intrinsically photosensitive ganglion cells also demonstrate blue-OFF responses generated through cones [10]. Do these signals travel through blue-sensitive amacrine cells or through the elusive blue-OFF bipolar cell? And if there are blue-sensitive amacrine cells, might there also be red- or green-sensitive amacrine cells involved in red/green colour opponency? The retina continues to surprise us.

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Bacteriophage Tubulins: Carrying Their Own Cytoskeleton Key

Cytoskeletal elements are well known to be widespread in eukaryotes and prokaryotes, providing important, diverse functions for cells large and small. Two new studies report that some bacteriophages encode their own tubulin homologs to facilitate phage reproduction within the host cell.

Daniel P. Haeusser and William Margolin

The last decades of research have uncovered a plenitude of prokaryotic homologs of eukaryotic actin, tubulin, and intermediate filaments in sundry organisms once thought devoid of an organized cytoskeleton [1]. To date, the identified prokaryotic tubulin super-family members consist of FtsZ, TubZ, and BtubA/B. Although the conservation of their primary sequence identity is limited to the GDP/GTP-binding motif (G box), their crystal structures show remarkable similarity between folds [2]. FtsZ is a highly conserved cell division protein found in most bacteria, several phyla of archaea, chloroplasts, and the mitochondria of certain protists [3]. TubZ is encoded within low-copy number plasmids of *Bacillus* species, where it functions in a plasmid segregation system. In this system, the TubR protein binds both to TubZ and to *tubS* centromeric sites on plasmid DNA to facilitate DNA segregation [4]. Phylogenetically closest to α/β-tubulin, BtubA/B of *Prosthecobacter* are unique among bacterial tubulin homologues in their ability to form large microtubule-like structures, but their biological role is unknown [5].

The proliferation of metagenomics has uncovered an additional reservoir of cytoskeletal proteins for characterization: bacteriophages. Research over a decade ago identified a protein, p1, from *Bacillus subtilis* phage ϕ29 that polymerizes into filaments that may play a role in anchoring the phage replication machinery to the cell membrane [6]. This small coiled-coil protein polymerizes in a nucleotide-independent manner, but lacks hallmarks of intermediate filament assembly [7]. More recently, researchers identified a phage actin homolog, Alp6A, in *Bacillus thuringiensis* phage 0305ϕ8-36 [8]. Alp6A forms filaments, but its function is unknown. Now, two new studies [9,10] show that some bacteriophage encode their own tubulin-like proteins. Kraemer *et al.* [9] report the presence of a family of proteins, named PhuZ for '*Ph*age t*u*bulin/Fts*Z*', and characterize a PhuZ from a *Pseudomonas chlororaphis* phage. Oliva *et al.* [10] report a protein structurally similar to TubZ from a phage of *Clostridium botulinum* that also encodes botulism toxin. Each of these phage-encoded tubulin homologs assembles into GTP-dependent two-stranded helical filaments, and it is likely that they both function to organize phage DNA.

Scanning genomic sequence databases, Kraemer *et al.* [9] identified novel tubulin homologs that clustered phylogenetically into two distinct groups. The first of these clusters has seven members present in different *Clostridium* species, with four encoded by the chromosome, one by a plasmid, and three by phages. One of these phages, called c-st, harbors the TubZ studied by Oliva *et al.* [10]. The second cluster, PhuZ, has four identified members, each encoded by a different *Pseudomonas* phage. Notably, the phage genomes represented in each of these phylogenetic clusters are unusually large, suggesting that phages with large genomes may benefit from encoding their own cytoskeletal protein.

The crystal structure of the monomeric GDP-bound form of PhuZ from phage 201ϕ2-1 of *P. chlororaphis* comprises an amino-terminal domain containing the G box, a long helical (H7) bridge domain and a small carboxy-terminal domain. Although it assembles into two-stranded helical filaments like TubZ, PhuZ's structure lacks a conserved interdomain helix (H6) that is important for the polymerization of other tubulin

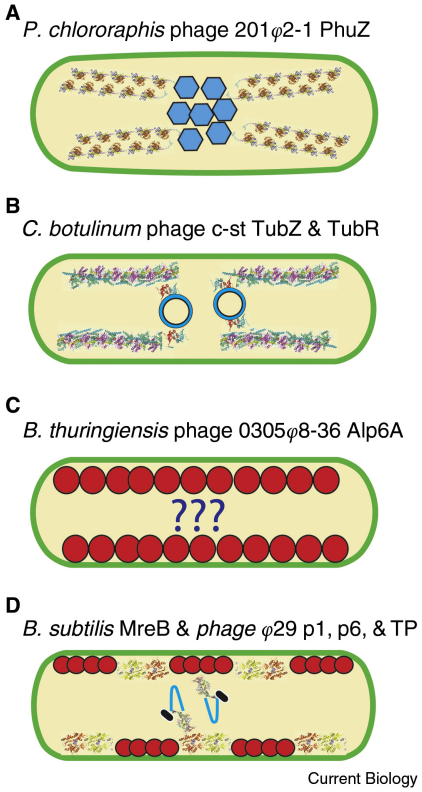

Figure 1. Comparison of phage-encoded cytoskeletal proteins.

(A) The tubulin homolog PhuZ (shown as crystal structure [9]) of *P. chlororaphis* lytic phage 201ϕ2-1 polymerizes into filaments that direct phage DNA replication to a rosette infection nucleoid at the bulged cell mid-point. It is unknown whether PhuZ filaments localize to the membrane or additional proteins are involved in phage DNA (blue hexagons) organization. (B) The tubulin homolog TubZ (shown as crystal structure [10]) of *C. botulinum* phage c-st polymerizes into filaments postulated to localize at the cell membrane. The TubR protein (shown as crystal structure [12]) binds to the carboxy-terminal tail of TubZ and to *tubS* on phage plasmid DNA (blue circle) to segregate the replicated plasmid DNA. (C) The actin homolog Alp6A (red circle) of *B. thuringiensis* phage 0305ϕ8-36 polymerizes into filaments *in vivo* [8]; however, the function of these filaments and their possible association with the membrane is unknown. (D) The small coiled-coil protein p1 (red circle) of *B. subtilis* phage ϕ29 polymerizes into membrane-associated filaments that are an important part of a complex directing linear phage DNA replication [6,7]. The phage terminal protein (TP; shown as crystal structure [15]) binds to the 5' ends of phage DNA (blue curve) and associates with both p1 and host-cell MreB (shown as crystal structure; 2D cross-section of helical filaments) and the bacterial nucleoid through its amino terminus [13,14]. Phage protein p6 (black oval) binds along the DNA and organizes TP [16].

homologs. Instead, PhuZ contains a unique acidic patch at its extreme carboxyl terminus, which is absent in TubZ, including TubZ from the c-st phage identified by Oliva *et al.* [10]. From their crystal data, Kraemer *et al.* [9] propose a model in which six acidic amino acids of the thirteen extreme carboxy-terminal residues of one PhuZ monomer form a 'knuckle' that nestles into a basic patch formed by helices H3–H5 on the adjacent monomer, creating an imbricated monomer pattern of polymerization. Deletion of the knuckle region or mutations that were predicted to disrupt interactions between these patches abolished PhuZ assembly *in vitro*, supporting the structural model of the acidic knuckle's singular role in polymerization.

Previous research on 201ϕ2-1 and related phage failed to observe any cytoskeletal structures in infected host cells. To visualize PhuZ *in vivo*, Kraemer *et al.* [9] expressed a GFP–PhuZ fusion protein from a plasmid-borne inducible promoter in *P. chlororaphis*. At low levels, GFP–PhuZ was diffusely localized in the cytoplasm, but at higher levels most cells contained multiple dynamic fluorescent filaments extending along the cell length, possibly associated with the membrane. Mutant PhuZ proteins, predicted to inhibit the interaction between the acidic knuckle and basic patch between PhuZ monomers, could not form filaments *in vivo*. As expected, mutations in the highly conserved tubulin T7 catalytic loop that are predicted to prevent GTP hydrolysis resulted in non-dynamic axial PhuZ filaments that interfered with host cell division.

The authors next addressed the role of PhuZ filament formation during infective lysis of *P. chlororaphis* using single-cell microscopy. To accomplish this, they monitored infection and lysis while expressing plasmid-borne GFP-PhuZ below the threshold level at which filaments are observed. As a result, fluorescent PhuZ polymers would only form when additional PhuZ was synthesized by the native phage. Time-lapse microscopy revealed that PhuZ filaments formed ~60 minutes post-infection and persisted dynamically for another ~175 minutes until host cell lysis. During this time, *P. chlororaphis* cells became elongated and bulged at mid-cell. Staining of DNA revealed that this bulged region contained a high concentration of phage-encapsidated DNA that formed a single rosette structure that had frequent contacts with the ends of PhuZ filaments.

How relevant are PhuZ polymers to phage reproduction? Using one of the T7 loop mutants that formed static polymers, the authors demonstrated that the resulting PhuZ filaments mislocalized the phage DNA from mid-cell to the cell poles, frequently scattering it into two or three smaller nucleoids. Moreover, single-cell infection assays revealed a significant decrease in phage burst size from cells expressing the catalytically defective mutant compared with overexpression of wild-type PhuZ. Although it is not yet clear how this compares to burst size with normal levels of PhuZ, it suggests that PhuZ filaments help to increase phage yield.

How might PhuZ direct phage DNA replication? One potential clue comes from the presence of a homolog of TubR in the *Clostridium* c-st phage genome [10]. It still remains to be determined whether c-st TubZ can form filaments *in vivo* and whether these filaments can enhance phage reproduction. However, phage c-st replicates as a plasmid [11], suggesting that c-st TubZ functions to position these plasmids, similar to canonical TubZ. This is not much different from the proposed centering function of 201ϕ2-1 DNA by PhuZ. It is possible that PhuZ, like TubZ, attaches to phage DNA via a TubR-like protein. However, the carboxy-terminal tail of c-st TubZ is involved in interactions with TubR [12], whereas the extended tail of PhuZ (containing the acidic knuckle) is involved in self-interaction during polymerization [9]. Another possible clue comes from the studies of *Bacillus* ϕ29 phage, where the membrane-associated p1 protein self-assembles to help organize the direction of phage DNA replication. Although details are still unclear, ϕ29 DNA replication requires additional phage-encoded DNA-binding proteins that are also dependent on the host cell MreB cytoskeleton [6,13,14]. By analogy, PhuZ may function as one component of a complex that includes both phage and host factors.

Taken together, these studies provide a compelling model for novel bacteriophage tubulins. With an overall filament morphology similar to TubZ (and ultimately to F-actin) [9], 201ϕ2-1 PhuZ and c-st TubZ seem important for forming a cytoskeleton within their host to organize the replication of their large genomes and to maximize their reproduction (Figure 1). Yet, many questions remain. As many of the *in vivo* experiments in the PhuZ study were done with overproduced protein, it will be important to assess the role of native PhuZ levels during the infection process, whether a *phuZ* null phage has significant defects, and whether cytoskeletal structures from native expression can be detected *in situ*. It will also be interesting to see how PhuZ interacts with other phage or host factors that might regulate phage DNA organization or PhuZ assembly. Indeed, Oliva *et al.* [10] found a gene adjacent to *tubZ* in phage c-st (*tubY*) that encodes a potent modulator of *in vitro* TubZ assembly. Finally, one major question is why these phages carry their own cytoskeletal tool with them, rather than make use of the host cell cytoskeleton as do eukaryotic viruses. One possibility is that large phage genomes require more stringent organization of their DNA and using a host factor for this purpose is too risky for the phage. Future studies will further illuminate this exciting new area of phage biology.

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Programmed Genome Rearrangements: In Lampreys, All Cells Are Not Equal

How can organisms silence deleterious gene loci? A recent study has shed light on a very brute mechanism in a jawless vertebrate: the irreversible deletion of massive chunks of genomic DNA.

Marie Sémon, Michael Schubert, and Vincent Laudet*

It is commonly accepted that, excepting the combinatorial diversity of immune cells, cells from the same individual share the same genome. However, this dogma has been challenged by recent work demonstrating that the cells of a given organism represent a mosaic of genomes with random abnormalities introduced, for example, during aging [1,2]. In contrast, clear cases of programmed genomic rearrangements, ranging from intra-chromosomal changes to the loss of complete chromosomes, albeit known for a long time, are still relatively rare. For example, in 1887 Boveri described the loss of chromatin during the development of the parasitic nematode worm *Ascaris megalocephala* [3]. This pioneering study was followed by similar descriptions in other parasitic nematodes, and also in copepods (crustaceans), dipteran flies (insects), hagfish (agnathan vertebrates), zebra finches (birds), bandicoots (marsupials) and even ciliates (protists) [4–12].

A particular case of specific genomic reorganization in animals is the so-called developmentally programmed genome rearrangement (PGR) leading to the elimination of portions of chromosomes (chromatin diminution) or the loss of entire chromosomes (chromosome elimination) during embryonic development [4]. PGR thus describes the loss of DNA in somatic cells