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The remarkable viral portal vertex: structure and a plausible model for mechanism

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Many icosahedral viruses including tailed bacteriophages and herpes viruses have a unique portal vertex where a dodecameric protein ring is associated with a fivefold capsid shell. While the peripheral regions of the portal ring are involved in capsid assembly, its central channel is used to transport DNA into and out of capsid during genome packaging and infection. Though the atomic structure of this highly conserved, turbine-shaped, portal is known for nearly two decades, its molecular mechanism remains a mystery. Recent high-resolution *in situ* structures reveal asymmetric interactions between the 12-fold portal and the fivefold capsid. These lead to a valve-like mechanism for this symmetry-mismatched portal vertex that regulates DNA flow through the channel, a critical function for high fidelity assembly of an infectious virion.

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Introduction

The 'portal' is a key structural component of icosahedral viruses [1·,2–5]. It is a 12-fold-symmetric dodecameric protein ring inserted into a fivefold-symmetric capsid vertex, making it the unique vertex of an icosahedral capsid [6–10]. In addition to creating a symmetry mis- match [11], this ringed structure provides a channel for DNA passage, for entry into the capsid during packaging and for exit out of the capsid during infection [12–17]. Hence, the name 'portal vertex'.

Despite having negligible sequence similarity, the portal vertex structure is highly conserved among viruses and 'universally' found among viruses from all three domains of life; bacteriophages (phages), eukaryotic (herpes) viruses [4,18] and archaeal viruses [19,20]. Phylogenetic analyses indicate that it is one of the most ancient structures, probably dating back to an early time in evolution when self-replicating entities appeared on Earth [21].

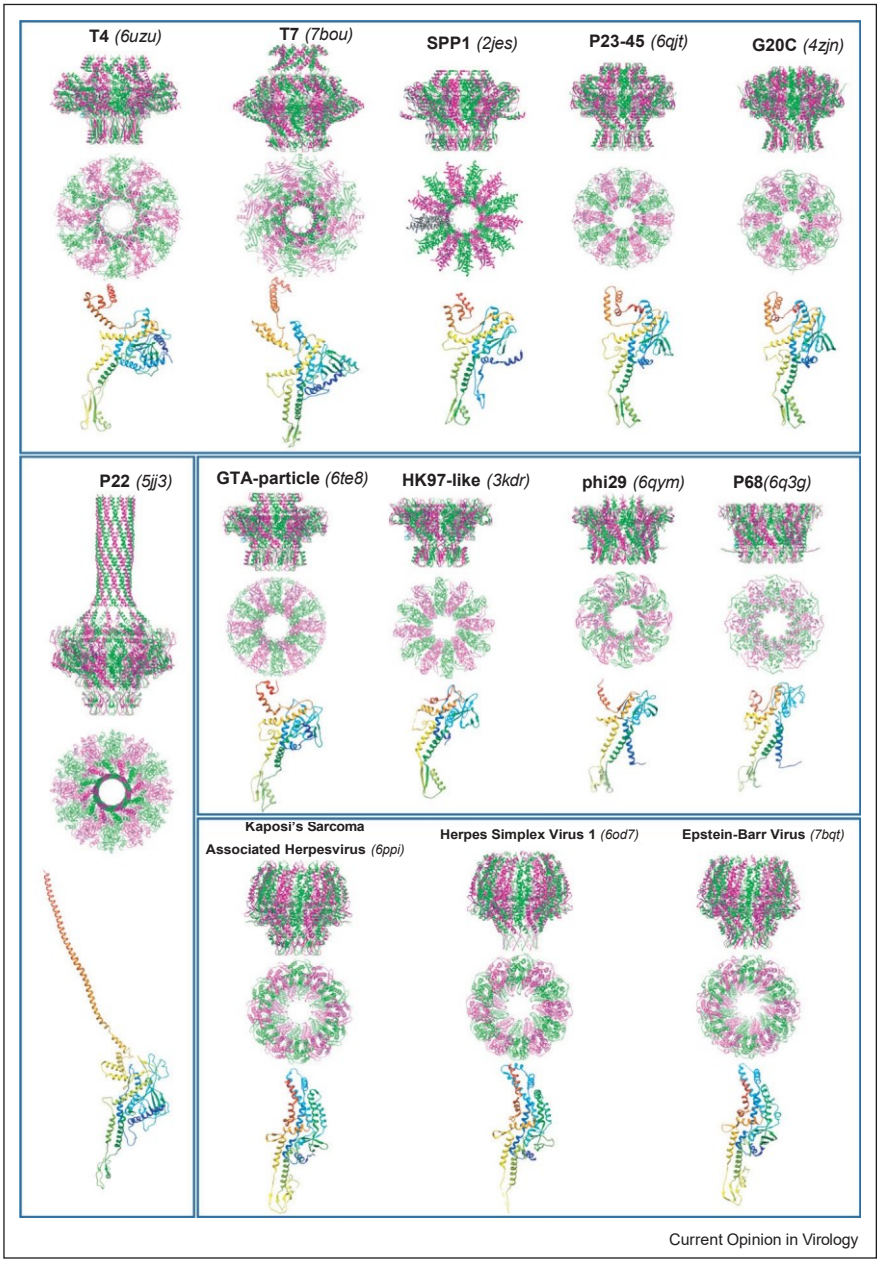
Portal vertex is the first-assembled structural unit in large icosahedral viruses on which the entire capsid is built [4,7,22,23]. In addition, the portal vertex is central to capsid maturation, DNA packaging [12–15,17], and assembly of head completion or 'neck' proteins that connect the head and tail structures in tailed phages [3,5,24].

Portal vertex, thus, acts from the very start of virus assembly to the very end, and beyond when the viral genome is delivered into a new host cell. Therefore, it is expected that the portal structure would be dynamic and goes through conformational transitions during virus mor- phogenesis. Although numerous atomic structures of por- tal proteins from different viruses have been determined by X-ray crystallography and cryo-electron microscopy (EM) [21,25··,26·,27·,28,29·,30,31,32·,33,34,35·,36·,37–40,41··,42,43], the dynamic aspects of the portal and their relationship to function and mechanism are poorly under- stood and only beginning to emerge. In this review, we will describe the common structural features of viral portals, then focus on structural and conformational states, and finally propose a model for the mechanism of the portal vertex in viral morphogenesis.

Structural features of the portal vertex

Portal vertices are turbine-shaped oligomers composed of twelve subunits arranged in a dodecameric symmetry (Figure 1) [21,25··,26·,27·,28,29·,30,31,32·,33,34,35·,36·, 37–40,41··,42,43]. The portals present in the capsid struc- tures are always dodecameric [1·]. However, some recom- binantly generated portal preparations do contain a mix- ture of different oligomers. For example, the recombinantly expressed Herpes simplex virus 1 (HSV- 1) portal can form multimers of 11–14 subunits [18]. The portal proteins differ substantially in size, from 35 kDa in phage phi29 [35·,36·] to 83 kDa in phage P22 [32·]. However, they exhibit remarkably similar folds, indicat- ing their common evolutionary origin and functional importance (Figure 1).

Figure 1

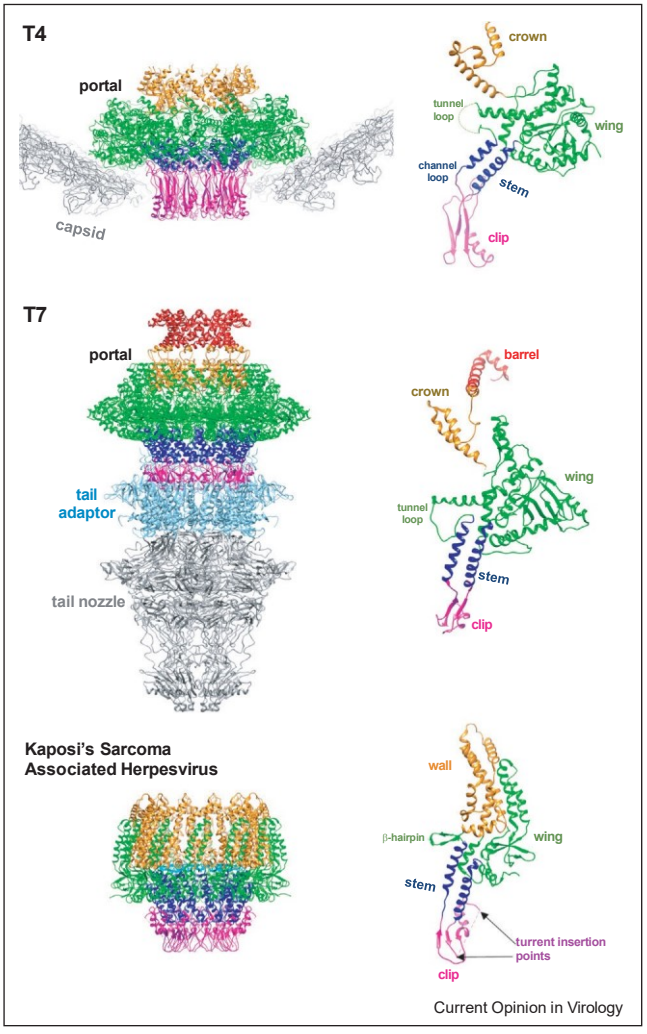


Structures of the portal assemblies from different tailed phages and herpes viruses. For each portal, the side and top views are shown with the subunits alternately colored green and magenta. In the case of the phage SPP1 portal, one subunit is colored black because the recombinantly produced portal used for structure determination was a 13-mer. The individual subunits of the portal proteins are shown in rainbow colors, ranging from blue at the N-terminus to red at the C-terminus. The Protein Data Bank identification numbers are shown in parentheses.

The structure of a portal protein subunit can be subdi- vided into 'clip', 'stem', 'wing', and 'crown' domains (Figure 2). In addition, there are two sets of loops, one near the narrower entrance to the portal ('channel loops') and another at the wider end connecting the stem and wing domains ('tunnel loops'), generating two constric- tions in an otherwise wider portal channel.

The clip domain has an a/b fold. It protrudes at the capsid exterior to varying degrees during viral morphogenesis and provides a docking platform for the pentameric DNA packaging motor ('large terminase') [12–15,17] and for neck proteins [3] that assemble as a dodecamer/hexamer post-packaging [44,45]. In phage T4, the clip domain contains two positively charged residues that surround

Figure 2



Domains of representative portal proteins from phage T4, phage T7, and Kaposi's sarcoma associated herpesvirus (KSHV). The crown region (referred to as 'wall' in the case of KSHV) of each portal is colored orange, wing domain is green, the stem region is blue, and the clip region is magenta. The barrel region of the T7 portal is colored red. For phage T4, the capsid protein subunits associated with the portal at the fivefold vertex are shown in gray. For phage T7, the tail adaptor ('neck') protein attached to the portal is shown in cyan, and the tail nozzle protein is shown in grey. The clip domain of KSHV portal contains a large ~130-aa 'turret' insertion consisting of tentacle helices. The points of this unmodelled insertion are indicated by arrows.

the portal entrance. These might be involved in capturing the genomic DNA end at the time of packaging initiation [21]. However, these residues are not universally con- served in other portal structures.

The clip region of phage T4 portal also contains an inner channel loop pointing into the DNA-translocation chan- nel. Mutational studies show that this loop might be important for function, and the cryo-EM structure and modelling of DNA inside the channel suggest that the channel loop might interact with DNA during genome packaging [21].

The stem domain consists of two antiparallel a-helices (Figure 2) that often span the thin capsid shell. Certain mutations in these helices affect DNA packaging, result- ing in overpackaging in phage P22 [46,47] and packaging abolition in phage SPP1 [48].

The wing domain of portal is located inside the capsid shell (Figure 2). It has an a/b fold that significantly varies in size in different viruses (Figure 1). However, the three wing domain helices including a long 'kinked helix' located closest to the portal channel, are present in all the structures determined. The twelve wing domains of the portal dodecamer interact with the inner capsid surface ('floor') of the five major capsid protein capsomers surrounding the portal [25••]. Because of the symmetry mismatch between the 12-fold-symmetric portal and fivefold-symmetric capsid, the wing domains encounter different capsid environments. The asymmetric recon- struction of the T4 portal vertex showed that the local flexible regions in the wing domain morph to compensate for the symmetry mismatch (see below) [25••].

The phage portals contain ~20-amino acid-long 'tunnel loops' (Figure 2), which connect the wing domain to one of the stem domain helices [21,28]. This loop protrudes into the portal channel and shows weak density in many portal structures probably because it is flexible and disor- dered (without DNA in the channel). Similar regions of herpes virus portals contain b-ribbons instead of loops [40,41••,43]. These create a constriction in the portal channel and its strict conservation among viruses impli- cates a common essential function, perhaps gripping DNA to prevent slipping. Mutational analyses showed that portals with large deletions in the tunnel loop could package short pieces of DNA, but not the complete genome because some of the last-packaged genome leaks out generating incompletely packaged and non-infectious viral particles [49,50].

The C-terminal regions of the portal proteins form a-heli- cal crowns exposed to the capsid interior (Figures 1 and 2). The crown region greatly varies in size in different viruses. It is expected to interact with the packaged DNA during genome translocation, and might act as a gauge to sense the amount of DNA pumped into the capsid [32•]. In phages containing internal cores (phage T7), the portal crown provides an attachment site for the core [27•].

The phage P22 portal contains an unusually long ~200-Å helical barrel [32•,33] (Figure 1), which is located on top of the crown and protrudes almost to the center of the mature capsid. However, this barrel is disordered in the P22 procapsid. The barrel probably helps to organize the

packaged DNA inside the capsid and may serve as a pressure gauge. Mutants with a shortened barrel produce properly assembled but noninfectious virions, indicating that the barrel is important to direct DNA delivery during phage infection [33].

Finally, phage and herpes virus structures show a ring of DNA density around the portal wing domains [41••,51]. This might represent one of the two ends of the packaged DNA that may be located in proximity to each other at the portal entrance [52,53]. Since the last packaged DNA is expected to be inside the portal channel, it is likely that the DNA encircling the portal belongs to the first pack- aged DNA that gets anchored to the portal.

Portal structural and conformational states

Local quasi-fivefold symmetric components

Although most of the reported portal structures display 12-fold symmetry, certain conformational snapshots of portals from viruses such as phage P22 [32•] and HSV- 1 [41••] include a quasi-fivefold symmetric component. The clip region of the P22 procapsid portal that also provides an interacting surface for attachment of the DNA packaging large terminase motor exhibits quasi- fivefold symmetry [32•]. Since the packaging motor is likely a pentamer [12,54,55], this asymmetric procapsid portal conformation might be important for efficient motor assembly and DNA translocation. Consistent with this hypothesis, strikingly higher binding affinity was observed for binding of the large terminase protein to P22 procapsid portal when compared to its binding to the 12-fold-symmetric mature virion portal [32•].

The *in situ* cryo-EM reconstruction of HSV-1 virion portal vertex shows 'tentacle' helices inserted into the portal clip domain [41••]. These helices adopt a quasi-fivefold symmetric arrangement, extending the clip region and forming a helix tunnel around the packaged DNA. It was postulated that these helices might provide an interacting surface for the packaging motor [41••]. In the mature virion, some of the tentacle helices interact with the fivefold capsid-penetrating triplex Tri1 protein and the pseudo-fivefold symmetric portal cap that seals off the packaged head. Notably, this connector complex struc- ture is distinct from that in tailed phages where a 12-fold- symmetric neck protein structure assembles on the portal after packaging termination.

It is not clear if similar portal asymmetries are a common feature among viruses because they have not been found in other phage portal structures. For instance, in phage f29, one of the best studied phages where the *in situ* structures of portals from procapsid, mature virion, and genome-emptied virion have been determined [35•], no quasi-fivefold symmetric regions have been found in the portal assembly.

Symmetry mismatch accommodation at the portal-capsid interface

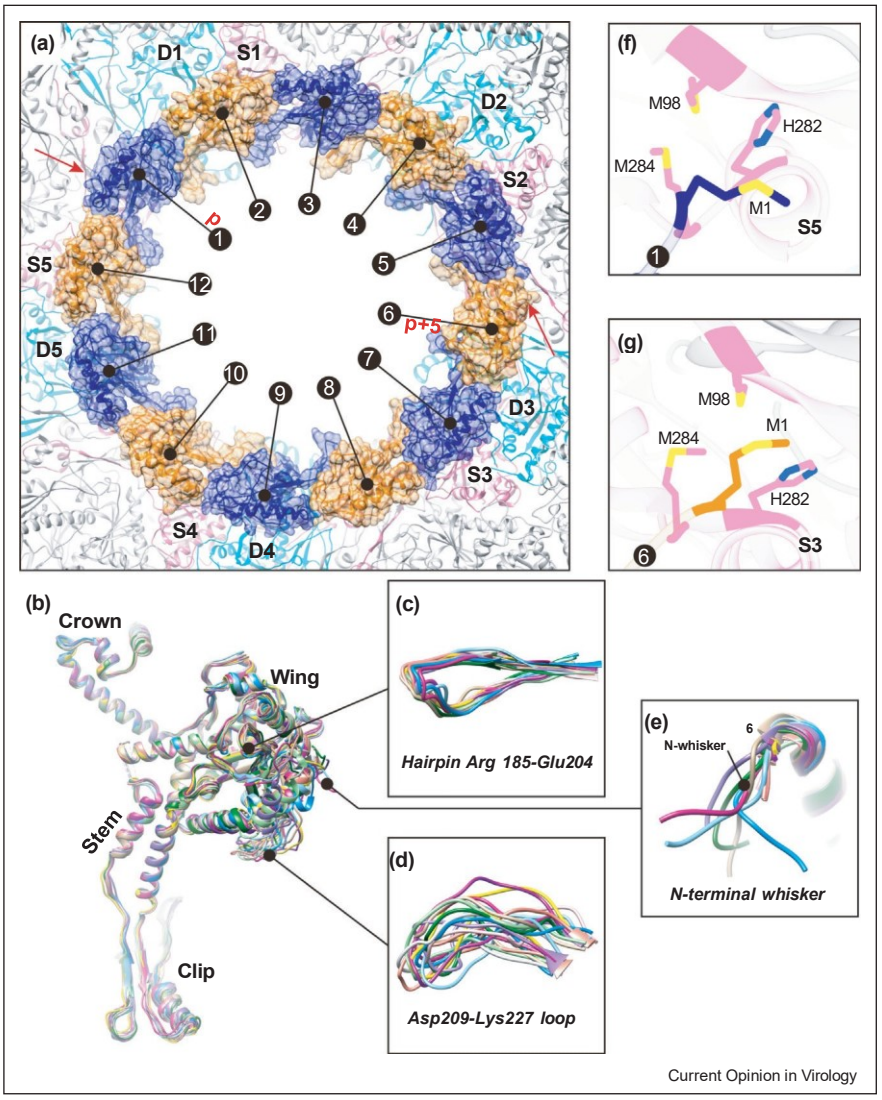
A severe symmetry mismatch exists between the dode- cameric portal and the fivefold capsid vertex. High-reso- lution *in situ* structures of the portal vertices that can resolve portal-capsid interfaces are required to reveal the mechanism by which this symmetry mismatch is accom- modated. However, this has been challenging, despite the profound developments in cryo-EM, mainly due to diffi- culties in data processing and flexibility in portal-capsid interfaces [25••].

To date, *in situ* portal vertex structures are available for T4, P23-45, and P68 phages [25••,29•,39], and for HSV-1 [41••]. Among these, the portal vertex structure of T4 shows relatively detailed structural information on the portal-capsid interface [25••] (Figure 3a–g). In this struc- ture, flexible components in the portal periphery display large conformational variations among the portal subunits (Figure 3b–g), whereas the capsid protein structures remain nearly unchanged when compared to their coun- terparts at the other icosahedral vertices. The flexible components include: the *hairpin* Arg185-Glu204, the *loop* Asp209-Lys227, and the N-terminal '*whisker*' Met1-Leu6 (Figure 3c–g). Despite the severity of the symmetry mismatch, nearly identical interactions between different portal subunits and the surrounding capsid protein mole- cules can occur repeatedly [25••]. For example, the N- whiskers of portal subunits 1 (p) and 6 (p + 5) attach to two fivefold-symmetry-related points in the capsid by forming potential methionine-metal clusters with the capsid pro- tein subunits (Figure 3a, f & g). In addition, similar charge interactions occur between fivefold-symmetry-related points in the capsid and portal subunits that has a subunit number relationship of p, p + 5, and p + 7. This is because the portal subunits having such numerical relationships face similar capsid environments rotated by either $+6^0$ or -6^0 due to the 12-fold symmetry of the portal and the fivefold symmetry of the capsid. The phage T4 portal uses the flexibility of the aforementioned peripheral components of the wing domains to morph locally and accommodate the symmetry mismatches while maintain- ing the 12-fold symmetry in the rest of the structure. As the symmetry of the portal and the capsid are strictly conserved, and the flexible components in the portal- capsid interface have been observed in both the *in situ* and 12-fold averaged portal vertex structures of many other phages and viruses [1•,2,3,5,56], a similar structural morphing mechanism might be in operation for symmetry mismatch accommodation.

Portal conformations

Recent structures reveal two conformational states of the portal: 'closed' and 'open'. In the thermophilic phage P23-45 [29•] and its close relative G20c [30], the diameter of the tunnel, the most constricted section of the portal channel, is ~22 A° in the open state and ~14 A° in the

Figure 3

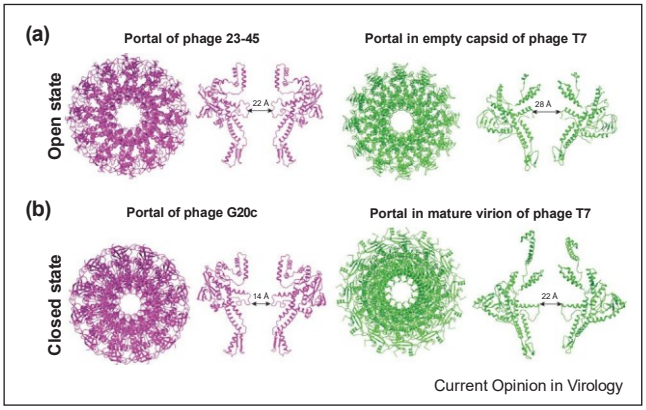


Symmetry mismatch accommodation in the portal-capsid interface of phage T4. (a) The flexible components of the portal subunits that make contacts with the capsid and their surrounding capsid environment. The capsid proteins are colored in pink, cyan or gray. The five surrounding capsid protein subunits (pink) are labeled as 'S' subunits and the five that are more distant to portal (cyan) are labeled as 'D' subunits. The portal subunits are shown in both surface view and ribbon diagram and labeled 1-12. The surface view is shown semitransparently. Alternate portal subunits are colored in blue or orange. For the portal subunits, only the regions that make contacts with the capsid are shown. The potential methionine-metal clusters that anchor the portal to the capsid are indicated using red arrows and the corresponding portal subunits are labeled as p (subunit 1) and p+5 (subunit 6). (b–e) Superimposition of the twelve portal subunits showing high conformational variations in their capsid-interfacing regions. (f, g) The potential methionine-metal clusters form between the N-whisker methionine (M1) of portal subunits p (subunit 1) or p +5 (subunit 6), and methionines M98 and M284 and histidine H282 of the capsid protein subunits. The figure is adapted from Fang *et al.* [25··].

closed state (Figure 4a). The open state is found in the *in situ* cryo-EM structure of the procapsid whereas the closed state is found in the crystal structure of the free portal. Modeling suggests that movements of portal's crown and wing domains can cause further constriction of the tunnel during which the tunnel loop and the kinked helix connected to it are remodeled [29·]. With the result, the diameter of the tunnel changes and the tunnel loop surface switches from exposing hydrophilic residues in the open state to one exposing hydrophobic residues in the closed state [29·].

Narrowing and widening of the tunnel has also been observed in the portal structures of other phages includ- ing T7 [26·,27·], f29 [35·], and P22 [32·]. In T7, major conformational differences were reported in the kinked

Figure 4



The Open and Closed conformational states of the portal. (a) The open states of the portals of thermophilic bacteriophage P23-45 (PDB ID: 6QJT) [29·] and phage T7 (PDB ID: 7BP0) [27·]. (b) The closed states of the portals of phage G20c (PDB ID: 6IBG) [31] and T7 (PDB ID: 7BOU) [27·].

helix-tunnel loop motif of two recombinant portal struc- tures [26·]. The tunnel widening seen here is extraordi- nary, from ~23 Å to ~53 Å. Docking of these structures into relatively low-resolution capsid structures showed
that the closed state corresponded to the portal in the procapsid and the open state to the mature virion [26·]. However, different results were obtained when the struc- tures were determined by *in situ* cryo-EM reconstruction
of portals present in the empty capsid and mature virion [27·]. Here, the tunnel diameter is ~28 Å in the open state found in the empty capsid and ~22 Å in the closed state found in the mature virion (Figure 4b). These differences suggest that the portal conformations, and in turn the
tunnel diameter, might be affected by sample preparation
and/or portal's association with other capsid components. However, a theme that is emerging from the above studies is one of a dynamic portal potentially having the ability to open and close the portal channel through movements of its domains, particularly the wing and crown domains that are in close contact with the capsid and the packaged DNA.

A model for the mechanisms of the portal vertex

It is remarkable that a single molecule, the portal protein, plays such key and diverse roles in capsid assembly, motor assembly, genome packaging, neck assembly, and infec- tion. It interacts with the major capsid protein and the scaffolding protein during capsid assembly, with the large terminase motor and the DNA during packaging, and with the head completion proteins during neck/tail assembly [1·,2,3]. Although the mechanisms still remain a mystery, a plausible model is emerging, however speculative.

A dodecameric portal, the first viral structure to be assem- bled, initiates capsid assembly [4,22,23]. The number of portals assembled, and kinetics of their assembly appear to be critical and tightly controlled, assisted by portal protein's interactions with the membrane, chaperone(s), and other host/viral components [57,58,59]. These have been documented in mutational studies of various phages [1·,23]. For instance, in T4, if the portal is missing during infection, icosahedral assembly is aborted, leading to accumulation of tubular polyheads after a lag period [23,60].

The *in situ* structure of the T4 capsid-portal interface suggests that the N-terminal whiskers of the portal might capture the gp23 major capsid protein molecules through formation of methionine-metal clusters [25··]. In the *in situ* structures of phage T7 [27·] and P22 capsids [61], density corresponding to the helix-turn-helix domain of the scaffolding protein is visible underneath the wing domains of the portal vertex suggesting that these might be the sites that bind the scaffolding protein subunits. The portal-capsid protein-scaffolding protein complex thus formed might serve as a nucleator and direct the newly synthesized major capsid protein and scaffolding protein subunits into correct icosahedral assembly path- ways by co-assembling the shell around the scaffolding core.

Following shell assembly and maturation, freed from scaffold interactions when the scaffolding protein either leaves (e.g. P22 [62]) or is degraded (e.g. T4 [63]), a conformational change likely occurs in the portal that allows docking of a pentameric packaging motor-DNA complex at the exposed clip region to initiate DNA translocation. Although low resolution structures are available for this complex, the nature of this symmetry- mismatched interface and the residues involved in the portal-motor interactions are yet unknown in any viral system [55,64]. The packaging force generated by the motor during DNA translocation and the residence of the newly packaged DNA near the portal vertex might alter its interactions with the capsomers, triggering capsid expansion that results in ~50% increase in the capsid volume. Expansion also greatly stabilizes the capsid structure [23,65] (Fang *et al*., unpublished).

Demonstration in several phages that the portal vertex exists in 'closed' and 'open' conformations implicates a 'valve-like' function for the portal channel. This might be required for DNA translocation, packaging termination, and neck assembly. It is clear from the *in situ* structures that re-modeling of the kinked helix and the tunnel loop can lead to various degrees of constriction of the portal tunnel. There is also a second constriction at the portal entrance which might also function as another valve, connected to the tunnel valve through two long stem helices [21,25··]. This means that DNA movement

through the portal channel can be regulated at both ends of the portal, at the entrance of the portal where it is connected to the packaging motor and at the exit where it is in contact with the capsid and the packaged DNA.

This establishes a mechanism of communication between the capsid exterior where the force generated by the motor translocates DNA into capsid and the capsid inte- rior where internal pressure builds as genome gets pack- aged [66–68]. This might be the key function of the portal; controlling the flow of the viral genome using a two-valve system regulated by signals received at each end through its interactions with the motor, the capsid, and the packaged DNA. The portal's crown, wing, and clip domains serve as sensors that generate the signals while the stem helices transmit the signals between the inside and the outside of the capsid. This would explain a number of portal's roles reported over the years through mutational, biochemical, and structural studies [1·,2]. These include: sensing and altering the fullness of the packaged head [47], regulation of the motor velocity, headful termination of packaging, DNA retention in the pressurized head, and attachment of the neck proteins [69,70].

Perhaps the best evidence for a dynamic valve is the closing of the channel when the head is full. The *in situ* structures indicate that the internal pressure of packaged DNA presses the portal downwards [27·,29·,35·], a signal to close the valve, disengage the motor, and not allow the packaged DNA to leak out. The detached large terminase motor can now make the packaging termination cut and is free to engage the newly generated DNA end with another empty capsid to continue processive packaging. At the same time, the exposed clip region provides sites for attachment of the neck proteins that seals off the packaged head [3,5]. This is perhaps one of the most vulnerable stages of virus assembly, hence these dynamic transactions must be orchestrated at a fast timescale such that the packaging arrest, termination cut, and sealing of the packaged head occur hand in hand, without any genetic information leaking out. Otherwise, escape of even a few nucleotides of packaged DNA could lead to generation of non-infectious virions.

Another key function of the proposed 'portal valving' might be to coordinate the packaging machine during DNA translocation. Dynamic movements of portal domains can allow unrestricted DNA flow when the motor ATPases fire and restrict it when the motor sub-units take turns in between firings to prevent DNA slippage [55], or regulate motor velocity in coordination with the kinetics of DNA organization inside the capsid, or help adjust the packaging machine force with increas- ing internal pressure during head filling [69,70]. These dynamic movements, especially of the crown and wing domains, might be supported by the observed morphing of the portal-capsid interface regions as well as the flexibility to tilt the portal disk through adjustment of portal's interactions with the capsid floor [25··].

The above working model thus serves as a framework to deeply analyze the portal functions at various stages of virus morphogenesis.

Conclusions

A wealth of atomic level information is now available on viral portal structures from a variety of phages and herpes viruses that point to a common functional theme. The more recent *in situ* structures point to a highly dynamic portal that is engaged in sensing and coordinating various aspects of virion assembly through interactions with other structural components. A working model is emerging in which the surface regions of the portal regulating virus assembly while the internal regions regulate genome packaging, through concerted movements of various por- tal domains. The proposed portal valving mechanism that can regulate DNA flow into and out of the virus capsid might turn out to be a necessary function for the survival of most icosahedral viruses, and is likely recapitulated in other biological systems. Future structural and single- molecule studies combined with biochemical and molec- ular genetic approaches should be able to tease out the details of the mechanism and lay out the complete picture of this remarkable nanomachine.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, have been highlighted as:

- · of special interest
- •• of outstanding interest

1. · Dedeo CL, Cingolani G, Teschke CM: Portal protein: the orchestrator of capsid assembly for the dsDNA tailed bacteriophages and herpesviruses. *Annu Rev Virol* 2019, 6:141-160

Comprehensive review of the role of the portal in capsid assembly.

2. Prevelige PE Jr, Cortines JR: Phage assembly and the special role of the portal protein. *Curr Opin Virol* 2018, 31:66-73.

3. Tavares P: The bacteriophage head-to-tail interface. *Subcell Biochem* 2018, 88:305-328.

4. Newcomb WW, Homa FL, Brown JC: Involvement of the portal at an early step in herpes simplex virus capsid assembly. *J Virol* 2005, 79:10540-10546.

5. Fokine A, Rossmann MG: Molecular architecture of tailed double-stranded DNA phages. *Bacteriophage* 2014, 4:e28281.

6. Murialdo H, Ray PN: Model for arrangement of minor structural proteins in head of bacteriophage lambda. *Nature* 1975, 257:815-817.

7. Murialdo H, Becker A: Head morphogenesis of complex double-stranded deoxyribonucleic acid bacteriophages. *Microbiol Rev* 1978, 42:529-576.

8. Muller-Salamin L, Onorato L, Showe MK: Localization of minor protein components of the head of bacteriophage T4. *J Virol* 1977, 24:121-134.

9. Driedonks RA, Engel A, tenHeggeler B, van D: Gene 20 product of bacteriophage T4 its purification and structure. *J Mol Biol* 1981, 152:641-662.

10. Casjens S, Hendrix R: *Control Mechanisms in dsDNA Bacteriophage Assembly*. New York: Plenum Press; 1988.

11. Parent KN, Schrad JR, Cingolani G: Breaking symmetry in viral icosahedral capsids as seen through the lenses of X-ray crystallography and cryo-electron microscopy. *Viruses* 2018, 10.

12. Rao VB, Feiss M: Mechanisms of DNA packaging by large double-stranded DNA viruses. *Annu Rev Virol* 2015, 2:351-378.

13. Feiss M, Rao VB: The bacteriophage DNA packaging machine. *Adv Exp Med Biol* 2012, 726:489-509.

14. Casjens SR: The DNA-packaging nanomotor of tailed bacteriophages. *Nat Rev Microbiol* 2011, 9:647-657.

15. Sun S, Rao VB, Rossmann MG: Genome packaging in viruses. *Curr Opin Struct Biol* 2010, 20:114-120.

16. Molineux IJ, Panja D: Popping the cork: mechanisms of phage genome ejection. *Nat Rev Microbiol* 2013, 11:194-204.

17. Bazinet C, King J: The DNA translocating vertex of dsDNA bacteriophage. *Annu Rev Microbiol* 1985, 39:109-129.

18. Trus BL, Cheng N, Newcomb WW, Homa FL, Brown JC, Steven AC: Structure and polymorphism of the UL6 portal protein of herpes simplex virus type 1. *J Virol* 2004, 78:12668-12671.

19. Pietila MK, Laurinmaki P, Russell DA, Ko CC, Jacobs-Sera D, Hendrix RW, Bamford DH, Butcher SJ: Structure of the archaeal head-tailed virus HSTV-1 completes the HK97 fold story. *Proc Natl Acad Sci U S A* 2013, 110:10604-10609.

20. Atanasova NS, Bamford DH, Oksanen HM: Haloarchaeal virus morphotypes. *Biochimie* 2015, 118:333-343.

21. Sun L, Zhang X, Gao S, Rao PA, Padilla-Sanchez V, Chen Z, Sun S, Xiang Y, Subramaniam S, Rao VB *et al.*: Cryo-EM structure of the bacteriophage T4 portal protein assembly at near-atomic resolution. *Nat Commun* 2015, 6:7548.

22. van Driel R, Couture E: Assembly of bacteriophage T4 head-related strucutres. II. In vitro assembly of prehead-like structures. *J Mol Biol* 1978, 123:115-128.

23. Black LW, Rao VB: Structure, assembly, and DNA packaging of the bacteriophage T4 head. *Adv Virus Res* 2012, 82:119-153.

24. Veesler D, Cambillau C: A common evolutionary origin for tailed-bacteriophage functional modules and bacterial machineries. *Microbiol Mol Biol Rev* 2011, 75:423-433 first page of table of contents.

25. •• Fang Q, Tang WC, Tao P, Mahalingam M, Fokine A, Rossmann MG, Rao VB: Structural morphing in a symmetry-mismatched viral vertex. *Nat Commun* 2020, 11:1713
Description of the *in situ* asymmetric structure of the phage T4 portal vertex showing the portal-capsid interface and structural morphing of the portal regions to compensate for the symmetry mismatch.

26. • Cuervo A, Fabrega-Ferrer M, Machon C, Conesa JJ, Fernandez FJ, Perez-Luque R, Perez-Ruiz M, Pous J, Vega MC, Carrascosa JL *et al.*: Structures of T7 bacteriophage portal and tail suggest a viral DNA retention and ejection mechanism. *Nat Commun* 2019, 10:3746
The open and closed conformational states of the phage T7 portal were described.

27. • Chen W, Xiao H, Wang X, Song S, Han Z, Li X, Yang F, Wang L, Song J, Liu H *et al.*: Structural changes of a bacteriophage upon DNA packaging and maturation. *Protein Cell* 2020, 11:374-379
The *in situ* cryo-EM structures of the phage T7 portal in the empty capsid and the mature virion were reported.

28. Lebedev AA, Krause MH, Isidro AL, Vagin AA, Orlova EV, Turner J, Dodson EJ, Tavares P, Antson AA: Structural framework for DNA translocation via the viral portal protein. *EMBO J* 2007, 26:1984-1994.

29. • Bayfield OW, Steven AC, Antson AA: Cryo-EM structure in situ reveals a molecular switch that safeguards virus against genome loss. *eLife* 2020, 9
The *in situ* cryo-EM structure of the portal from the procapsid of thermophage P23-45 was reported at near atomic resolution.

30. Cressiot B, Greive SJ, Si W, Pascoa TC, Mojtabavi M, Chechik M, Jenkins HT, Lu X, Zhang K, Aksimentiev A *et al.*: Porphyrin-assisted docking of a thermophage portal protein into lipid bilayers: nanopore engineering and characterization. *ACS Nano* 2017, 11:11931-11945.

31. Bayfield OW, Klimuk E, Winkler DC, Hesketh EL, Chechik M, Cheng N, Dykeman EC, Minakhin L, Ranson NA, Severinov K *et al.*: Cryo-EM structure and in vitro DNA packaging of a thermophilic virus with supersized T=7 capsids. *Proc Natl Acad Sci U S A* 2019, 116:3556-3561.

32. • Lokareddy RK, Sankhala RS, Roy A, Afonine PV, Motwani T, Teschke CM, Parent KN, Cingolani G: Portal protein functions akin to a DNA-sensor that couples genome-packaging to icosahedral capsid maturation. *Nat Commun* 2017, 8:14310
Comparison of the phage P22 portal structures in the procapsid and the mature virion.

33. Olia AS, Prevelige PE Jr, Johnson JE, Cingolani G: Three-dimensional structure of a viral genome-delivery portal vertex. *Nat Struct Mol Biol* 2011, 18:597-603.

34. Bardy P, Fuzik T, Hrebik D, Pantucek R, Thomas Beatty J, Plevka P: Structure and mechanism of DNA delivery of a gene transfer agent. *Nat Commun* 2020, 11:3034.

35. • Xu J, Wang D, Gui M, Xiang Y: Structural assembly of the tailed bacteriophage varphi29. *Nat Commun* 2019, 10:2366
Portal structures of the phage phi29 prohead, mature virion, and genome-emptied virion were described to near-atomic resolution.

36. • Simpson AA, Tao Y, Leiman PG, Badasso MO, He Y, Jardine PJ, Olson NH, Morais MC, Grimes S, Anderson DL *et al.*: Structure of the bacteriophage phi29 DNA packaging motor. *Nature* 2000, 408:745-750
The first atomic structure of a viral portal protein.

37. Simpson AA, Leiman PG, Tao Y, He Y, Badasso MO, Jardine PJ, Anderson DL, Rossmann MG: Structure determination of the head-tail connector of bacteriophage phi29. *Acta Crystallogr D Biol Crystallogr* 2001, 57:1260-1269.

38. Guasch A, Pous J, Ibarra B, Gomis-Ruth FX, Valpuesta JM, Sousa N, Carrascosa JL, Coll M: Detailed architecture of a DNA translocating machine: the high-resolution structure of the bacteriophage phi29 connector particle. *J Mol Biol* 2002, 315:663-676.

39. Hrebik D, Stverakova D, Skubnik K, Fuzik T, Pantucek R, Plevka P: Structure and genome ejection mechanism of *Staphylococcus aureus* phage P68. *Sci Adv* 2019, 5:eaaw7414.

40. Gong D, Dai X, Jih J, Liu YT, Bi GQ, Sun R, Zhou ZH: DNA-packing portal and capsid-associated tegument complexes in the tumor herpesvirus KSHV. *Cell* 2019, 178:1329-1343.e12.

41. •• Liu YT, Jih J, Dai X, Bi GQ, Zhou ZH: Cryo-EM structures of herpes simplex virus type 1 portal vertex and packaged genome. *Nature* 2019, 570:257-261
The *in situ* asymmetric structure of the herpes simplex virus type 1 portal vertex was reported at near-atomic resolution, showing interactions of the portal with the capsid proteins and genomic DNA.

42. Li Z, Zhang X, Dong L, Pang J, Xu M, Zhong Q, Zeng MS, Yu X: CryoEM structure of the tegumented capsid of Epstein-Barr virus. *Cell Res* 2020, 30:873-884.

43. Machon C, Fabrega-Ferrer M, Zhou D, Cuervo A, Carrascosa JL, Stuart DI, Coll M: Atomic structure of the Epstein-Barr virus portal. *Nat Commun* 2019, 10:3891.

44. Chaban Y, Lurz R, Brasiles S, Cornilleau C, Karreman M, Zinn-Justin S, Tavares P, Orlova EV: Structural rearrangements in the phage head-to-tail interface during assembly and infection. *Proc Natl Acad Sci U S A* 2015, 112:7009-7014.

45. Fokine A, Zhang Z, Kanamaru S, Bowman VD, Aksyuk AA, Arisaka F, Rao VB, Rossmann MG: The molecular architecture of the bacteriophage T4 neck. *J Mol Biol* 2013, 425:1731-1744.

46. Bedwell GJ, Prevelige PE Jr: Targeted mutagenesis of the P22 portal protein reveals the mechanism of signal transmission during DNA packaging. *Virology* 2017, 505:127-138.

47. Casjens S, Wyckoff E, Hayden M, Sampson L, Eppler K, Randall S, Moreno ET, Serwer P: Bacteriophage P22 portal protein is part of the gauge that regulates packing density of intravirion DNA. *J Mol Biol* 1992, 224:1055-1074.

48. Cuervo A, Vaney MC, Antson AA, Tavares P, Oliveira L: Structural rearrangements between portal protein subunits are essential for viral DNA translocation. *J Biol Chem* 2007, 282:18907-18913.

49. Padilla-Sanchez V, Gao S, Kim HR, Kihara D, Sun L, Rossmann MG, Rao VB: Structure-function analysis of the DNA translocating portal of the bacteriophage T4 packaging machine. *J Mol Biol* 2014, 426:1019-1038.

50. Grimes S, Ma S, Gao J, Atz R, Jardine PJ: Role of phi29 connector channel loops in late-stage DNA packaging. *J Mol Biol* 2011, 410:50-59.

51. Lander GC, Tang L, Casjens SR, Gilcrease EB, Prevelige P, Poliakov A, Potter CS, Carragher B, Johnson JE: The structure of an infectious P22 virion shows the signal for headful DNA packaging. *Science* 2006, 312:1791-1795.

52. Black LW: Old, new, and widely true: the bacteriophage T4 DNA packaging mechanism. *Virology* 2015, 479–480:650-656.

53. Ray K, Ma J, Oram M, Lakowicz JR, Black LW: Single-molecule and FRET fluorescence correlation spectroscopy analyses of phage DNA packaging: colocalization of packaged phage T4 DNA ends within the capsid. *J Mol Biol* 2010, 395:1102-1113.

54. Morais MC: The dsDNA packaging motor in bacteriophage ϕ29. *Adv Exp Med Biol* 2012, 726:511-547.

55. Sun S, Kondabagil K, Draper B, Alam TI, Bowman VD, Zhang Z, Hegde S, Fokine A, Rossmann MG, Rao VB: The structure of the phage T4 DNA packaging motor suggests a mechanism dependent on electrostatic forces. *Cell* 2008, 135:1251-1262.

56. Ahi YS, Mittal SK: Components of adenovirus genome packaging. *Front Microbiol* 2016, 7:1503.

57. Yap NL, Rao VB: Novel mutants in the 5' upstream region of the portal protein gene 20 overcome a gp40-dependent prohead assembly block in bacteriophage T4. *J Mol Biol* 1996, 263:539-550.

58. Bazinet C, Villafane R, King J: Novel second-site suppression of a cold-sensitive defect in phage P22 procapsid assembly. *J Mol Biol* 1990, 216:701-716.

59. Quinten TA, Kuhn A: Membrane interaction of the portal protein gp20 of bacteriophage T4. *J Virol* 2012, 86:11107-11114.

60. In "Molecular Biology of Bacteriophage T4'' (J.M. Karam, ed.), pp. 218-258. ASM Press, Washington, DC.

61. Chen DH, Baker ML, Hryc CF, DiMaio F, Jakana J, Wu W, Dougherty M, Haase-Pettingell C, Schmid MF, Jiang W *et al.*: Structural basis for scaffolding-mediated assembly and maturation of a dsDNA virus. *Proc Natl Acad Sci U S A* 2011, 108:1355-1360.

62. Fuller MT, King J: Purification of the coat and scaffolding proteins from procapsids of bacteriophage P22. *Virology* 1981, 112:529-547.

63. In "Molecular Biology of Bacteriophage T4'' (J.M. Karam, ed.), pp. 209-212. ASM Press, Washington, DC.

64. Woodson M, Pajak J, Mahler BP, Zhao W, Zhang W, Arya G, White MA, Jardine PJ, Morais MC: A viral genome packaging motor transitions between cyclic and helical symmetry to translocate dsDNA. *Sci Adv* 2021, 7.

65. Ray K, Oram M, Ma J, Black LW: Portal control of viral prohead expansion and DNA packaging. *Virology* 2009, 391:44-50.

66. Smith DE, Tans SJ, Smith SB, Grimes S, Anderson DL, Bustamante C: The bacteriophage straight phi29 portal motor can package DNA against a large internal force. *Nature* 2001, 413:748-752.

67. Evilevitch A, Lavelle L, Knobler CM, Raspaud E, Gelbart WM: Osmotic pressure inhibition of DNA ejection from phage. *Proc Natl Acad Sci U S A* 2003, 100:9292-9295.

68. Bauer DW, Huffman JB, Homa FL, Evilevitch A: Herpes virus genome, the pressure is on. *J Am Chem Soc* 2013, 135:11216-11221.

69. Liu S, Chistol G, Hetherington CL, Tafoya S, Aathavan K, Schnitzbauer J, Grimes S, Jardine PJ, Bustamante C: A viral packaging motor varies its DNA rotation and step size to preserve subunit coordination as the capsid fills. *Cell* 2014, 157:702-713.

70. Berndsen ZT, Keller N, Smith DE: Continuous allosteric regulation of a viral packaging motor by a sensor that detects the density and conformation of packaged DNA. *Biophys J* 2015, 108:315-324.

71. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE: UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem* 2004, 25:1605-1612.