Molecular Microbiology (2005) **55**(5), 1300–1314 doi:10.1111/j.1365-2958.2005.04509.x

MicroMeeting

2004 ASM Conference on the New Phage Biology: the 'Phage Summit'

Sankar Adhya,[1] Lindsay Black,[2] David Friedman,[3] Graham Hatfull,[4] Kenneth Kreuzer,[5] Carl Merril,[6] Amos Oppenheim,[7] Forest Rohwer[8] and Ry Young[9]*

[1]*Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, 37 Convent Dr., Rm 5138, Bethesda, MD 20892-4264, USA.*
[2]*Department of Biochemistry and Molecular Biology, University of Maryland Medical School, 108 N. Greene Street, Baltimore, MD 21201-1503, USA.*
[3]*Department of Microbiology and Immunology, University of Michigan, 5641 Medical Science Building II, Ann Arbor, MI 48109-0620, USA.*
[4]*Pittsburgh Bacteriophage Institute, 4249 5th Avenue, University of Pittsburgh, Pittsburgh, PA 15260, USA.*
[5]*Department of Biochemistry, Duke University Medical Center, Box 3711, Durham, NC 27710, USA.*
[6]*Section on Biochemical Genetics, National Institute of Mental Health, NIH, Bethesda, MD 20892, USA.*
[7]*Department of Molecular Genetics and Biotechnology, The Hebrew University – Hadassah Medical School, PO Box 12272, Ein Karem, Jerusalem, Israel 91120.*
[8]*Department of Biology, San Diego State University, 5500 Campanile Dr., San Diego, CA 92182-4614, USA.*
[9]*Department of Biochemistry and Biophysics, Texas A&M University, 2128 TAMU, College Station, TX 77843-2128, USA.*

Summary

In August, more than 350 conferees from 24 countries attended the ASM Conference on the New Phage Biology, in Key Biscayne, Florida. This meeting, also called the Phage Summit, was the first major international gathering in decades devoted exclusively to phage biology. What emerged from the 5 days of the Summit was a clear perspective on the explosive resurgence of interest in all aspects of bacteriophage biology. The classic phage systems like λ and T4, reinvigorated by structural biology, bioinformatics and new molecular and cell biology tools, remain model systems of unequalled power and facility for studying fundamental biological issues. In addition, the New Phage Biology is also populated by basic and applied scientists focused on ecology, evolution, nanotechnology, bacterial pathogenesis and phage-based immunologics, therapeutics and diagnostics, resulting in a heightened interest in bacteriophages *per se*, rather than as a model system. Besides constituting another landmark in the long history of a field begun by d'Herelle and Twort during the early 20th century, the Summit provided a unique venue for establishment of new interactive networks for collaborative efforts between scientists of many different backgrounds, interests and expertise.

Accepted 1 December, 2004. *For correspondence. E-mail ryland@tamu.edu; Tel. (+1) 979 845 2087; Fax (+1) 979 862 4718.

Introduction

The ASM Conference on the New Phage Biology was held on 1–5 August in Key Biscayne, Florida. This meeting, known as the 'Phage Summit', was the first major international meeting dedicated to the biology of bacteriophage in decades. There were more than 350 registrants from 24 countries and 188 universities, research institutions, companies and government agencies. The strong response to the announcement of the Summit reflects a vigorous and very broad-based re-emergence of interest in phage biology, beyond its historical role as the facile model system used to establish the fundamentals of molecular genetics. As was made evident at the Conference, the classical phage systems like λ, T4 and T7, with their unparalleled genetic power, have themselves been energized by the admixture of new biophysical, spectroscopic and fluorescence technologies and by the enhanced context provided by the explosion of sequence and structural information. In addition, the Summit revealed that the 'new phage biology' spans a number of other areas, including ecology, evolution, structural biology and molecular pathogenesis. Moreover, applications of our burgeoning knowledge of bacteriophage are featured in nanotechnology, vaccine design and phage-based therapeutics, diagnostics and prophylactics.

The Conference, co-chaired by S. Adhya (National Cancer Institute, Bethesda) and R. Young (Texas A&M), was

constituted as a series of keynote addresses and platform sessions structured on the traditional Phage Meetings at Cold Spring Harbor, with short talks followed by sometimes contentious question periods. In addition, there were more than 250 posters, which remained displayed throughout the entire conference. Platform speakers were asked to provide posters with supporting information, a device which helped the conferees from so many distinct disciplines negotiate the widely divergent terminologies and diverse experimental systems. Because of the short talk format and an audience with such heterogeneous expertise, the presentations were distilled to their most arresting scientific content, leading to highlight after highlight and vibrant question periods after each talk. Several conferees noted how many times during the sessions there were audible gasps and even spontaneous outbreaks of applause. Moreover, the frequent nucleation of discussion groups around posters during breaks in the platform talk schedule lent an air of ferment and excitement to the entire proceedings.

Adding to this stimulating, almost revivalist atmosphere, the first keynote talk, by W.C. Summers (Yale University), reminded everyone that the origins of modern biological science are grounded in phage biology, showing vintage pictures from a 'phage summit' of 1952 with Nobelists Delbrück, Hershey and Luria, among others, in attendance (Fig. 1). Summers' fascinating talk examined in detail the laboratory notebooks of Seymour Benzer from the period when the famous T4 *rII* studies were conceptualized and begun. Benzer discovered that T4 *rII* mutants would not plate on *Escherichia coli* K-12 carrying the λ prophage. The non-permissive phenotype was so tight that recombination frequencies between individual base pairs could be measured, a fact exploited by Benzer in his paradigm work in constructing the first fine-structure map of a gene. Summers' analysis showed that, although the conceptualization of the system was later chronicled to be a sudden flash of inspiration, in reality the *rII* system's utility became apparent over months of indirect and often tangential experimentation. By demystifying this legendary achievement and relating it to the realities of laboratory science, Summers' presentation provided a perfect historical backdrop to the proceedings, connecting everyone present to the golden era of classical phage genetics.

Regulation, development and phage–host interactions

Regulation, development and phage–host interactions continue to be dynamic areas of intensive study in phage biology, as was evident from the 73 abstracts, three keynote talks and the first session of oral presentations, presided over by A. Oppenheim (Hebrew University, Jerusalem). A number of talks centred on the λ lysis-lysogeny paradigm, which, although after more than 50 years is easily the most intensively studied molecular developmental decision in biology, continues to reward its investigators. The keynote speaker in the session, J. Little (University of Arizona), took a systems biology approach relating to the gene regulation circuitry of phage λ and its evolution. He discussed two sets of findings. First, his group constructed phages in which a crucial regulatory protein, Cro, is replaced with the Lac repressor; several of these phages behaved like the wild type in their ability to support the lysis-lysogeny decision and in their UV

Fig. 1. A previous 'Phage Summit' at Royaumont, France in 1952. Left to right: Alfred Hershey, Jacques Monod, Elie Wollman, Gunther Stent, Max Delbrück. (From W. Summers' talk on the history of the *rII* genetic system.) Photograph used with permission from S. Benzer.

dose–response for prophage induction (Atsumi and Little, 2004). Second, his group individually removed features of the circuitry previously thought crucial to its proper operation: differential affinity of CI and Cro for the O_R operators (Little *et al*., 1999), positive autoregulation of *cI* gene expression and cooperative DNA binding by CI. In the latter two cases, suppressors were needed to restore near-wild-type behaviour. Little put these findings in the context of a two-stage model for evolution of a complex circuit. In the first step, simple modules recombine to create a more complex wiring diagram. The ability to replace Cro with Lac repressor supports a modular organization for the existing circuit. In the second stage, refinements (such as autoregulation) are added to a simpler functional circuit, yielding the more elaborate circuitry found in modern phages. The ability to remove certain refinements without destroying the circuitry supports this aspect of the model. A. Oppenheim described constructs using various fluorescence protein fusions that allow the continuous kinetic monitoring of phage regulatory functions. The results beautifully showed that when lysogeny is favoured, the transcription activator protein CII plays a key role in activating repressor synthesis and in inhibiting late gene expression. This experimental tool should allow better mathematical description of the lysis–lysogeny decision process. Another approach in studying the systems behaviour of phage was presented by L. Chan (D. Endy group, Massachusetts Institute of Technology), who reported the use of experimentally determined parameters in making a mathematical model for phage T7 development (Endy *et al*., 1997). The Endy group is making progress in engineering a re-factored T7 by separating genes and regulatory elements, making a new phage that is simpler to study and model (Endy *et al*., 2000).

Lambdoid phages usually encode two anti-terminators, the N and Q proteins, which act by modifying host RNA polymerase (RNAP) in the early and late operons, respectively, and work on these systems continues to be exciting. A. Das (University of Connecticut, Farmington) reported that his group has identified the target of N in RNAP to be in the beta subunit flap, a flexible domain that constitutes part of the RNA exit channel. He also presented evidence that N binds a RNA:DNA hybrid and showed that the hybrid-binding domain in N is distinct from those that bind *nut* site RNA and the RNAP flap (Das *et al*., 2003). These results lead to a new concept of anti-termination in which N is proposed to form a stable sliding clamp on the RNA:DNA hybrid that impedes RNA release by either Rho helicase or the terminator hairpins. A contrasting story was told by R. Weisberg (National Institute of Child Health and Human Development, Bethesda), who related that in the lambdoid phage HK022 RNAP is modified by transcription through anti-termination sequence called a *put* site (for polymerase utilization, analogous to the *nut* site required for *N*-dependent anti-termination in phage λ; King *et al*., 1996) and that the modification persists as long as the *put* RNA is tethered to RNAP. Cleaving an RNA loop that tethers *put* RNA to the active centre of RNAP eliminates anti-termination *in vitro* (Sen *et al*., 2001). Terminators located as far as 10 kb from *put* are efficiently anti-terminated *in vivo*, suggesting that the tether functions by increasing the local concentrations of the interacting partners.

Temperate coliphage 186, a member of the P2 family, is unrelated in sequence to phage λ but the two phages are remarkably similar in lifestyles. By comparing the genetic switches of the two phages, K. Shearwin (University of Adelaide, Australia) reported how different solutions can evolve to solve a single biological problem. In contrast to the divergent lytic and lysogenic promoters of the λ switch, the corresponding promoters of 186 are arranged face-to-face, giving rise to convergent transcription. Such an arrangement leads to transcriptional interference, with the strong lytic *pR* promoter inhibiting the weak lysogenic promoter *pL*. The interference is critical for the lysogenic switch. *In vivo* results showed that the interference needs the passage of the converging RNAP over the weak promoter. Shearwin suggested a 'sitting duck' model (Callen *et al*., 2004) in which open complexes at the weak promoter are sensitive to head-on collisions with elongating RNAP (Fig. 2).

Although the historic focus on the classic coliphages was critical for the vertical development and unparalleled rigor of phage molecular genetics, workers in new phage systems are now finding diverse and equally compelling regulatory stories. E. Semenova (K. Severinov group, Waksman Institute, Piscataway, NJ) described an unusual *Xanthomonas* phage, Xp10, with a genome organized similarly to lambdoid phages. Surprisingly, Xp10 relies on both host RNAP and a phage-encoded T7-like single-subunit RNAP for expression of its late genes (Semenova *et al*., 2004). Such conjoint action by different RNAPs in a single regulatory phase is unprecedented for a bacteriophage. Rapid progress in the detailed molecular characterization of Gram-positive hosts was reported. For example, M. Pedersen (K. Hammer group, Technical University of Denmark, Lyngby) reported that Alt, the late gene activator of lactococcal phage TP901-1, has a C-terminal motif that causes binding to four direct repeats upstream of the late promoter. In the Lactobacillus phage A2, the genes for both the major capsid and major tail proteins as well as the gene that encodes the lysogenic repressor Cro are expressed as two products, by virtue of efficient −1 frame-shifting mediated by 'slippery sequences' and RNA stem-loop structures, as reported by I. Rodriguez (J.E. Suarez group, Universidad de Oviedo, Oviedo) (Garcia *et al*., 2004). Finally, M. Clokie (N. Mann group, University of Warwick, UK) reported that

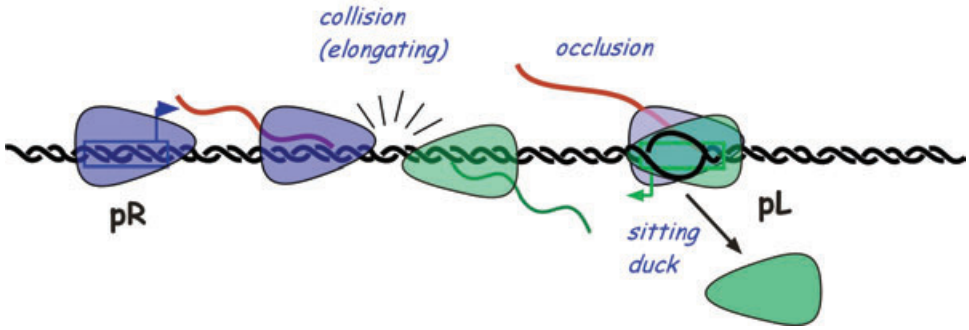

Fig. 2. 'Sitting duck' model for the regulation of the lysogenic promoter of phage 186. Three potential mechanisms of transcriptional interference by the convergent promoters of bacteriophage 186 are shown. These are: (i) collisions between elongating polymerases, (ii) occlusion of the weak promoter (pL) by passage across it of RNAP originating from the opposing strong promoter (pR) and (iii) the 'sitting duck' mechanism, where initiation intermediates waiting to fire at pL are removed by collision with polymerase coming from pR. At the wild-type interpromoter distance of 62 bp, the 'sitting duck' mechanism makes by far the major contribution to the observed 5.6-fold interference. From the presentation of K. Shearwin (University of Adelaide).

more than half of cyanobacterial phages examined contained photosynthetic genes that are expressed throughout the phage infection cycle. These phages may have important implications for the communal photosynthetic physiology of the picophytoplankton and consequently on biogeochemical cycles. Surprisingly, sequence comparisons indicated that the phage-encoded photosynthetic genes are closely related to but distinct from those of their cyanobacterial hosts (Millard *et al*., 2004).

In a keynote address, L. Rothman-Denes (University of Chicago) described the virion-associated RNAP (vRNAP) of coliphage N4 (Kamtekar *et al*., 2002). Unlike all other double-stranded DNA (dsDNA) phages characterized to date, N4 does not use the host RNAP to transcribe its early genes. Instead, vRNAP is injected into the host at the onset of infection and is required for injection of the early half of the genome. Limited proteolysis revealed three domains in the vRNAP polypeptide: the N-domain, required for the early DNA injection; the C-terminal domain required for vRNAP encapsidation; and the central 1100 aa domain (mini-vRNAP) that has all the transcriptional properties of full length vRNAP. Rothman-Denes described the crystal structure of mini-vRNAP, determined by her collaborator, K. Murakami (Pennsylvania State, PA), showing that the alpha-carbon backbone at the active site is completely superimposable with T7 RNAP. vRNAP interactions with its single-stranded, hairpin-containing DNA promoter were mapped to the same domain structures used by T7 RNAP to interact with its dsDNA promoter. She also reported results showing that the role of host single-strand DNA binding protein is to help template recycling by binding to the RNA product (Davydova and Rothman-Denes, 2003).

Phage replication, recombination and repair

The session on Phage Replication, Recombination and Repair, chaired by K. Kreuzer (Duke University), began with a keynote talk about the phage T4 replication fork by N. Nossal (National Institutes of Health, Bethesda). Using chain-terminating nucleotides and a special 450 bp circular substrate, her group showed that inhibition of the lagging-strand polymerase does not block leading strand synthesis, arguing against a tight coupling of the leading and lagging-strand enzymes. She showed electron microscopy studies performed in collaboration with J. Griffith (University of North Carolina, Chapel Hill) in which the entire replication complex was visualized and analysed (Chastain *et al*., 2003). Nossal's group is pioneering a novel technique involving 'biopointers', in which proteins are tagged with biotinylation epitopes and then visualized by adding a 0.2 kb DNA molecule covalently linked to streptavidin (Fig. 3). As an example of the power of this technique, Nossal showed electron micrographs revealing that the helicase loading protein, gp*59*, stays with the travelling replication fork.

Other intriguing aspects of T4 DNA metabolism were also highlighted. D. Shub (State University of New York, Albany) discussed the arrangement of the topoisomerase genes *39* and *60*, separated by about 1000 bp in T4, and in related phages, where the two reading frames are fused. He presented evidence that the intervening DNA in T4 encodes a special endonuclease, MobA, which directs DNA mobility reactions. Most intron mobility systems involve an endonuclease that makes double-stranded breaks, but MobA makes site-specific single-stranded breaks, which apparently initiate the recombination reaction involved in DNA mobility (also see Landthaler *et al*., 2004). Kreuzer described experiments that utilize the 'awesome power of T4 *rII* genetics' demonstrating that in double-stranded break repair, the two ends both interact with the same homologous chromosome (see Shcherbakov *et al*., 2002; Stohr and Kreuzer, 2002). Interestingly, mutations that greatly reduce the amount of gp*46*, which

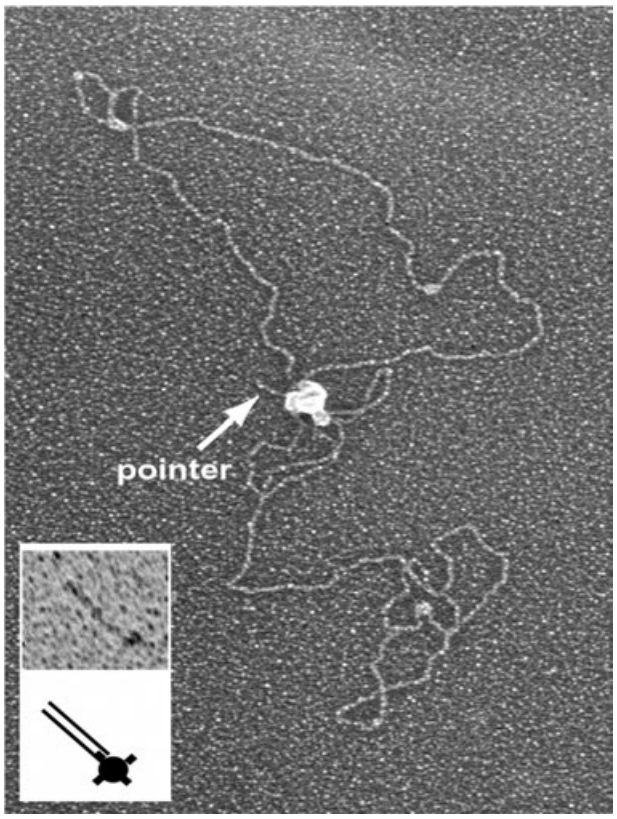

Fig. 3. Electron micrograph of a replication fork with bacteriophage T4 proteins. The locations of the biotin-tagged leading and lagging-strand DNA polymerases are shown by biopointers composed of streptavidin attached to 179 bp biotinylated DNA. Rolling circle replication of M13 DNA by T4 DNA polymerase, clamp, clamp-loader, helicase, helicase loader, primase, RNaseH, ligase and 32 ssDNA-binding protein gives a long linear product. The nascent lagging-strand fragment is folded into a duplex DNA loop at the fork. The dense complex at the fork (enclosed in the dashed circle) contains the replication proteins, as well as the protein-covered single-stranded regions of the lagging-strand that are folded into a compact structure (Chastain *et al*., 2003). There is a single biotin tag at the N-terminus of each T4 DNA polymerase. Unbound replication proteins were removed by gel filtration before addition of the streptavidin DNA pointers. (Unpublished experiments of N.G. Nossal, P. Chastain, A.M. Makhov and J.D. Griffith; from the presentation by N.G. Nossal.)

is the T4 homologue of eukaryotic Rad50 protein, appear to debilitate this end co-ordination. The eukaryotic Rad50 protein and its partner Mre11 (T4 homologue is gp*47*) are likewise implicated in end co-ordination in eukaryotic cells.

The highlight of another keynote presented by M. Salas (Universidad Autonoma, Madrid) was new insight on the structure–function relationships in the DNAP of *Bacillus subtilis* phage ϕ29. This remarkably processive enzyme has protein priming, DNA polymerization, proof reading 3′–5′ exonuclease and strand displacement activities (Blanco and Salas, 1996). Its crystal structure, determined in collaboration with the T. Steitz group (S. Kamtekar, *et al*., submitted) shows that one of the two domains that are conserved in both eukaryotic and prokaryotic protein-primed DNAPs is part of a loop that covers the DNA-binding cleft. Indeed, a mutant polymerase lacking the region is impaired in both processivity and strand displacement.

The short talks in the remainder of the session had many highlights worthy of mention. Two talks were concerned with the application of new technologies. In an overview of his revolutionary 'recombineering' technology (Court *et al*., 2002), D. Court (National Cancer Institute, Frederick, MD) detailed how genes can be directly mutated at high efficiency (>30%) by electroporation of ≈70 nt single-stranded oligonucleotides targeted to the lagging-strand template in cells deficient for mismatch repair (Costantino and Court, 2003). A. Segall (San Diego State) summarized interesting work on the isolation of oligopeptide inhibitors that bind the Holliday junction and thereby inhibit enzymes such as λ Int and the *E. coli* proteins RecG and RuvABC (Boldt *et al*., 2004), including one class isolated from peptide libraries and found to inhibit bacterial growth and to generate DNA breaks (Cassell *et al*., 2000; Cassell and Segall, 2003; Boldt *et al*., 2004) (K.V. Kepple, J.L. Boldt and A.M. Segall, submitted).

Unusual DNA transactions were the focus of talks by W.-M. Huang (University of Utah) and D. Manna (P. Higgins laboratory, University of Alabama at Birmingham). Huang reported on chromosomes with hairpin ends, such that the entire chromosome is a continuous single-stranded DNA (ssDNA) folded back on itself. These DNAs, which are found in bacteria (e.g. *Borrelia* spp. and *Agrobacterium tumefaciens*), phages (e.g. N15) and plasmids, require protelomerase (Huang *et al*., 2004), an enzyme in the type IB topoisomerase/tyrosine-recombinase family of proteins. Huang's group has been able to clone the duplex version of the end sequence into a plasmid and to demonstrate *in vitro* conversion of the plasmid into a hairpin molecule via the protelomerase. Manna described genomic analyses of phage Mu transposition sites, which have hot and cold spots that could be correlated with high-affinity binding sites for the Mu B protein and with areas of intense transcription respectively (Manna *et al*., 2004).

DNA partitioning and entry into the cell was also a focus. R. Edgar (M. Yarmolinsky group, National Institutes of Health, Bethesda) presented *in vivo* studies on the partitioning of the plasmid prophage form of phage P1. In DNA gyrase-inhibited cells, the observed blockage by ParB of rotational diffusion of DNA in dimeric plasmids carrying two centromeres was taken as evidence for ParB-mediated centromere pairing (Edgar *et al*., 2001). The blockage of rotational diffusion of DNA in monomeric plasmids carrying a single centromere to which ParB binds was found to depend on the presence of ParA, suggesting that ParA enhances pairing or, more likely, tethers a ParA–ParB–DNA complex to an unidentified cellular component (Edgar *et al*., 2001). W. Robins (I. Molineux group, Univer-

sity of Texas at Austin) showed convincing experiments that transcription is the driving force for the translocation of T7 DNA into the host, namely that transcriptional terminators and anti-terminators modulate both the rate and efficiency of entry of phage T7 DNA into the cell.

Taken together, the talks in this area clearly showed that phage continue to provide some of the best model systems for investigating the fundamental mechanisms of DNA metabolism. Many connections of these talks to DNA transactions in eukaryotes, including aspects relevant to human disease, were obvious throughout the session. The session was also marked by incisive questions and comments from a highly participatory audience.

Structural biology and morphogenesis

A number of provocative findings were reported at the Morphogenesis and Assembly session, chaired by L. Black (University of Maryland, Baltimore). Among these was a report by D. Bamford (University of Helsinki) of the structure of the tectivirus PRD1, which has an external protein coat surrounding a biological membrane enclosing a linear dsDNA genome. The high-resolution structure of this unusual phage supports the interesting proposal that only a relatively low number of protein folds are capable of assembling an icosahedral virus coat (Abrescia *et al*., 2004; Cockburn *et al*., 2004). Accordingly, it is proposed that viruses fall into distinct groups, each having a common ancestor and sharing similar structural and assembly principles, bringing phylogeny to virus classification. Seen in this light, one can question whether the extraordinarily large number of phylogenetically disputatious phage genomes are an illusion in the larger scheme of things.

Reports on DNA packaging in tailed dsDNA phages showed how complex this terminal DNA transaction can become. M. Feiss (University of Iowa) presented results showing that the baroque interaction of λ terminase with the *cos* packaging control sequences and host factors can be replaced in a near relative with a much simpler sequence recognition process that is independent of the Integration Host Factor. This rigorous analysis of packaging is consistent with other findings that increasing complexity can be layered onto, and perhaps is subsequently removed from, a basic working evolutionary plan. S. Casjens (University of Utah) described the head-full packaging strategy of the *Salmonella* generalized transducing phage ES18. This phage is like P22 and other phages in recognition of a specific *pac* site to initiate DNA packaging but is unlike them in that it then makes packaging initiation cleavages in the concatemeric substrate DNA anywhere within a region of about 2 kb centred on the putative *pac* site. Casjens' model for this is that, after recognition, the ES18 terminase can slide around on the DNA for up to 1 kb in either direction before cleavage. Phylogenetic analysis of these and the other known terminases indicated that the different types of terminases largely fall into separable and robust groups, suggesting that terminase sequences can be used to predict the type of ends virion DNAs will have. Black reported that T4 DNA, packaged into capsids at ≈500 mg ml^{-1}, can have a number of unusual twists, such as more than 1000 embedded but invisible proteins, and offered data suggesting that the popular rotary portal mechanism of packaging is far from established.

Talks by B. Fane (University of Arizona) and A. Kuhn (University of Hohenheim, Germany) focused on protein factors that are required for capsid assembly. Fane discussed the role of ϕX174 internal scaffolding protein, D, in facilitating several steps during morphogenesis without being absolutely required for any of them. Selections for assembly-competent mutants that can use successively smaller fragments of D have identified several functions of the protein. Kuhn described the role of the host membrane protein YidC in insertion of the major procoat of filamentous phage M13 into the bilayer. YidC protein, which has homologues in mitochondria and chloroplasts, was reconstituted into proteoliposomes where, in the presence of a transmembrane electrochemical potential, it supported efficient membrane insertion of microgram quantities of procoat in a topologically correct orientation. F. Arisaka (Tokyo Institute of Technology), R. Duda (R. Hendrix laboratory, University of Pittsburgh) and E. Goldberg (Tufts School of Medicine, Boston) reported structure–function studies of some of the sophisticated proteins involved in virion function, from lysozymes to proteases to nano-worthy adsorption fibre materials. Arisaka described newly determined features of the previously published structure of the T4 tail lysozyme, which undergoes proteolytic processing at a specific site. By replacing the residue at the cleavage site and thus blocking the cleavage, a structure that, after cleavage, had been too flexible to be visible in the crystal structure was revealed. Surprisingly, although acquiring a cold-sensitive phenotype, the phage with the uncleavable lysozyme remained infection competent. Duda has found that a C-terminal peptide from the HK97 maturation protease is necessary and sufficient to direct the protease or a heterologous protein into the interior of the HK97 prohead. Except for this peptide, the protease appears to be related to capsid proteases from viruses as distant as the Herpesviruses. This encapsidation signal might be used to generate a capsid-like protein coat around enzymes or other biomolecules for storage, protection and targeted delivery. Goldberg described initial experiments aimed at exploiting the basic structure of the T4 tail fibre as struts for nanodevices. The length of these struts can be adjusted and they can also be decorated with peptide

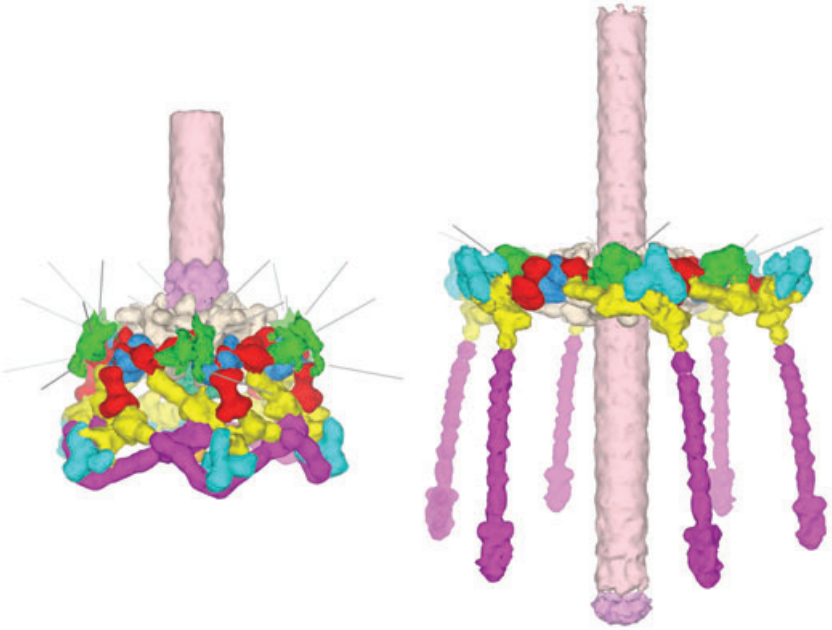

Fig. 4. The T4 baseplate structure in the hexagonal (left) and star (right) conformations. Colours identify different proteins: gp*7* (red), gp*8* (blue), gp*9* (green), gp*10* (yellow), gp*11* (cyan) and gp*12* (magenta). For clarity, the full-length short-tail fibres are shown in the star-shaped baseplate. The model of the disordered receptor-binding domain is based on the crystal structure and the corresponding density from the hexagonal baseplate map. For consistency, the long-tail fibres are shown as rods in both conformations. From the presentation by P. Leiman.

display ligands (Hyman *et al*., 2002). In a recent development, a heat-stable coiled-coil motif has been engineered into the strut, eliminating the need for the gp*38* chaperone that is required for the gp*37* trimerization in T4 long tail fibre maturation (Qu *et al*., 2004)

P. Leiman (M. Rossman laboratory, Purdue) presented a truly remarkable study, using a combination of X-ray crystallography and electron cryo-microscopy (cryoEM), revealing the structural changes that occur in bacteriophage T4 on attachment to the host cell (Leiman *et al*., 2004). The cryoEM reconstructions of the T4 baseplate were obtained in the pre- and post-attachment conformations and interpreted in terms of its component proteins, integrating atomic structures of baseplate proteins, genetics and biochemical data (Fig. 4). This analysis suggests that re-orientation of the long-tail fibres following specific interactions of the tips with the cell surface up to 1450 Å away changes the network of protein interactions in the baseplate, resulting in the hexagon to star transition. The fibres, acting through their baseplate attachment proteins, act as levers that move the baseplate towards the cell surface. During these transitions, most baseplate proteins rotate as rigid bodies with little change in their overall structure. The presentation culminated in a movie simulation that, while almost frighteningly realistic, was a highlight of the meeting.

Phage genomics and evolution

Mosaicism of phage genomes was a major theme of this session chaired by G. Hatfull (University of Pittsburgh). The keynote speaker, R. Hendrix (University of Pittsburgh), described how modules containing one or more genes are shared among phage genomes and suggested recombination mechanisms that could give rise to these mosaic relationships. These processes include assortment of modules by homologous recombination between shared modules, and the generation of new mosaic boundaries by illegitimate recombination and selection for function (Hendrix, 2002). Hendrix also described the identification of 'morons', segments of DNA, typically containing an open reading frame (ORF) flanked by a promoter and a terminator, that are present in one genome but absent in a close relative. Many morons appear to encode functions that may be beneficial to the host, suggesting that these are acquired by phages as one means of 'paying their rent' when housed as prophages in the chromosomes of their hosts. Hatfull reviewed the structure of 30 mycobacteriophage genomes, typically ≈70 kb and highly diverse at the nucleotide and protein levels, with a pervasive mosaicism in which single genes are frequently modules (Pedulla *et al*., 2003). The predicted ORFs can be grouped into approximately 1500 protein families, but fewer than 15% of these are homologous to proteins described outside this mycobacteriophage group. The seven complete genome sequences of *Listeria* phages described by M. Loessner (Swiss Federal Institute of Technology, Zurich) are clearly also built as mosaics, and exhibit a number of unusual features. For example, phage A118 integrates into the gene encoding a homologue of the *B. subtilis* ComK competence regulator, and phage PSA, like phage A2 mentioned above, utilizes unusual frame-shifting schemes to express its structural proteins (Zimmer *et al*., 2003).

A contrast to the diversity of these *Listeria* and *Mycobacteria* phages was presented by A. Nilsson (Stockholm

University) and his collaborator and keynoter, E. Haggård-Ljungquist (Stockholm University), who described the P2 phage family, which has prophage representatives in a wide spectrum of proteobacteria, including almost 30% of the strains in the ECOR library (Ochman and Selander, 1984; Nilsson *et al*., 2004). Bacteriophage P2 belongs to a family of related phages that can be found in different proteobacteria. An analysis of the late genes of P2-like coliphages has revealed such a high degree of identity that they may be regarded as slight variants of the same phage (Nilsson and Haggård-Ljungquist, 2001). Phylogenetic analyses of P2 relatives from different host species show that they are a conservative group of phages without modular evolution. The take-home-lesson here is that these phages may have evolved clonally, as they follow the evolution of their respective hosts. Moreover, dramatic new findings illustrated how P2-like phages evolve with their *E. coli* host and compete with each other. At one specific DNA site, 10 out of 11 analysed prophages in the ECOR library contain different horizontally transferred gene cassettes, probably encoding proteins benefiting the host and integrated by a site-specific recombination event (Nilsson *et al*., 2004). In addition, the P2-like coliphages can be divided into at least five immunity classes which contain different developmental switches.

K. Stedman (Portland State University, OR) and J. Karam (Tulane Medical School, New Orleans) further emphasized the diversity emerging from the current wave of phage genomics. Karam reported a comparative genomic analysis of T4-like phages, for which six complete genome sequences are available (see http://phage.bioc.tulane.edu/), revealing interesting diversity of genome size and organization, the functions for genome modification and DNA metabolism, and content of mobile elements (Bebenek *et al*., 2002). Stedman described viruses of extreme archaeal thermophiles that, by electron microscopy, were found to have many bizarre and unusual morphologies. The complete sequences of *Sulfolobus solfataricus* phages SSV1, SSV2 and SSV3 have been compared, providing insights into their essential and non-essential functions (Wiedenheft *et al*., 2004). Interestingly closely related viruses appear to be abundant and some have been characterized from both Yellowstone and Kamchatka.

Work by A.J. Clark (University of Arizona), in collaboration with C. Dale (University of Utah), and A. Hodes (J. Miller laboratory, University of California at Los Angeles School of Medicine) dealt with specific mechanisms by which diversity is generated. Hodes described a variation-generating process in the *Bordetella* phage BPP-1 and its relatives, in which a phage-encoded reverse transcriptase copies information from an invariant template cassette into the *mtd* tail gene (Doulatov *et al*., 2004). In the process, nearly every adenine residue in the template is a site of random replacements in the expression locus. Interestingly, related systems appear to exist in a wide variety of other phages and bacteria, although it remains unclear as to whether the bacterial systems are in fact prophage-associated. Clark discussed an intriguing plasmid prophage of the *Sodalis glossinidius*, the secondary endosymbiont of the tsetse fly. The plasmid appears to be a 'heterodimeric' genome related to ancestral phages resembling the lambdoid phage HK620 and the *Salmonella* phage epsilon-15. It has two sets of virion structural genes, one set complete and presumably active, the other set reduced and consisting of pseudogenes. Clark speculated that endosymbionts are relatively isolated from phage infection and selective pressure on resident phage is reduced, allowing the persistence of evolutionary intermediate forms which would be eliminated more rapidly in free-living bacteria exposed to frequent infection.

M. Sullivan (S. Chisholm group, Massachusetts Institute of Technology) described the genomes of three *Prochlorococcus* phages representing a T7-like podovirus, P-SSP7, and two T4-like myophages, P-SSM2 and P-SSM4 (Sullivan *et al*., 2003). All three of these phages contain the gene encoding the D1 protein of the photosystem II reaction centre. While the role of this phage gene is not clear, it is speculated that it might compensate for the host D1 function, which is rapidly turned over, thus maintaining photosynthesis during phage infection. This and other observations (integrase gene in the T7-like phage; phosphate-response genes and carbon metabolic genes in the T4-like phages) suggest that these phage genomes adapted to infect open ocean photosynthetic hosts by co-opting genes from their hosts.

Both the R. Young and Hatfull laboratories emphasized the utility of phage discovery and genomics as educational tools. E. Summers (R. Young group, Texas A&M) reported on a programme of genomic sequencing of *Burkholderia* phages, in which the bulk sequencing and bioinformatic analyses are performed by teams of undergraduates. A new branch of the Mu family is represented by the phage BcepMu, which is a prophage in a Burkholderia lineage that infects human cystic fibrosis patients (Summer *et al*., 2004). The BcepMu genome carries potential pathogenesis determinants. Besides providing serious scientific advances, these reports illustrate the suitability of phage genomics as educational facilitators that may play an important role in generating the next generation of phage biologists.

Phage in pathogens and phage–bacteria interactions

The keynote speaker in this session, chaired by D. Friedman (University of Michigan), was M. Waldor (Tufts School of Medicine, Boston) who led off with a provocative report on the nature of the immunity (repressor/operator) system

of *Vibrio cholerae* phage CTXϕ. Unusual in being a temperate filamentous phage, CTXϕ carries the gene encoding cholera toxin, the major virulence factor of this important pathogen (Waldor and Mekalanos, 1996; Kimsey and Waldor, 2004). Waldor and colleagues found that like the λ family of phages, the CTX prophage is induced by the SOS response. In that response, RecA, activated by ssDNA produced by DNA damage and subsequent repair activity, stimulates the auto-cleavage of the LexA protein, the master regulator that controls expression of a large number of enzymes involved in DNA repair. λ CI repressor is the only protein involved in repression of the λ prophage and its auto-cleavage is also stimulated by activated RecA. The Waldor group found that CTXϕ differs from λ in that expression from the prophage is repressed by both a prophage-encoded repressor, RstR, and by the host LexA protein. Thus, unlike λ, expression of the CTXϕ prophage is, in part, directly controlled by a bacterial protein that serves as a sensor of the physiological state of the bacterium.

Friedman presented studies on the physiology of lysogens with prophages that carry the genes encoding shiga toxin (Stx). His group had shown that the toxin is produced and released from a subpopulation of spontaneously induced cells (Wagner *et al*., 2001). Using a reporter system designed to quantify the fraction of spontaneously induced lysogens (Livny and Friedman, 2004), it was found that Stx-encoding lambdoid phages induce more readily than do non-Stx-encoding lambdoid phages, suggesting that the former may have evolved a more sensitive trigger for Stx production and release. The next application of this reporter system is to determine whether there is increased induction *in situ* leading to high levels of Stx in the animal gut.

Dean Scholl (National Institutes of Health, Bethesda) discussed a newly isolated T7-like coliphage, M59-2, that recognizes and degrades the extracellular polysaccharide colanic acid. Cells that produce this structure are resistant to many LPS-specific phages such as T7 but are susceptible to M59-2. Like many other polysaccharide-specific phages, M59-2 encodes a tail enzyme that is probably responsible for this activity. As colanic acid is important for biofilm formation in *E. coli*, this phage and/or the tail enzyme could be useful in biofilm control.

L. Thomason (National Cancer Institute, Frederick, MD) reported on studies re-examining a legacy phenomenon, the physiological role of the λ *rex* gene products. In a series of studies originating in the work by S. Benzer noted above, Rex proteins have been shown to be responsible for the exclusion of T4 *rII* mutants by the λ prophage. Thomason found that overexpression of the *rex* genes affects the physiology of the bacterium, resulting in overproduction of NADH when the bacteria are grown in the presence of certain sugars such as mannitol and fructose. Rex also appears to play a role in optimizing the development of the induced λ prophage.

The co-opting of host proteins by *B. subtilis* phage ϕ29 to implement its strategy for adapting to a changing physiological bacterial environment was the subject of a talk by W. Meijer (Universidad Autonoma, Madrid). In collaboration with M. Salas, Meijer found that the bacterial machinery involved in segregation of the bacterial genome into the prespore is also responsible for spore-entrapment of the phage genome. In addition, the bacterial Spo0A protein, the master regulator for entry in sporulation, silences the early phage promoters. The end result is phage DNA in the spore awaiting germination to begin its life cycle.

The consequences of the interminable struggle for dominance between the phage and its bacterial host were brought home by the talk of G. Kaufmann (Tel Aviv University). Phage T4 infection results in the inactivation of the bacterial restriction modification enzyme EcoPrrI and that, in turn, activates an associated $tRNA^{Lys}$-specific anticodon nuclease (ACNase) termed PrrC (Amitsur *et al*., 2003). Cleavage of $tRNA^{Lys}$ by this ACNase occurs 5′ to the wobble base. Unless thwarted, this activity would stop the progression of the progression of the phage, but the phage-encoded RNA repair enzymes polynucleotide kinase and RNA ligase normally offset the damage. Structural studies reveal ACNase to be a hexamer of PrrC. An NTPase domain that interacts with EcoPrrI occupies the ≈260 aa amino-proximal portion of PrrC while the ACNase activity resides in the remaining ≈130 aa. Known PrrC homologues are sporadically distributed among distantly related bacterial groups. All of them feature the catalytic ACNase residues but anti-codon recognition is found only in a subset. Thus, the various PrrC restriction RNases must differ in substrate specificity.

Another talk by M. Smith (University of Aberdeen, Scotland) dramatized the intricate interactions possible in the battles for survival between phages and bacteria. Protection from infection by phage ϕ31 is afforded *Streptomyces coelicolor* by the Pgl system (Sumby and Smith, 2002). Unlike classical restriction systems, the Pgl^+ bacterium supports one round of growth of ϕ31, but the progeny of this infection is unable to go through any further rounds of growth in *S. coelicolor*. Thus, initially infected bacteria form a defensive line that blocks further infection of the developing *S. coelicolor* hyphae. Although one of the genes involved in this exclusionary process encodes a putative DNA adenine methyltransferase, suggesting modification of the ϕ31 DNA during replication in *S. coelicolor*, evidence for such a process has as yet not been obtained. Moreover, the obvious genetic approach has not been successful, in that it has not been possible to select mutants of ϕ31 that escape the exclusionary process. It seems likely that multiple sites on the ϕ31 DNA are

involved, requiring multiple mutations for the DNA to escape this mysterious 'bait and switch' strategy of the bacterium.

Phage ecology and taxonomy

The overarching theme of this session, chaired by F. Rohwer (San Diego State), was repeated reminders about just how much is left to learn about phage. C. Suttle (University of British Columbia), R. Sandaa (University of Bergen) and Rohwer started the session with talks showing that the vast majority of phage diversity, as assessed at the sequence level, remains uncharacterized. Sandaa's work in solar salterns showed that phage diversity appears to be highest at the intermediate salinities. This is consistent with patterns found in macroorganisms and highlights the emergence of classical ecological theory with microbial studies. The saltern work also directly connected to a major theme of the section, namely viruses of extremophiles. For example, A. Kropinski (Queen's University, Kingston, Ontario) presented the first genome and genetic characterization of an alkaliphilic phage, BCJA1. In his presentation, truly a high-point in the conference, D. Prangishvili (Institut Pasteur, Paris, France) literally stunned the audience with his description of the exceptional novel morphotypes and diversity of phages of *Crenarchaeota* growing geo-thermally heated areas of high volcanic activity (i.e. acidic environments above 80°C) (Prangishvili, 2003; Prangishvili and Garrett, 2004). Seven novel virus families have been established based on unique features of morphotype and genome organization of these viruses. About 95% of genes of the sequenced genomes show no similarity to genes in public databases, suggesting unusual solutions to biological problems. Most remarkably, some of these phages have capsids that change morphology over a period of days after being released from their hosts.

The addition of all these new phage morpho- and genotypes highlighted the need of the phage community to update our taxonomy systems. R. Edwards (University of Tennessee, Memphis, TN) presented work suggesting that phage taxonomy systems based on free-phage properties versus genomic information are essentially compatible. This presentation was followed by a lively discussion in which the difficulties of the various options for phage taxonomy were thoroughly examined.

Members of well-known phage groups are also exceptionally diverse and contain significant amounts of novel sequence. H. Krisch (Centre National de la Recherche Scientifique, Toulouse, France) showed that two T4-like phages, RB49 and Aeh1, both have large segments in their genomes with sequences that are unrelated to the T4 sequence, to each other, and to anything else in the databases. N. Mann (University of Warwick, UK) described another T4-like phage genome, SPM2, which carries the rate-limiting genes for cyanobacterial photosynthesis, providing a context for the earlier presentation by Clokie, in which physiological data support the notion that these photosynthesis genes are important for phage replication. This story is also similar to that presented by M. Sullivan in the Phage Genomics and Evolution session (i.e. other cyanophage genomes carry photosynthesis genes).

In fact, lateral gene transfer by phages was a recurring theme within the session and throughout the meeting. Suttle and Rohwer, for example, presented data showing that phage genes are moving between environments and over large geographical distances. A critical new concept with health implications is that phage-encoded genes involved in pathogenesis also appear to be moving through the environment, possibly independent of their microbial hosts.

Significant progress is being made in our understanding of phage ecology. E. Wommack (University of Delaware) and R. Sandaa showed how viral populations change over environmental and seasonal gradients (Wommack and Colwell, 2000). These data sets are essential for the modelling efforts of investigators, like A. Rabinovitch (Ben-Gurion University of the Negev, Israel), a physicist who, in collaboration with colleagues A. Zaritsky and I. Aviram, has proposed that bacteria in a culture might be protected by the bodies of their dead compatriots (Rabinovitch *et al*., 2003). Mathematical modelling and real-time detection of phage infection using green fluorescence protein is being used by I. Calvo-Bado (L. Wellington laboratory, University of Warwick, UK) to study phage ecology in soil environments. These system biology approaches to phage biology are likely to bring dramatic changes in the field of phage ecology in the near future.

Phage in therapeutics and biotechnology

With 59 abstracts, this popularity of this area rivalled even that of the classical topics of gene regulation and phage–host interactions. C. Merril (National Institutes of Health, Bethesda) chaired the platform session and reviewed the potential of phage as an anti-bacterial agent for clinical infections. He stressed the need for quantitative data concerning interactions between phage, pathogenic bacteria and the infected animal, as therapeutic phage concentrations need to be sufficient to lower titres of pathogens before the latter reach a lethal concentration for the mammalian host. Merril reviewed experiments showing that λ phage administered to animals can avoid being cleared by the mammalian host defence system by single missense changes in the capsid protein (Merril *et al*., 1996). This provides a tool for exploring the molecular and cellular basis of this clearance, which would be a critical

consideration in any systemic application of phage therapeutics. Merril also emphasized that for the realization of clinical phage therapy applications in the USA there is a need for enhanced methods of identification of infecting bacterial strains, availability of useful therapeutic phage strains, appropriate methods of phage purification and the development of protocols for phage administration. Information on phage genetics and physiology, which is known in detail for only a few phage strains, needs to be extended to phage strains of therapeutic interest for helping in the elimination of any phage-encoded toxin genes and enhancing therapeutic properties of phage strains (Merril *et al*., 2003).

The keynote speaker for the session, V. Fischetti (Rockefeller University), demonstrated the effectiveness of phage endolysins in destroying bacteria *in vitro* and predicted that these 'enzybiotics' will provide new anti-bacterial agents for the treatment of antibiotic-resistant bacteria. Fischetti noted that phage endolysins are in general specific for their host species and genera, offering a potential for avoiding damage to commensal organisms. *In vivo* mouse experiments demonstrated that a single dose of specific endolysin was sufficient to clear either streptococcal or pneumococcal bacterial strains colonized in the nasal or oral cavities of the mice. In addition, a single intravenous dose of enzyme given to mice made septicemic with *Streptococcus pneumoniae* reduced the bacterial titres in the blood by two orders of magnitude. Fischetti reported that phage enzymes specific for *Streptococcus pyogenes*, *S. pneumoniae* and *Bacillus anthracis* are available. D. Donovan (United States Department of Agriculture, Beltsville, MD) discussed his efforts to express phage endolysins in the mammary gland of transgenic dairy cows. The goal is to control and prevent mastitis, a disease that costs the dairy industry approximately $2 billion annually. His group has developed an expression cassette in which the gene for the endolysin of a Group B *Streptococcus* phage endolysin is inserted between the 5′ and 3′ flanking regions of the ovine β-lactoglobulin gene and is testing its expression in cultured cells. The phage endolysin is highly specific for streptococcal species and thus would be expected to have no effect on the enteric bacteria of humans.

Development of a phage endolysin for staphylococcal infections was presented by S. O'Flaherty (P. Ross group, Teagasc, Cork, Ireland). The Ross laboratory has successfully expressed an intron-containing endolysin gene of phage K, a lytic phage with broad host range among *Staphylococcus aureus* strains, in both *E. coli* and *Lactococcus lactis*. In both cases zymograms with dead staphylococcal cells indicated that active recombinant LysK product was produced, so it may be possible to use these bacteria for endolysin production for both medical and veterinary applications.

Phage and their components can also be used as vaccines and to enhance vaccine systems. R. Calendar (University of California, Berkeley) and colleagues used phage-based integration vectors (Lauer *et al*., 2002) to enhance a *Listeria monocytogenes* vaccine system. The capacity of *L. monocytogenes* to grow as a facultative intracellular bacterium in mammalian cells provides its proteins direct access to the T cell-mediated immunity system. By using phage-based integration vectors, antigenic target genes can be introduced into two different neutral sites on the *L. monocytogenes* chromosome. The use of this system to express tumour antigens resulted in significant tumour regression and long-term survival in tumour-bearing mice (Brockstedt *et al*., 2004). Calendar also modified the system to make it safer for human applications by replacing a drug-resistance marker with the *L. monocytogenes* alanine racemase (*dal*) gene.

Research on the use of whole-phage particles as a delivery vehicle for a DNA vaccine against *Yersinia pestis* was presented by J.R. Clark (J. March group, Moredun Research Institute, Penicuik, UK). The gene for the V-antigen, which has been shown to give protection against *Y. pestis* infection, was cloned into plasmid and bacteriophage vectors under the control of a eukaryotic expression cassette. The V-antigen DNA vaccine which was delivered using the bacteriophage vector gave IgG2a responses significantly higher than that from the plasmid-borne vaccine, following intramuscular delivery. Interestingly, while phages delivered orally (by gavage needle) were not as efficacious as phages given by intramuscular inoculation, the orally administered phage preparation still matched the performance of the intramuscular plasmid vaccine. Similarly, λ and plasmid vectors containing the gene for the hepatitis B surface antigen (HBSAg) under the control of the eukaryotic cytomegalovirus promoter were used for intradermal vaccination in cannulated sheep. In the case of phage administrations, effective anti-phage titres were found in the draining lymph after the second inoculation, along with a significant IgM and IgG anti-HBSAg response. The authors suggested that the virus-like properties of the phage particles result in them being taken up by professional antigen presenting cells (such as dendritic cells) where efficient expression of the vaccine genes can occur (Clark and March, 2004).

The capacity of phage to deliver genes in mammalian hosts was graphically demonstrated by C. Gorman-Zanghi (S. Dewhurst group, University of Rochester, NY) with images of light emission from mice inoculated intradermally with λ phage carrying a luciferase gene. A similar delivery system is being used with a λ construct in which there is a C-terminal fusions between the gp*D* external virion protein and the IgG-binding domains of staphylococcal protein A and streptococcal protein G. Purified λ phage with both fusion types are being used

in conjunction with antibodies specific for common dendritic cell receptors to target human and murine dendritic cells *in vitro*. Successful gene transductions are evaluated by luciferase and green fluorescent protein expression.

By using two 'non-essential proteins' displayed at high density on the outer capsid surface of the phage T4, T. Sathaliyawala (V. Rao laboratory, Catholic University of America, Washington, DC) has adapted this phage as a vaccine vector. He presented evidence that the capsid 'decoration' proteins Hoc (155 copies per capsid), a highly immunogenic 39 kDa molecule, and Soc (810 copies per capsid), a 9.7 kDa molecule, can be used for display fusions. Several full-length foreign proteins fused to these proteins have been displayed on the surface of the T4 capsid, demonstrating the potential of using such phage-based nanoparticles as customized multicomponent vaccines. The idea was further pursued with a phage displaying the HIV capsid protein P24 fused to either the N-terminus or the C-terminus of Hoc. Humoral and cellular responses showed high P24-specific IgG antibody that lasted for 6 weeks after the last booster dose. In addition, P24-specific INFγ and IL4 secreting cells were detected in the spleen and lymph nodes.

In a novel application of phage components, P. Guo (Purdue University, West Lafayette, IN) proposed the use of the self-assembling motor pRNA of bacteriophage ϕ29 as a targeting and delivery vector for therapeutic RNAs. The natural function of pRNA is to form a hexameric ring as part of a motor complex which facilitates ϕ29 DNA packaging. As hexamer formation occurs by interaction of pRNA interlocking loops, it was possible to make pRNAs that self-assemble *in vitro* to form dimers or trimers efficiently by making complementary mutations in these loops. Engineering a CD4 receptor-binding RNA aptamer into the structure helped delivery of siRNA or a ribozyme moeity to a CD4 overexpressing T cell line by receptor-mediated endocytosis. Guo noted that the use of 30–40 nm complexes reduces the problem of short half-life that confronts experimenters wishing to use the siRNAs in mammalian systems.

Because of the limited time available for oral presentations in this area, many compelling stories were presented as posters. For example, there were posters on the alteration of restriction/modifications systems in lactococcal starter cultures to protect them from phage interference for cheese production (O. McAuliffe, Teagasc, Cork, Ireland), an extensive review of the clinical application of phage therapy in Poland (A. Gorski, Institute of Immunology and Experimental Therapy, Warsaw), the use of phage to treat respiratory and systemic colibacillosis in poultry (W. Huff, United States Department of Agriculture/Agricultural Research Service, Fayetteville, AR), and the isolation of phage for the control of bacterial diseases in sugar-beet (I. Kolonyuk, Kyiv National University, Ukraine). Throughout the duration of the conference, meetings with discussions and interactions at these and other posters were especially energized by the evident momentum in biotechnological and therapeutic applications of phage biology.

The view from the Summit

The 2004 ASM Conference on the New Phage Biology was a unique opportunity for scientists from many different areas of specialization but with a common interest in bacteriophage biology to come together in one place and gain a sense of the new, vital currents in the field. Many new networks of contact and collaboration were established as attendees from these diverse areas met and mingled with each other and with scientists from the traditional phage systems. Everone benefited from the energetic participation of G. Bertani (Fig. 5), who in 1951 published the first paper on temperate phages (Bertani, 1951). The phages P1 and P2 were both carried in the Lisbonne strain of *E. coli* and became two of the most fertile systems for phage molecular genetics (Bertani, 2004). With S. Luria, Bertani also furnished the acronym LB to the most widely used growth medium in bacteriology.

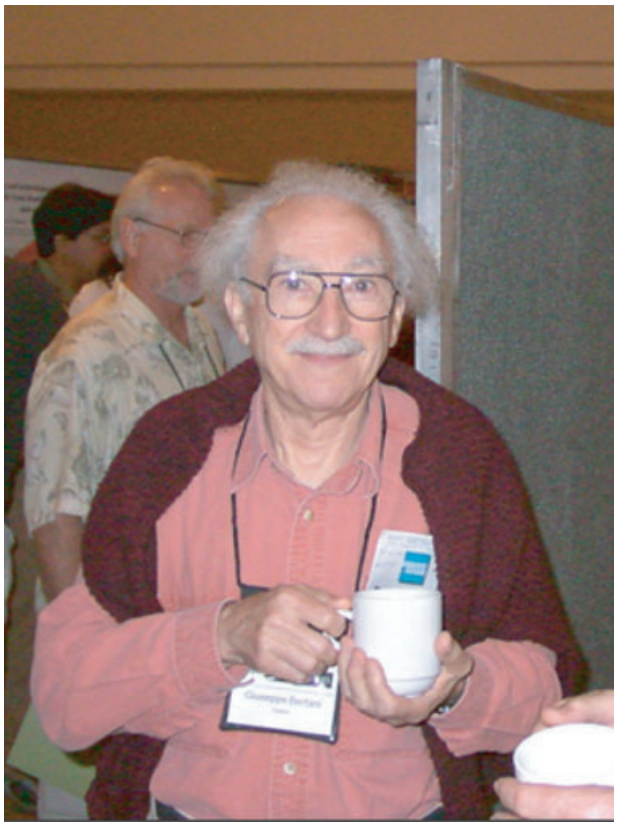

Fig. 5. G. Bertani, more than 50 years after the first paper on lysogenic phage (P1 and P2) (Bertani, 1951; 2004).

Highlights of the Conference will be displayed on the Phage Summit web pages on the Bacteriophage Ecology Group website. To help cement the networking experience, a Phage People database assembled for promulgating the Conference will also be available and can be obtained in various formats by downloading from the site or by emailing the author (R.Y.). The database contains not only contact information but also a summary of research expertise and interests for many scientists interested in phage.

In view of the overwhelming response to the announcement of the meeting and the widespread enthusiasm with which every keynote talk, platform session and poster session was received, there is no doubt that the Phage Summit should be repeated. Plans are now under active discussion, and suggestions for leadership, venue and infrastructure are earnestly solicited (phagesummit@tamu.edu).

Acknowledgements

The authors wish to thank Daisy Wilbert for clerical assistance in the preparation of the manuscript, and the Conference office of the American Society for Microbiology for their tireless efforts in helping to organize the meeting. Essential support for the Conference was provided by a grant from NIAID and funds from NICHD; we thank Milton Hernandez of NIAID and Robert A. Weisberg of NICHD for their invaluable assistance in securing these resources. We are very grateful to colleagues from the private sector who support phage biology with their enthusiasm and encouragement and who arranged for funding for this conference: J. Ramachandran, GangaGen (Palo Alto); David Martin, Avidbiotics (San Francisco); Tony Smithyman, Special Phage Services (Brookvale, New South Wales), Gregory Bogosian, Monsanto (St. Louis) and Deanna Hancock, Elanco Animal Health (Greenfield, IN).

References

Abrescia, N.G., Cockburn, J.J., Grimes, J.M., Sutton, G.C., Diprose, J.M., Butcher, S.J., *et al.* (2004) Insights into assembly from structural analysis of bacteriophage PRD1. *Nature* **432:** 68–74.

Amitsur, M., Benjamin, S., Rosner, R., Chapman-Shimshoni, D., Meidler, R., Blanga, S., and Kaufmann, G. (2003) Bacteriophage T4-encoded Stp can be replaced as activator of anticodon nuclease by a normal host cell metabolite. *Mol Microbiol* **50:** 129–143.

Atsumi, S., and Little, J.W. (2004) Regulatory circuit design and evolution using phage lambda. *Genes Dev* **18:** 2086–2094.

Bebenek, A., Carver, G.T., Dressman, H.K., Kadyrov, F.A., Haseman, J.K., Petrov, V., *et al.* (2002) Dissecting the fidelity of bacteriophage RB69 DNA polymerase: site-specific modulation of fidelity by polymerase accessory proteins. *Genetics* **162:** 1003–1018.

Bertani, G. (1951) Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. *J Bacteriol* **62:** 293–300.

Bertani, G. (2004) Lysogeny at mid-twentieth century: P1, P2, and other experimental systems. *J Bacteriol* **186:** 595–600.

Blanco, L., and Salas, M. (1996) Relating structure to function in ϕ29 DNA polymerase. *J Biol Chem* **271:** 8509–8512.

Boldt, J.L., Pinilla, C., and Segall, A.M. (2004) Reversible inhibitors of lambda integrase-mediated recombination efficiently trap Holliday junction intermediates and form the basis of a novel assay for junction resolution. *J Biol Chem* **279:** 3472–3483.

Brockstedt, D.G., Giedlin, M.A., Leong, M.L., Bahjat, K.S., Gao, Y., Luckett, W., *et al.* (2004) *Listeria*-based cancer vaccines that segregate immunogenicity from toxicity. *Proc Natl Acad Sci USA* **101:** 13832–13837.

Callen, B.P., Shearwin, K.E., and Egan, J.B. (2004) Transcriptional interference between convergent promoters caused by elongation over the promoter. *Mol Cell* **14:** 647–656.

Cassell, G.D., and Segall, A.M. (2003) Mechanism of inhibition of site-specific recombination by the Holliday junction-trapping peptide WKHYNY: insights into phage lambda integrase-mediated strand exchange. *J Mol Biol* **327:** 413–429.

Cassell, G., Klemm, M., Pinilla, C., and Segall, A. (2000) Dissection of bacteriophage lambda site-specific recombination using synthetic peptide combinatorial libraries. *J Mol Biol* **299:** 1193–1202.

Chastain, P.D., Makhov, A.M., Nossal, N.G., and Griffith, J. (2003) Architecture of the replication complex and DNA loops at the fork generated by the bacteriophage T4 proteins. *J Biol Chem* **278:** 21276–21285.

Clark, J.R., and March, J.B. (2004) Bacterial viruses as human vaccines? *Expert Rev Vaccines* **3:** 463–476.

Cockburn, J.J., Abrescia, N.G., Grimes, J.M., Sutton, G.C., Diprose, J.M., Benevides, J.M., *et al.* (2004) Membrane structure and interactions with protein and DNA in bacteriophage PRD1. *Nature* **432:** 122–125.

Costantino, N., and Court, D.L. (2003) Enhanced levels of lambda Red-mediated recombinants in mismatch repair mutants. *Proc Natl Acad Sci USA* **100:** 15748–15753.

Court, D.L., Sawitzke, J.A., and Thomason, L.C. (2002) Genetic engineering using homologous recombination. *Annu Rev Genet* **36:** 361–388.

Das, A., Garcia, M.J., Jana, N., Lazinski, D., Michaud, G., Sengupta, S., and Zhang, Z. (2003) Genetic and biochemical strategies to elucidate the architecture and targets of a processive transcription antiterminator from bacteriophage lambda. *Meth Enzymol* **371:** 438–459.

Davydova, E.K., and Rothman-Denes, L.B. (2003) *Escherichia coli* single-stranded DNA-binding protein mediates template recycling during transcription by bacteriophage N4 virion RNA polymerase. *Proc Natl Acad Sci USA* **100:** 9250–9255.

Doulatov, S., Hodes, A., Dai, L., Mandhana, N., Liu, M., Deora, R., *et al.* (2004) Tropism switching in *Bordetella*

bacteriophage defines a family of diversity-generating retroelements. *Nature* **431:** 476–481.

Edgar, R., Chattoraj, D.K., and Yarmolinsky, M. (2001) Pairing of P1 plasmid partition sites by ParB. *Mol Microbiol* **42:** 1363–1370.

Endy, D., Kong, D., and Yin, J. (1997) Intracellular kinetics of a growing virus: a genetically structured simulation for bacteriophage T7. *Biotechnol Bioeng* **55:** 375–389.

Endy, D., You, L., Yin, J., and Molineux, I.J. (2000) Computation, prediction, and experimental tests of fitness for bacteriophage T7 mutants with permuted genomes. *Proc Natl Acad Sci USA* **97:** 5375–5380.

Garcia, P., Rodriguez, I., and Suarez, J.E. (2004) A −1 ribosomal frameshift in the transcript that encodes the major head protein of bacteriophage A2 mediates biosynthesis of a second essential component of the capsid. *J Bacteriol* **186:** 1714–1719.

Hendrix, R.W. (2002) Bacteriophages: evolution of the majority. *Theor Popul Biol* **61:** 471–480.

Huang, W.M., Joss, L., Hsieh, T., and Casjens, S. (2004) Protelomerase uses a topoisomerase IB/Y-recombinase type mechanism to generate DNA hairpin ends. *J Mol Biol* **337:** 77–92.

Hyman, P., Valluzzi, R., and Goldberg, E. (2002) Design of protein struts for self-assembling nanoconstructs. *Proc Natl Acad Sci USA* **99:** 8488–8493.

Kamtekar, S., Berman, A.J., Wang, J., Lazaro, J.M., de Vega, M., Blanco, L., Salas, M., and Steitz, T.A. (2004) Insights into strand displacement and processivity from the crystal structure of the protein-primed DNA polymerase of bacteriophage ϕ29. *Molec. Cell* **16:** 609–618.

Kazmierczak, K.M., Davydova, E.K., Mustaev, A.A., and Rothman-Denes, L.B. (2002) The phage N4 virion RNA polymerase catalytic domain is related to single-subunit RNA polymerases. *EMBO J* **21:** 5815–5823.

Kimsey, H.H., and Waldor, M.K. (2004) The CTXphi repressor RstR binds DNA cooperatively to form tetrameric repressor–operator complexes. *J Biol Chem* **279:** 2640–2647.

King, R.A., Banik-Maiti, S., Jin, D.J., and Weisberg, R.A. (1996) Transcripts that increase the processivity and elongation rate of RNA polymerase. *Cell* **87:** 893–903.

Landthaler, M., Lau, N.C., and Shub, D.A. (2004) Group I intron homing in *Bacillus* phages SPO1 and SP82: a gene conversion event initiated by a nicking homing endonuclease. *J Bacteriol* **186:** 4307–4314.

Lauer, P., Chow, M.Y., Loessner, M.J., Portnoy, D.A., and Calendar, R. (2002) Construction, characterization, and use of two *Listeria monocytogenes* site-specific phage integration vectors. *J Bacteriol* **184:** 4177–4186.

Leiman, P.G., Chipman, P.R., Kostyuchenko, V.A., Mesyanzhinov, V.V., and Rossmann, M.G. (2004) Three-dimensional rearrangement of proteins in the tail of bacteriophage T4 on infection of its host. *Cell* **118:** 419–429.

Little, J.W., Shepley, D.P., and Wert, D.W. (1999) Robustness of a gene regulatory circuit. *EMBO J* **18:** 4299–4307.

Livny, J., and Friedman, D.I. (2004) Characterizing spontaneous induction of Stx encoding phages using a selectable reporter system. *Mol Microbiol* **51:** 1691–1704.

Manna, D., Breier, A.M., and Higgins, N.P. (2004) Microarray analysis of transposition targets in *Escherichia coli*: the impact of transcription. *Proc Natl Acad Sci USA* **101:** 9780–9785.

Merril, C.R., Biswas, B., Carlton, R., Jensen, N.C., Creed, G.J., Zullo, S., and Adhya, S. (1996) Long-circulating bacteriophage as antibacterial agents. *Proc Natl Acad Sci USA* **93:** 3188–3192.

Merril, C.R., Scholl, D., and Adhya, S.L. (2003) The prospect for bacteriophage therapy in Western medicine. *Nat Rev Drug Discov* **2:** 489–497.

Millard, A., Clokie, M.R.J., Shub, D.A., and Mann, N.H. (2004) Genetic organization of the *psbAD* region in phages infecting marine *Synechococcus* strains. *Proc Natl Acad Sci USA* **101:** 11007–11012.

Nilsson, A.S., and Haggård-Ljungquist, E. (2001) Detection of homologous recombination among bacteriophage P2 Relatives. *Mol Phylogenet Evol* **21:** 259–269.

Nilsson, A.S., Karlsson, J.L., and Haggard-Ljungquist, E. (2004) Site-specific recombination links the evolution of P2-like coliphages and pathogenic enterobacteria. *Mol Biol Evol* **21:** 1–13.

Ochman, H., and Selander, R.K. (1984) Standard reference strains of *Escherichia coli* from natural populations. *J Bacteriol* **157:** 690–693.

Pedulla, M.L., Ford, M.E., Houtz, J.M., Karthikeyan, T., Wadsworth, C., Lewis, J.A., *et al.* (2003) Origins of highly mosaic mycobacteriophage genomes. *Cell* **113:** 171–182.

Prangishvili, D. (2003) Evolutionary insights from studies on viruses of hyperthermophilic archaea. *Res Microbiol* **154:** 289–294.

Prangishvili, D., and Garrett, R.A. (2004) Exceptionally diverse morphotypes and genomes of crenarchaeal hyperthermophilic viruses. *Biochem Soc Trans* **32:** 204–208.

Qu, Y., Hyman, P., Harrah, T., and Goldberg, E.B. (2004) *In vivo* bypass of chaperone by extended coiled-coil motif in T4 tail fiber. *J Bacteriol* **186:** 8363–8369.

Rabinovitch, A., Aviram, I., and Zaritsky, A. (2003) Bacterial debris – an ecological mechanism for coexistence of bacteria and their viruses. *J Theor Biol* **224:** 377–383.

Semenova, E., Djordjevic, M., Shraiman, B., and Severinov, K. (2004) The tale of two RNA polymerases: transcription profiling and gene expression strategy of bacteriophage Xp10. *Mol Microbiol*. doi:10.1111/j.1365-2958.2004.04442.x

Sen, R., King, R.A., and Weisberg, R.A. (2001) Modification of the properties of elongating RNA polymerase by persistent association with nascent antiterminator RNA. *Mol Cell* **7:** 993–1001.

Shcherbakov, V., Granovsky, I., Plugina, L., Shcherbakova, T., Sizova, S., Pyatkov, K., *et al.* (2002) Focused genetic recombination of bacteriophage T4 initiated by double-strand breaks. *Genetics* **162:** 543–556.

Stohr, B.A., and Kreuzer, K.N. (2002) Coordination of DNA ends during double-strand-break repair in bacteriophage T4. *Genetics* **162:** 1019–1030.

Sullivan, M.B., Waterbury, J.B., and Chisholm, S.W. (2003) Cyanophages infecting the oceanic cyanobacterium *Prochlorococcus*. *Nature* **424:** 1047–1051.

Sumby, P., and Smith, M.C. (2002) Genetics of the phage growth limitation (Pgl) system of *Streptomyces coelicolor* A3(2). *Mol Microbiol* **44:** 489–500.

Summer, E.J., Gonzalez, C.F., Carlisle, T., Mebane, L.M.,

Cass, A.M., Savva, C.G., *et al.* (2004) *Burkholderia cenocepacia* phage BcepMu and a family of Mu-like phages encoding potential pathogenesis factors. *J Mol Biol* **340:** 49–65.

Wagner, P.L., Neely, M.N., Zhang, X., Acheson, D.W., Waldor, M.K., and Friedman, D.I. (2001) Role for a phage promoter in Shiga toxin 2 expression from a pathogenic *Escherichia coli* strain. *J Bacteriol* **183:** 2081–2085.

Waldor, M.K., and Mekalanos, J.J. (1996) Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* **272:** 1910–1914.

Wiedenheft, B., Stedman, K., Roberto, F., Willits, D., Gleske, A.K., Zoeller, L., *et al.* (2004) Comparative genomic analysis of hyperthermophilic archaeal *Fuselloviridae* viruses. *J Virol* **78:** 1954–1961.

Wommack, K.E., and Colwell, R.R. (2000) Virioplankton: viruses in aquatic ecosystems. *Microbiol Mol Biol Rev* **64:** 69–114.

Zimmer, M., Sattelberger, E., Inman, R.B., Calendar, R., and Loessner, M.J. (2003) Genome and proteome of *Listeria monocytogenes* phage PSA: an unusual case for programmed +1 translational frameshifting in structural protein synthesis. *Mol Microbiol* **50:** 303–317.