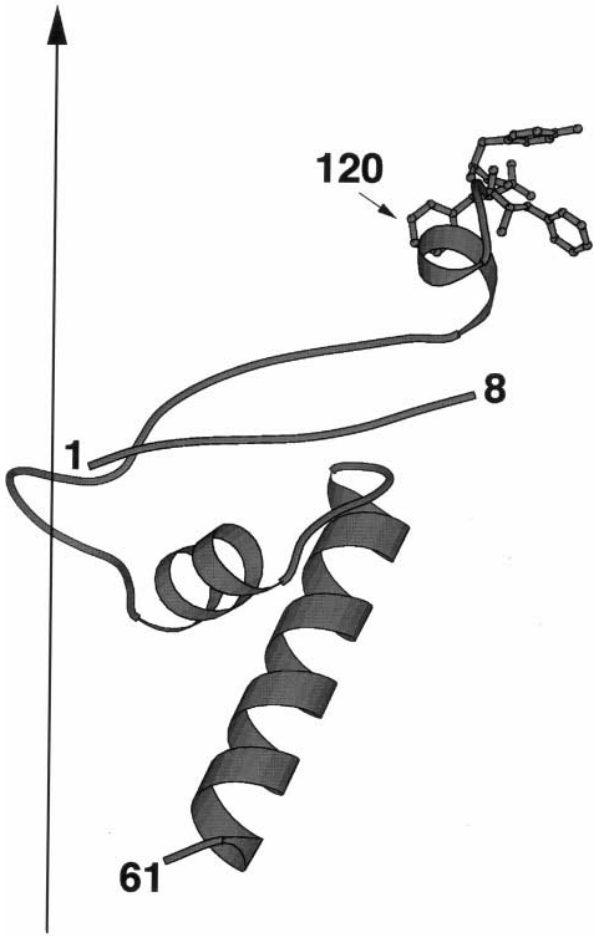

Figure 7. Ribbon diagram of the part of the ϕX174 internal scaffolding protein B that is visible in the X-ray map, namely residues 1–8 and 61–120 [20]. The side chains are indicated for the important aromatic residues at the C-terminus, where the B protein makes contact with the F capsid protein. The nearest twofold axis (arrow) is shown pointing radially outwards.

Scaffolding proteins of adenovirus

The assembly pathway of adenovirus, a large dsDNA animal virus, has been relatively well worked out [147, 148]. The adenovirus virion is a complex structure, consisting of 720 copies of the major capsid protein, the hexon, arranged as trimers in a pseudo-T = 25 lattice [149]. A number of minor capsid proteins are found which mediate the interactions between the hexons [147, 149]. Assembly intermediate capsids are found during a wild-type infection [150, 151] and accumulate in some mutant infections [152, 153]. A number of proteins are found associated with these intermediate particles which are not present in mature particles and could therefore have a scaffolding function [147, 148, 151, 154]. One of these proteins, the 52/55 kDa protein, has more recently been shown to be involved in genome packaging rather than capsid assembly [155].

Another protein, the 100 kDa protein, however, has a more clearly defined role as a scaffolding protein. This multifunctional protein is required for trimerisation of the hexon as well as transport of hexons to the nucleus. A scaffolding protein function for 100 kDa is evidenced by the failure of temperature-sensitive mutants to assemble capsids at the nonpermissive temperature, whereas intermediate particles and virions are assembled upon shift to permissive temperatures, in the absence of de novo protein synthesis [156–158]. The hexon trimerisation function of the 100 kDa protein is located in the carboxy-terminal part; the scaffolding function resides in the central part of the protein (residues 360–400). In the intermediate particles, 100 kDa is associated with protein IIIa near the fivefold vertex. A 90 kDa cleavage product of 100 kDa associates with the hexons at the capsid faces.

Unfortunately, not much else is known about the 100 kDa scaffolding protein, and even less is known about the other presumptive scaffolding proteins, the 32–33 kDa and the 40 kDa proteins [147, 148, 154].

More scaffolding proteins?

Many closely related viruses to the ones already described exist, which, by inference, are assumed to contain homologous scaffolding proteins, even though they have not been positively identified. This includes e.g. dsDNA phages P1 [159], Mu [160] and *B. subtilis* phage SPP1 [161, 162]. (An interesting exception to the almost ubiquitous use of scaffolding proteins in dsDNA phages is seen in HK97, which is structurally very similar to phage λ, and yet assembles without the use of scaffolding proteins [163].)

Many other viruses are clearly able to assemble without the use of scaffolding proteins, or at least do not absolutely require them. Nevertheless, several less well described virus groups of complex morphology probably use them for their morphogenesis. For example, it is very likely that the complex dsDNA viruses of the *Iridoviridae* and the structurally related African swine fever virus assemble via intermediates which require scaffolding proteins [164].

Proteins with a similar function to scaffolding proteins also exist in enveloped viruses. The nucleocapsid protein of vesicular stomatitis virus could be reassembled in vitro only in the presence of M protein [165]. The M protein appears to act as a scaffold for nucleocapsid assembly in addition to its role in mediating nucleocapsid-membrane interactions [166, 167]. In this case, however, the M protein remains inside the capsid

after virus assembly has been completed. Similar roles may be envisaged for the M proteins of other viruses, such as the influenza viruses.

Scaffolding proteins are, however, not restricted to viral capsid assembly. One example of a nonviral scaffolding protein is the FlgD protein of *Salmonella* [168]. This 27-kDa protein is required for flagellar hook assembly, but is absent from the mature hook. Several other proteins are known which can be considered de facto scaffolding proteins, although they are not generally thought of in this context. This includes a number of chaperones, such as PapD, which is involved in the assembly of the bacterial pili [169] or gp57A of bacteriophage T4, which is involved in phage tail fibre assembly [170]. Many other, more traditional chaperones also affect the assembly of their substrates in addition to their more familiar role in protein folding [13]. Another large group of proteins that could be considered scaffolding proteins are the many microtubule-associated and actin-binding proteins of the cytoskeleton, which satisfy the criteria for scaffolding proteins, in that they promote or regulate an assembly process (polymerisation) and are only transiently associated with their substrate. These systems have been the subject of a number of reviews [171–173]. Many other, more complex assembly systems are now being characterized, promising to reveal novel types of scaffolding proteins [174, 175].

Structural themes

Structures of scaffolds and scaffolding proteins

Direct structural information on scaffolding proteins is scarce. Only the external and internal scaffolding proteins of bacteriophage ϕX174 have been resolved to high resolution by X-ray crystallography [18, 20]. The external scaffolding protein Sid of bacteriophage P4 is the only one that has been observed by electron microscopy and 3D reconstruction [31]. No nuclear magnetic resonance (NMR) or X-ray crystal structure of an isolated scaffolding protein is yet available.

The difficulty in obtaining structures of scaffolding proteins is partly due to the intrinsic instability of assembly intermediates, and hence the difficulty of obtaining suitable material for structural analysis. Scaffolds are by definition only transient structures, and it may therefore be impossible to isolate significant amounts of scaffolding-containing structures from a wild-type infection. Furthermore, the scaffolding protein may not be organized with the same symmetry as the capsid shell and be present only in substoichiometric amounts. In addition, the scaffolding proteins themselves appear to have a generally high intrinsic flexibility (see below).

The largest coherent group of scaffolding proteins are the internal corelike scaffolds. For a few of these, P22 [49], herpesvirus [85, 89] and ϕ29 [132], 3D reconstructions of scaffolding-containing procapsids have been obtained. None of these structures show the scaffolding protein structure, presumably due to lack of icosahedral order of the core. Radial profiles display a relatively dense central region, a low-density connecting region, and a small, ordered domain forming specific interactions with the shell. Low-angle X-ray scattering measurements in P22 and λ reveal a similar organisation [48, 69]. If these three regions represent functional domains of the scaffolding protein, it would have a highly elongated shape, which is consistent with solution measurements for a number of internal scaffolding proteins, including P22 gp8 [50, 56] and λ gpNu3 [71] (fig. 5). Biochemical, genetic and structural studies both of the P22 and herpes scaffolding proteins demonstrate a two-domain structure, where one domain forms the intrascaffolding interactions, and the other makes contact with the shell, consistent with the available structural information [50, 53, 54, 87, 97, 99, 100].

An icosahedrally ordered internal scaffolding protein like the B protein of ϕX174 appears to be unusual. Even here, however, only about one-half of the protein is visible in the X-ray structure, probably due to disorder in the protein structure itself [20]. In this protein, the C-terminal tail forms specific interactions with the capsid protein, whereas the N-terminal part is apparently involved in intrascaffolding interactions [20, 146].

The organization of the T4 core is superficially not unlike those of the spherical cores, except that it is prolate rather than isometric [105]. (The T4 core, of course, also contains several other proteins in addition to the main scaffolding protein gp22.) Interestingly, the main protein of the T4 core, gp22, seems to share several structural properties with the scaffolding core proteins of the P22-like phages [121, 123].

In contrast to the internal scaffolding cores, the external scaffolds of P4 and ϕX174 both do display strict icosahedral order. This may represent a fundamentally distinct mode of operation of these scaffolding proteins. The external scaffold of P4 is made up of lofty arches, which span the two- and threefold axes (fig. 3B). This suggests that Sid has an elongated shape and that the scaffold is made up of a parallel, quasi-symmetric arrangement of 60 dimers or alternatively a strictly symmetric arrangement of 60 elongated monomers [31]. For ϕX174, on the other hand, the organisation is quite different: the external scaffold is composed of 30 asymmetric rhomboids each containing eight copies of protein D (figs 3A, 4). Within each rhomboid, the compact and globular subunits are arranged in a completely nonsymmetrical fashion, and exhibit great variability in conformation and in protein-protein

interactions. This remarkably nonsymmetrical organization of the ϕX174 D protein emphasises the structural versatility of these proteins and opens enormous possibilities in the interpretation of structures which have so far been interpreted in light of a simple, symmetric model according to the principles of Caspar and Klug [30]. It will be very interesting to see the structure of the P4 external scaffold at high resolution and whether it is also arranged with such lack of local symmetry.

Secondary structure and structural flexibility

The scaffolding core proteins of P22 [52], ϕ29 [139] and T4 [121] all have a high predicted or measured α helical content. All these functionally similar proteins also share other features, such as an elongated shape and a high charged amino acid composition, suggesting perhaps a structural relationship also at a higher resolution.

Perhaps by coincidence, the external scaffolding proteins of ϕX174 (protein D) and P4 (Sid) also have predominantly α helical structures [18, 40]. As there is unlikely to be an evolutionary relationship between these very different proteins, perhaps there is a specific structural advantage of using α helical structures for this purpose.

The most advantageous property for a scaffolding protein to have is probably that of conformational flexibility. This allows the structural adaptability that is necessary to bind to several, conformationally nonequivalent capsid proteins, as well as to accommodate both capsid protein binding and subsequent release and removal from the capsid. Flexibility in proteins include domain movements and hinge rotations as well as changes in secondary structure and transitions between ordered and disordered structures [176–180]. Sometimes, structural proteins are kept in an unfolded state until completion of assembly, such as the VP23 triplex proteins of herpesvirus [181] or the FlgM protein of the bacterial flagella [182, 183].

In viruses, conformational switching is ubiquitous. For example, the N-termini of several plant virus capsid proteins switch between ordered and unordered conformations upon assembly, depending on their structural context [184, 185]. In the polyomaviruses, the capsid protein pentamers have long C-terminal arms that are disordered in solution, but takes on a set of specific conformations when they form the quasi-T = 7 capsid shell [186]. Wholesale conformational changes are observed upon capsid maturation in λ, P22, herpesvirus and T4 [64, 98, 187, 188].

Several bacteriophage capsid and scaffolding proteins have been fund to undergo secondary structure changes during capsid maturation [57–59, 189, 190]. The gp8 scaffolding protein of P22 comprises multiple α helical domains and exhibits high flexibility in solution. In the P22 procapsid structure, however, the protein is highly ordered [57].

In ϕX174, the D scaffolding protein exhibits at last three distinctly different conformations depending on the structural environment, including switching between order and disorder and between types of secondary structure [18, 20]. These conformational differences presumably reflect adaptive changes during assembly. It is not known what the solution structure of protein D is, but it is likely to be quite different from the structure in the procapsid [18, 20, 29].

The P4 Sid protein is also predicted to have large disordered regions [unpublished results] even though it forms a highly ordered, regular scaffold in the P4 procapsid structure [31]. Such disordered regions may reflect conformational flexibility and be important for scaffold and capsid assembly.

Functional roles of scaffolds

Several roles have been proposed for the viral scaffolding proteins, including initiation of assembly, stimulation of shell growth, capsid size determination, mechanical stability of the shell and capsid maturation. In general, scaffolding proteins can act in three main ways: (i) in the active establishment of correct interactions between substrate molecules; (ii) a more catalytic role, in the promotion and nucleation of assembly reactions; (iii) a chaperone-like function in preventing the formation of incorrect interactions.

Scaffolding proteins and specificity of the assembly

The generally high specificity of scaffolding proteins for their 'substrate' suggests an active role in defining the final assembly. The role of the scaffolding proteins may be to actively switch the capsid precursors from a non-active (non-sticky) to an assembly-active sticky conformation. By linking this switch to the binding of scaffolding proteins, the reaction is prevented from occurring randomly, and instead delayed until the precursors have been brought in the correct spatial relationship. The scaffolding proteins may be able to induce conformational changes in the capsid or other structural proteins, which would not normally occur due to energetic barriers. Although ATP is generally not utilised, the required energy can be stored in the scaffolding proteins themselves as 'conformational energy' [8]. The P22 scaffolding protein gp8 is only seen to interact with the capsid protein at some of the several quasi-equivalent positions, suggesting that it is actively involved in the conformational switching of the gp5 capsid protein [191]. These observations are consistent

with a model in which assembly is driven by alternating scaffolding-scaffolding, scaffolding-capsid and capsid-capsid interactions. In P22, the formation of scaffold-capsid protein interactions as well as scaffold removal are also coupled to folding and unfolding of the gp8 protein [57, 190, 192]. Such coupling of the folding and assembly reactions may provide more control over the assembly process, linking the energetical expense of folding to the scaffolding-induced conformational switching and assembly processes.

The highly charged surfaces of the internal scaffolding proteins suggest that the scaffold-capsid protein interaction is dominated by charged and polar interactions. By contrast, capsid protein interactions are largely of a nonpolar nature, and the assembly reaction is generally considered to be entropy-driven [17, 115, 193–195]. In ϕX174, most of the contacts between capsid and scaffold are of a nonpolar nature [20]. Polar interactions are likely to be important to generate specificity and stability of the assembly reaction [195].

One of the functions of the scaffolding proteins is to bring functional subunits together by encouraging interactions between proteins that do not interact by themselves. Examples of this kind of function are found in ϕ29, λ and P22, where the connector and capsid proteins only interact in the presence of scaffolding protein [62, 66, 139].

There is usually a close relationship between the assembly of the capsid and the scaffold, and many scaffolding proteins do not have a propensity for assembling in the absence of capsid protein. Conversely, in many viruses, such as ϕX174, no capsids or capsid-related structures at all are formed in the absence of scaffolding proteins. Presumably, the scaffolding protein is required to bind the capsid precursors (in the case of ϕX174, pentamers of F and G proteins) and bring them together spatially. In P22, binding of gp8 scaffolding protein to capsid protein monomers appears to lead to gp8 dimerization, which thereby brings the capsid proteins together in the correct relationship for shell assembly in a sequential manner [51, 191].

In other systems, however, scaffold and capsid assembly are apparently completely independent. In T4, complete scaffolding cores are formed in vivo and in vitro in the absence of capsid protein [106, 110–112]. The cores are subsequently required as a template for the capsid proteins.

Size determination

One of the main functions attributed to the scaffolding proteins is that of size determination. This may be accomplished by a number of mechanisms: The internal corelike scaffolds work by a somewhat unspecific fashion, by providing a scaffolding core of a defined size, dependent mostly on the physical shape of the protein. Thus, the core-forming domains of HSV and CMV scaffolding proteins could be swapped without loss of function [100, 101]. In P22, only correctly sized scaffolding-containing shells are produced in the presence of scaffolding protein. Small shells can only be produced at low efficiency in vivo in the absence of scaffolding protein [60]. Presumably the length ($\sim$200 Å) of the gp8 scaffolding protein prohibits the formation of a smaller scaffolding core. In phage λ, on the other hand, small-head mutants of the capsid proteins also contain cores [72]. gpNu3 is only about 100 Å long, however, and is therefore able to accommodate the smaller core size [71]. In contrast, phage ϕ29 scaffolding and capsid proteins coassemble into closed capsid-like particles of variable size, demonstrating that scaffolding protein alone is not sufficient for size determination in this system [139].

In T4, mutants in the scaffolding proteins can generate shells of aberrant size [119, 120]. In the absence of scaffolding protein, only unclosed, tubular polyheads are formed. These tubular forms require no conformational switching of the capsid protein, suggesting that one of the roles of the scaffolding protein may be to enable this kind of switching. Alternatively, the T4 core only provides a template of appropriate size for the shell protein to assemble upon. The core and shell together are thought to specify shell length through a Vernier mechanism [118, 125], but there must be additional factors involved, as scaffolding-containing polyheads are produced when connector protein is absent [105]. The T4 case is more complicated, as there are several gene products involved in construction of the core.

The external scaffolding proteins, on the other hand, apparently operate by a more specific mechanism of sequential scaffolding-capsid interactions and hence have a more ordered structure, symmetrically coupled to the capsid itself.

Phage P4 represents a particularly interesting case, where perfectly formed capsids are formed in the absence of the external scaffolding protein Sid, as long as the internal scaffolding protein, gpO, is present. The presence of Sid, however, changes the size specifically to a different type of capsid. Apparently, Sid overrides any size-determining effect that gpO may have, although gpO may still be required to prevent unspecific aggregation of capsid protein [31, 39].

Nucleation and catalysis of the assembly

Even though capsid proteins often do not form structures in the absence of scaffolding proteins at low protein concentrations, they will often do so at elevated protein concentrations in vitro or during protein expres-

sion. These structures usually comprise mostly aberrant, unclosed shells of undefined size. In the presence of scaffolding proteins, however, assembly often occurs at lower concentrations. The scaffolding proteins in this case act as a nucleator or a promoter of assembly, lowering the concentration required for assembly to a point where nonspecific aggregation is not predominant.

A similar function has been ascribed to the bacteriophage connector, generally considered to act as an initiator of assembly in vivo. In this respect, the two protein functions may be complementary. The connector may in some cases even be redundant in terms of assembly, and indeed perfectly symmetric shells of T7, P22 and ϕ29 are produced in the absence of connector.

In phage λ, particles containing complete scaffolding cores are never seen in wild-type infections. Likewise, in P22 and T7 only substoichiometric amounts of scaffolding protein are required for capsid assembly. In both systems, assembly is compatible with a ratio of scaffolding to capsid protein ranging from about 0.2:1 to more than 1:1 [44, 45, 77]. Lower ratios than this results in the formation of various aberrant capsid protein aggregates, including polycapsids in T7. This amount is still more than that expected for a simple nucleation event, however, suggesting that, unlike the connector, the scaffolding protein is required at more than one point in the shell. This suggests that the scaffolding protein subunits are reused at several points within the growing shell, e.g. at the growing edge of the shell, where there would be a transient, but required specific interaction between scaffolding and capsid protein [44, 45]. Furthermore, electron microscopy has shown than the scaffolding protein only interacts with capsid protein at certain quasi-equivalent positions, reducing the amount of protein needed [191]. Particles with complete cores, such as seen in some λ mutant infections, may be caused by a failure to release the scaffolding protein after use [66–68].

A truly catalytic mode of action would imply that the scaffolding proteins are recycled and reused for subsequent rounds of assembly. The only scaffolding proteins known to be recycled, however, are those of P22 [42] and ϕ29 [131]. Other scaffolds, like those of T4, λ, herpes and P2 are irreversibly changed by proteolytic degradation after assembly and therefore do not function in a catalytic way per se.

This assembly-promoting function of scaffolding proteins is similar to that of certain microtubule-associated proteins, which either increase the rate of nucleation or decrease the rate of 'catastrophe', the transition from a growing to a shrinking state, resulting in a greater overall length of microtubules [172]. (Other proteins work in the opposite way, reducing the overall microtubule length, obviously important to prevent the entire cell from filling up with microtubules.)

Preventing improper interactions

The capsid proteins of large viruses need to have a high intrinsic flexibility in order to attain the several nonequivalent conformations that are required [30, 196]. In addition, many viruses, most notably the dsDNA bacteriophages, undergo major structural transitions during maturation, which also need to be accommodated by flexibility in the structural proteins [64, 187].

This high intrinsic flexibility is evident in the propensity for many capsid proteins to assemble into structures of variable size and shape. During normal morphogenesis, this flexibility needs to be curtailed to ensure that only the distinct subset of possible conformations that are required for a specific capsid structure are generated. One role of scaffolding proteins may therefore be to restrict this natural variability.

In this light, the role of the scaffolding proteins is not unlike that of traditional chaperones, in inhibiting improper interactions from forming by preventing assembly or delaying it to the right moment. It is necessary to have sufficient scaffolding protein present to ensure that all the protein is caught before it assembles incorrectly. The fact that many scaffolding proteins, such as the D protein of ϕX174 or Sid protein of P4, are produced in a large excess, suggests a chaperone-like, rather than a catalytic role for these proteins.

Why use scaffolding proteins?

As these different examples have shown, scaffolding proteins have many different modi operandi. which are not necessarily mutually exclusive. The unifying principle is that subsequent assembly steps depend on their presence (or their action), and that they associate only transiently with the nascent assembly.

When are scaffolding proteins needed? It is obviously not always necessary to have scaffolds, as many quite complex structures assemble perfectly well in the absence of scaffolding proteins, e.g. ribosomes [197] or T4 tailplates [198]. Typically, scaffolding proteins are invoked in viruses that contain many identical subunits, in which case scaffolding proteins may be needed to simplify the regulation of conformational switching. Yet many large viruses, such as the Reoviridae, apparently assemble without a need for scaffolding proteins. The Reoviridae, however, do assemble their complex T = 13 shell onto a core template, which then effectively functions as a scaffold for shell assembly [199]. On the other hand, some quite simple viruses, like ϕX174, where the T = 1 structure of shell requires no conformational variation to be generated, do require a scaffold. (The scaffold is in this case strangely enough more complex than the viral shell itself!)

Internal scaffolding may be primarily required when a large, hollow space needs to be constructed, either due to structural limitations of the shell proteins or simply to exclude other proteins from entering the unfinished shell [16]. However, at least one virus even from the classical group of scaffold-utilising dsDNA phages, HK97, does not require a scaffolding protein [163].

In conclusion, one cannot find a single fundamental characteristic that distinguishes scaffolding proteins from other structural proteins. Perhaps it is most useful to think of scaffolding proteins as just another structural protein, which is removed because there is a requirement for releasing the binding sites for additional proteins. Clearly, using scaffolding proteins offers an additional level of control over the assembly pathway, thus enabling the assembly of structures that would not otherwise be possible. Whether this extra level of control is required will be dependent on a number of factors, including the complexity of the system and the properties of the structural proteins themselves.

Acknowledgments. I am grateful to Roman Tuma, Björn Lindqvist and Ben Fane for making data available prior to publication, and to Michael Rossmann and Björn Lindqvist for their helpful comments on the manuscript. A special thanks to Michael Rossmann for offering me the opportunity to write this review.

1 Alberts B. (1998) The cell as a collection of protein machines: preparing the next generation of molecular biologists. Cell **92**: 291–294
2 Edgar R. S. and Wood W. B. (1966) Morphogenesis of bacteriophage T4 in extracts of mutant-infected cells. Proc. Natl. Acad. Sci. USA **55**: 498–505
3 Epstein R. H., Bolle A., Steinberg E. M., Kellenberger E., Boy de le Tour E., Chevalier R. et al. (1963) Physiological studies of conditional lethal mutants of bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. **28**: 375–394
4 Fraenkel-Conrat H. and Williams R. C. (1955) Reconstitution of active tobacco mosaic virus from its inactive protein and nucleic acid components. Proc. Natl. Acad. Sci. USA **41**: 690–698
5 King J. (1980) Regulation of structural protein interactions as revealed in phage morphogenesis. In: Biological Regulation and Development, pp. 101–132, Goldberger R. F. (ed.), Plenum Press, New York
6 Kellenberger E. (1976) DNA viruses. Cooperativity and regulation through conformational changes of phage assembly. Phil. Trans. Roy. Soc. London **B 276**: 3–13
7 Kellenberger E. (1990) Form determination of the heads of bacteriophages. Eur. J. Biochem. **190**: 233–248
8 Steven A. C. (1993) Conformational change—an alternative energy source? Exothermic phase transition in phage capsid maturation. Biophys J **65**: 5–6
9 King J., Lenk E. V. and Botstein D. (1973) Mechanism of head assembly and DNA encapsulation in *Salmonella* phage P22. II. Morphogenetic pathway. J. Mol. Biol. **80**: 697–731
10 Showe M. K. and Black L. W. (1973) Assembly core of bacteriophage T4: an intermediate in head formation. Nature New Biol. **242**: 70–75
11 King J., Griffin-Shea R. and Fuller M. T. (1980) Scaffolding proteins and the genetic control of virus shell assembly. Quart. Rev. Biol. **55**: 369–393
12 Casjens S. and Hendrix R. (1988) Control mechanisms in dsDNA bacteriophage assembly. In: The Bacteriophages, pp. 15–91, Calendar R. (ed.), Plenum Press, New York
13 Gething M.-J. (ed.) (1997) Guidebook to Molecular Chaperones and Protein-Folding Catalysis. Oxford University Press, New York
14 Hendrix R. W. and Garcea R. L. (1994) Capsid assembly of dsDNA viruses. Seminars in Virology **5**: 15–26
15 Murialdo H. and Becker A. (1978) Head morphogenesis of complex double-stranded deoxyribonucleic acid bacteriophages. Microbiol. Rev. **42**: 529–576
16 Casjens S. (1997) Principles of virion structure, function and assembly. In: Structural Biology of Viruses, pp. 3–37, Chiu W. (ed.), Oxford University Press, New York
17 Casjens S. (1985) An introduction to virus structure and assembly. In: Virus Structure and Assembly, pp. 2–28, Casjens S. (ed.), Jones and Bartlett, Boston
18 Dokland T., McKenna R., Ilag L. L., Bowman B. R., Incardona N. L., Fane B. A. et al. (1997) Structure of a viral procapsid with molecular scaffolding. Nature **389**: 308–313
19 Dokland T., McKenna R., Sherman D. M., Bowman B. R., Bean W. L. and Rossmann M. G. (1998) Structure determination of the ϕX174 closed procapsid. Acta Cryst. **D 54**: 878–890
20 Dokland T., Bernal R. A., Burch A., Pletnev S., Fane B. A. and Rossmann M. G. (1999) The Role of scaffolding proteins in the assembly of the small, single-stranded DNA virus ϕX174. J. Mol. Biol. **288:** 595–608
21 Ilag L. L., Olson N. H., Dokland T., Music C. L., Cheng R. H., Bowen Z. et al. (1995) DNA packaging intermediates of bacteriophage ϕX174. Structure **3**: 353–363
22 Hayashi M. (1978) Morphogenesis of the isometric phages. In: The Single-Stranded DNA phages, pp. 531–547, Denhardt D. T., Dressler D. and Ray D. S. (eds), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
23 Hayashi M., Aoyoma A., Richardson D. Jr and Hayashi M. (1988) Biology of the bacteriophage ϕX174. In: The Bacteriophages, pp. 1–71, Calendar R. (ed.), Plenum Press, New York
24 Fujisawa H. and Hayashi M. (1977) Functions of gene C and gene D products of bacteriophage ϕX174. Virology **21**: 506–515
25 Fujisawa H. and Hayashi M. (1977) Two infectious forms of bacteriophage ϕX174. J. Virol. **23**: 439–442
26 Tonegawa S. and Hayashi M. (1970) Intermediates in the assembly of ϕX174. J. Mol. Biol. **48**: 219–242
27 Siden E. J. and Hayashi M. (1974) Role of the gene B product in bacteriophage ϕX174 development. J. Mol. Biol. **89**: 1–16
28 Weisbeek P. J. and Sinsheimer R. L. (1974) A DNA-protein complex involved in bacteriophage ϕX174 particle formation. Proc. Natl. Acad. Sci. USA **71**: 3054–3058
29 Farber M. B. (1976) Purification and properties of bacteriophage ϕX174 gene D product. J. Virol. **17**: 1027–1037
30 Caspar D. L. D. and Klug A. (1962) Physical principles in the construction of regular viruses. Cold Spring Harb. Symp. Quant. Biol. **27**: 1–24
31 Marvik O. J., Dokland T. E., Nøkling R. H., Jacobsen E., Larsen T. and Lindqvist B. H. (1995) The capsid size-determining protein Sid forms an external scaffold on phage P4 procapsids. J. Mol. Biol. **251**: 59–75
32 Christie G. E. and Calendar R. (1990) Interactions between satellite bacteriophage P4 and its helpers. Annu. Rev. Genet. **24**: 465–490
33 Bertani L. E. and Six E. (1988) The P2-like phages and their parasite, P4. In: The Bacteriophages, pp. 73–143, Calendar R. (ed.), Plenum Press, New York
34 Lindqvist B. H., Deho G. and Calendar R. (1993) Mechanisms of genome propagation and helper exploitation by satellite phage P4. Microbiol. Rev. **57**: 683–702
35 Dokland T. E., Lindqvist B. H. and Fuller S. D. (1992) Image reconstruction from cryo-electron micrographs reveals the morphopoietic mechanism in the P2-P4 bacteriophage system. EMBO J. **11**: 839–846

36 Barrett K. J., Marsh M. L. and Calendar R. (1976) Interactions between a satellite bacteriophage and its helper. J. Mol. Biol. **106**: 683–707
37 Shore D., Deho G., Tsipis J. and Goldstein R. (1978) Determination of capsid size by satellite bacteriophage P4. Proc. Natl. Acad. Sci. USA **75**: 400–404
38 Six E. W., Sunshine M. G., Williams J., Haggård-Ljungquist E. and Lindqvist B. H. (1991) Morphopoietic switch mutations of bacteriophage P2. Virology **182**: 34–46
39 Marvik O. J., Sharma P., Dokland T. and Lindqvist B. H. (1994) Bacteriophage P2 and P4 assembly: alternative scaffolding proteins regulate capsid size. Virology **200**: 702–714
40 Nilssen Ø., Six E. W., Sunshine M. G. and Lindqvist B. H. (1996) Mutational analysis of the bacteriophage P4 capsid-size-determining gene. Virology **219**: 432–442
41 Botstein D., Waddell C. H. and King J. (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
42 King J. and Casjens S. (1974) Catalytic head assembling protein in virus morphogenesis. Nature **251**: 112–119
43 King J., Botstein D., Casjens S., Earnshaw W., Harrison S. and Lenk E. (1976) Structure and assembly of the capsid of bacteriophage P22. Phil. Trans. Roy. Soc. Lond. **B 276**: 37–49
44 Prevelige P., Thomas D. and King J. (1988) Scaffolding protein regulates the polymerization of P22 subunits into icosahedral shells in vitro. J. Mol. Biol. **202**: 743–757
45 Prevelige P., Thomas D. and King J. (1993) Nucleation and growth phases in the polymerization of coat and scaffolding subunits into icosahedral procapsid shells. Biophys. J. **64**: 824–835
46 Fuller M. T. and King J. (1980) Regulation of coat protein polymerization by the scaffolding protein of bacteriophage P22. Biophys J. **32**: 381–401
47 Earnshaw W. and King J. (1978) Structure of phage P22 coat protein aggregates formed in the absence of the scaffolding protein. J. Mol. Biol. **126:** 721–747
48 Earnshaw W. C., Casjens S. and Harrison S. C. (1976) Assembly of the head of bacteriophage P22: X-ray diffraction from heads, proheads and related structures. J. Mol. Biol. **104:** 387–410
49 Thuman-Commike P. A., Greene B., Jakana J., Prasad B. V. V., King J., Prevelige P. E. et al. (1996) Three-dimensional structure of scaffolding-containing phage P22 procapsids by electron cryo-microscopy. J. Mol. Biol. **260:** 85–98
50 Parker M. H., Stafford W. F. and Prevelige P. E. (1997) Bacteriophage P22 scaffolding protein forms oligomers in solution. J. Mol. Biol. **268:** 655–665
51 Parker M. H. and Prevelige P. E. (1998) Electrostatic interactions drive scaffolding/coat protein binding and procapsid maturation in bacteriophage P22. Virology **250:** 337–349
52 Parker M. H., Jablonsky M., Casjens S., Sampson L., Krishna N. R. and Prevelige P. E. (1997) Cloning, purification and preliminary characterization by circular dichroism and NMR of a carboxyl-terminal domain of the bacteriophage P22 scaffolding protein. Protein Science **6:** 1583–1586
53 Parker M. H., Casjens S. and Prevelige P. E. (1998) Functional domains of bacteriophage P22 scaffolding protein. J. Mol. Biol. **281:** 69–79
54 Tuma R., Parker M. H., Weigele P., Sampson L., Sun Y., Krishna N. R. et al. (1998) A helical coat protein recognition domain of the bacteriophage P22 scaffolding protein. J. Mol. Biol. **281:** 81–94
55 Fuller M. T. and King J. (1982) Assembly in vitro of bacteriophage P22 procapsids from purified coat and scaffolding subunits. J. Mol. Biol. **156:** 633–665
56 Fuller M. T. and King J. (1981) Purification of the coat and scaffolding proteins from procapsids of bacteriophage P22. Virology **112:** 529–547
57 Tuma R., Prevelige P. E. and Thomas G. J. (1996) Structural transitions in the scaffolding and coat proteins of P22 virus during assembly and disassembly. Biochemistry **35:** 4619–4627
58 Prevelige P. E., Thomas D., King J., Towse S. A. and Thomas G. J. (1990) Conformational states of the bacteriophage P22 capsid subunit in relation to self-assembly. Biochemistry **29:** 5626–5633
59 Prevelige P., Thomas D., Aubrey K. L., Towse S. A. and Thomas G. J. (1993) Subunit conformational changes accompanying bacteriophage P22 capsid maturation. Biochemistry **32:** 537–543
60 Thuman-Commike P. A., Greene B., Malinski J. A., King J. and Chiu W. (1998) Role of the scaffolding protein in P22 procapsid size determination suggested by T = 4 and T = 7 procapsid structures. Biophys. J. **74:** 559–568
61 Greene B. and King J. (1994) Binding of scaffolding subunits within the P22 procapsid lattice. Virology **205:** 188–197
62 Bazinet C. and King J. (1988) Initiation of P22 procapsid assembly in vivo. J. Mol. Biol. **202:** 77–86
63 Casjens S. and Hendrix R. W. (1974) Locations and amounts of the major structural proteins of bacteriophage lambda. J. Mol. Biol. **88:** 535–545
64 Dokland T. and Murialdo H. (1993) Structural transitions during maturation of bacteriophage lambda capsids. J. Mol. Biol. **233:** 682–694
65 Kochan J. and Murialdo H. (1983) Early intermediates in bacteriophage lambda prohead assembly. II. Identification of biologically active intermediates. Virology **131:** 100–115
66 Ray P. and Murialdo H. (1975) The role of gene Nu3 in bacteriophage lambda head morphogenesis. Virology **64:** 247–263
67 Hohn T., Flick H. and Hohn B. (1975) Petit λ a family of particles from coliphage lambda infected cells. J. Mol. Biol. **98:** 107–120
68 Murialdo H. (1979) Early intermediates in bacteriophage lambda prohead assembly. Virology **96:** 341–367
69 Earnshaw W. C., Hendrix R. W. and King J. (1979) Structural studies of bacteriophage lambda heads and proheads by small angle X-ray diffraction. J. Mol. Biol. **134:** 575–594
70 Murialdo H. and Becker A. (1977) Assembly of biologically active proheads of bacteriophage lambda in vitro. Proc. Natl. Acad. Sci. USA **74:** 906–910
71 Ziegelhoffer T., Yau P., Chandrasekhar G. N., Kochan J., Georgopoulos C. and Murialdo H. (1992) The purification and properties of the scaffolding protein of bacteriophage lambda. J. Biol. Chem. **267:** 455–461
72 Katsura I. (1983) Structure and inherent properties of the bacteriophage lambda head shell. IV. Small-head mutants. J. Mol. Biol. **171:** 297–317
73 Steven A. C. and Trus B. (1986) The structure of bacteriophage T7. In: Electron Microscopy of Proteins, vol. 5: Viral Structure, pp. 1–35, Harris J. R. and Horne R. W. (eds), Academic Press, London
74 Serwer P. (1976) Internal proteins of bacteriophage T7. J. Mol. Biol. **107:** 271–291
75 Serwer P. and Watson R. H. (1982) Function of an internal bacteriophage T7 core during assembly of a T7 procapsid. J. Virol. **42:** 595–601
76 Serwer P. (1979) Fibrous projections from the core of a bacteriophage T7 procapsid. J. Supramol. Struct. **11:** 321–326
77 Cerritelli M. E. and Studier F. W. (1996) Assembly of T7 capsids from independently expressed and purified head protein and scaffolding protein. J. Mol. Biol. **258:** 286–298
78 Rixon F. J. (1993) Structure and assembly of herpesviruses. Semin. Virol. **4:** 135–144
79 Steven A. C. and Spear P. G. (1997) Herpesvirus capsid assembly and envelopment. In: Structural Biology of Viruses, pp. 312–351, Chiu W., Burnett R. M. and Garcia R. L. (eds), Oxford University Press, New York
80 Baker T. S., Newcomb W. W., Booy F. P., Brown J. C. and Steven A. C. (1990) Three-dimensional structures of maturable and abortive capsids of equine herpesvirus 1 from cryoelectron microscopy. J. Virol. **64:** 563–573
81 Booy F. P., Newcomb W. W., Trus B. L., Brown J. C., Baker T. S. and Steven A. C. (1991) Liquid-crystalline,

phage-like packing of encapsidated DNA in herpes simplex virus. Cell **64:** 1007–1015
82 Schrag J. D., Prasad B. V. V., Rixon F. J. and Chiu W. (1989) Three-dimensional structure of the HSV1 nucleocapsid. Cell **56:** 651–660
83 Newcomb W. W., Trus B. L., Booy F. P., Steven A. C., Wall J. S. and Brown J. C. (1993) Structure of the herpes simplex virus capsid. Molecular composition of the pentons and the triplexes. J. Mol. Biol. **232:** 499–511
84 Person S. and Desai P. (1998) Capsids are formed in a mutant virus blocked at the maturation site of the UL26 and UL26.5 open reading frames of herpes simplex virus type 1 but are not formed in a null mutant of UL38 (VP19C). Virology **242:** 193–203
85 Zhou Z. H., Macnab S. J., Jakana J., Scott L. R., Chiu W. and Rixon F. J. (1998) Identification of the sites of interaction between the scaffold and outer shell in herpes simplex virus-1 capsids by difference electron imaging. Proc. Natl. Acad. Sci. USA **95:** 2778–2783
86 Nicholson P., Addison C., Cross A. M., Kennard J., Preston V. G. and Rixon F. J. (1994) Localization of the herpes simplex virus type 1 major capsid protein VP5 in the cell nucleus requires the abundant scaffolding protein VP22a. J. Gen. Virol. **75:** 1091–1099
87 Wood L. J., Baxter M. K., Plafker S. M. and Gibson W. (1997) Human cytomegalovirus capsid assembly protein precursor (pUL80.5) interacts with itself and with the major capsid protein (pUL86) through two different domains. J. Virol. **71:** 179–190
88 Matusick-Kumar L., Hurlburt W., Weinheimer S. P., Newcomb W. W., Brown J. C. and Gao M. (1994) Phenotype of the herpes simplex virus type 1 protease substrate ICP35 mutant virus. J. Virol. **68:** 5384–5394
89 Rixon F. J., Addison C., MacGregor A., Macnab S. J., Nicholson P., Preston V. G. et al. (1996) Multiple interactions control the intracellular localization of the herpes simplex virus type 1 capsid proteins. J. Gen. Virol. **77:** 2251–2260
90 Tatman J. D., Preston V. G., Nicholson P., Elliott R. M. and Rixon F. J. (1994) Assembly of herpes simplex virus type 1 capsids using a panel of recombinant baculoviruses. J. Gen. Virol. **75:** 1101–1113
91 Thomsen D. R., Roof L. L. and Homa F. L. (1994) Assembly of herpes simplex virus (HSV) intermediate capsids in insect cells infected with recombinant baculoviruses expressing HSV capsid proteins. J. Virol. **68:** 2442–2457
92 Newcomb W. W., Homa F. L., Thomsen D. R., Ye Z. and Brown J. C. (1994) Cell-free assembly of the herpes simplex virus capsid. J. Virol. **68:** 6059–6063
93 Newcomb W. W., Homa F. L., Thomsen D. R., Booy F. P., Trus B. L., Steven A. C. et al. (1996) Assembly of the herpes simplex virus capsid: characterization of intermediates observed during cell-free capsid formation. J. Mol. Biol. **263:** 432–446
94 Spencer J. V., Newcomb W. W., Thomsen D. R., Homa F. L. and Brown J. C. (1998) Assembly of the herpes simplex virus capsid: preformed triplexes bind to the nascent capsid. J. Virol. **72:** 3944–3951
95 Desai P., Watkins S. C. and Person S. (1994) The size and symmetry of B capsids of herpes simplex virus type 1 are determined by the gene products of the UL26 open reading frame. J. Virol. **68:** 5365–5374
96 Rixon F. J., Cross A. M., Addison C. and Preston V. G. (1988) The products of herpes simplex virus type 1 gene UL26 which are involved in DNA packaging are strongly associated with empty but not with full capsids. J. Gen. Virol. **69:** 2879–2891
97 Newcomb W. W. and Brown J. (1991) Structure of the herpes simplex virus capsid: effects of extraction with guanidine-HCl and partial reconstruction of extracted capsids. J. Virol. **65:** 613–620
98 Trus B. L., Booy F. P., Newcomb W. W., Brown J. C., Homa F. L., Thomsen D. R. et al. (1996) The herpes simplex virus procapsid: structure, conformational changes upon maturation, and roles of the triplex proteins VP19c and VP23 in assembly. J. Mol. Biol. **263:** 447–462
99 Hong Z., Beaudet-Miller M., Durkin J., Zhang R. and Kwong A. D. (1996) Identification of a minimal hydrophobic domain in the herpes simplex virus type 1 scaffolding protein which is required for interaction with the major capsid protein. J. Virol. **70:** 533–540
100 Beaudet-Miller M., Zhang R., Durkin J., Gibson W., Kwong A. D. and Hong Z. (1996) Virus-specific interaction between the human cytomegalovirus major capsid protein and the C terminus of the assembly protein precursor. J. Virol. **70:** 8081–8088
101 Oien N. L., Thomsen D. R., Wathen M. W., Newcomb W. W., Brown J. C. and Homa F. L. (1997) Assembly of herpes simplex virus capsids using the human cytomegalovirus scaffold protein: critical role of the C terminus. J. Virol. **71:** 1281–1291
102 Preston V. G., Kennard J., Rixon F. J., Logan A. J., Mansfield R. W. and McDougall I. M. (1997) Efficient herpes simplex virus type 1 (HSV-1) capsid formation directed by the varicella-zoster virus scaffolding protein requires the carboxy-terminal sequences from the HSV-1 homologue. J. Gen. Virol. **78:** 1633–1646
103 Haanes E. J., Thomsen D. R., Martin S., Homa F. L. and Lowery D. E. (1995) The bovine herpesvirus 1 maturational proteinase and scaffold proteins can substitute for the homologous herpes simplex virus type 1 proteins in the formation of hybrid type B capsids. J. Virol. **69:** 7375–7379
104 Pelletier A., Do F., Brisebois J. J., Lagace L. and Cordingley M. G. (1997) Self-association of herpes simplex virus type 1 ICP35 is via coiled-coil interactions and promotes stable interaction with the major capsid protein. J. Virol. **71:** 5197–5208
105 Black L. W., Showe M. K. and Steven A. C. (1995) Morphogenesis of the T4 head. In: Bacteriophage T4, pp. 218–258, Mathews C. K. (ed.), ASM Press, Washington, DC
106 Traub F. and Maeder M. (1984) Formation of the prohead core of bacteriophage T4 in vivo. J. Virol. **49:** 892–901
107 Traub F., Keller B., Kuhn A. and Maeder M. (1984) Isolation of the prohead core of bacteriophage T4 after crosslinking and determination of protein composition. J. Virol. **49:** 902–908
108 Onorato L., Stirmer B. and Showe M. K. (1978) Isolation and characterization of bacteriophage T4 mutant preheads. J. Virol. **2:** 409–426
109 van Driel R., Traub F. and Showe M. K. (1980) Probable localization of the bacteriophage T4 prehead proteinase zymogen in the center of the prehead core. J. Virol. **36:** 220–223
110 Traub F., Maeder M. and Kellenberger E. (1981) Bacteriophage T4 head assembly. In vivo characterisation of the morphopoietic core. Prog. Clin. Biol. Res. **64:** 127–137
111 van Driel R. and Couture E. (1978) Assembly of bacteriophage T4 head-related structures. II. In vitro assembly of prehead-like structures. J. Mol. Biol. **123:** 115–128
112 van Driel R. and Couture E. (1978) Assembly of the scaffolding core of bacteriophage T4 preheads. J. Mol. Biol. **123:** 713–719
113 Yanagida M., Boy de la Tour E., Alff-Steinberger C. and Kellenberger E. (1970) Studies on the morphopoiesis of the head of bacteriophage T-even. 8. Multilayered polyheads. J. Mol. Biol. **50:** 35–58
114 van Driel R. (1977) Assembly of T4 head-related structures. Assembly of polyheads in vitro. J. Mol. Biol. **114:** 61–72
115 Caldentey J. and Kellenberger E. (1986) Assembly and disassembly of bacteriophage T4 polyheads. J. Mol. Biol. **188:** 39–48
116 Caldentey J., Lepault J. and Kellenberger E. (1987) Isolation and reassembly of bacteriophage T4 core proteins. J. Mol. Biol. **195:** 637–647
117 Kato H. and Baschong C. (1997) Isolation of a gp20-complex and its role in in vitro assembly of both prohead and core of bacteriophage T4. Virology **227:** 400–408

118 Paulson J. R., Lazaroff S. and Laemmli U. K. (1976) Head length determination in bacteriophage T4: the role of the core protein P22. J. Mol. Biol. **103:** 155–174
119 Keller B., Maeder M., Becker-Laburte C., Kellenberger E. and Bickle T. A. (1986) Amber mutants in gene 67 of phage T4. Effects on formation and shape determination of the head. J. Mol. Biol. **190:** 83–95
120 Keller B., Dubochet J., Adrian M., Maeder M., Wurtz M. and Kellenberger E. (1988) Length and shape variants of the bacteriophage T4 head: mutations in the scaffolding core genes 68 and 22. J. Virol. **62:** 2960–2969
121 Mesyanzhinov V. V., Sobolev B. N., Marusich E. I. and Efimov V. P. (1990) A proposed structure of bacteriophage T4 gene product 22—a major prohead scaffolding core protein. J. Struct. Biol. **104:** 24–31
122 van Driel R. (1980) Assembly of bacteriophage T4 head-related structures. IV. Isolation and association properties of T4 prehead proteins. J. Mol. Biol. **138:** 27–42
123 Engel A., van Driel R. and Driedonks R. (1982) A proposed structure of the prolate phage T4 prehead core. An electron microscopic study. J. Ultrastruct. Res. **80:** 12–22
124 Paulson J. R. and Laemmli U. K. (1977) Morphogenetic core of the bacteriophage T4 head: structure of the core in polyheads. J. Mol. Biol. **111:** 459–485
125 Berger B. and Shor P. W. (1998) On the structure of the scaffolding core of bacteriophage T4 and its role in head length determination. J. Struct. Biol. **121:** 285–294
126 Driedonks R. A., Engel A., ten Heggeler B. and van Driel R. (1981) Gene 20 product of bacteriophage T4, its purification and structure. J. Mol. Biol. **152:** 641–662
127 Black L. W. and Brown D. T. (1976) Head morphologies in bacteriophage T4 head and internal protein mutant infections. J. Virol. **17:** 894–905
128 Showe M. K., Isobe E. and Onorato L. (1976) Bacteriophage T4 prehead proteinase. II. Its cleavage from the product of gene 21 and regulation in phage-infected cells. J. Mol. Biol. **107:** 55–69
129 Showe M. K., Isobe E. and Onorato L. (1976) Bacteriophage T4 prehead proteinase. I. Purification and properties of a bacteriophage enzyme which cleaves the capsid precursor proteins. J. Mol. Biol. **107:** 35–54
130 Anderson D. L., Hickman D. D. and Reilly B. E. (1966) Structure of *Bacillus subtilis* bacteriophage ϕ29 and the length of ϕ29 deoxyribonulecic acid. J. Bacteriol. **91:** 2081–2089
131 Anderson D. and Reilly B. (1993) Morphogenesis of bacteriophage ϕ29. In: *Bacillus subtilis* and Other Gram-Positive Bacteria: Biochemistry, Physiology and Molecular Genetics, pp. 859–867, Sonenshein A. L., Hoch J. A. and Losick R. (eds), American Society for Microbiology, Washington, DC
132 Tao Y., Olson N. H., Xu W., Anderson D. L., Rossmann M. G. and Baker T. S. (1998) Bacteriophage ϕ29: a complex biological machine studied by cryo-electron microscopy. Cell **95:** 431–437
133 Nelson R. A., Reilly B. E. and Anderson D. L. (1976) Morphogenesis of bacteriophage ϕ29 of *Bacillus subtilis*: preliminary isolation and characterization of intermediate particles of the assembly pathway. J. Virol. **19:** 518–532
134 Vinuela E., Camacho A., Jimenez F., Carrascosa J. L., Ramirez G. and Salas M. (1976) Structure and assembly of phage ϕ29. Philos. Trans. Roy. Soc. Lond. B **276:** 29–35
135 Camacho A., Jimenez F., De La Torre J., Carrascosa J. L., Mellado R. P., Vasquez C. et al. (1977) Assembly of *Bacillus subtilis* phage ϕ29. 1. Mutants in the cistrons coding for the structural proteins. Eur. J. Biochem. **73:** 39–55
136 Bjornsti M.-A., Reilly B. E. and Anderson D. L. (1985) Morphogenesis of bacteriophage ϕ29 of *Bacillus subtilis*: prohead restoration for DNA-gp3 packaging and assembly. J. Virol. **53:** 858–861
137 Guo P., Erickson S., Xu W., Olson N., Baker T. S. and Anderson D. (1991) Regulation of phage ϕ29 prohead shape and size by the portal vertex. Virology **183:** 366–373
138 Lee C. S. and Guo P. (1995) In vitro assembly of infectious virions of ds-DNA phage ϕ29 from cloned gene products and synthetic nucleic acids. J. Virol. **69:** 5018–5023
139 Lee C. S. and Guo P. (1995) Sequential interactions of structural proteins in phage ϕ29 procapsid assembly. J. Virol. **69:** 5024–5032
140 Lee C. S. and Guo P. (1994) A highly sensitive system for the assay of in vitro viral assembly of bacteriophage ϕ29 of *Bacillus subtilis*. Virology **202:** 1039–1042
141 Lengyel J. A., Goldstein R. N., Marsh M., Sunshine M. G. and Calendar R. (1973) Bacteriophage P2 head morphogenesis: cleavage of the major capsid protein. Virology **53:** 1–23
142 Agarwal M., Arthur M., Arbeit R. D. and Goldstein R. (1990) Regulation of icosahedral virion capsid size by the in vitro activity of a cloned gene product. Proc. Nat. Acad. Sci. USA **87:** 2428–2432
143 Marvik O. J., Jacobsen E., Dokland T. and Lindqvist B. H. (1994) Bacteriophage P2 and P4 morphogenesis: assembly precedes proteolytic processing of the capsid proteins. Virology **205:** 51–65
144 Rishovd S., Marvik O. J., Jacobsen E. and Lindqvist B. H. (1994) Bacteriophage P2 and P4 morphogenesis: identification and characterization of the portal protein. Virology **200:** 744–751
145 Rishovd S., Holzenburg A., Johansen B. V. and Lindqvist B. H. (1998) Bacteriophage P2 nad P4 morphogenesis: structure and function of the connector. Virology **245:** 11–17
146 Burch A. D., Ta J. and Fane B. A. (1999) Cross-functional analysis of the Microviridae internal scaffolding protein. J. Mol. Biol. **286:** 95–104
147 Philipson L. (1983) Structure and assembly of adenoviruses. Curr. Topics Microbiol. Immunol. **109:** 1–52
148 D'Halluin J. C. (1995) Virus assembly. In: The Molecular Repertoire of Adenoviruses. I. Structure and Function, pp. 47–66, Doerfler W. and Böhm P. (eds), Springer, Berlin
149 Burnett R. M. (1997) The structure of adenovirus. In: Structural Biology of Viruses, pp. 209–238, Chiu W., Burnett R. M. and Garcia R. L. (eds), Oxford University Press, New York
150 Sundquist B., Everitt E., Philipson L. and Höglund S. (1973) Assembly of adenoviruses. J. Virol. **11:** 449–459
151 Edvardsson B., Everitt E., Jörnvall H., Prage L. and Philipson L. (1976) Intermediates in adenovirus assembly. J. Virol. **19:** 533–547
152 Edvardsson B., Ustacelebi S., Williams J. and Philipson L. (1978) Assembly intermediates among adenovirus type 5 temperature-sensitive mutants. J. Virol. **25:** 641–651
153 D'Halluin J.-C., Milleville M., Boulanger P. A. and Martin G. R. (1978) Temperature-sensitive mutant of adenovirus type 2 blocked in virion assembly: accumulation of light intermediate particles. J. Virol. **26:** 344–356
154 D'Halluin J. C., Martin G. R., Torpier G. and Boulanger P. A. (1978) Adenovirus type 2 assembly analyzed by reversible cross-linking of labile intermediates. J. Virol. **26:** 344–356
155 Gustin K. E. and Imperiale M. J. (1998) Encapsidation of viral DNA requires the adenovirus L1 52/55-kilodalton protein. J. Virol. **72:** 7860–7870
156 Oosterom-Dragon E. A. and Ginsberg H. S. (1981) Characterization of two temperature-sensitive mutants of type 5 adenovirus with mutations in the 100,000-Dalton protein gene. J. Virol. **40:** 491–500
157 Morin N. and Boulanger P. (1984) Morphogenesis of human adenovirus type 2: sequence of entry of proteins into previral and viral particles. Virology **136:** 153–167
158 Morin N. and Boulanger P. (1986) Hexon trimerization occuring in an assembly-defective, 100K temperature-sensitive mutant of adenovirus 2. Virology **152:** 11–31
159 Walker J. T. and Walker D. H. (1983) Coliphage P1 morphogenesis: analysis of mutants by electron microscopy. J. Virol. **45:** 1118–1139
160 Grimaud R. (1996) Bacteriophage Mu head assembly. Virology **217:** 200–210

161 Alonso J. C., Luder G., Stiege A. C., Chai S., Weise F. and Trautner T. A. (1997) The complete nucleotide sequence and functional organization of *Bacillus subtilis* bacteriophage SPP1. Gene **204:** 201–212

162 Becker B., de la Fuente N., Gassel M., Gunther D., Tavares P., Lurz R. et al. (1997) Head morphogenesis genes of the *Bacillus subtilis* bacteriophage SPP1. J. Mol. Biol. **268:** 822–839

163 Duda R. L., Mertincic K. and Hendrix R. W. (1995) Genetic basis of bacteriophage HK97 prohead assembly. J. Mol. Biol. **247:** 636–647

164 Cobbold C. and Wileman T. (1998) The major structural protein of African swine fever virus, p73, is packaged into large structures, indicative of viral capsid or matrix precursors, on the endoplasmatic reticulum. J. Virol. **72:** 5215–5223

165 Newcomb W. W., Tobin G. J., McGowan J. J. and Brown J. C. (1982) In vitro reassembly of vesicular stomatitis virus skeletons. J. Virol. **41:** 1055–1062

166 Barge A., Gaudin Y., Coulon P. and Ruigrok R. W. (1993) Vesicular stomatitis virus M protein may be inside the ribonucleocapsid coil. J. Virol. **67:** 7246–7253

167 Mebatsion T., Weiland F. and Conzelmann K.-K. (1999) Matrix protein of rabies virus is responsible for the assembly and budding of bullet-shaped particles and interacts with the transmembrane spike glycoprotein G. J. Virol. **73:** 242–250

168 Ohnishi K., Ohto Y., Aizawa S., Macnab R. M. and Iino T. (1994) FlgD is a scaffolding protein needed for flagellar hook assembly in *Salmonella typhimurium*. J. Bacteriol. **176:** 2272–2281

169 Lombardo M.-J., Thanassi D. G. and Hultgren S. J. (1997) *Escherichia coli* PapD. In: Guidebook to Molecular Chaperones and Protein-Folding Catalysis, pp. 463–465, Gething M.-J. (ed.), Oxford University Press, Oxford

170 Marusich E. I., Kurochkina L. P. and Mesyanzhinov V. V. (1998) Chaperones in bacteriophage T4 assembly. Biochemistry (Moscow) **63:** 399–406

171 Drewes G., Ebneth A. and Mandelkow E.-M. (1998) MAPs, MARKs and microtubule dynamics. Trends Biochem. Sci. **23:** 307–311

172 Mandelkow E. and Mandelkow E.-M. (1995) Microtubules and microtubule-associated proteins. Curr. Opin. Cell Biol. **7:** 72–81

173 Salmon E. and Way M. (1999) Cytoskeleton. Curr. Opin. Cell Biol. **11:** 15–17

174 Gregorio C. C., Granzier H., Sorimachi H. and Labeit S. (1999) Muscle assembly: a titanic achievement? Curr. Opin. Cell Biol. **11:** 18–25

175 Fowkes M. E. and Mitchell D. R. (1998) The role of preassembled cytoplasmic complexes in assembly of flagellar dynein subunits. Mol. Biol. Cell **9:** 2337–2347

176 Huber R. (1979) Conformational flexibility and its functional significance in some protein molecules. Trends Biochem. Sci. **4:** 271–276

177 Huber R. and Bennett W. S. (1983) Functional significance of flexibility in proteins. Biopolymers **22:** 261–279

178 Subbiah S. (1996) Protein Motions, R. G. Landes Company, Georgetown, TX

179 Romero P., Obradovic Z., Kissinger C. R., Villafranca J. E., Garner E., Guilliot S. et al. (1998) Thousands of proteins likely to have long disordered regions. Pac. Symp. Biocomput. 437-448

180 Dunker A. K., Garner E., Guilliot S., Romero P., Albrecht K., Hart J. et al. (1998) Protein disorder and the evolution of molecular recognition: theory, predictions and observations. Pac. Symp. Biocomput. 473-484

181 Kirkitadze M. A., Barlow P. N., Price N. C., Kelly S. M., Boutell C. J., Rixon F. J. et al. (1998) The herpes simplex virus triplex protein, VP23, exists as a molten globule. J. Virol. **72:** 10066–10072

182 Daughdrill G. W., Hanely L. J. and Dahlquist F. W. (1998) The C-terminal half of the anti-sigma factor FlgM contains a dynamic equilibrium solution structure favoring helical conformations. Biochemistry **37:** 1076–1082

183 Daughdrill G. W., Chadsey M. S., Karlinsey J. E., Hughes K. T. and Dahlquist F. W. (1997) The C-terminal half of the anti-sigma factor, FlgM, becomes structured when bound to its target, sigma 28. Nature Struct. Biol. **4:** 285–291

184 Harrison S. C., Olson A. J., Schutt C. E., Winkler F. K. and Bricogne G. (1978) Tomato bushy stunt virus at 2.9 Å resolution. Nature **276:** 368–373

185 Abad-Zapatero C., Abdel-Meguid S. S., Johnson J. E., Leslie A. G. W., Rayment I., Rossmann M. G. et al. (1980) Structure of southern bean mosaic virus at 2.8 Å resolution. Nature **286:** 33–39

186 Liddington R. C., Yan Y., Moulai J., Sahli R., Benjamin T. L. and Harrison S. C. (1991) Structure of simian virus 40 at 3.8-Å resolution. Nature **354:** 278–284

187 Prasad B. V. V., Prevelige P. E., Marietta E., Chen R. O., Thomas D., King J. et al. (1993) Three-dimensional transformation of capsids associated with genome packaging in a bacterial virus. J. Mol. Biol. **231:** 65–74

188 Steven A. C., Bauer A. C., Bisher M. E., Robey F. A. and Black L. W. (1991) The maturation-dependent conformational change of phage T4 capsid involves the translocation of specific epitopes between the inner and the outer capsid surfaces. J. Struct. Biol. **106:** 221–236

189 Steven A. C., Greenstone H., Bauer A. C. and Williams R. W. (1990) The maturation-dependent conformational change of the major capsid protein of bacteriophage T4 involves a substantial change in secondary structure. Biochemistry **29:** 5556–5561

190 Tuma R. and Thomas G. J. (1997) Mechanisms of virus assembly probed by Raman spectroscopy: the icosahedral bacteriophage P22. Biophys. Chem. **68:** 17–31

191 Thuman-Commike P. A., Greene B., Malinski J. A., Burbea M., McGough A., Chiu W. et al. (1999) Mechanism of scaffolding-directed virus assembly suggested by comparison of scaffolding-containing and scaffolding-lacking P22 procapsids. Biophys. J. **76:** 3267–3277

192 Tuma R., Prevelige P. E. and Thomas G. J. (1998) Mechanism of capsid maturation in a double-stranded DNA virus. Proc. Natl. Acad. Sci. USA **95:** 9885–9890

193 Lauffer M. A. (1975) Entropy-Driven Processes in Biology, Springer, New York

194 da Poian A. T., Oliveira A. C. and Silva J. L. (1995) Cold denaturation of an icosahedral virus. The role of entropy in virus assembly. Biochemistry **34:** 2672–2677

195 Ross P. D. and Subramanian S. (1981) Thermodynamics of protein association reactions: forces contributing to stability. Biochemistry **20:** 3096–3102

196 Caspar D. L. D. (1980) Movement and self-control in protein assemblies: quasi-equivalence revisited. Biophys. J. **32:** 108–138

197 Nomura M. (1973) Assembly of bacterial ribosomes. Science **179:** 864–873

198 Wood W. B. (1980) Bacteriophage T4 morphogenesis as a model for assembly of subcellular structure. Quart. Rev. Biol. **55:** 353–367

199 Grimes J. M., Burroughs J. N., Gouet P., Diprose J. M., Malby R., Zientara S. et al. (1998) The atomic structure of the bluetongue virus core. Nature **395:** 470–478