The Phage Meeting: Classical Venue, New Momentum

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“Molecular Genetics of Bacteria and Phages” is the longest-running series of molecular biology meetings, originating in the Phage Meetings, which began in 1940. The 58th meeting, held at the Wisconsin Union, Madison, Wisconsin, from 2 to 7 August 2005, attracted more than 300 participants and had 10 sessions with a total of 90 talks and two poster sessions with 170 presenters. In such a brief review, we can give only a sampling of the many highlights of the exciting and very broadly based science that was presented. The meeting opened with the announcement of Ishita Shah as the winner of the annual Nat Sternberg Thesis Prize for the most outstanding Ph.D. thesis on prokaryotic molecular genetics. Shah, who trained with Richard Wolf (University of Maryland, Baltimore County), presented the opening talk in the “Global Regulation and Stress Response” session, chaired by Tania Baker (MIT). Shah’s talk described a new prerecruitment mechanism within the SoxRS two-component regulatory system of *Escherichia coli* in which SoxS binds to RNA polymerase (RNAP) prior to scanning the chromosome for SoxS-dependent promoters (Fig. 1). Shah’s presentation also introduced the topic of proteolysis as a means of adaptation in *E. coli*, which was the central theme of several talks in this session. For example, Elizabeth Oakes (MIT) investigated the *in vivo* role of the Lon protease by using a proteomic approach to identify substrates, which were detected by their ability to associate with a proteolytically inactive “trap” form of Lon. The results indicate that Lon does not indiscriminately associate with proteins but instead binds preferentially to proteins whose degradation may be important for adaptation. Other talks in this session covered a variety of global control mechanisms in *E. coli*, including the GASP (growth advantage in stationary phase) phenotype described by Steven Finkel (University of Southern California). This phenotype is often conferred by mutations in *rpoS*, the gene for the so-called stationary-phase sigma factor. It was discovered that a single *rpoS* mutant does not dominate aged cultures, contrary to previous models. Instead, there are selective pressures to maintain the wild-type *rpoS* allele and also to accumulate multiple mutant *rpoS* alleles.

Session II, entitled “RNA Polymerase Structure and Function,” was chaired by Dmitry Vassylyev (University of Alabama at Birmingham). Vassylyev used recent crystal structures of RNAPs and their transcription elongation complexes to reveal key features of the transcription process, such as a 9-bp RNA-DNA hybrid, base stacking in the RNA exit channel, and a two-step substrate-loading mechanism for both single-subunit

and multisubunit RNAPs. The high-resolution structures of bacterial RNAPs were also used to examine transcription inhibition by small molecules. Vassylyev proposed allosteric mechanisms of inhibition by rifamycins, streptolydigin, and tagetitoxin, while Richard Ebright (Rutgers University) described possible mechanisms of action of the inhibitors myxopyronin, coralopyronin, and ripostatin, all of which interact with the switch region of RNAP. The switch region mediates the opening and closing of the active center cleft of RNAP and represents an attractive new antibiotic target. Several talks in this session focused on the synergistic regulation of *E. coli* transcription initiation by ppGpp and DksA. Especially interesting was the demonstration by Catherine Vrentas (University of Wisconsin) that the ω subunit of RNAP contributes to ppGpp-mediated regulation in the absence of DksA, providing a new function for this RNAP subunit. Numerous other aspects of transcription were discussed, with presentations ranging from a description by Scotty Kyzer (University of Wisconsin) of the *in vitro* reconstitution of a hairpin-stabilized paused transcription complex to the discovery by Innokenti Toulkounov (University of Wisconsin) that deleting the RNAP lid region, which is located at the upstream end of the RNA-DNA hybrid of elongation complexes, inhibits transcription initiation.

Session III, entitled “Transcription Regulation,” included talks on a variety of transcription control mechanisms. The chair was Steve Busby (University of Birmingham), who described the use of chromatin immunoprecipitation and high-density microarrays to monitor the *in vivo* distribution of cyclic AMP receptor protein and RNAP on the *E. coli* chromosome. This methodology provides an alternative to transcriptome approaches for studying global transcription patterns. The crystal structure of the first MarR family member, OhrR of *Bacillus subtilis*, was presented by Richard Brennan (Oregon Health and Science University). OhrR represses expression of *ohrA*, which encodes a thiol peroxidase, and is activated by oxidation of its lone cysteine residue. Structures of both reduced apo-OhrR and an OhrR-*ohrA* operator complex were described; these revealed the structural basis for OhrR activation and multiple DNA binding mechanisms of OhrR, including an extended eukaryote-like winged helix-turn-helix motif that binds distal minor grooves. Unusual positive autoregulation of the activator protein Rns of enterotoxigenic *E. coli* was described by George Munson (University of Miami). This regulation requires Rns binding to a downstream site to allow RNAP to form an open complex at the *ms* promoter, Rns binding to an upstream site to allow RNAP to escape from the *ms* promoter, and Fis binding between the Rns binding sites to bend DNA into a conformation that allows necessary protein-

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FIG. 1. Sternberg Award presentation: mechanisms of transcription activation. In recruitment, the activator first binds its DNA target in the promoter (catabolite activator protein [CAP]) and then recruits RNAP through protein-protein interactions. In postrecruitment, RNAP forms a stable closed complex, and then the activator, usually DNA bound, makes protein-protein contacts that stimulate isomerization of the closed complex to the open complex. In prerecruitment, the activator forms a binary complex with RNAP, and the complex then scans the chromosome for activator-dependent promoters. Prerecruitment provides a means to overcome the deficit in information content of an activator, like SoxS, that binds to a highly degenerate site, because it uses the sigma factor as well as the activator binding site for DNA recognition. In this respect, the activator functions as a co-sigma factor.

protein contacts. Other interesting talks included the description by Ido Golding (Princeton University) of a novel method of measuring mRNA levels in a single *E. coli* cell and its use to show burst-like transcription and a description by Tim Miyashiro (University of Pennsylvania) of how an *E. coli* two-component regulatory system subject to positive feedback operates as a self-tuning amplifier to achieve a graded output.

Session IV focused on posttranscriptional regulatory events and the translational machinery. Mathias Springer (CNRS, Paris, France) described the translational operator site for the *E. coli rpmI-rplT* operon, which is subject to autogenous repression by ribosomal protein L20, encoded by *rplT*. Only the C-terminal 60 amino acids of L20, which comprise a globular domain, are required for repression, despite the complexity of the operator site, which includes 10 hairpins and a long-range tertiary interaction. In contrast, the binding site for the global regulator CsrA, described by Paul Babitzke (Pennsylvania State University), consists of a conserved primary sequence element that is often present within a simple stem-loop. While the details of these molecular interactions remain to be determined, it is evident that there is much to learn about the rules for protein-RNA recognition and about how binding can inhibit translation. A second major topic was the role of small RNAs in gene regulation. New RNAs that regulate outer membrane structure were discussed in reports from Maude Gillier (NIH) and Rajeev Misra (Arizona State University), and the mechanism of small RNA-dependent mRNA destabilization by recruitment of the degradosome was described by Teppei Morita (Nagoya University). A special small RNA, tmRNA, is involved in rescue of blocked translational complexes. Ken Keiler (Pennsylvania State University) discussed intriguing features of tmRNA tagging in *Caulobacter*, where tmRNA activity is cell cycle regulated and loss of tmRNA

results in a delay of initiation of DNA replication. Other ribosomal stalling and recycling systems were also discussed.

The fifth session, on DNA metabolism and transactions, was chaired by Anna Karls (University of Georgia) and spanned the scale from organismal to single-molecule studies. Among the many highlights was the report from Aliaa Abdelhakim (MIT) that in the context of the transpososome, the MuA subunit has an "adaptor" domain that binds ClpX and thus facilitates the dissociation of the otherwise extremely stable transpososome. More-mechanistic insight on a transposition system came from single-molecule studies of the nonspecific interaction of Tn5 transposase with DNA. Transposase binding is associated with discrete and rather slow shortening of the DNA, suggesting loop formation, according to Christian Adams (University of Wisconsin). A surprising finding was reported by Jue Wang (MIT), who described microarray studies indicating that *B. subtilis* can slow or stop its replication forks during amino acid starvation in a RelA/ppGpp-dependent manner. Maria Schumacher (Oregon Health and Science University) described the crystal structure of the P1 partition protein ParB bound to centromere DNA containing the partition elements BoxA and BoxB. Remarkably, in this structure ParB is an asymmetric dimer with two DNA binding modules, a globular central domain that binds BoxB and an extended helix-turn-helix domain that binds BoxA. The structure indicates that the binding modules can rotate freely, allowing ParB to bridge the arms of a looped partition site.

Effects of translational arrest were also discussed in the "Bacterial Cell Biology" session, which was chaired by Kenn Gerdes (University of Southern Denmark). Gerdes described the effects of inactivation of toxin-antitoxin systems that are responsible for cleavage of mRNA within translation complexes during nutritional stress, under conditions where tmRNA is important for translational accuracy. Most of this session focused on genetic and cell biology approaches to the analysis of cell division and DNA segregation. The formation of the FtsZ ring at the division site is a crucial event for initiation of division. Richard Weart (Washington University) described a novel role for the ClpX chaperone in maintaining the pool of unassembled FtsZ and showed that this activity, unlike the normal function of ClpX in conjunction with its partner ClpP, is independent of ATP hydrolysis and does not involve FtsZ degradation. Aaron Handler (Harvard University) reported on a newly identified small peptide that affects Z-ring formation during sporulation in *B. subtilis*. The ability of the overexpression of FtsQ, FtsB, or FtsN to compensate for the deletion of the essential division gene *ftsK*, reported by Brett Geissler (University of Texas Medical School, Houston, TX), suggests that a number of cell division proteins have overlapping functions. Time-lapse fluorescence microscopy of live cells was used by Toshiyuki Hatano (National Institute of Genetics, Japan) to demonstrate that the SopA partitioning protein forms a helical structure within the cell that appears to guide the positioning of the F plasmid at the cell poles.

Session VII, entitled "Surfaces and Signaling," was chaired by David Thanassi (Stony Brook University) and described studies of the PapC usher protein, which serves as a platform in the outer membrane for the assembly and secretion of the P pilus of uropathogenic *E. coli*. A pathway for pilus growth in which pilus chaperone subunit complexes initially target to an

-terminal domain of the usher, leading to interaction with the usher C terminus and assembly of the subunits into the pilus fiber, was presented. David Zusman (University of California, Berkeley) described FrzS, which is essential for the S-motility swarming of *Myxococcus xanthus* and has an N-terminal response regulator domain with a C-terminal coiled-coil domain. The audience was enthralled by spectacular images of dynamic intracellular localization of FrzS-green fluorescent protein, which reverses every 7 to 8 min when the cell reverses direction and, based on its intracellular trajectory, may be associated with a helical filament in the cytosol. Several speakers addressed regulatory systems that involve sensors in the envelope. Albert Siryaporn (University of Pennsylvania) discussed two factors that limit cross talk between the EnvZ-OmpR and CpxA-CpxR two-component systems in *E. coli*: competition between CpxR and OmpR for CpxA and direct inhibition by the cognate sensor kinase, EnvZ. Sarah Ades (Pennsylvania State University) reported that the role of σ^E in the cell goes beyond its recognized extracytoplasmic stress response, as σ^E activity is regulated by the alarmone ppGpp in response to nutritional stress, and showed cell biology experiments indicating that σ^E is crucial for maintaining the integrity of the cell envelope.

The "Genomics and Proteomics" session introduced novel technologies for the analysis of a wide range of experimental problems. The chair, Janine Maddock (University of Michigan), discussed the advantages of isobaric tags in quantitative simultaneous comparisons of protein distributions in multiple samples, and the application of this approach to *Bacillus anthracis* germination was presented. The analysis of low-abundance proteins represents a special problem in proteomics, one solution to which is subfractionation; Lili Niu (Texas A&M University) utilized heparin binding to enrich for *E. coli* nucleic acid binding proteins as an example of this type of approach. Small RNAs were discussed in this session as well, with the description by Jonathan Livny (Tufts Medical School) of a new computer program, sRNAPredict, for the identification of candidate small RNA coding regions based on conservation among genomes and location upstream of transcription termination signals. Application of this approach to the *Vibrio cholerae* genome yielded nearly all of the known small RNAs from this organism as well as a number of new candidates, several of which were confirmed by Northern analysis. Several interesting studies in which a variety of approaches was used to study gene expression were also described.

Session IX, chaired by Karen Ottemann (University of California, Santa Cruz), focused on the molecular biology of pathogens. Ottemann reported proteomic experiments to identify *cheZ* as the site of a suppressor mutation that allowed nonchemotactic *cheW* mutants of the ulcer-causing bacterium *Helicobacter pylori* to regain chemotaxis. CheZ is a previously unidentified homolog of the CheY phosphatase, suggesting that suppression results from the stabilization of small amounts of CheY phosphate. Brian Stevenson (University of Kentucky) discussed the regulation of proteins on the surface of the Lyme disease spirochete, *Borrelia burgdorferi*. His group has identified a chromosomally encoded protein, BifC, which may be a transcriptional regulatory protein controlling differential expression in the mammalian and flea hosts. Li Tan (University of Alabama at Birmingham) described the use of a unique

Yersinia pestis-*Caenorhabditis elegans* biofilm system to study the mechanism of plague transmission by fleas infected with *Y. pestis*. With this system, he has been able to show that sialic acid is required for biofilm formation on *C. elegans*, that *Y. pestis* can take up sialic acid or synthesize it, and that a *Y. pestis* protein, NeuX, is a novel sialic acid synthase. Several presentations dealt with unusual secretion systems important for pathogenesis. Barbara Bensing (UCSF VA Medical Center) talked about the accessory Sec system of *Streptococcus gordonii*, which is required for the transport of the large glycoprotein GspB to the cell surface. Recent results indicate that the N-terminal 90 amino acids of GspB function as an export signal that targets GspB to the accessory Sec system and, in conjunction with glycosylation, inhibits its export via the canonical Sec pathway. Patricia DiGiuseppese-Champion (UCSF) reviewed experiments aimed at understanding the ESX-1 alternate secretion pathway of *Mycobacterium tuberculosis*. She and her coworkers in the laboratory of Jeff Cox have results indicating that the signal for recognition and export resides at the extreme C terminus of the secreted molecules and suggesting that the ESX-1 machine shares features common to those of type IV secretion machines used by pathogens to manipulate host cell response.

The last session, entitled "Bacteriophage Development and Host Interactions," began with a description of a historic milestone for phage biology and molecular biology in general. The session chair, Carlos Catalano (University of Colorado at Denver), revealed that his group has achieved the complete de novo assembly of infectious phage lambda particles from purified host and viral proteins, procapsids, and tails and has used this defined system to package commercially available lambda DNA. Among numerous other interesting talks was that presented by Laura Marinelli (University of Pittsburgh) on studies of the mycobacteriophage tape measure protein (TMP), which determines the length of the phage tail. In phage Barnyard, TMP is proteolytically processed and is associated with cell wall and membrane fractions. These results, combined with the presence of a domain that confers murein hydrolase activity, suggest that phage TMPs play a pivotal role in DNA injection. J. Rodney Brister (NIH) reported the use of a PCR-amplified array covering the entire genome of bacteriophage T4 to identify the five origins used for early DNA replication. Phage evolution was considered by Ian Molineux (University of Texas), who described a T7 mutant that overcomes a deletion of gene 1, which encodes the essential T7 RNA polymerase, by activating a novel replication origin. Molineux suggested that the mutant corresponds to an ancestral phage that later acquired more efficient pathways for gene expression and DNA replication. Regulatory decisions in lambdoid phages were the subject of talks by Gerry Koudelka (SUNY—Buffalo) and Taehyun Park (Texas A&M University). Koudelka reported that environmental salt increases the spontaneous induction frequency of the lambdoid phage 434 by directly affecting the stability of repressor-operator complexes. Park described unprecedented "topological gymnastics" of the lambdoid phage 21 holin in the regulation of lysis. Using cysteine cross-linking and protease accessibility experiments, Park found that hole formation is triggered when one of the two transmembrane helices completely exits the bilayer and enters the periplasm.

There were many other equally compelling presentations, and it is important to note that the great preponderance of them were delivered by students and postdocs, continuing one of the meeting's signature traditions. It was striking to the authors of this review how rapidly the breadth of the meeting is expanding. It was certainly clear that the canonical molecular genetics systems of *E. coli* and *B. subtilis* are still vibrant, but many other prokaryotic systems were vigorously discussed. Moreover, no other meeting has such a wide-ranging field of view, spanning from basic phage genetics to organismal genomics/proteomics, from atomic-level structure-function

analyses to microbial cell biology, and from single-molecule biochemistry to viral self-assembly. We think this continues to reflect the problem-oriented philosophy of the physicists, chemists, and microbiologists who founded the original Phage Meetings more than 60 years ago. There can be no doubt that during the 5 days of the 2005 session of the "Molecular Genetics of Bacteria and Phages" meeting, the faculty, postdocs, and students who gathered in the Wisconsin Union on the shore of Lake Mendota could attest to the new momentum of this classic venue.

We thank the National Science Foundation for its support.