Meeting report of the European Molecular Biology Organization (EMBO) Symposium 'Viruses of Microbes II', Brussels, July 2012.

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Meeting report

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1. Meeting location and purpose

Nearly 400 scientists from 49 countries gathered last July in Brussels, Belgium, for a five-day conference on diversity, evolution, ecology and the environmental impact of microbial viruses, as well as new developments in fundamental, biotechnological, industrial and medical aspects of virus research (Viruses of Microbes II: http://events.embo.org/12-virus-microbe/). The conference, hosted by the Belgian Royal Military Academy, was the first in a series supported by the European Molecular Biology Organization (EMBO), but stemming from the "Viruses of Microbes" meeting initiated at the Institut Pasteur in June 2010. Belgian grants from FWO Vlaanderen, the FNRS and the FWO research community Phagebiotics (WO.022.09), as well as grants from the Moore Foundation (UK) and sponsoring from several commercial companies, enabled the organization of this meeting.

A total of 60 oral contributions, spread over 15 sessions and two specific workshops, along with 243 posters, focused on five main themes: 1) virus–host interactions; 2) bacteriophage-based antibacterials; 3) (meta) genomics, viral diversity and evolution; 4) structure–function relationship in virions; and 5) viruses in biotechnology. The Symposium aimed to bring together the bacterial virus and lower eukaryotic virus researchers, which is also the core aim for the establishment of the "International Society for Viruses of Microorganisms" (ISVM, http://www.isvm.org, chair Markus Weinbauer (CNRS, France)), which held its first general assembly. Also linked to the conference, the PHAGE non-profit organization (Phage for Human Application Group Europe, http://www.p-h-a-g-e.org/) advocates the potential use of phage therapy for human applications, and a special workshop was dedicated to this theme, reported elsewhere in this issue.

In the plenary talk, Graham Hatfull (University of Pittsburg, PA) focused on the exploration and exploitation of phages infecting mycobacteria. Through a research-education program adopted by more than 70 US colleges and universities, his laboratory managed to collect 2532 phages, among which 373 have been fully sequenced, infecting the common host strain *Mycobacterium smegmatis* mc2155. This collection provides a unique tool for studying viral diversity, which is wide and not homogeneous, meaning that there are groups with many representatives as well as singleton groups, most likely reflecting the diversity of the host.

By highlighting the role of lateral gene transfer in phage genome architecture and the appearance of singleton phages, Hatfull suggested that the viral genomic landscape represents a continuum which is still poorly sampled. Through this massive effort in phage isolation and sequencing, over 40,000 novel genes were discovered, offering a plethora of possible new insights, such as the presence of genetic switches based on integration-dependent phage immunity.

2. Sessions

2.1. Virus–host interactions

The 'Virus–host interactions' sessions covered bacterial host defence systems, novel ways of host overtake by viral proteins and studies of recombination and horizontal transfer events.

The study of clustered, regularly interspaced short palindromic repeat (CRISPR) loci and the highly diverse Cas (CRISPR-associated) proteins is a relatively new field in phage research. This bacterial adaptive immune system functions by the genomic incorporation of short stretches of phage or plasmid DNA (spacers) in between stretches of identical repeats. CRISPR transcripts (crRNAs) are processed to small RNAs, which work alongside Cas proteins to rapidly cleave invading DNA of the same source, e.g., the same phage.

Sylvain Moineau (University Laval, Quebec, Canada) was among the first to describe this system. In his talk, he focused on the acquisition of novel spacers in the Type IIa CRISPR locus of *Streptococcus thermophilus*. By studying bacteriophage-insensitive mutants and phage revertants, he unravelled the relationship between spacer acquisition and bacterial immunity. Moreover, he provided in vivo evidence that the CRISPR/Cas system specifically cleaves plasmid and bacteriophage double-stranded DNA within the proto-spacer and in a sequence-specific manner.

Another key event in CRISPR/Cas activation is the maturation of crRNAs, which was the focus of Emmanuelle Charpentier (University Umeå, Sweden). She described a novel pathway of *Streptococcus pyogenes* crRNA maturation, which is mediated by base pairing of the precursor crRNA with a trans-

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encoded RNA (tracrRNA) located 210 bp upstream from the CRISPR locus. The resulting double-stranded RNA is then cleaved by the bacterial RNase III, the first example of the involvement of a non-Cas protein in CRISPR activity. In the next step, Cas9 introduces tracrRNA:crRNA-guided double-stranded breaks in target DNA by means of its HNH (homing endonuclease) and RuvC-like endonuclease domains. This Cas9 endonuclease activity can be programmed with guide RNA, engineered as a single transcript, to target and cleave any dsDNA sequence of interest, thus offering considerable potential for gene targeting and genome editing applications.

Apart from being derived from invading phage and plasmid DNA, a significant portion of spacers consist of sequences complementary to bacterial genes. Peter Fineran (University Otago, New Zealand) focused on this intriguing observation in CRISPR loci of the plant pathogen *Pectobacterium atrosepticum*. Using a controllable, engineered CRISPR/Cas system, his group showed that the presence of these potentially toxic (or "suicidal") sequences can only be sustained when accompanied by mutations which inactivate the CRISPR/Cas system. He concluded that, most likely, the chromosomally derived spacers do not serve a regulatory function, but were merely incorporated by accident during the course of evolution.

A number of groups reported on the functional studies of unknown viral proteins. Pieter-Jan Ceyssens (University Leuven, Belgium) used protein–protein interaction (PPI) assays in the study of uncharacterized bacteriotoxic proteins produced by a diverse clade of virulent *Pseudomonas aeruginosa* phages. Similar assays were used by the group of Mark Young (Montana State University, Bozeman, MT) to analyse C92, a protein synthesized by the archaeal virus STIV, infecting the Archaeon *Sulfolobus*. This protein forms remarkable pyramid-like structures on the archaeal cell wall (also discussed by Tessa Quax), which open upon the release of newly formed STIV virions. Jamie Snyder described how STIV recruits a host ATPase to this egress point on the cell membrane by hijacking the cellular ESCRT-III (endosomal sorting complex required for transport) paralogue. This provides a fundamental link between viruses of Archaea and Eukarya (e.g., HIV), which use a similar subset of cellular proteins.

Chloë Danioux (Institut Pasteur, Paris, France) described a novel transcriptional repressor, SvtR, of the archaeal virus SIRV2. This protein binds upstream of its target DNA and polymerizes towards the promoter, causing the disaggregation of the archaeal transcription initiator complex in a novel "run and kick" mechanism of transcriptional repression.

Many temperate phages are integrated into their host chromosome to form a lysogen, and lysogenization plays a key role in bacterial evolution. Using an *Escherichia coli*/λ phage mouse model system, Marianne De Paepe (INRA Jouy-en-Josas, France) showed that lysogenization occurred even more efficiently in the mouse than in Petri dishes. During induction, Red-β-mediated recombination of λ with defective prophages occurred frequently, which might be a pervasive force in lateral gene transfer.

Maggie Smith (University of Nottingham, UK) presented novel work on the serine integrase of *Streptomyces* phage ΦC31. This enzyme catalyses both integration and excision of the phage genome. These events are controlled by conformational changes of the Int protein, which forms a flat interface allowing rotation around the DNA substrates. The auxiliary ΦC31 protein Gp3 specifically activates excision by forming synaptic complexes between *attL* and *attR* sites. She concluded that this system of integration and excision has proven very valuable in genetic engineering, as it is a controllable system which only requires short stretches of DNA to mediate recombination.

Superantigen-encoding pathogenicity islands (SaPIs) of *Staphylococcus aureus* "pirate" phages to spread among their hosts. SaPI1 is de-repressed by its 80α helper phage, and specifically packaged into T = 4 capsids, which are smaller than the capsids made by the helper (T = 7). Gail Christie's laboratory (Virginia Commonwealth University, Richmond, VA) unravelled the molecular mechanisms behind the differential assembly of the two capsid forms. Her group identified two unique SaPI-encoded proteins in the SaPI procapsid, both of which are necessary and sufficient to direct small capsid formation. One of these proteins, CpmB, constitutes internal scaffolding, which promotes efficient redirection of capsid size, driven by the action of CpmA.

Bradley Hillman (State University of New Jersey, Rutgers, NJ) presented an overview of the large diversity of viruses infecting the chestnut blight fungus, *Cryphonectria parasitica*, and he mainly focused on the genome content and gene expression analysis of representatives of the hypoviruses and reoviruses.

Human transcriptome analysis led to the remarkable identification of a higher frequency of chlorovirus sequences in brains of patients with mental disorders compared to the control group. The results of James Van Etten (University Nebraska, Lincoln, NE), indicating the presence of the algae virus PBCV-1 in human tissues and blood samples and its persistence in mice up to 4 weeks after viral inoculation, strongly suggest the existence of a novel class of viruses capable of infecting mammals.

2.2. Bacteriophage-based antibacterials

The lack of new antibiotics and the increasing public health issues caused by bacteria resistant to antibiotics led to renewed interest in the use of bacterial viruses as antibacterials.

Applications of bacteriophages or of bacteriophage-derived enzymes have been reported during the past years for prevention of food contamination and medical devices or treatment of human and animal bacterial infections. As an example, the demand for alternative drugs and growth promoters for livestock is now increasing in response to restrictions on the use of antibiotics for animal production.

Paul Barrow (University of Nottingham, UK) recalled that in the UK, several experiments performed back in the 80s demonstrated that bacteriophages were efficient in reducing bacterial infections in cattle. He reported on more recent applications for the control of *Campylobacter* and *Salmonella* infections in poultry. However, despite these convincing data,

there are currently no bacteriophage-based products in use in veterinary settings.

To develop bacteriophage applications for humans, it is important to learn more about the immune response to bacteriophages, a vast question that Krystyna Dabrowska (Institute of Immunology and Experimental Therapy, Wroclaw, Poland) tried to answer using bacteriophage T4 head proteins gp23, gp24, Hoc and Soc. She looked at antibody production in mice in response to injection of purified T4 proteins or T4 viral particles, and at the presence of antibodies recognizing these proteins in normal human blood samples. Gp23, the major capsid protein and the most abundant in the T4 virion, was the most antigenic protein in mice, but also the only protein for which antibodies were found in human blood. Whether major capsid proteins from other bacteriophages would be the most antigenic virion protein remains to be analysed.

Until recently, the food industry mainly ignored bacteriophages, except for the dairy sector where phages represent a source of significant economic loss when they infect and destroy bacterial cultures used for fermentation. The usual method for preventing bacteriophage contamination, i.e. rotation of starter cultures, is still in use. However, as detailed by Douwe van Sideren (University College, Cork, Ireland), recent research aimed at improving the control of phages in starter cultures has focused on the molecular understanding of the relationship between bacteriophages and their hosts. The base plate assembly process of two *Lactococcus* bacteriophages carrying host protein recognition domains was reported, including the tail fibres, which can harbour endopeptidase activity used for degrading the host peptidoglycan.

In contrast to the dairy industry, vegetable production could benefit from bacteriophages used as antibacterials, as vegetables grown in fields are often exposed to multiple bacterial pathogens. Evelien Adriaenssens (University Leuven, Belgium) reported on the efficacy of bacteriophages to reduce soft rot caused by *Dickeya solani* on potatoes in the field, resulting in a cost-effective yield increase. Only two different T4-related bacteriophages were sufficient to obtain these results, using experimentally infected potatoes. To reach sufficiently high titres, the phages were produced with CIM columns (BIA Separations, Ajdovščina, Slovenia; see also talk by Milos Barut), a process that could be scaled up for industrial production.

As detailed by Martin Loessner (Institute of Food Science and Nutrition, Zurich, Switzerland), bacteriophages could also be used to prevent bacterial food contamination. Bacteriophages targeting *Listeria monocytogenes,* for instance, greatly reduced the level of contamination in cheese.

In the line of preventive application, early detection of pathogens is highly beneficial not only for the food industry, but also for human health in general. Taking advantage of the cell-binding domain (CBD) of phage endolysins, several tools were developed as green fluorescent hybrid proteins allowing for the specific visualization of *Listeria* cells. Another application was described, for which magnetic beads were coated with several copies of CBD domains, allowing the isolation of specific bacteria from various complex media using magnets, followed by the subsequent detection of these bacteria.

Not only bacterial cells, but also spores should be taken into account when pathogen detection is considered. Tarek El-Arabi (University Guelph, Canada) reported on a new bacteriophage, member of the Myoviridae, which targets spores of the *Bacillus cereus* group. Using PCR with a probe highly specific for the bacteriophage genome, less than 10 spores per ml could be successfully detected under laboratory conditions.

The whole endolysin can also be used to target and kill Gram-positive bacteria. The most potent among these enzymes, PlyC, was isolated from a bacteriophage infecting streptococcal species. Two talks, by Yang Shen and Daniel Nelson (Institute Bioscience and Biotechnological Research, University of Maryland, Rockville, MD), shed light on two different properties of this enzyme. Its X-ray crystal structure was recently obtained. The active enzyme is composed of one copy of the PlyCA polypeptide, tethered to a ring-like structure formed by eight copies of the PlyCB polypeptide. The first 8 amino acids of the latter are involved in the binding of the former. Interestingly, two catalytic domains (CHAP and glycoside hydrolase) not studied before were revealed and experimentally confirmed. Together with eight CBD moieties, these domains may explain the higher potency of this enzyme compared to other endolysins. These eight CBDs display several positive charges that are involved in another particularity of PlyC, its ability to penetrate eukaryotic cells. PlyCB alone was sufficient to be internalized, as revealed by 3D electron microscopy. Epithelial cells containing intracellular *Streptococcus* cells were also used to show that purified PlyC could efficiently sterilize this co-culture system, demonstrating the potential use of this enzyme to target and kill intracellular pathogens.

A workshop open to a larger audience of medical doctors and nurses and chaired by Isabelle Huys (University of Leuven, Belgium) and Martin Zizi (President of P.H.A.G.E (Phages Human Applications Group Europe)), was dedicated to key issues related to human phage therapy. More details on this workshop can be found elsewhere in this issue.

2.3. Metagenomics, viral diversity and evolution

The ‘metagenomics, viral diversity, and evolution’ sessions spanned the tiniest (ssDNA phages) to the largest (Mimiviruses) viral genomes, with discoveries and surprises in nearly every talk. The emerging theme centred on viral diversity, but demonstrated that the field is now clearly moving beyond ‘counts’ and ‘marker studies’ to develop a broader understanding of the myriad of viral forms and ways that viruses in nature succeed.

Christelle Desnues (Université de la Méditerranée, Marseille, France) documented the diverse mobile elements (provirophage and transpovirons) that appear prominent in the evolution of giant viruses (‘Giruses’); Matthias Fischer (Max Planck Institute for Medical Research, Heidelberg, Germany) focused on the structural characterization and evolutionary history of CroV (*Cafeteria roenbergensis* virus) particles, and Steven Wilhelm (University of Tennessee, Knoxville, TN) shared technical challenges and tremendous progress in mapping approximately 20 new Girus genomes (challenging genomes to obtain!),

derived from flow cytometry sorting and sequencing. Phytoplankton viruses were highlighted by Keizo Nagasaki (Fisheries Research Agency, Yokohama, Japan), who summarized 20 years of harmful algal bloom research while outlining promising future research directions, as well as by Hervé Moreau (Oceanological Observatory, Banyuls-sur-Mer, France), who detailed findings on phytoplankton resistance to viruses. At the smaller end of the spectrum, Sioban Duffy (State University New Jersey, Rutgers, NJ) explored the molecular mechanism driving high mutation rates in ssDNA phages (almost 1/3 of RNA virus mutation rates), which was due predominantly to C→T deaminations resulting from oxidative damage of ssDNA that is commonly in a highly unordered state. Matthew Sullivan (University Arizona, Tucson, AZ) combined new methods (viral-tagging and quantitative electron microscopy) with metagenomics to experimentally link viruses to hosts in a high-throughput manner and to identify which viral reference genomes are needed to map 'unknown' viral metagenome sequence space. Forest Rohwer discussed the necessity to revise evolutionary theories in the light of viruses being the winners in the game of life and the Viral Information Hypothesis, which states that genetic information replicates itself to the detriment of system energy efficiency (for details, see http://www.youtube.com/watch?v=GOixYJfwUgA&feature=plcp).

2.4. Structure–function relationship in virions

The structure–function relationship sessions made it clear that the structural deciphering of whole or parts of viruses can provide insight into a wide variety of standing issues, ranging from viral and cellular evolution to virion assembly and infection dynamics.

By comparing capsid structures and scrutinizing the way viruses are constructed, Dennis Bamford (University of Helsinki, Finland) demonstrated that there are only a limited number of pathways to fold proteins that assemble into a virion. As a result, a higher-order classification of viruses can be established into distinct structural lineages or morphotypes regardless of their genomic similarity or of the hosts they infect. Bamford further advocated that viruses might have a polyphyletic origin that pre-dates the monophyletic divergence of their cellular hosts into bacteria, archaea and eukarya. As an illustration of the paradigm that the vast viral sequence diversity is reduced to only a limited structure–space, Hanna Oksanen (University of Helsinki, Finland) reported isolation from a hypersaline environment of an icosahedral, membrane-containing, double-stranded DNA bacteriophage (designated Salisaeta icosahedral phage 1 or SSIP-1) that shared its architectural principles with the PRD1–adenovirus structural lineage. With the addition of a novel extremophilic bacteriophage, this structural lineage includes members that infect cells belonging to each of the three domains of life regardless of ecological constraints, and supports the view that they might share an ancient common origin. Furthermore, through recapitulating the current knowledge on the physical and structural aspects of the elaborate viral factory that is mounted by giant Mimiviruses in the cytoplasm of their amoeba host, Abraham Minsky (Weizmann Institute Science, Rehovot, Israel) proposed that these highly organized factories might have acted as precursors of eukaryotic nuclei and thus have played a role in the emergence of eukaryotic cells.

In addition to evolutionary insights, structural analysis of viruses and their components revealed a number of intricacies concerning the different steps of the viral infection cycle. Regarding adsorption and entry steps, Lasha Gogokhia (University of Utah, Salt Lake City, UT) presented data on a capsid decoration (Dec) protein of the L phage of *Salmonella enterica serovar Typhimurium*, which seems to act as an accessory stabilizer of the virion in addition to allowing for weak binding to the host cell surface. This Dec protein was proposed to play a role in initial and reversible attachment of the virion to the host, likely in cases where the initial collision of the phage particle with the bacterial cell is not tail-first.

In the context of adsorption, some viruses have to degrade their way through the host's capsule before reaching their cognate receptor. Some capsules contain polysialic acid and attract phages whose tailspike proteins contain unique endosialidases that can specifically recognize and degrade polysialic acid present in the glycocalyx of bacterial cells. However, polysialic acids are important onco-developmental glycotopes in mammalian cells and are also present in the capsules of some neuroinvasive bacteria. Rita Gerardy-Schahn (Hannover Medical School, Hannover, Germany) demonstrated that these highly specific phage endosialidases could lead to new ways of targeting cytotoxic agents to malignant and/or pathogenic cells.

Further dealing with adsorption and infection, Ian Molineux (University of Texas, Austin, TX) described the intricate dynamics of phage T7 as it adsorbs to *E. coli* and then penetrates the cell membranes.

Petr Leiman (École Polytechnique Fédérale de Lausanne, Switzerland) reported more on the structural and functional similarities between contractile phage tails and type VI secretion systems. The cell-puncturing tip of the internal tube that is extruded by these contractile secretion systems was shown to contain a needle-shaped structure formed by a long β-helical domain, an oligonucleotide/oligosaccharide binding fold (OB-fold) and a domain that is orthologous to gp27 of phage T4. This tip is responsible for the initial interaction with the host membrane and different distinct classes of such tips are likely to exist. The exact organization of the tip is determined by the structure of the host cell envelope, which is targeted by the secretion system or by phage.

Paulo Tavares (CNRS, Gif-sur-Yvette, France) focused on interactions between the portal and head completion proteins that make up the genome gatekeeper in phage SPP1. Structural and functional analysis of the interactions within this gatekeeper, in the mature virion before tail attachment, as well as after DNA release, provided a model for how this protein complex prevents premature leakage of the viral genome while allowing its ejection upon infection.

Structural insights into the replication of RNA viruses stemmed from the presentation by Fleur Dolman (University Aarhus, Aarhus, Denmark) on the virus-encoded RNA-dependent RNA polymerase (RdRP), which interacts extensively with

host-encoded proteins during infection to form a complex that ensures template specificity and polymerase activity. Building further on the recently elucidated structure of the RdRP core complex of the Qβ RNA phage, the importance of key residues in the enzyme's palm and dimerization regions was discussed, together with their effects on the kinetics of the enzyme.

Once the viral genome is replicated, virion assembly and maturation take place. Pascale Boulanger (Institut Biochimie Biophysique Moléculaire et Cellulaire, Orsay, France) discussed this process in *E. coli* phage T5 and emphasized the central role for the pb11 head protease in procapsid maturation. Time-resolved small angle X-ray scattering revealed that procapsid expansion upon packaging of the phage DNA is a two-state event that depends on cooperative conformational changes within the capsid, leading to its stabilization without the need for either cross-linking between the coat protein subunits or additional cementing proteins.

Finally, several viral release strategies were detailed from a structural point of view. Before secretion of the Pf3 filamentous phage, for example, its coat protein inserts itself into the *E. coli* host membrane with the help of the YidC membrane insertase. Studying this interaction both in vitro and in vivo, Andreas Kuhn (University Hohenheim, Stuttgart, Germany) revealed that the C-terminus of the Pf3 coat protein binds the cytoplasmic portion of YidC, after which the coat protein's N-terminal domain translocates and contacts the periplasmic domain of YidC. Moreover, YidC residues important for this interaction have been identified, increasing our understanding of how YidC provides a hydrophobic surrounding for inserting substrate proteins into the membrane. These data clearly underscore how the analysis of viral components can improve our understanding of the host components they interact with.

A totally different form of viral release was highlighted by Tessa Quax (Institut Pasteur, Paris, France), who elaborated on the genesis of baseless hollow pyramids that emerge on the surface of *Sulfolobus islandicus* cells when infected with the *S. islandicus* rod-shaped virus 2 (SIRV2). These virus-associated pyramids (VAPs) consist of multiple copies of the SIRV2-encoded P98 membrane protein and adopt a seven-fold rotational symmetry that can open up to release the mature virions from the cell. Furthermore, since these VAPs are capable of self-assembly even when expressed in a heterologous host such as *E. coli*, they represent a novel viral-encoded self-assembling entity that is distinct from the capsid.

2.5. Viruses in biotechnology

The biotechnology session presented a wide range of technological advances ranging from novel *Clostridium difficile* phages as candidates for phage therapy to very new technologies, including a reversible phage element that could allow the DNA to 'count' the cell's exposure to various environmental cues and stack crystallized filamentous phages into hierarchically organized structures for electricity generation. Presentations on bioinformatic approaches to identify nuclear localization signals and their potential roles in gene transfer and on the use of phage lysins and advances in large scale phage purification strategies rounded out this diverse session.

Martha Clokie (University Leicester, UK) described the characterization of the temperate bacteriophages that are associated with clinical and environmental strains of *C. difficile*, including many that lyse numerous clinical strains of *C. difficile*. This pathogen is difficult to eradicate from hospitals where it is generally associated with a patient history of broad-spectrum antibiotic therapy. Patients either become colonized by asymptomatic strains or they become infected by persistent spores from strains that dominate health care settings. *C. difficile* infection is responsible for up to 25,000 deaths per year in the USA alone. Clokie expanded her studies to include a hamster model in collaboration with her colleague Gill Douce (University Glasgow, Scotland), showing that phage application enabled significant reduction in the gut pathogen.

Maarten Walmagh (University Leuven, Belgium) described the secondary structures of bacteriophage endolysins originating from phages infecting Gram-negative bacteria as being either globular (T4-like) or modular. The latter are unique as they consist of an N-terminal peptidoglycan binding domain and a C-terminal catalytic domain, an architecture which is the opposite of the modular structures frequently found in endolysins from phages infecting Gram-positive hosts. Comparison of representative enzymes with these structures (globular vs. modular) demonstrated the modular ones to be more active against permeabilized cells of three different Gram-negative species. A strategy was developed for exogenous application of these modular endolysins, based on the fusion of different outer membrane destabilising/disrupting tags to the wild type protein. These tags are: 1) cationic, disrupting the ionic stabilizing forces of the lipopolysaccharide layer; 2) hydrophobic, intercalating in the hydrophobic stacking of the fatty acid chains in the phospholipid bilayer of the outer membrane; or 3) amphipathic, allowing for efficient passage of the endolysins through the outer membrane to their peptidoglycan substrate. Up to 40 different tag-fused constructs were tested for their in vitro antibacterial potential and the most active ones were able to protect both human keratinocyte cultures and *Caenorhabditis elegans* SS104 nematodes from the lethal effects of the highly cytotoxic *P. aeruginosa* PA14 strain. These results are promising for the use of these tag-fused proteins for protection or eradication of Gram-negative infections.

A very interesting presentation by Modesto Redrejo-Rodriguez, from Margarita Salas' lab at the Centro de Biologia Molecular "Severo Ochoa" (Madrid, Spain), described a bioinformatic search and experimental verification that identified sequences that behave as eukaryotic nuclear localization signals on the φ29, PRD1 (and other) terminal proteins (TP). These elements prime DNA replication of some phages and become covalently linked to their genomes. It was proposed that nuclear targeting might be an evolutionarily conserved feature of phage terminal proteins, potentially facilitating horizontal gene transfer between distantly or unrelated organisms. Moreover, heterologous DNA can be amplified in vitro in order to incorporate the φ29 TP, providing DNAs with enhanced delivery for downstream applications.

Drew Endy (Stanford University, CA) presented work regarding genetically encoded memory systems. His team has bioengineered chromosomal data storage devices by using serine integrases from bacteriophages. For example, they implement a binary digit chromosomal data register wherein the orientation of a reversible phage element could allow the DNA to 'count' the cell's exposure to various environmental cues. Unpublished work from the new BIOFAB project (biofab.org) was also presented, with elegant measures to precisely regulate gene expression with high reliability by using nested and overlapping expression control elements, as found in many phage genomes. Endy finally pointed to a growing global effort, known as the iGEM (International Genetically Engineered Machine) Foundation, that has thus far enabled over 10,000 young would-be bioengineers to discover and invent the future of biotechnology by working together (http://www.igem.org).

Seung-Wuk Lee (University of California, Berkeley, CA) described fascinating work wherein phages have been used to design materials with a wide range of functions. He terms his novel virus-based material design approaches "virotronics". His work includes specific biosensors and photonic and energy-producing devices using genetically engineered viruses. His system takes advantage of M13 filamentous phage as a basic building block and stacks the phages into hierarchically organized structures. By exploiting the resulting structures, Lee's group can fabricate multiple functional materials including piezoelectric-based electric energy generators. He envisions small portable energy-generating biomachines that will be worn or inserted into the human body at positions where normal flexing of muscles will generate small amounts of energy to power such things as pacemakers.

Dave Donovan (Agricultural Research Service (ARS), US Department of Agriculture, Beltsville, MD)) presented work by Dwayne Roach, a Fellow in his group, who investigates *Lactobacillus* phage endolysins as antimicrobial agents expressed in yeast to protect fuel ethanol fermentations from bacterial contamination. Lactobacilli contaminate commercial fuel ethanol cultures and reduce yields by competing with yeast for nutrients and releasing organic acids that lower pH and inhibit yeast growth. The presented data demonstrated that purified lysins kill several species of *Lactobacillus* isolated from commercial fuel ethanol facilities. The lysins were active under conditions similar to the fermentation environment, including both mildly acidic pH and up to 5% ethanol. When purified lysin was added exogenously to a mock fermentation that was experimentally infected with lactobacilli, the bacterial load was reduced by 1.5 log CFUs. At least one gene, encoding a lysin, has been cytoplasmically expressed in yeast by ARS colleagues Steve Hughes, Ken Bischoff and Piyum Khatibi (ARS, Peoria, IL).

Milos Barut (BIA Separations, Ajdovščina, Slovenia) described methylacrylate-based monolithic columns that are engineered to purify large biomolecules, including phage particles. High purity of phage particles is a hurdle faced by all human phage therapy applications and thus purification is an essential and much-needed technology. This presentation focused in part on how to design an efficient small-scale purification process of various phages based on their biophysical properties and to then transfer that pilot work to a large preparative scale. Other advantages of these monolithic columns include near-real-time analytics of what is happening in the biomanufacturing process as a tool to help process developers determine the appropriate time for product extraction.

3. Additional information

The information exchange has now been further substantiated by a special issue published in Virology (Elsevier, 2012, 'Viruses of Microbes', editors Molineux, Toussaint, Prangishvili). In addition, several presenters at the conference have given permission to make the video of their lecture publically available through the conference website, or directly at http://www.youtube.com/user/EMBOVoM2012.

The four poster awards went to:

Metagenomics: Tomohiro Mochizuki (Institut Pasteur, Paris, France): An archaeal virus ASPV with exceptional virion architecture and the largest single-stranded DNA genome, a first member of the proposed family "Spiraviridae".

Structure–function relationship: Nikki Dellas (Montana State University, Bozeman, MT) Insights into structural and functional biomolecular machinery of thermophilic archaeal viruses.

Virus–host interactions: Isabel Holguera López (Instituto de Biología Molecular "Eladio Viñuela" (CSIC), Madrid, Spain). Disclosing the in vivo organization of a viral histone-like protein in *Bacillus subtilis* mediated by its capacity to recognize the viral genome.

Biotechnology and antibacterials: Aleksandra Glowacka (University Life Sciences, Warsaw, Poland). Staphylococcal nematode infections as a model system for pre-testing of potential therapeutic phages.

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