The XXIIIrd Phage/Virus Assembly Meeting

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The XXIIIrd Phage/Virus Assembly (PVA) meeting returned to its birthplace in Lake Arrowhead, CA on September 8–13, 2013 (**Fig. 1**). The original meeting occurred in 1968, organized by Bob Edgar (Caltech, Pasadena, CA USA), Fred Eiserling (University of California, Los Angeles, Los Angeles, CA USA) and Bill Wood (Caltech, Pasadena, CA USA). The organizers of the 2013 meeting were Bill Gelbart (University of California, Los Angeles, Los Angeles, CA USA) and Jack Johnson (Scripps Research Institute, La Jolla, CA USA). This meeting specializes in an egalitarian format. Students are distinguished from senior faculty primarily by the signs of age. With the exception of historically based introductory talks, all talks were allotted the same time and freedom. This tradition began when the meeting was phage-only and has been continued now that all viruses are included. Many were the animated conversations about basic questions. New and international participants were present, a sign that the field has significant attraction, as it should, based on details below. The meeting was also characterized by a sense of humor and generally good times, a chance to both enjoy the science and forget the funding malaise to which many participants are exposed. I will present some of the meeting content, without attempting to be comprehensive.

Figure 1. The UCLA Lake Arrowhead Conference Center, ca. 1968.

Introductory Talks: Historical Background

The introductory talks focused on the historical importance of 1) working toward comprehensive genetics, which began with phage T4 via attempts to obtain conditional lethal mutations of all essential genes (Jon King, Massachusetts Institute of Technology, Cambridge, MA USA, with citation of the visionary, leading-from-the-top influence of Bob Edgar), and which led to the finding of protein assembly pathways regulated by conformational changes of previously assembled protein molecules, 2) accelerating generalizable concept development, as illustrated by the concept of a viral procapsid that is assembled before packaging of nucleic acid, a development assisted by the use of assembly intermediate kinetics (Jon King), 3) serving by the discipline of phage assembly as a "point discipline" for the development biochemical and biophysical techniques, as illustrated by the development of discontinuous buffer systems and detergent binding to fractionate proteins by molecular weight (Jon King, describing the advancement of SDS-polyacrylamide gels by Uli Laemmli), 4) finding of a literally astronomical scale of phage presence in the biosphere, with some home-produced astronomy to illustrate scale (Fred Eiserling), and 5) generating spin-offs in the area of education, as illustrated by the introduction of a problems-oriented approach (Fred Eiserling, with citation of the work of Bill Wood).

To continue, I note the following. A major manuscript from the laboratory of Bill Wood initiated the use of kinetics with procapsids/DNA packaging.[1] The Bill Studier laboratory, in post-Edgar studies, performed the most organized systematization of phage genetics during work on the smaller phage, T7.[2] This laboratory also brought electrophoresis in both SDS-polyacrylamide gels for protein[2] and agarose gels for DNA[3] to approximately the current state. This work on T7 culminated in the generation of the major expression vectors used today and one of the major display vectors.[4] Michael Showe and collaborators initiated immunological detection on either an SDS-polyacrylamide or any other gel during studies of the scaffold-cleaving, procapsid-associated protease of phage T4.[5] Several biophysical disciplines have cut teeth on phage assembly, including 1) the analytical physics-based description of packaged DNA, as represented by Bill Gelbart,[6] and 2) the computer-modeling-based counterpart, as represented by Steve Harvey (Georgia Tech, Atlanta, GA USA)[7] at the meeting, with Harvey's current computer modeling in the area of packaged RNA. That is not all (I have not yet discussed

cryo-electron microscopy, for example), but the above is enough to firmly establish the broadly defined field of this meeting as a, if not the, "point discipline" (as in point guard in basketball) for all biology.

In the text below, I will use the word, shell, to refer to the viral protein component that forms the cavity in which nucleic acid is packaged. I will use the word, capsid, to refer to the entire membrane-independent protein component, which includes tail and internal proteins in the case of double-stranded DNA phages. I will discuss proteins by function and will not use encoding gene numbers.

Translational Studies

Translational merging of basic assembly-based studies with vaccine development was performed with both eukaryotic viruses and a phage. Abhay Kotecha (University of Oxford, UK, with contributions from the Pirbright Institute, UK, Onderstepoort Veterinary Institute, South Africa and University of Reading, UK) reported the use of computer modeling to obtain stabilized capsid- and virus-vaccines for Foot and Mouth Disease Virus. Paul Gottlieb (from CUNY, New York, USA) showed that nucleic acid-free, aberrant assemblies of both influenza and respiratory syncytial virus have potential to be used as improved vaccines. Michael Rossmann (Purdue University, West Lafayette, IN USA) presented a cryo-EM analysis of the temperature-dependence of the structure of dengue virus. A phase transition of the outer membrane was observed at human body temperature (37 °C), suggesting design of vaccine antigens to maintain the 37 °C structure. Venigalla Rao (Catholic University, Washington, DC USA) reported the formation of an extensive investigator network to develop anti-microbial vaccines based on antigen display in phage T4 shell proteins called Hoc and Soc, with data that indicate a major success in the area of plague vaccines.

Additional points for reflection
This advanced work on T4-based vaccines would become even more advanced if dry vaccines for both ambient temperature transport and ambient temperature use were developed. One might do this by either 1) learning to dry phage T4-based vaccines or 2) displaying antigens in the shell of a phage that is already drying-resistant.

The translational research was in a more futuristic (but no less important) mode in the area of drug delivery via in vitro-assembled capsids of plant viruses. The basic idea is to reduce the toxicity and increase the targeting of anti-cancer and other drugs by encapsulation in a viral capsid-delivery vehicle that releases the drug at a target. The major advantage of using plant viral capsids is that they 1) can be inexpensively produced in very large (multi-gram) amounts, 2) are relatively uniform in structure and, presumably, other properties, such as permeability, and 3) can be simply re-assembled in vitro after dissociation to subunits. In the areas of particle amount and in vitro assembly simplicity, plant viruses expand the translational, "point discipline" character of the more general discipline of viral assembly. In efforts to capitalize on the advantages of plant viruses, Nicole Steinmetz (Case Western Reserve, Cleveland, OH USA) reported that viral capsid

accumulation in tumors is improved with filamentous versions of plant viral capsids (also called viral nanoparticles, or VNPs). For gene delivery, Rees Garmann (University of California, Los Angeles, Los Angeles, CA USA) reported a Cowpea Chlorotic Mottle Virus capsid in vitro-assembled around recombinant Sindbis virus RNA; the former is a plant virus, the latter, an animal virus. This viral chimera is to be used as a vector for gene delivery (from the laboratory of Bill Gelbart).

An independent approach is to fuse phage phi29 packaging RNA (pRNA), which is part of the phi29 DNA packaging apparatus, to other RNAs to be delivered.[8] Zhengyi Zhao (Laboratory of Peixuan Guo, University of Kentucky, KY USA) showed whole-body images of mice with fluorescent pRNA selectively accumulating in solid tumors.

Additional points for reflection
One anticipates pursuing drug targeting (and other delivery objectives) with one or more of the following basic strategies: 1) analytical, rational design, 2) computer modeling-based rational design, and 3) mutation/selection. Mutation/selection appears to be an undervalued choice. Mutation/selection, in most scenarios, means the use of phages or, better, the capsids of evolved phages. The basic idea is to let the phages evolve to target neoplasms and to outrun metastasis.

Finally, in the area of translational research, the author notes that the topic of phage therapy/prophylaxis of bacterial infections was basically absent from the meeting. This absence is significant when one considers (especially in California) the potential of phage therapy for solving problems caused by bacterial diseases of citrus trees (citrus greening disease, for example), grape vines (Pierce's disease, for example) and people (carbapenem antibiotic-resistant gastrointestinal infections, for example). These problems often occupy space in news broadcasts, without even a hint that a phage-based strategy might be used. The reasons for this absence in the meeting are that 1) phage therapy has not yet reached the point at which the structure and assembly of the phages involved is perceived to have translational utility, and 2) in Western Europe and the US, the research objectives of phage therapy studies often do not match the clinical objectives. Eastern Europe, especially Poland and Georgia (former USSR), presents a much more focused and successful picture of phage therapy, which is perhaps an example of hardship improving focus.

Moving on to the basic science, the author notes that the biology-based topics of the meeting fall in the following, sometimes overlapping categories: 1) genome delivery, which means injection in the case of double- (and now single-) stranded DNA phages, 2) assembly of procapsids, 3) conversion of procapsids to nucleic acid-containing heads, including the process of packaging of either RNA or DNA, and 4) assembly of the tails that are used by double-stranded DNA phages to inject DNA. Sometimes the procedure used for investigation of 1)–4) becomes the primary focus of the work. I will discuss some of these procedure-focused presentations in a separate section.

Nucleic Acid Injection

The formation of non-tail, nucleic acid-injecting "injection tubes" was a theme that was played at the meeting with several variations. Injection tubes are typically cylindrical and extend

from a virus to inject DNA or RNA into a cellular compartment. In the case of double-stranded DNA phages, injection tubes form after host cell contact initiated by tail fibers. After several decades of almost no detailed, structure-based investigation of injection tubes (an isolation-of-intermediates-derived exception is in reference 9, including citation of work from 1978), suddenly structure-based variations on the injection tube theme, were presented for 1) adeno-associated virus-induced, liposome-derived tubes formed during injection into (eukaryotic) nuclei (Brian Bothner, Montana State University, Bozeman, MO USA), 2) phage PRD1 internal membrane-formed tubes that extend from one of 12 capsid vertices and inject DNA through the envelopes of Gram-negative bacterial cells (Nicola Abrescia, Derio and Bilbao, Spain, with support from the Dennis Bamford laboratory in Helsinki, Finland), 3) protein-only tubes that do the same and are made of proteins that apparently pass, pre-DNA injection, through the central channel of tails of phages lambda, P22, T4 and T7 (Ian Molineux, University of Texas, Austin, TX USA), and 4) a protein-only tube, with 10 coiled-coil α helices (α-helical barrel) at the internal tube surface, assembled in vitro from the pilot protein of phage phiX174 (single-stranded DNA genome), a protein that exists unassembled in the mature viral shell, while occupying the center of each of 12 shell protein pentamers (Aaron Roznowski and Lei Sun, representing a collaboration of the Bently Fane laboratory, University of Arizona, Tucson AZ USA and the Michael Rossman laboratory). The work on PRD1 earned Nicola Abrescia one of three awards for graduate student/postdoctoral work.

Additional points for reflection

The range of virus types falling under the injection tube theme suggests that this theme reflects evolutionary constraints that have also existed for viruses of other types.

Moving the focus to the DNA injected, Francoise Livolant (CNRS, Orsay, France) reported a new wrinkle in the DNA injection story, when she described the use of both single-molecule light microscopy and cryo-electron microscopy to find that in vitro phage T5 injection underwent pausing (time scale, seconds). The interpretation was that the pauses were caused by reversible DNA conformation-derived injection blockage. This theme will re-appear under DNA packaging, below. However, the assumption should not be made that all stalls are DNA-generated. The author's laboratory reported stalled (i.e., partial) DNA expulsion that was so stable (> 1 y) and precisely quantized that the source of the stalling/quantization has to be capsid proteins (presumably the shell and connector proteins), not DNA. A phage T3 mutant was used in the latter study.

Returning to the injection proteins, Gino Cingolani (Thomas Jefferson University, Philadelphia, PA USA) and Sherwood Casjens (University of Utah, Salt Lake City, UT USA) described genetic/structural analysis of the phage P22 tail needle found to be unstructured at the DNA exit-blocking N-terminus in the mature phage particle, but to have a N-terminal trimeric helical bundle when isolated at low pH and presumably also after DNA injection (Cingolani). Structural variations at the host cell-contacting C-terminus caused variation in cellular potassium ion release, thought to be a proxy for DNA injection (Casjens).

Procapsid (and Mature Capsid) Assembly

A past theme in procapsid assembly is the requirement for a scaffold that directs assembly of the outer shell of the procapsid and that prevents aberrant shell protein assembly. The scaffold is usually made of a protein separate from the major shell protein. Variations on this theme include 1) several fates of the scaffold, including cleavage (T4, for example), expulsion (P22 and T3/T7, for example), re-cycling (P22, for example) (reviewed in ref. 10) and non-recycling (T7, for example, author's work, unpublished) and 2) inclusion of the scaffold at the N-terminus of the major shell protein (delta domain), with subsequent cleavage by a procapsid-associated protease (HK97).[10]

After procapsid assembly, double-stranded DNA enters capsids via a 12-membered ring (connector or portal) that is embedded in the shell.[10] In contrast to what one might initially think, the connector is not part of the nucleus for the assembly of the icosahedral procapsids of phages T7[11] and P22.[12] Carol Teschke (University of Connecticut, Storrs, CT USA) reported incorporation of P22 connector protein during in vitro procapsid assembly. To incorporate, the connector protein had to be monomeric. Incorporation slowed assembly. Might connector protein incorporation be changing the shell assembly nucleus (perhaps by changing un-skewed hexamers into the skewed hexamers that are found in procapsids), so that only one connector incorporates?

David Veesler (laboratory of Jack Johnson) found, by cryo-electron microscopy (cryo-EM), that phage HK97 procapsid-associated protease molecules interacted directly with procapsid-associated scaffolding domains that were not well ordered. The number of protease molecules per procapsid varied. Terje Dokland (University of Alabama, Tuscaloosa, AL USA) presented evidence that a host protease cleaves the scaffold of transducing particles that are the product of the pirating of a helper phage capsid by two scaffolding proteins in a host pathogenicity island. A developing theme was procapsid-associated shell protein that had secondary structure near the N-terminus that was not present in the mature shell, as found for phage T5 (Alexis Huet, from the University of Pittsburgh, USA with collaboration of Pascale Boulanger, CNRS, Orsay, France), D3 (below) and T7 (Wen Jiang, Purdue, West Lafayette, IN USA and the author).

The skewing of the hexamers of procapsids[10] has raised the question of how strain is distributed in the shell. William Klug (University of California, Los Angeles, Los Angeles, CA USA) and colleagues are approaching this question from the perspective of macroscopically derived theory of continuum elasticity. They find that some of the strain is implicit in the capsomers (pentamers and hexamers); all strain is not generated by quasi-equivalence.

The finding of capsid assembly intermediates has been chronically difficult, in part because of the high speed of assembly after multi-protein nucleation. Elizabeth Pierson (in the group of Martin Jarrold, University of Indiana, Bloomington, IN USA) used whole particle mass spectrometry to detect assembly intermediates of the 240-subunit Hepatitis B capsid, with confirmation of particle type by cryo-EM; 104, 111, and 115 subunit

intermediates were observed. This work earned Elizabeth Pierson the second award for graduate student/postdoctoral work.

Bonnie Oh (laboratory of Roger Hendrix, University of Pittsburgh, Pittsburgh PA USA) reported that, for procapsid assembly from plasmid-encoded phage HK97 shell protein, deletion of the entire delta domain caused loss of assembly, while various point mutations caused one of the following: aberrant assembly, defects in connector incorporation, loss of protease incorporation.

Noroviruses (single-stranded RNA) presumably undergo simultaneous capsid assembly and nucleic acid packaging in vivo (no nucleic acid-free procapsids). Guillaume Tresset (CNRS, Orsay, France) used time-resolved small-angle X-ray scattering to find the following phases for in vitro capsid assembly (no RNA): 1) dimerization of pentamers, 2) more rapid formation of larger intermediates, and 3) a slower re-arrangement of inter-subunit contacts.

Nonetheless, some single-stranded RNA virus capsids undergo major maturation reactions after initial formation. Tatiana Domitrovic (laboratory of Jack Johnson) found that generation and exposure of peptide maturation products of the shell protein of non-enveloped, single-stranded RNA virus, NωV, was correlated with lytic activity against membranes.

Nucleic Acid Packaging

Double-stranded DNA viruses, double-stranded RNA phages and single-stranded DNA phage, ϕX174, package genomes via a nucleic acid-free procapsid. A universal theme for both double-stranded DNA viruses and double-stranded RNA phages is packaging via a ring of ATPase molecules. The ATPase ring is connector-attached for double-stranded DNA phages, and has been 4-, 5-, or 6-membered, depending on the phage and the investigator. This multi-molecular character has lead to the terms, "DNA or RNA packaging motor," to describe the nucleoprotein complex that packages a phage genome.

Some DNA packaging ATPases (called terminases) have a C-terminal domain that serves as an endonuclease that cuts intracellular DNA concatemers to mature genomes; concatemers are linear polymers of mature genomes. Other double-stranded DNA packaging ATPases, such as the ATPase of phage phi29, do not have endonuclease activity and package a genome that does not need to be cut.

DNA and RNA packaging motors have attracted research interest because they provide a relatively simple model system for understanding the mechanisms and evolution of biological motors in general, with a major assist from both high-resolution structure determination and single-molecule-based procedures. In addition, phage genetics provides thus far unmatched capacity to perturb motors. The working assumption is that all phage DNA packaging motors use basically the same mechanism (common theme), although variations on the common theme will exist.

In contrast, single-stranded RNA viruses package RNA without a procapsid and without a motor, i.e., the shell assembles around a partially condensed RNA molecule. Eric Dykeman (University of York, in collaboration with the University of Leeds, UK) reported that recently demonstrated packaging signals (typically stem-loop structures, several per genome) are conserved among different animal viruses and phages (MS2, for example) and plant viruses (satellite tobacco necrosis virus, or STNV, for example). Computer modeling suggests that optimization of packaging via optimization of packaging signals exerts significant selective pressure on the evolution of single-stranded RNA viruses. Peter Stockley (York and Leeds, UK) presented single-molecule-based confirmation of the role of the packaging sequences.

For cowpea mosaic virus, Pooja Saxena (John Innes Center, Norwich, UK) added that genetically blocking RNA replication blocks RNA packaging in vivo, although the reason is not known; empty capsids are assembled. Boon Chong Goh (University of Illinois, USA) performed molecular dynamic simulation of the apparent locking, by a cleavage-produced peptide, of a shell contraction-type maturation event that occurs after positive strand RNA packaging by *Nudarella capensis* ω Virus.

Roman Tuma (University of Leeds, UK) used fluorescence correlation spectroscopy (which determines diffusion coefficient and effective radius in solution) to observe the early stages of assembly of single-stranded RNA viruses, including phage MS2 and satellite tobacco necrosis virus. He detected an RNA collapse that occurred after addition of shell protein, but before assembly.

Influenza A virus has, in contrast, eight unique single-stranded RNA segments, raising the question of how the virus "knows" how to assemble with one copy of each. Sergey Venev (University of Massachusetts, Worcester, MA USA) investigated the details by observing the sequence variability for both packaged RNA and RNA left unpackaged during an infection. Some regions near both 5′ end and 3′ end were less variable for packaged RNA. These regions overlapped known packaging signals.

The following are new observations that were presented at the meeting and that constrain models of how double-stranded DNA packaging motors work. 1) Li Dai (laboratories of Taejip Ha, University of Illinois, Urbana-Champaign, IL USA, and Venigalla Rao) reported single-molecule observation of the serial packaging by a single procapsid of numerous, identical, fluorescently labeled, short oligonucleotides. When active packaging ATPase was mixed with fluorescently labeled, mutationally inactivated (dead) packaging ATPase, packaging was observed when one, but not more, "dead" ATPase molecule was present in the motor, as judged by the fluorescence of the dead ATPase. 2) Paolo Tavares (CNRS, Gif-sur-Yvette, France) extended his laboratory's genetic analysis of the role of the connector in phage SPP1 packaging. He has found that some connector mutations make the cutting activity of the packaging ATPase premature (shorter genome produced) and that the 12 subunits all contribute to this effect. 3) Lindsay Black (University of Maryland, College Park, MD USA) reported that a) during packaging, the phage T4 packaging motor expels DNA-bound molecules of the bi-functional intercalating dye, YOYO-1, based on loss of DNA fluorescence, which supports previous data that indicate crunching, possibly to A-form DNA, of the motor-embedded DNA segment and b) FRET measurements support the idea that, like the packaging

ATPases of other double-stranded DNA phages[13] (and unlike the packaging ATPase in a proposed model of the T4 DNA packaging motor), the packaging ATPase of phage T4 binds to the connector at its endonuclease-carrying C-terminus. 4) Marc Morais (University of Texas, Galveston, TX USA) found, by cryo-EM, that the phage phi29 DNA packaging motor had "extra" density, i.e., density not accounted for in the packaging ATPase structure. The extra density (part of the connector?) contacted the DNA molecule. This study begins the detailed, structure-based analysis of the various states of a functioning DNA packaging motor.

Additional points for reflection

Informatics-based analysis supports the presence of a partial endonuclease domain in the non-endonuclease-containing phi29 packaging ATPase.[13] The simplest corollary is that the partial phi29 and complete SPP1 and T4 endonuclease domains all participate in the motor. The inter-suppression of C-terminal ATPase and portal clip mutations, for SPP1[14] and T4,[15] therefore, raises the possibility that the connector participates in the motor also (see ref. 13). However, we now are faced with explaining the consensus finding that neither the phi29 ATPase nor the N-terminal domain of the SPP1 and T4 ATPases has a known DNA binding site (see refs. 13 and 15). This finding would be explained if the ATPase clamped the DNA molecule, rather than bound it. If so, then packaging in the presence of a dead ATPase molecule (above) is easily explained in that the components of the ATPase ring need not all be alive to generate a clamp.

A major advance in the study of DNA packaging and other motors is the single-molecule measurement of velocities and forces of packaging. Zachary Berndsen (representing a collaboration between the laboratories of Shelly Grimes/Paul Jardine, University of Minnesota, Minneapolis, MN USA and Douglas Smith, University of California, San Diego, CA USA) stopped phage phi29 DNA packaging and then re-started it. Single-molecule measurement of the DNA packaging rate revealed that packaging was made less prone to stall and 70% faster after packaging had been delayed by 10 min. The proposed reason was stall-associated relaxation of the conformation of the partially packaged DNA molecule, which is reminiscent of what was observed during DNA injection by Francoise Livolant (above). In support, Nicholas Keller (same collaboration) exhibited evidence that polyvalent cation sufficient to condense DNA slowed packaging via the generation of altered packaged DNA conformation. Douglas Smith reported that, based on molecular dynamics simulations, observed differences in relative orientation of the two domains of the phage T4 packaging ATPase were associated with sufficient energy difference to account for the single-molecule dynamics observed. Genetic perturbations produced energy perturbations that correlated with motor function perturbations.

Shelley Grimes, in collaboration with the Marc Morais laboratory, reported that binding of the phi29 pRNA to the DNA packaging motor induced a conformational change in the shell hexamers that surround the connector. Might the phi29 shell also be involved in motor function (see ref. 13)? She further suggested communication between the connector and ATPase during DNA packaging (see ref. 13 and also the Additional Points for Reflection, above). Paul Jardine (University of Minnesota, Minneapolis, MN USA) emphasized initially that 1) the subunits

of the phi29 packaging ATPase ring are coordinated in their activity (see author's Additional Points for Reflection, above) and 2) that the previous nanometry-measured forces are a little high, by about 20 pN. He then presented the observation that the phase of the motor in which DNA is still (dwell; ATP bound) increases in length as the motor-countering force increases. If this is a programmed (clock-like) process, then the following question arises. What is moving during the dwell, in order to generate the clock-effects? (See refs. 13 and 16 for a description of the possibility that this movement involves transfer of energy from ATPase to connector.) Nano-scale and larger clocks do not work without movement.

Analogy of phage with Adenovirus DNA packaging has been questioned because packaged Adenovirus DNA, unlike packaged phage DNA, is bound to proteins that produce a beads-on-a-string appearance when observed by electron microscopy. Philomena Ostapchuk (laboratory of Patrick Hearing, State University of New York, Stony Brook, NY USA) reported that 1) genetically removing the major "bead" protein does not prevent DNA packaging, although the resultant particles are non-infectious and 2) immune-detection has revealed a unique vertex, even though this vertex has not yet been distinguished by cryo-EM. Might the "bead" proteins enter through pores in the capsid's shell as DNA is packaged via a traditional DNA packaging motor (see ref. 17 for the proposal that a back-up cycle exists to expel such proteins for phage DNA packaging motors)?

Viral double-stranded DNA is packaged so tightly that internal, DNA-generated pressure is a, if not the, major factor in stability to DNA expulsion. If one osmotically counters the internal pressure with high concentrations of capsid impermeant molecules (thereby generating external pressure), then double-stranded DNA phages are stabilized, as shown long ago.[18] More recently, the dependence of stabilization on water activity (osmotic pressure) has been used to determine the pressure vs. packaged DNA amount for phages.[19] Dave Bauer (laboratory of Alex Evilevitch, Carnegie Mellon University, Pittsburgh, PA USA) found that, like phages, Herpes simplex virus has a packaged genome that generates significant pressure, in this case 18 atmospheres. Cathy Yan Jin (laboratories of Bill Gelbart and Charles Knobler, with help form Kristin Parent, Michigan State University, East Lansing, MI USA) described the use of a polyvalent cation to inhibit DNA expulsion from phage P22 by reducing the force exerted by the DNA molecule on the capsid.

Tail Assembly and Function

Phages are classified via tail structure: 1) short tail, *podovirus*, 2) long, non-contractile tail, *siphovirus*, and 3) long, contractile tail, *myovirus*.[20] The length of a highly α-helical protein, called the tape measure protein, determines the length of *siphovirus* and *myovirus* tails. The tail is shortened when the tape measure protein is shortened.[21,22] Nichole Cumby (laboratories of Alan Davidson and Karen Maxwell, University of Toronto, Toronto, ON, CA) reported that the phage HK97 tape measure protein also functions during the process of injection. She transferred injection phenotype by making hybrid tape measure protein. She

also raised the question of whether injection is reversible, which, if true, implies feedback to the injection apparatus. Senjuti Saha, from the same laboratories, investigated pyocins, which are tail-like bacteriocidal secretion products, possibly weapons used by one bacterial strain against others and possibly useful for anti-bacterial therapy. She found that tails from several phages did not have bacteriocidal activity. The hunt is on for the cause of the difference in lethality between tails and pyocins. The work on HK97 earned Nicole Cumby the third award for graduate student/postdoctoral work.

Bacterial type VI secretion systems resemble contractile phage tails with reverse direction of payload. Petr Leiman (École Polytechnique Fédérale, Lausanne, Switzerland) reported a critical metal binding region in the membrane-"attacking" tip protein of both the type VI secretion system (zinc bound) and phage tail/pyocin spikes (iron bound). Peng Ge (University of California, Los Angeles, Los Angeles, CA USA) reported an R-type pyocin structure that was obtained via cryo-EM and the imposing of helical symmetry. The structure indicates that charge-charge interactions are the primary source of force for sheath contraction.

Structure and Procedures for Obtaining It

One poorly kept secret is that work on phage/viral assembly both has been and is critical to development of procedures of electron microscopy. Among the advanced procedure-dependent studies were the following. 1) Alasdair Steven (National Institutes of Health, Bethesda, MD USA) reported 3D reconstructions of phage T7 "bubblegrams," generated by gas bubbles (probably hydrogen gas) released from proteins, but not DNA, by exposure of cryo-EM specimens to the electron beam. This study found that an end of the packaged T7 DNA genome was embedded in an internal roughly cylindrical, tail- and connector-co-axial core. The core is a stack of four rings, each of a different protein. 2) Wen Jiang (Purdue, West Lafayette, IN USA) reported the use of the first focused alignment during asymmetric 3D cryo-EM reconstruction (abbreviated FAR). FAR is alignment with particles masked so that only a limited region is used for alignment. FAR was used for each of several regions of the T7 core stack, thereby revealing that 1) next-nearest neighboring T7 core stack layers varied in relative orientation, a phenomenon that generates numerous assembly isomers and 2) the core stack was slightly tilted, possibly to generate precession during DNA packaging and, thereby, to minimize DNA tangling. Discussions at the meeting suggest that the use of FAR is anticipated in future 3D reconstruction of the various DNA packaging motors. In this area, the race is on to achieve the first asymmetric reconstructions of the motor in its various functional states.

Continuing with the advanced procedure-dependent studies, Chuan Hong (laboratory of Wah Chiu, Baylor College of Medicine, Houston, TX USA, with the collaboration of the laboratory of Dennis Bamford) reported asymmetric reconstruction of the phage PRD1 ATPase and connector rings, both 12-mers. The inner membrane of PRD1 had made this accomplishment difficult; the reconstruction also revealed the connection of the ATPase-connector rings to the inner membrane.

In the realm of archaeal viruses, Chi-yu Fu (laboratory of Jack Johnson) described the cryo-EM- and X-ray diffraction-based structure (4.5 Å resolution) of phage STIV (host: *Sulfolobus solfataricus*), which 1) grows optimally at pH 3 and 80 °C, 2) has an internal membrane, and 3) has turrets at its 5-fold vertices. The turrets connect the internal membrane to the capsid's shell. All capsid proteins have a β-jelly roll fold, also common to several other viruses (including phage PRD1 and Adenovirus) that all possibly have a common ancestor. Mark Young (Montana State University, Bozeman MO USA) reported that STIV has a packaging ATPase for which the crystal structure reveals a multimeric state, in analogy with what has been seen with phage packaging ATPases.

Al Katz (City University of New York, NY USA) reported incomplete occupancy by shell-associated RNA-directed RNA polymerase and shell-associated protein assembly cofactor in procapsids of the double-stranded RNA phage, phi6. Intensities from difference maps, based on symmetric cryo-EM reconstructions, were used to determine occupancy. Mohamed Zairi (laboratory of Paolo Tavares) reported that the center of phage SPP1 shell-associated hexons has a protein that has a central region that is collagen-like, based on sequence, melting temperature and trimerization.

Amelie Leforestier (CNRS, Orsay, France) eliminated reconstruction for the cryo-EM-based analysis of the conformation of packaged DNA, because of both intra- and inter-particle variability of DNA conformation. In mature T5 and SPP1 phage, she reported liquid crystal-like regions connected as though in a Twist Grain Boundary liquid crystal, a possibility that reproduces the structures observed during 3D reconstruction. In the case of partially filled capsids, the DNA conformation varied among disordered, liquid crystalline and wound conformations, with the results depending on the experimental conditions. Dependence of packaged DNA state on temperature was also implicit in a talk by Alex Evilevitch.

Some new structures reported at the meeting are the following. The long tail fibers of phage T4 are known to have two halves, connected at an elbow. Shuji Kanamaru (representing a collaboration among investigators in Madrid, Spain, Yokohama, Japan and Santiago de Compostela, Spain) reported X-ray crystallographic determination of the structure of the tail-proximal half fiber, including the finding that the straight (shaft) regions are rich in β-helices. Thomas Smith (Danforth Plant Science Center, St. Louis, MO USA) reported the atomic structure of cucumber necrosis virus, including the presence of a zinc-binding site not present in a structural homolog, tomato busy stunt virus. Bob Duda (University of Pittsburgh, Pittsburgh, PA USA) reported 3D reconstruction of an HK97-homologous phage D3 capsid, which was a partially processed procapsid (procapsid II). Kamel Omari (University of Oxford, UK) reported that the capsid of double-stranded RNA phage, phi8, has a dimeric asymmetric subunit (120 total) with non-equivalent environments for the members of a dimer. The clustering is pentameric, with the possibility of assembly via pentamers. Preeti Gipson (laboratory of Wah Chiu, in collaboration with the laboratory of Jon King) reported another form of symmetry breaking in the shell

of a double-stranded DNA marine virus, Syn5. In this case, additional proteins break the symmetry, while producing holes at 3-fold, but not pseudo-3-fold, axes. Finally, work is in progress on refining the structures of 1) the packaged single-stranded RNA molecule of phages like MS2 (James Geraets, from the University or York, UK) and 2) the capsid of large eukaryotic viruses, including Adenovirus (Vijay Reddy, Scripps Research Institute, La Jolla, CA USA) and herpesvirus (James Conway, University of Pittsburgh, Pittsburgh, PA USA).

Membrane-Associated Capsid Assembly

Membrane-associated assembly is the dominant feature of the assembly of single-stranded DNA, filamentous phages. To simplify analysis, Andreas Kuhn (University of Hohenheim, Germany) described the use of proteoliposomes that had incorporated the protein that inserts the major capsid protein in membranes. Membrane-insertion of the phage M13 and Pf3 capsid protein was detected by use of a fluorescence-based technique (FRET).

Concluding Thoughts

Virus assembly is complicated enough to be biological, but simple enough to be investigated at the level of either analytical physics or physics-via-computer modeling. That is part of the attraction of the field of the PVA meeting, in the areas of both basic and translational science. One envisages that the basic biology will, in part, go backward in time, so that we achieve a reasonable rendition of evolution. On the other hand, one envisages that the biology will go forward in time, so that we achieve genetic perturbations that provide the assembly (motor) intermediates that chemists and physicists need to investigate dynamics. One also envisages going forward in the direction of using both the high speed and the genetic aspects to improve both vaccines and other pathogenic cell-managing devices. For both basic science and medicine, high-speed viral genetics, via biochemically-managed mutagenesis, needs to be developed beyond what now exists, especially for phages. Or, one might say, as in the beginning, so also in the end, with a boost from advances in biochemistry. The next stop for the PVA meeting is, Lausanne, Switzerland where Petr Leiman will be the organizer in 2015.

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