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Virus maturation: dynamics and mechanism of a stabilizing structural transition that leads to infectivity

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Abstract

For many viruses, the final stage of assembly involves structural transitions that convert an innocuous precursor particle into an infectious agent. This process — maturation — is controlled by proteases that trigger large-scale conformational changes. In this context, protease inhibitor antiviral drugs act by blocking maturation. Recent work has succeeded in determining the folds of representative examples of the five major proteins —major capsid protein, scaffolding protein, portal, protease and accessory protein — that are typically involved in capsid assembly. These data provide a framework for detailed mechanistic investigations and elucidation of mutations that affect assembly in various ways. The nature of the conformational change has been elucidated: it entails rigid-body rotations and translations of the arrayed subunits that transfer the interactions between them to different molecular surfaces, accompanied by refolding and redeployment of local motifs. Moreover, it has been possible to visualize maturation at the submolecular level in movies based on time-resolved cryo-electron microscopy.

Introduction

In virus assembly — particularly for large and complex viruses — the sequence of events that yield progeny virions proceeds in three phases. First, a precursor particle — the provirion or procapsid — is formed. Second, the viral genome is packaged. The final phase — maturation, which overlaps with packaging — consists of programmed changes that convert the provirion into a complete, infectious, virion. The idea of first assembling an inert precursor and then activating it is not new (see http://mv.vatican.va/3_EN/pages/x-Select/20select/20select_03.html). Nevertheless, the paradigm applies well to the process of virus maturation and recent developments have yielded insight into the underlying molecular mechanisms.

Maturation is typically controlled by a viral protease that is incorporated into the provirion as a zymogen and then activated to process specific sites in the provirion interior, including itself (each other). The primary role of proteolysis is not degradative elimination of certain components but, rather, to trigger structural changes. These changes, and the altered pattern of interactions that they bring about, stabilize the fragile precursor and/or they may actuate certain downstream reactions. For enveloped viruses, maturation tends to be based on processing the glycoproteins that recognize receptors and subsequently cause the viral envelope to fuse with a host cell membrane, delivering the nucleocapsid into the cytoplasm (e.g. [1,2]).

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For many viruses, both enveloped and non-enveloped, the procapsid is the key site of maturation. In effect, its surface shell is induced to undergo a solid-state phase transition that stabilizes the particle, enabling it to withstand the high pressure imposed by the densely packed genome [3] as well as other challenges; moreover, sites are generated that allow the capsid to interact with other components, completing maturation. The role of allosteric control in virus assembly has long been appreciated [4,5]. However, it is only recently that detailed information has emerged concerning the structures of the proteins, the nature of the conformational changes, and the dynamics of maturation. Acknowledging advances made with another ancient family of double-stranded DNA viruses (e.g. [6,7•]), this review will focus on maturation phenomena shared by the tailed bacteriophages, particularly HK97, and a family of animal viruses, the herpes-viruses, particularly herpes simplex virus type 1 (HSV1). In conclusion, to underscore that maturation is a widespread phenomenon, a cursory summary is given of maturation-related studies in other systems.

Assembly pathway and building blocks

A generic assembly pathway is shown in Figure 1. Formulating such schemes is an exercise in frustration as there are exceptions to every rule. Nevertheless, the emerging picture is that much the same set of proteins —with deep evolutionary roots — is used, in differing numbers and combinations, to assemble capsids of diverse sizes and shapes. Although there tends to be little or no indication of common antecedents from sequence similarity, the folds have remained true to type, while being elaborated in some cases with additional domains or through gene duplication. In the past few years, structures have been determined for examples of each of these proteins (Figure 2).

One of the few features for which there is no documented exception (it is probably only a matter of time) is the portal/connector protein, a dodecameric ring that occupies one of the 12 vertices of the icosahedral capsid, and serves as the conduit via which DNA enters and exits the capsid [8]. It is plausible that the portal also initiates procapsid assembly and, after packing is complete, presents the template on which the tail is mounted. The structure of the φ29 connector has been determined [9,10], and its basic architecture is reproduced in SPP1 [11•] and T7 [12••].

Expression strategies: in pieces or as polyproteins?

Scaffolding proteins are employed to direct procapsid assembly with due fidelity and are subsequently expelled, either intact or in fragments. Structures have been determined for the φ29 scaffolding protein, a dimeric coiled coil [13••], and for a fragment of the P22 protein [14]. At first sight, HK97 provides the inevitable exception in having no scaffolding protein. However, its capsid protein has an unusually long propeptide — the Δ domain (102 residues) — that probably plays this role [15]. Certainly, it has a high α-helical content [16], a property of scaffolding proteins [17]. The fact that the T4 major capsid protein (MCP) also has a long propeptide while not dispensing with scaffolding proteins, of which it has two essential ones [18], does not invalidate this hypothesis; it may simply be that T4, with typical panache, has three, not two, essential scaffolding proteins. Variations in expression strategy, whereby, in one system, two or more proteins are synthesized as a polyprotein that is subsequently dissected, whereas in another system, they are expressed as separate gene products, are quite common. For example, the HSV protease enters the procapsid as a fusion with the scaffolding protein, later to release itself (see below).

The MCP of HK97 has been solved as a component of the mature capsid [19,20••]. Its fold turned out to be quite different from the β barrel of the first several capsids to be determined. The P22 MCP appears to have essentially the same fold as that of HK97, in the absence of discernible sequence similarity [21•]. Will this trend be maintained throughout this class of

virus or will other MCPs exhibit different folds (in all, some half-dozen folds have been recognized to serve as building blocks for icosahedral capsids [22])? In particular, it will be interesting to know whether a version of the HK97 MCP fold is present in the floor layer of herpesvirus capsids, as seems a distinct possibility.

Once the procapsid has been assembled, it has to be matured. Facilitated by the action of the viral protease, this transformation is further promoted by electrostatic repulsive forces exerted by packaged DNA on the inner capsid surface [23]. These forces are outwards directed and should grow in strength as packaging proceeds.

However, P22 and T7 have no proteases and serve as examples of phages that have devised other ways to control this transition (e.g. to restrain the procapsid from starting to mature before it is complete). As yet, there are no high-resolution structures for bacteriophage proteases, but there are several for herpesvirus proteases (e.g. [24]), which are likely to be structurally similar [25••].

The principal effect of maturation is structural transformation. For most phages, this involves a substantial expansion — typically by ~20% in linear dimensions, a near doubling of enclosed volume. At the same time, the capsid changes shape, from round to polyhedral. Although the wall thins by ~50%, the expanded capsid is more robust than the precursor. In some systems (e.g. P22, T7), expansion stabilizes the capsid sufficiently to resist the pressure of encapsidated DNA. In others, further measures are taken: λ and T4 bind clamp-like accessory proteins, whose structure has been determined in the case of λ [26], whereas HK97 establishes a network of covalent cross-links [27].

Maturation of bacteriophage HK97

The HK97 system lends itself to the study of maturation. Its capsid protein, gp5, has been characterized comprehensively [15] and its structure is known [19]. Even in the absence of the portal, 60 hexamers and 12 pentamers of gp5 self-assemble to form a procapsid (Prohead I), while incorporating the correct quota (~60 copies) of protease. Interestingly, a hexamer-only structure with empty vertices, called the ‘whiffleball’, may be reassembled from mutant hexamers [28]. Maturation begins with cleavage of the Δ domains (gp5 to gp5*), producing Prohead-II, which may be expanded *in vitro* by acidification, then restoring to neutrality.

The structural changes between Prohead II and Head II lead to the formation of a remarkable network of intermolecular cross-links. Cryo-EM analysis combined with model building revealed that Prohead II expansion is accomplished by rigid-body rotations of ~40° and translations of up to 50 Å of the cores of gp5* subunits, accompanied by the refolding of two extended motifs — the N-arm and E-loop ([23]; Figure 2, bottom).

The controllability of HK97 maturation *in vitro* has allowed its dynamics to be studied in initially synchronized populations by cryo-EM and small-angle X-ray scattering (SAXS) [29,30], leading to the identification of three transition states or expansion intermediates (EIs) (Figure 3). At pH 4.15, Prohead II rapidly converts into a semi-expanded state (EI-I), then to the structurally similar EI-II and finally to a larger, thin-walled, spherical particle (EI-III). Upon neutralization, EI-III rapidly assumes the polyhedral head structure. The term Head II refers to the mature, fully cross-linked capsid. Initially, expansion was thought to be complete before cross-linking began, but closer investigation established that cross-linking starts at the EI-II stage; EI-III preparations are mixtures of structurally similar but variably cross-linked particles, of which the extreme case, EI-IV, has all cross-links in place except those donated by the penton subunits [31•].

Determination of the structures of the successive states by cryo-EM allowed the creation of maturation movies, initially by morphing [29] and subsequently in terms of pseudo-atomic models (WR Wikoff *et al*., unpublished; http://mmtsb.scripps.edu/viper/MOVIES/hk97movies.html). The finding that most particles captured in cryo-micrographs of populations of maturing capsids could be used for image reconstruction based on icosahedral symmetry indicated that this symmetry is observed, to a close approximation and for most of the time, by maturing capsids. Switching from one state to the next, when it occurs, is rapid and cooperative but not synchronized over the population (i.e. the transitions are stochastically triggered). Possible pathways between these staging posts have been explored computationally [32].

As HK97 matures, covalent cross-links form between K169 at the tip of the E-loop and N356 of a neighboring subunit. This reaction is autocatalytic (i.e. it occurs without the involvement of an enzyme). The cross-linking pattern is such that each subunit in a given hexamer or pentamer connects to subunits in two (different) neighboring capsomers, creating an interlinked network or 'chain mail' [19,27]. The effects of cross-linking on capsid stability have been investigated by calorimetry [33••], showing that, in this system (unlike T4 or P22), expansion *per se* results in a slight destabilization, which is more than compensated by the emphatic stabilization that accompanies cross-linking. Given that complementation experiments showed a cross-link-incompetent mutant to be non-viable, indeed dominant negative, cross-linking is an essential function for HK97.

Capsid maturation of herpes simplex virus

Herpesviruses are a family of large DNA viruses that, despite having diversified to infect a wide range of vertebrate hosts, have maintained a fixed architecture consisting of a nucleocapsid surrounded by a lipoprotein envelope and, between them, the tegument — a compartment for delivering proteins to the host cell [34]. Although all herpesviruses have a T=16 capsid geometry that, to our knowledge, is yet to be documented among bacteriophages, they exhibit many phage-like behaviors in assembly [35]. (For a recent review of HSV assembly and DNA packaging, see [36].) Procapsid assembly is directed by an internal scaffolding protein ('assembly protein'), which forms an inner shell, and a peripheral scaffolding protein ('triplex') (Figure 4a). Maturation, normally coupled with DNA packaging, is initiated by the protease clipping the terminal peptide from the assembly protein, disrupting its interaction with the outer shell. Finally, the mature nucleocapsid exits the nucleus and proceeds along a pathway on which it acquires tegument and envelope.

As noted above, the structures of several herpesvirus proteases have been determined (Figure 2). They are dimers, like the scaffolding protein to which they are initially attached. HSV1 has a portal protein [37] that resembles phage portals in symmetry and structure [38•]. The triplex is a heterotrimer that coordinates capsomers at threefold lattice sites. Unlike assembly protein, it is not discarded during maturation but remains *in situ*, switching its role from morphogenesis (scaffold) to structural reinforcement (accessory protein). The enormous HSV1 capsid (1250 Å diameter) has been visualized at 8.5 Å resolution in its mature state [39]. Its MCP has a very high mass for a capsid protein, 150 kDa, about half of which is in the 'protrusion domain', which extends 80 Å outwards in an elaboration reminiscent of the excrescences on adenovirus hexon [6]. A crystal structure has been determined for the protrusion domain, revealing a highly α-helical fold [40••].

The procapsid, which is short lived, was first detected in *in vitro* assembly experiments with recombinant baculovirus-expressed HSV1 proteins [41]. It was then realized that the capsids that accumulate in cells infected with a virus carrying a temperature-sensitive mutation in the protease are, in fact, procapsids. When cells infected with this mutant are switched to the

permissive temperature, the proteases in assembled procapsids are activated and maturation ensues. This remarkable property has enabled study of downstream events in synchronized populations [42]. The *in vitro* assembled procapsids were validated when they were shown to be indistinguishable from particles extracted from mutant-infected cells [43]. In HSV1 procapsids, maturation results in enhanced interactions in the hexameric rings of protrusion domains as they convert from highly asymmetric to symmetric configurations, and the establishment of strong interactions between the floor domains of adjacent capsomers (Figure 4b). These movements involve substantial rigid-body rotations, pointing to a mechanism similar to that of HK97.

Procapsids mature spontaneously, albeit slowly, under ambient conditions, even without proteolysis. This property facilitated the analysis of its dynamics by time-resolved cryo-EM, whereby 17 states could be reconstructed, temporally ordered, and assembled into movies [44••]. The complexity of this transition is generated, in part, by the staggering of the same events taking place at different quasi-equivalent sites. In this way, the integrity of the capsid is maintained as it progresses through incremental changes that ultimately transform the pattern of intermolecular interactions. One by-product is the creation of binding sites on the outer surface for a small protein, VP26; this protein binds to hexamers of the MCP but not to the pentamers (Figure 4a, right). VP26 binding appears to involve the interfaces between adjacent protrusion domains and thus may require these domains to be appropriately juxtaposed. The role of VP26 remains somewhat mysterious. It does not confer additional stability [45] and it is dispensable, although its absence somehow decreases infectious virion production [46]. Another consequence of HSV1 procapsid maturation is weakening of the interaction between the inner (scaffold) shell and the MCP. On the normal pathway, the scaffold is expelled, but in capsids that mature without packaging DNA, it is retained in a shrunken form in particles called B-capsids.

Maturation of other viruses

In this section, we touch on some of the other systems in which capsid maturation has been detected. Early studies on tailed phages have been reviewed [47]. Studies of the P22 system have continued in considerable depth (e.g. [48]) and this *Salmonella* phage shares many aspects of the paradigm to which HK97 subscribes.

The double-stranded RNA viruses have several notable properties, including that they must select and package one copy each of multiple (up to 11) linear segments; their capsids serve not only as a repository for nucleic acid but also as a functional compartment where transcription and replication take place. It is envisaged that, in the three-segment phage φ6 system, packaging proceeds sequentially and each successful packaging event elicits a change in the maturing procapsid, exposing a binding site that serves as a receptor for the next segment [49]. This pathway may emulate the succession of EIs in the HK97 system.

Tetraviruses are small (T=4) ssRNA viruses of insects whose assembly and maturation have been investigated for NωV. Recombinant expression of the MCP led to the identification of a procapsid that is ~25% larger than the mature capsid — the inverse of the phage paradigm [50]. Its maturation (a condensation process) has been characterized [51]. Autocatalytic cleavage of the MCP is required to render maturation irreversible, whereas the transition may be induced, reversibly, by pH change in the absence of cleavage.

Papillomaviruses are non-enveloped DNA viruses that propagate in mammalian epithelia and consist of a T=7 all-pentamer capsid [52] containing the chromatinized circular dsDNA genome. Several have been implicated in cervical carcinoma, and encouraging progress has been achieved with vaccines of recombinant capsids or virus-like particles (VLPs) [53].

Recently, a considerably larger provirion has been identified that, in maturing, undergoes compaction correlated with the formation of disulfide bonds [54••].

Lentiviruses, including HIV, follow elaborate assembly/ maturation pathways that include the envelopment (budding) of a spheroidal precursor shell of Gag and Gag-Pol polyproteins [55•], whose principal components, once separated, reorganize to form the conical capsid (CA) [56] and a thin-walled surface shell or matrix (MA) [57]. Maturation also involves processing of the glycoprotein precursor (by some other protease), activating it to a fusion-competent state.

The ability to probe precursor-product relationships depends on having in hand both the procapsid and the mature form. *In vivo*, procapsids mature rapidly and therefore do not accumulate: accordingly, their existence may not be suspected unless the system is perturbed so as to impede maturation. Two such situations are mutations in the maturational protease(s) and the expression of combinations of capsid protein genes in heterologous systems. It is likely that precursors exist, and maturation plays a comparably important role, in systems in which they have not yet been discovered.

Finally, we note that comparable structural transitions are undergone by the capsids of some viruses (e.g. picornaviruses) during cell entry [58•,59•]. In such cases, the mature infectious virion — although endowed with long-term stability — does not represent a global minimum free-energy state but, rather, a local minimum, from which it shifts upon interaction with the receptor [60].

Conclusions

Maturation, the crucial final phase of virus assembly, involves large cooperative conformational changes that either directly render the virion infectious or enable it to bind additional components needed for infectivity. For the class of viruses that encompasses the tailed bacteriophages and the herpesviruses, high-resolution structures have now been determined for examples of each of the five proteins that are typically involved in capsid assembly, either as building blocks or as morphogenic factors. Maturation is usually triggered by a protease whose activity triggers a structural transformation of the precursor procapsid by rigid-body rotations of the capsid protein subunits that transfer the intersubunit interactions to different, mutually complementary, regions of molecular surface. Refolding of local motifs also takes place in at least some cases. In addition to providing enhanced stability, these changes abolish binding sites that are no longer needed and create new binding sites required for downstream reactions. As a physical process, virus maturation appears similar to and may cast light on other multi-protein assemblies that also undergo structural changes in their reaction cycles. For bacteriophage HK97 and HSV, it has been possible to capture metastable intermediates of the maturation transformation in cryo-electron micrographs and, consequently, to visualize maturation as a dynamic event.

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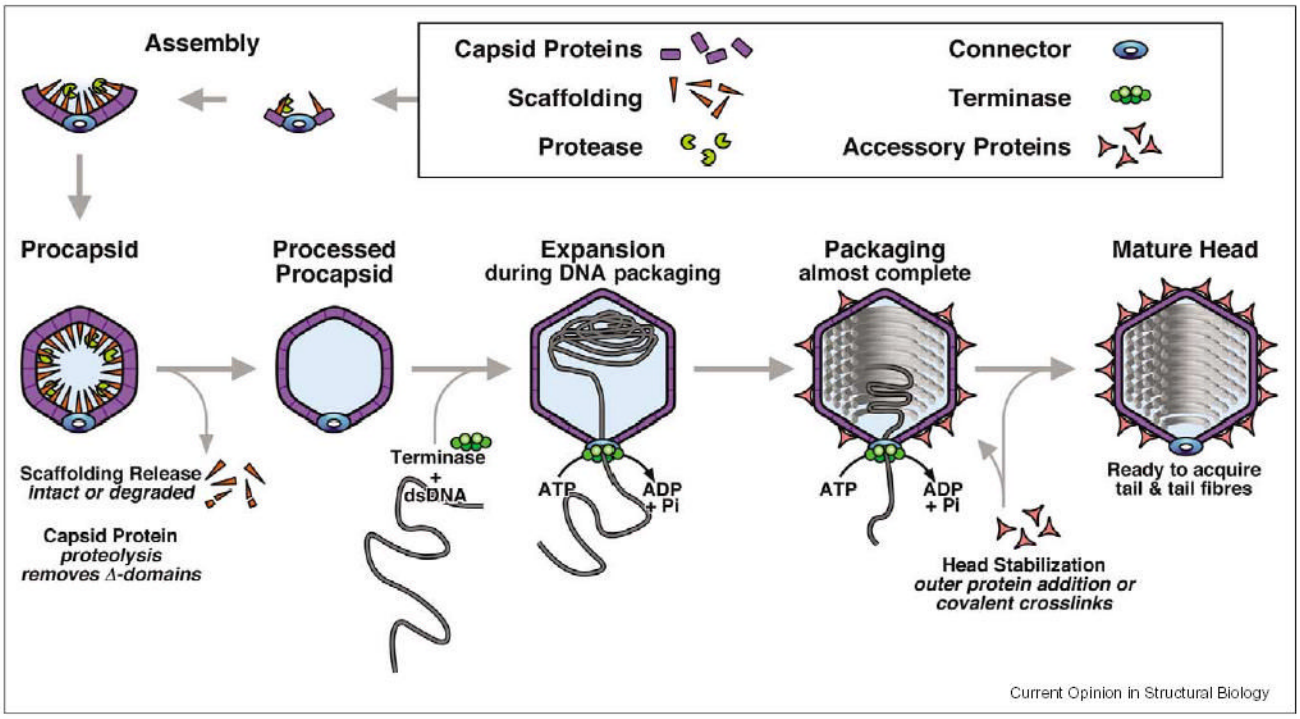

Figure 1.
Generic assembly pathway for the heads of tailed bacteriophages.

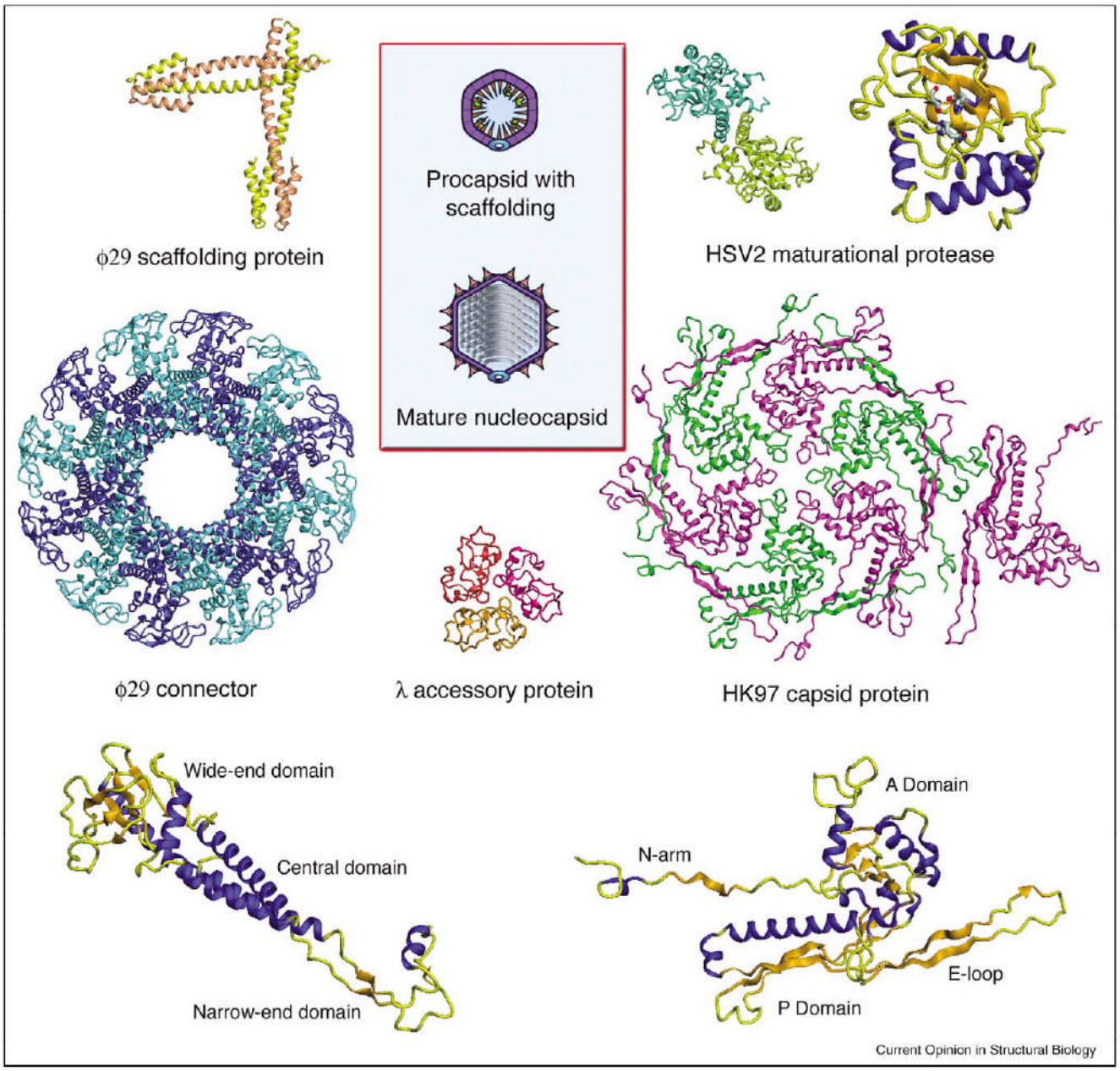

Figure 2.

Structural genomics of capsid assembly. Diagrams of the procapsid and mature head (capsid plus internally coiled DNA) are shown at center (from Figure 1). Ribbon diagrams are shown for the φ29 scaffold (PDB code 1N04, [13••]) and portal/connector (PDB code 1IJG, [9]), the HSV2 protease (PDB code IAT3, [61]), the λ accessory protein (PDB code 1TCZ, [26]) and the HK97 MCP (PDB code 1OHG, [19]). It remains to be seen whether system-to-system variation is accomplished by embellishment of the same basic fold (e.g. by adding additional domains, as with portals, whose molecular masses vary widely [12••]) or by substituting proteins with entirely different folds. We conjecture that accessory proteins and scaffolding proteins are the likeliest candidates for variations of the latter kind.

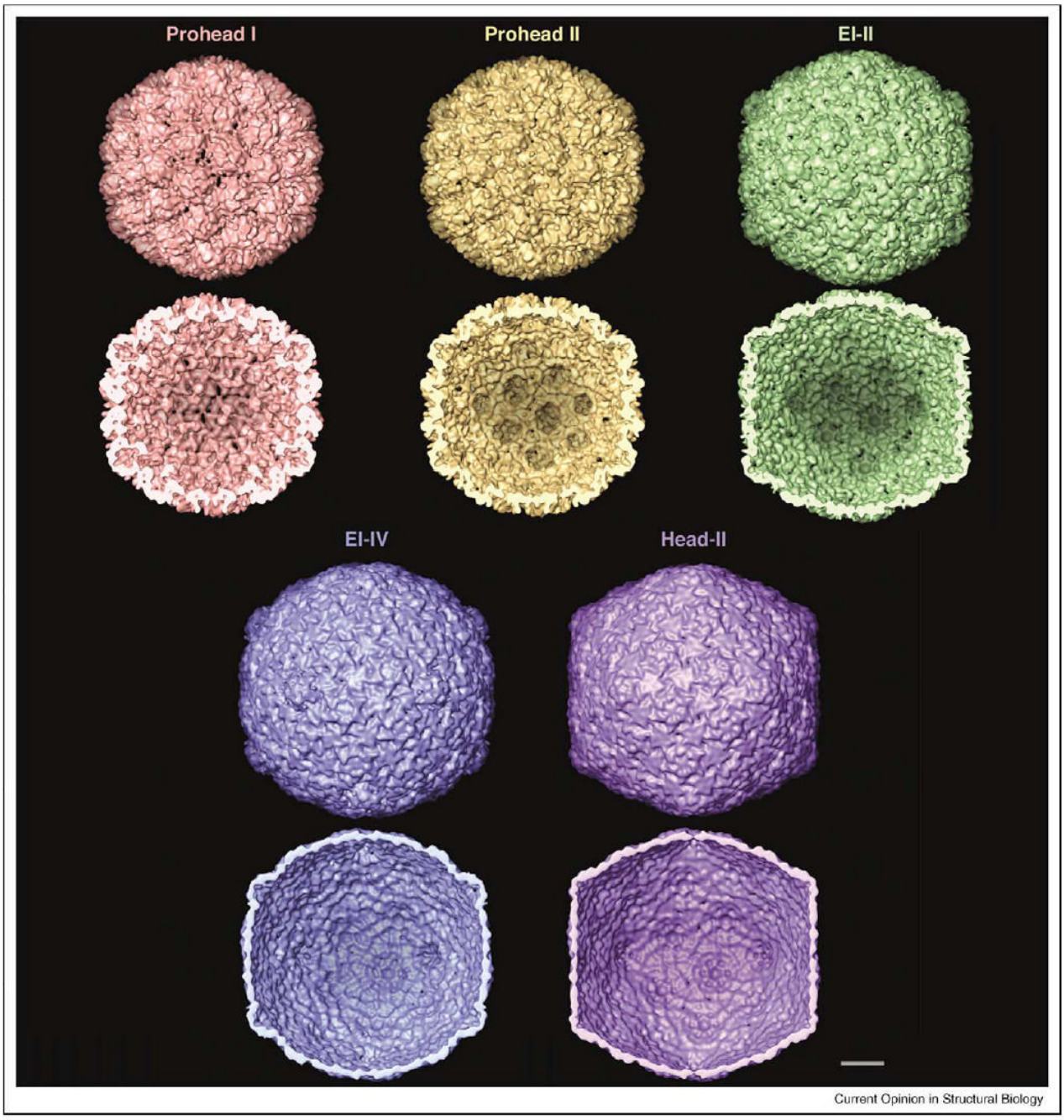

Figure 3.

Maturation pathway of the HK97 capsid, visualized in surface renderings (outer surface, upper rows; inner surface, lower rows) at ~14 Å resolution. All images are from cryo-EM reconstructions, except Head II, which is a resolution-limited rendition of the crystal structure [19]. The capsid has icosahedral geometry (T=7 *laevo*) and is viewed along a twofold axis of symmetry. Prohead I is composed of 420 copies of gp5. In Prohead II, the N-terminal Δ domains have been removed from the inner surface. In acid-induced maturation *in vitro*, the first transition state, EI-I (not shown), is about 10% bigger than Prohead II [29] and very similar to EI-II [33••] (shown). The main difference between EI-I and EI-II is that EI-II has some covalent cross-links [31•]. The next structural state is a thin-walled spherical particle called the 'balloon'.

Balloons vary in their extents of cross-linking, as in EI-III (variable partial cross-linking) and EI-IV (almost complete cross-linking). The balloon structure is very similar to that of the end-state Head, except for the positions of its pentons, which move ~30 Å outwards in the final transition. Bar = 100 Å .

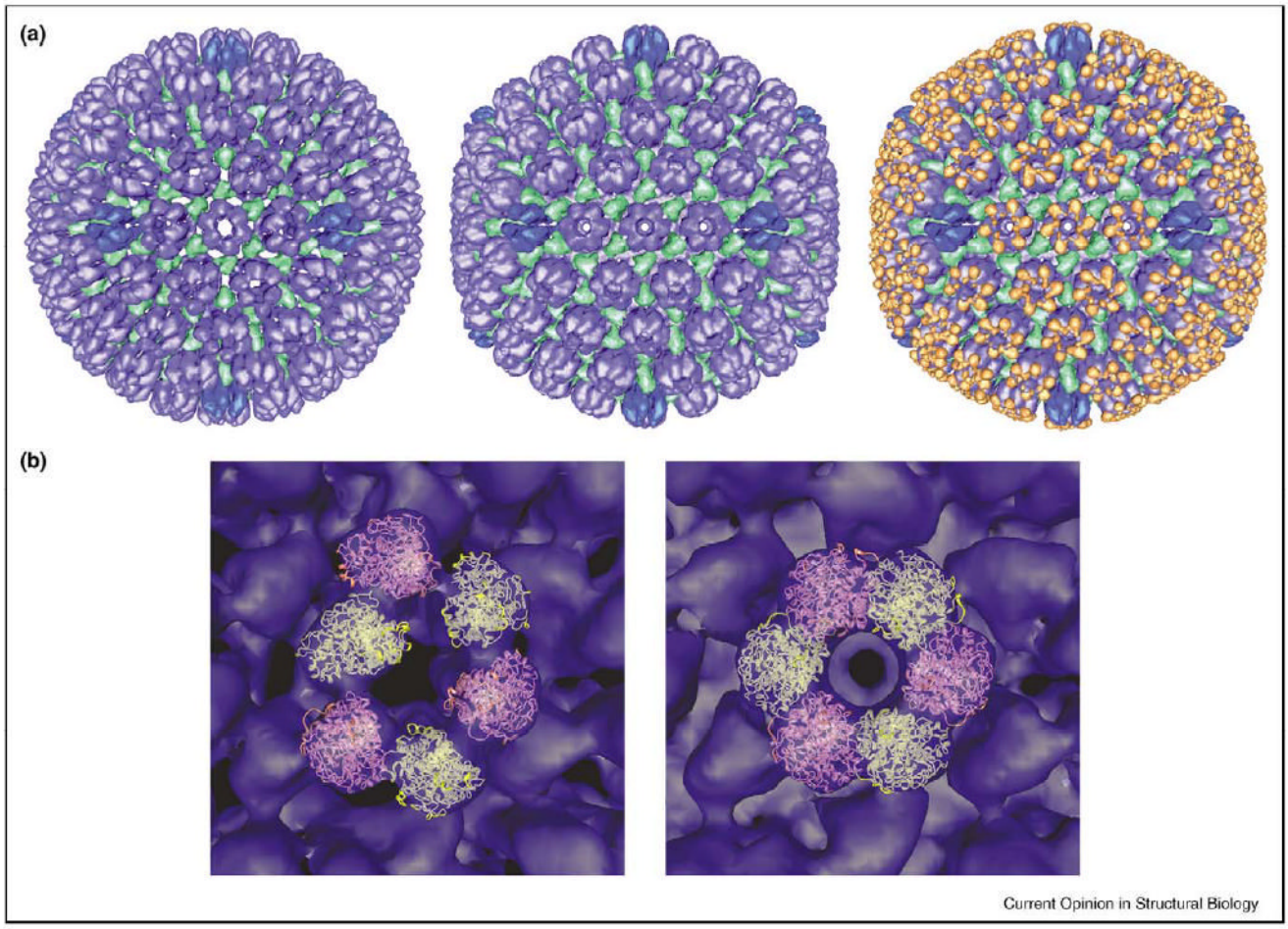

Figure 4.

Movements of and VP26 binding by the protrusion domains of HSV1. **(a)** Maturation of the 1250 Å diameter HSV1 procapsid (left), whose shell contains VP5 (the MCP, blue) and the triplex proteins, VP19c and VP23 (green). If the portal protein, UL6, is available, it occupies one vertex. The scaffolding protein, preUL26.5, and the protease–scaffold fusion, preUL26, form an inner shell that is not shown. The matured capsid (middle) exposes binding sites around the tips of the VP5 hexamers that bind six copies of VP26 (orange) — right. VP26 does not bind to penton VP5. The differing conformations of penton and hexon VP5 are denoted by differing shades of blue in all three panels. The binding site for VP26 on hexons involves two adjacent subunits of VP5: inappropriate juxtaposition of these subunits offers an explanation for the failure of VP26 to bind to procapsid hexons or pentons (either state). The left and middle images were adapted from [44••], and the right from [62]. **(b)** The crystal structure of the protrusion domain (PDB code 1NO7, [40••]) has been fitted into the P-hexons (i.e. the peripentonal hexons) of the procapsid (left) and the mature capsid (right). The initial fitting was done by hand and then refined with an automated program (JB Heymann, unpublished). In the precursor state, there is essentially no contact between adjacent protrusion domains. In maturation, they swivel about hinges at the top of the underlying floor domain to make extensive nearest-neighbor contacts in a now highly sixfold symmetric hexon protrusion. The cryo-EM envelope of the capsid is blue. Ribbon diagrams of alternating protrusion domains around a hexon are pink and yellow.