Meeting report

Interdisciplinary bacteria and phages

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A report of the meeting 'Molecular Genetics of Bacteria and Phages', Cold Spring Harbor, USA, 20-24 August 2008.

It is fashionable to talk about interdisciplinary meetings - as opposed to conferences dedicated to a single discipline, process, or molecule. The 'Phage' meeting at the Cold Spring Harbor Laboratory has been cross-disciplinary since its inception, 59 years ago, and the 2008 meeting was no exception. One of the hallmarks of the microbial world is diversity, both in terms of the organisms and their array of remarkable processes. That was indeed the take-home message from this summer's meeting.

Stress

If you think you're stressed, it's nothing compared to bacteria! They are constantly bombarded by a multitude of stressors both from the environment and from within. Successful species have learned how to adapt to, dodge or ameliorate such stresses. For instance, just as none of us would hit the beach for a day in the sun without sunscreen, neither would the cyanobacterium *Nostoc punctiforme*. Tanya Soule (Arizona State University, Tempe, USA) presented work showing that the 18 genes within the cluster responsible for synthesizing the ultraviolet A sunscreen scytonemin, a heterocyclic indol alkaloid, are differentially induced under ultraviolet A stress compared with white light. Through comparative genomics, Soule showed that there is an additional five-gene satellite cluster that operates in response to ultraviolet A stress. Thus, when faced with prolonged ultraviolet A exposure, these cyanobacteria protect themselves with sunscreen.

The formation of spores is inherent to surviving harsh conditions for some bacterial species. Kumaran Ramamurthi (Harvard University, Cambridge, USA) described work on the *Bacillus subtilis* protein SpoVM (VM) showing that this protein responds to a geometric signal within the cell. VM is a small polypeptide that localizes to the surface of the developing spore, which is the only convex surface within the mother cell. Experiments utilizing *B. subtilis* mutants that do not develop the convex curvature fail to localize VM. Furthermore, experiments in yeast showed that VM only adheres to the surface of convex organelles, and purified VM preferentially adheres to vesicles displaying a strongly positive curvature. Who would have guessed that a peptide can sense geometry to begin the assembly of a supramolecular structure?

Temperature can be both a stressor and an environmental cue. Whereas *Listeria monocytogenes* can survive at temperatures ranging from 3°C to 43°C, it is only motile using flagella below 30°C. Heather Kamp (Harvard Medical School, Boston, USA) showed that the temperature-dependent control of flagellar motility is due to both transcriptional and post-transcriptional regulation of GmaR, an anti-repressor that controls genes for flagellar synthesis. She has found that the downregulation of flagellar genes at 'body temperature', when the pathogen is preparing for intracellular existence, and the corresponding upregulation of virulence genes, allow this pathogen to invade host cells.

Nutritional stress has long been known to reprogram gene expression, through the cAMP-catabolite regulator protein (CRP) and ppGpp regulatory systems. One of us (MB) described how carbon-source or amino-acid starvation, acting through cAMP-CRP and ppGpp, respectively, induce movement of group II introns in *Escherichia coli*. These small-molecule regulatory effectors act at the level of the target DNA, rather than at the level of the ribonucleoprotein that is usually involved in mobilizing these introns. Remarkably, by moving to new sites during periods of nutritional stress, these RNA elements serve to generate genetic diversity within the DNA genome.

Small RNAs (sRNAs) can also be sensitive to cAMP-CRP signaling in response to carbon-source starvation. Nicholas DeLay (National Cancer Institute, Bethesda, USA) reported on the post-transcriptional silencing of several *E. coli* genes by the sRNA CyaR (RyeE), which is activated by cAMP-CRP. Two such genes are *ompX*, encoding an outer membrane protein involved in adhesion, and *luxS*, encoding auto-inducer-2 synthase, a stimulator of biofilm formation. DeLay speculated that reduced expression of *ompX* and *luxS* in response to starvation-induced CyaR activation can inhibit adhesion and biofilm formation, allowing cells to escape from a nutrient-deprived environment.

Crowds can be stressful! Quorum sensing, the method used by bacteria to detect cell density, plays a role in the movement of ICE*Bs1* (integrative and conjugal element) in *B. subtilis*, the subject of the keynote talk by Alan Grossman (Massachusetts Institute of Technology, Cambridge, USA). PhrI is a secreted quorum-sensing peptide, encoded within ICE*Bs1*. Its expression is stimulated by low levels of nutrients and high cell density, and the secreted peptide is taken up both by the cells that make it and by neighboring cells. At the same time, expression of *rapI*, a gene in ICE*Bs1* that encodes the regulatory protein RapI, is also stimulated. RapI helps regulate the transfer of ICE*Bs1*. However, PhrI inhibits RapI, so in a dense cell population that already contains ICE*Bs1*, as gauged by PhrI uptake, the movement of ICE*Bs1* into neighboring cells that already contain the element is prohibited. Thus, during the stresses of crowding and low nutrients, the energy required to move ICE*Bs1* is conserved.

Life or death

Microbes seem always to be teetering on the brink between life and death, and have amassed numerous fail-safes to aid survival. To defend themselves against phage infection, they have developed a phage-resistance system termed CRISPR (cluster of regularly interspaced short palindromic repeats). This defense system entails integration of fragments of viral nucleic acid into the bacterial genome as clusters of short palindromic repeats. Matthijs Jore (Wageningen University, the Netherlands) presented work showing how the viral sequences within CRISPRs are used, by the CRISPR-associated (Cas) proteins, to mount a host defense. Upon transcription of the CRISPR, Cas proteins cleave an RNA precursor in each repeat, and hold the cleavage product containing the virus-derived sequence. The helicase Cas3 then interacts with these mature CRISPR RNAs, using them as 'guide' RNAs to prevent phage proliferation, in a mechanism similar to RNA interference in eukaryotic cells.

Another group of mechanisms to resist phage are the abortive infection (Abi) systems, like the Abi system on a cryptic plasmid of *Erwinia carotovora* subspecies *atroseptica*. The work presented by Tim Blower (University of Cambridge, UK) showed that this Abi system can protect effectively against multiple phage infections. Interestingly, the system works like the toxin-antitoxin (TA) systems; ToxN, a toxin, and ToxI, which inhibts ToxN, are responsible for the abortive infection phenotype. However, like known toxin-antitoxin systems, ToxN is lethal to the cell unless ToxI is also expressed, thus blurring the line between these systems and phage-resistance systems.

The function of bacterial toxin-antitoxin systems is still mysterious, although theories abound. Holly Ramage (University of California, San Francisco, USA) revealed that the worldwide pathogen *Mycobacterium tuberculosis* possesses many such systems. Bioinformatics revealed an astounding 87 potential toxin-antitoxin gene pairs, of which 24 were found to be functional in *Mycobacterium smegmatis*. Of these genes, subsets are upregulated or downregulated during hypoxia or macrophage infection of *M. tuberculosis*, an expression pattern that will contribute to understanding how this organism persists within the macrophage.

Nancy Woychik (University of Medicine and Dentistry of New Jersey, Piscataway, USA) discussed work on the *phd-doc* toxin-antitoxin system *encoded by E. coli* bacteriophage P1. Unlike most other bacterial toxin-antitoxin systems, in which the toxin cleaves mRNA, the toxin Doc stabilizes mRNA, while blocking translational elongation. It does so by binding to the 30S ribosomal subunits, in a similar way to the translational inhibitor hygromycin B (HygB). Doc was also shown to cleave the 16S rRNA, thus inhibiting elongation in two ways.

RNA synthesis and processing

Transcription often entails a complex interplay of many factors. The bacteriophage λ Q antiterminator protein (λQ) is responsible for the expression of the λ late genes, playing a role in transcription elongation. Ann Hochschild (Harvard Medical School, Boston, USA) described how λQ accomplishes this by direct interaction with the RNA polymerase at the β-flap of the polymerase, facilitating elongation of the nascent transcript. She also showed that the β-flap of the polymerase can be a direct target of transcriptional elongation factors.

Another presentation on RNA polymerase, by Richard Ebright (Rutgers University, Piscataway, USA), discussed the use of fluorescence resonance energy transfer (FRET) to measure the distance between the RNA polymerase β' pincer, which moves as a clamp, relative to the stationary β-pincer. FRET analysis showed that in the 'open' complex of promoter and RNA polymerase the clamp is partially closed; this conformation allows single-stranded, but not double-stranded, DNA to enter the polymerase. During elongation, the clamp closes further. Interestingly, the α-pyrone antibiotic myxopyronin binds the RNA polymerase and locks the clamp into a partially-to-fully closed position, which only allows single-stranded DNA to

enter the polymerase. Therefore, myxopyronin works by preventing the interaction between the RNA polymerase and the double-stranded promoter region of the DNA.

Many biological molecules undergo processing before becoming fully functional. One such case is tRNA. The current model for tRNA processing in *E. coli* invokes RNase E to initiate processing. However, Bijoy Mohanty (University of Georgia, Athens, USA) described results showing that processing of the *leuQPV* tRNA transcript is dependent on RNase P, and that processing of the monocistronic *leuX* tRNA transcript relies on PNPase, a 3' to 5' exonuclease. This is the first known example of removal of a stem-loop structure by a 3' to 5' exonuclease. Thus, there are multiple tRNA-processing pathways in *E. coli*.

Is there anything microbes can't do?

Scientists have been exploiting microbes for decades. 'Recombineering' - genetic engineering utilizing the Red recombination system of phage λ - has enabled the quick and efficient tinkering of genes, from clean deletions, to point mutations to insertions. Lynn Thomason (SAIC-Frederick, Inc., Frederick, USA) showed that *in vivo* genetic engineering using this system and single-stranded oligonucleotides is replication dependent. Targeting the lagging strand of the DNA yields more recombinants, suggesting that Red-mediated recombination takes place concurrently with DNA replication by annealing within the replication fork. Indeed, PolA deletion strains showed a 100-fold decrease in λ Red-mediated recombineering.

One of the most exciting talks of the meeting focused on *Shewanella oneidensis*, an odd bacterium that can be used to create microbial fuel cells. This organism has the remarkable ability to 'breathe' solid iron and can use a variety of compounds, including Fe(III), as terminal electron acceptors. As described by Steven Finkel (University of Southern California, Los Angeles, USA), if *S. oneidensis* is incubated in the presence of an electrode that serves as the terminal electron acceptor, electrons will flow from the bacterium to the electrode, effectively creating a current. Microscopic inspection showed that nanowire networks, essentially comprising the pili of bacteria, form on the electrodes, and that the conductivity of these nanowires can be directly measured. Can it be long before we are relying on bacteria as a source of electricity?

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