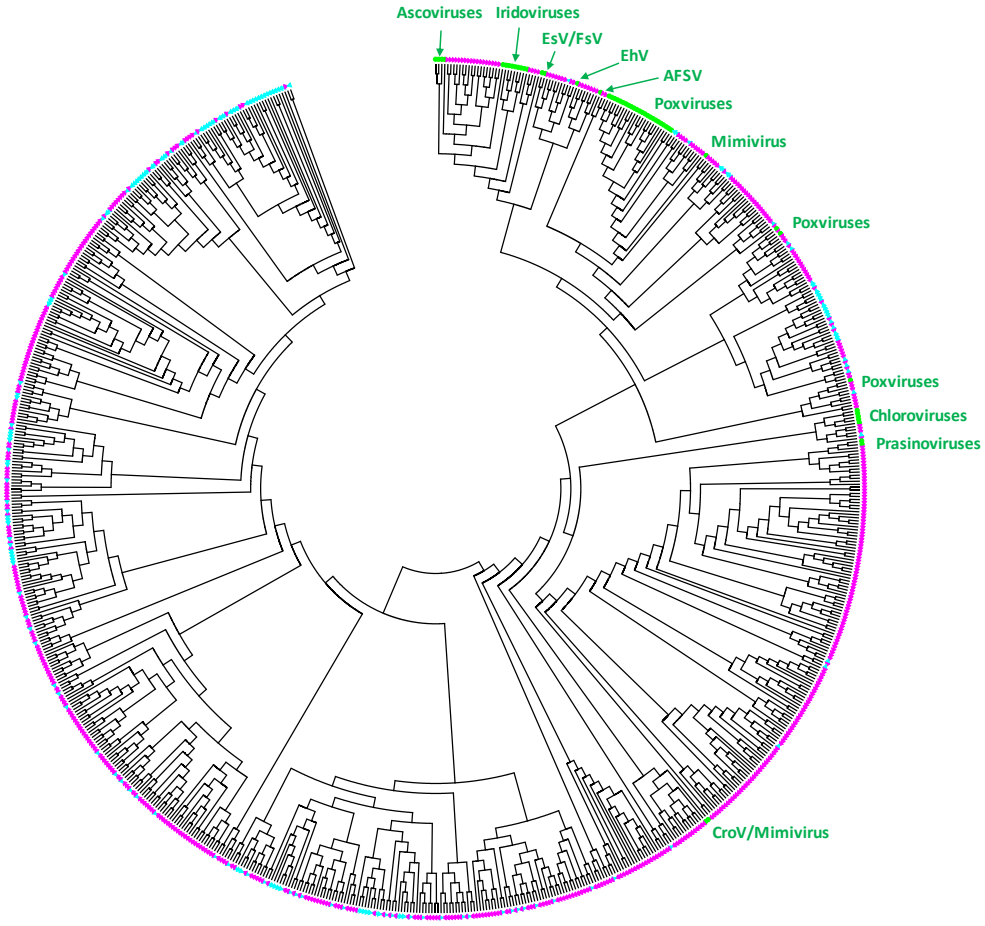
International conference on the

Viruses of the Environment

Challenges in Viral Ecology and Evolution

March 22-23, 2011
Heidelberg, Germany



Organizers:

Hiroyuki Ogata, Jean-Michel Claverie, Eric Karsenti,
Stefanie Kandels-Lewis, Maurice Heral

Meeting venue:

ISG Hotel, Im Eichwald 19, 69126 Heidelberg, Germany
Tel: +49 (0) 6221 38 61-0

International conference on the Viruses of the Environment:
Challenges in Viral Ecology and Evolution

March 22-23, 2011
Heidelberg, Germany
ISG Hotel, Im Eichwald 19, 69126 Heidelberg, Germany, Tel: +49 (0) 6221 38 61-0, Fax: +49 (0) 6221 3861-200, E-mail: isg@embl.de

Theme and objectives:

Viruses are ubiquitous on our planet. They are found in all cellular life forms from Bacteria, Archaea to Eukaryotes. They show spectacular diversity at both molecular and morphological levels. They play important ecological roles and take part in global biogeochemical cycle. Viruses are alive. They played active roles in the evolution of cells. All these discoveries and recent ideas point to the importance of the ongoing efforts to study viruses of the environment. The international conference entitled “Viruses of the Environment: Challenges in Viral Ecology and Evolution” gathers leading scientists studying viruses from different ecological and evolutionary perspectives, provides enhanced opportunities to exchange recent discoveries/ideas and aims to identify important issues towards advancing this growing field of life science. Themes to be discussed at the meeting include fundamental problems in viral ecology, evolution, genomics and metagenomics. The meeting is organized in collaboration between the TARA-OCEANS consortium and the French National Agency for Research (ANR) as a series of meetings entitled “Atelier de Reflexion Prospective (ARP)”, with a specific goal to build on recent results and observations to make a state-of-the-art position report and suggestions to the ANR committees and other funding agencies for future lines of research in the viral research field that should be funded in priority. The size of the meeting will be a moderate one (with the maximum of 40 participants) which we consider appropriate for having a successful meeting with lively contributions from all participants.

Organizers:

Hiroyuki Ogata (CNRS, France)
Jean-Michel Claverie (CNRS, France)
Eric Karsenti (EMBL, Germany)
Stefanie Kandels-Lewis (EMBL, Germany)
Maurice Heral (ANR, France)

Cover figure: A phylogenetic tree of NCLDVs including environmental sequences (DNA packaging ATPase)

Scientific program

Tuesday, 22nd of March 2011

8:30-8:40 **Eric Karsenti** (EMBL) / Welcome address

Session 1: New dimensions of the virus world (chair: Willie Wilson)

8:40-9:20 **Luis Villarreal** (Univ. California, USA)
9:20-10:00 **Patrick Forterre** (Inst. Pasteur, France)
10:00-10:40 **Dennis H. Bamford** (Univ. Helsinki, Finland)

Coffee break

11:00-11:40 **Jean-Michel Claverie** (Univ. Méditerranée, France)
11:40-12:20 **David Prangishvili** (Inst. Pasteur, France)

Lunch

Session 2: Ecological impacts (chair: Hiroyuki Ogata)

14:00-14:40 **Gunnar Bratback** (Univ. Bergen, Norway)
14:40-15:20 **Keizo Nagasaki** (Fisheries Research Agency, Japan)
15:20-16:10 **Tristan Renault** (Ifremer, France)

Coffee break

16:30-17:10 **Jean-Robert Bonami** (CNRS, France)
17:10-17:50 **Matthew Sulivan** (Univ. Arizona, USA)
17:50-18:30 **Telesphore Sime-Ngando** (CNRS, France)

Dinner (19:30-)

Organizers kindly request to speakers:

- **to keep the timing of your talk**
- **to provide your presentation slide (.ppt, .pdf) to Stefanie Kandels-Lewis/Hiroyuki Ogata at the meeting** (this would be extremely useful for the organizers to make reports of the meeting)

Wednesday, 23rd of March 2011

Session 3: Diversity in the biology of viruses (chair: Pascal Hingamp)

8:30-9:10 **Willie Wilson** (Bigelow Laboratory, USA)
9:10-9:50 **Mike Allen** (Plymouth Marine Lab., UK)
9:50-10:40 **Hiroyuki Ogata** (CNRS, France)

Coffee break

11:00-11:40 **Jean-Michel Drezen** (CNRS, France)
11:40-12:20 **Matthias Fischer** (Univ. British Columbia, Canada)

Lunch

Session 4: Diversity in the biology of viruses (chair: Mike Allen)

14:00-14:40 **Nigel Grimsley** (CNRS, France)
14:40-15:20 **Pascal Hingamp** (Univ. Méditerranée, France)
15:20-15:40 **Mart Krupovic** (Inst. Pasteur, France)
15:40-16:00 **Guenther Witzany** (Telos-Philosophische Praxis, Austria)
16:00-16:20 **Simon Roux** (Clermont Université, France)

Coffee break

16:40-17:40 Discussion: *"Thematics/projects: What should be financed?"*

Dinner (19:30-)

“Why should virus contribute to host evolution?”
(or better yet; why life needs viruses to evolve)

Luis P. Villarreal

Center for Virus Research
Department of Molecular Biology and Biochemistry
University of California, Irvine, USA

In the last two decades, we have come to realize that our world is a vast viral habitat. Metagenomics informs us that not only do viruses dominate most habitats, but most genomes also harbor a complex mix of viruses and other mobile genetic parasites. All life must have attained stability with its virus populations. We generally think of viruses as the ultimate selfish agents and viruses can ‘pirate’ host genes. But we are now realizing that the persistence of virus derived information has had big consequences for the evolution of life. The stability of virus persistence, however, is not inherent to selfish DNA concepts. My interest has long been to understand the mechanisms and consequence of virus persistence to the evolution of life. I will thus outline various concepts that can explain how viruses affect host populations. These concepts include virus mediated immunity, addiction modules and horizontal population based selection. I argue that these persistence mechanisms inherently promote group identity. Consortial based evolution has long been dismissed by evolutionist. Yet viruses have experimentally been shown to display cooperative fitness, consistent with modern quasi species theory. In addition, more recently biocommunication theory (pragmatics) informs us that any real code (e.g. genetic) requires population based agents to edit the code. Mechanisms based on fittest type individuals will fail as code editors. Viruses (and TEs) appear to provide such populations. Given these distinct conceptual foundations, we now examine several examples of virus mediated evolution. The ion channel genes of chlorellavirus virus appear associated with virus identity/competition. I will present phylogenetic analysis by my colleagues which indicate these viral genes are likely ancestral to host genes. I then consider the eukaryotes, which have a distinct virus-host equilibrium that involves much more retroviral activity, resulting in a regulatory, not gene centric based evolution. I briefly consider mammalian reproduction and why sets of retrovirus genes were ‘pirated’ by the host (placenta). ERV colonization and TE disturbances seems to be a defining event in recent evolution of mammals which can be better understood with the above concepts.

Viruses, plasmids and membrane vesicles in gene transfer: what can we learn from Archaea?

Marie Gaudin, Nicolas Soler, Evelyne Marguet and Patrick Forterre

Institut de Génétique et Microbiologie, Univ Paris-Sud, UMN CNRS 8621 and Institut Pasteur, 25 rue du Dr Roux. 75015, Paris.

Beside a few genes with viral signatures, most genes in environmental viromes are ORFans (genes without homologues in database). This testifies for the creativity of virocells (1) in which new specific viral proteins are constantly produced. Viromes also contain cellular genes whose origin is unclear. It is often assumed that these genes testify for a major role of viruses in lateral gene transfer (LGT) between cellular organisms. However, this traditional view remains to be validated by comparative genomic analysis. This is not an easy task, since it is not easy to distinguish viral genes from cellular genes in global analyses. Here, I will argue that we can learn much in focusing on specific groups of organisms, presenting preliminary analyses of viruses and plasmids (VP) from hyperthermophilic archaea of the order Thermococcales. We conclude from this analysis that VP are not major agents of LGT between cells, but play a major role in evolution by introducing new genes into cellular genomes (2,4,5).

We have recently started to investigate in Thermococcales a new phenomenon that could be involved in LGT between cells: the production of membrane vesicles (MV). All ribocells (Archaea, Bacteria, Eukarya) produce MV that ressemble small virion size (50-200 nm). In Archaea, MV are produced by budding of the cytoplasmic membrane, resembling eukaryotic ectosomes (6). Importantly, archaeal MV are associated to cellular DNA and produce fluorescent spots that cannot be distinguish from virions by epifluorescence microscopy (6). This suggests that many putative virions detected in environmental studies by epifluorescence microscopy could by MV and that cellular DNA present in viromes could originated from MV. Accordingly, MV, instead of viruses, could be major vehicles for gene transfer in the environment. Interestingly, we recently identify MV-containing plasmid and viral genomes in *Thermococcus nautilus*, suggesting that MV could be also used as vehicles for the transfer of PV. Considering the striking morphological similarity between some virions and MV, one can also wonder if production of MV has been involved in the origin of some viruses.

Until now, MV have been only studied in a handful of laboratories and we lack an integrated view of the phenomenon. For a long time, MV have been considered as cellular dusts without significance. Only recently the importance of MV in human physiology has been realized. For instance, it is now recognized that human MV can transfer microRNA to recipient cells, modulating their activity (7). The presence and roles of MV in the environment have rarely been studied, and the molecular mechanisms of MV formation in the three domains of life are poorly known. These are promising areas of research that should be supported by funding agencies.

1. Forterre, P., Manipulation of cellular syntheses and the nature of viruses : the virocell concept C.R. Chimie, doi:10.1016/j.crci. (2010)
2. Soler N, Gaudin M, Marguet E, Forterre P. Plasmids, viruses and virus-like membrane vesicles from Thermococcales. Biochem Soc Trans. 2011 39:36-44.
3. Krupovic, M., Gribaldo, S., Bamford, D., Forterre, P. The evolutionary history of archaeal MCM helicases: a case study of vertical evolution combined with hitch-hiking of mobile genetic elements. Molecular Biology and Evolution (2010) doi: 10.1093/molbev/msq16
4. Krupovic, M , Forterre P , Bamford DH . Comparative Analysis of the Mosaic Genomes of Tailed Archaeal Viruses and Proviruses Suggests Common Themes for Virion Architecture and Assembly with Tailed Viruses of Bacteria. J Mol Biol. (2010) 397:144-160
5. Cortez, D., Forterre P. and Gribaldo, S.;A hidden viral reservoir is the major source of foreign genes and ORFans in archaeal and bacterial genomes. Genome Biology, 10:R65 (2009)
6. Soler, N. Marguet, E. VerBavatz, J.M. and Forterre, P. Virus-like vesicles and extracellular DNA produced by hyperthermophilic archaea of the order Thermococcales. Res Microbiol., 159:390-399 (2008).
7. Gibbings DJ, Ciaudo C, Erhardt M, Voinnet O. Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. Nat Cell Biol 2009; 11:1143-9.

Organization of the viral universe

Dennis.H. Bamford

Institute of Biotechnology and Department of Biological Sciences.
Biocenter 2, P.O. Box 56, University of Helsinki, Finland

Viruses are the most abundant living entities in the biosphere outnumbering their host organisms by one to two orders of magnitude. It is conceivable that they cause the highest selective pressure their hosts encounter. As obligate parasites viruses are dependent on their hosts but their origins seem to deviate from that of cellular life.

What are the possible principles to build viruses is an open question. However, structural studies on virus capsids and coat protein folds propose that there are only a limited number of ways to construct a viral protein coat. Consequently, relatedness of viruses is not connected to the type of cells they infect and the same architectural principle of the capsid has been observed in viruses infecting bacteria as well as humans. Using the viral capsid structure it is possible to group viruses to several lineages that may have existed before the three cellular domains of life (bacteria, archaea and eukarya) were separated. This would mean that viruses are ancient and that early cells were infected with many different types of viruses proposing that the origin of viruses is polyphyletic opposing the monophyletic origin of cellular life. Comparing currently available viral high resolution structures agree with the proposal that in spite of the enormous number of viruses in the virosphere the very limited protein fold space limits strongly the number possible capsid architectures.

The important current issues in virology include a further understanding of the functions of viruses in the entire biosphere and how they modulate cellular life. Understanding of how viruses are constructed and how that relates to the origin and evolution of viruses (and even life) is a related important topic.

Mimivirus and other Giruses: freaks of nature, or key players in Evolution?

Jean-Michel Claverie

Laboratory of Structural and Genomic Information, CNRS - UPR2589, Parc Scientifique de Luminy - 163 Avenue de Luminy - Case 934, FR- 13288, Marseille cedex 09, France
Email: claverie@igs.cnrs-mrs.fr

The wonder world of viruses of the Archaea

David Prangishvili

Institut Pasteur, Department of Microbiology, 25 rue du Dr. Roux, 757245 Paris Cedex 15, France

The discovery of the third domain of life, the Archaea, has led to the replacement of the prokaryote/eukaryote dichotomy by a trinity of domains, the Archaea, Bacteria and Eukarya, and initiated studies on specific aspects of this domain, including the associated virosphere.

From extreme geothermal environments at different geographical locations we have isolated and described 22 different viruses infecting hyperthermophilic members of the Archaea (about half of all known archaeal viruses). The viruses have double-stranded (ds) DNA genomes and exceptionally diverse morphotypes which differ dramatically from those of dsDNA viruses of Bacteria and Eukarya. Due to their unique morphological and genomic properties, the viruses have been assigned to eight families, including rod-shaped *Rudiviridae*, filamentous *Lipothrixviridae*, spindle-shaped *Fuselloviridae*, bottle-shaped *Amplullaviridae*, two-tailed *Bicaudaviridae*, spherical *Globuloviridae*, droplet-shaped *Guttaviridae,* and bacilliform *Clavaviridae*.

In my talk I will summarize the state of art of our studies on archaeal viruses, their morphological, genomic, and biological properties, and lay emphasis on the impact of these studies on our understanding of the world of viruses and its evolution.

Virus Ecology - From VLP to Metagenomes

Gunnar Bratbak

University of Bergen

Viruses has been described as a major cause of mortality, a driver of global geochemical cycles and a reservoir of the greatest genetic diversity on Earth, but still their abundance may be underestimated, accurate estimates of activity are elusive and we don't know what is regulating their activity. The viral community in seawater is presumably dominated by bacteriophages since bacteria are the most abundant host. Viruses infecting algae and protozoa have hosts that can be recognized by their morphology and much of the research has been focused on specific host virus system where the ecological significance of the host is known. Our knowledge about these viruses is thus often far more detailed that for the marine phages. Viruses are counted as Viral Like Particles by electron microscopy, epifluorescence microscopy or flow cytometry, but it may indeed be difficult to recognize and include all viruses and to exclude all non-viral particles. No method for measuring viral activity have yet become standard and rates obtained using different methods vary widely. Moreover, we don't know what is regulating their activity. The diversity of the viral community in natural waters, as assessed by PFGE and metagenomics suggest that a viral community may include thousands of genotypes but be dominated by 10-30 populations each making up less than few percent of the total abundance. Nevertheless, the ecological significance of viruses relate to their ability to transfer genetic material, their effect on carbon and nutrient flow, host population dynamics and community diversity.

Viruses as harmful algal bloom eliminator

Keizo Nagasaki, Natsuko Nakayama, Yuji Tomaru

Fisheries Research Agency, Japan

So far, we have revealed that virus infection is one of the important factors having a significant impact on the dynamics of algal blooms; the impact is not only quantitative (affecting biomass) but also qualitative (changing host clonal composition). Especially, our recent studies are focused on the two major algal groups, dinoflagellates and diatoms. In both cases, we made several host-virus systems into laboratory culture; the viruses were all shown to be considerably different from any known terrestrial viruses. These findings, as such, are of much interest from the viewpoint of pure science concerning virology, marine biology, and studies of evolution. Still, because authors belong to fisheries science field, we are always exposed to a kind of pressure to consider the possible elimination method for harmful algal blooms (HABs). In this talk, we will introduce the outline of our studies on algal viruses for about 20 years and our recent efforts to confront the HAB problem.

Virus infections among marine molluscs: herpes viruses causes today extensive losses in shellfish farming industry worldwide

Tristan Renault

Laboratoire de Génétique et Pathologie, Ifremer, 17390 La Tremblade, France

The interest of studying viruses infecting marine molluscs is twice. Primarily, such studies are needed in order to increase basic knowledge on viruses in aquatic environments. Moreover, mollusc production is the second most important aquaculture activity in the world by quantity and by value. The world production in 2006 was estimated at 14.1 million tonnes, representing 27% of the total world aquaculture production valued at US$ 11.4 billion. By volume, oysters (Ostreidae) are the second most important aquaculture taxonomic group to cyprinids at 4.6 million tonnes. The Pacific cupped oyster, C. gigas, itself had the greatest contribution with a world-wide production volume of 4.4 million tonnes. Although mollusc culture is steadily growing in importance in the aquaculture sector, cultivated molluscs may suffer from severe mortality outbreaks. Among the possible causes is the occurrence of infectious diseases due to a variety of pathogens including viruses.

Since 2008, massive mortality outbreaks are reported in several farming areas among Pacific oysters, Crassostrea gigas, not only in Europe including France, Ireland and the Channel Islands, but also in New Zealand and Australia. These are attributed to a combination of adverse environmental factors together with the presence of the Ostreid herpesvirus 1 (OsHV-1). Mortalities are considerable, particularly in seed stocks resulting in a shortage in supplies of the shellfish over next years.

Multiple mollusc species of economic importance are being also affected as herpes virus or herpes-like viruses have been detected in various marine bivalve species including the European flat oyster Ostrea edulis, Antipodean flat oyster Ostrea angasi, chilean oyster Tiostrea chilensis, carpet shell clam Ruditapes decussatus, Manila clam Ruditapes philippinarum, great scallop Pecten maximus. Infections are often associated with massive mortality outbreaks among larvae and juveniles. Moreover, herpes-like viruses were also reported in marine gastropods. Highly pathogenic herpes-like viruses were reported in several abalone species including Haliotis diversicolor supertexta, H. laevigata and H. ruber associated to massive mortality outbreaks.

In this context, the necessity of studying herpes viruses and herpes-like viruses infecting marine molluscs is confirmed. More knowledge on such viruses is needed in order to develop suitable strategies to minimize their impact on shellfish production.

Viral diseases of aquatic Crustacean

Jean-Robert Bonami

C.N.R.S.
Pathogens and Environment, EcoLag, UMR 5119, cc 093
Université Montpellier 2, Place E. Bataillon, 34095 Montpellier Cedex 5

Since the first report in 1966 of a virus infecting crab in south of France, the number of viral diseases reported in aquatic crustacean has dramatically increased in parallel with aquaculture development. These diseases were described in marine and freshwater crabs, in shrimp and freshwater prawn, in crayfish species and lobsters. To date, the listed viruses in crabs, more than 30 viruses, are at least as numerous as shrimp and prawn viruses. The viral diversity is very large as some particles were characterized as members of the Parvoviridae family (20 nm in diameter) and the largest as a newly created virus family, the Nimaviridae (300 X 100 nm). Of course, shrimp/prawn viruses were the most investigated due to their negative impact on shrimp farming. In fact, losses in shrimp culture attributed to viral diseases are estimated in billions of US $.

As immunity in invertebrates is quite only limited to innate response, vaccination cannot be used and consequently preventive methods or selection of healthy broodstock (or specific pathogen free) were developed to prevent diseases or to limit their impact. These methods are principally based on nucleic acid detection by PCR or RT-PCR.

Among these viral diseases, 3 are listed by the OIE and have to be reported: the WSS (White spot syndrome), the IHHN (Infectious haemocytic and hematopoietic necrosis) and the WTD (White tail disease).

If epizooties were localized in farms or in some producing area, i.e. in under control area, recently it was noted that some viruses were able to infect other groups of crustacean and eventually able to develop in natural environment. The most important example is the WSSV (White spot syndrome virus), which is able to invade quite all crustacean, crabs, shrimp and prawn, crayfish, lobsters as well in marine as in freshwater environment. What can we do if this virus develops in European coasts (production of crabs and lobsters) or rivers (crayfish)? To my knowledge, in European countries, no financial support is for the moment available and the need of crustacean virologists is flagrant.

Ocean viruses: Towards unraveling population genome variability, understanding virus-host interactions and accessing the uncultured

Matthew Sullivan

University of Arizona, Ecology and Evolutionary Biology Department, Tucson, AZ, USA

Viruses are ubiquitous, abundant, and play key roles in the biosphere including mortality, horizontal gene transfer, and manipulation of host metabolism important to global biogeochemical cycles. Despite the advent of numerous new molecular techniques, viral ecology will continue to benefit from novel methods to better study viral-microbe interactions, and circumvent 'cultivation bottleneck' limitations. The Tucson Marine Phage Lab seeks to identify and alleviate these bottlenecks in our ability to interpret and understand phage biology in the wild. In this talk, I will focus on our work with the abundant ocean T4 phages -- from genomes to metagenomes, ecology to molecular evolution -- with particular emphasis on our recent, unpublished molecular evolutionary studies. Beyond T4 phages, I hope to share my vision for future funding calls by emphasizing the need for transformative methods in viral ecology. Specifically, I will present briefly on new methods that we have developed (e.g., a new chemistry-based method for viral concentration) that are or should greatly enable high-throughput studies to examine genomic variability among wild viral populations and to link these observations to hosts and mechanism.

Viruses from unexplored environments and other unexplored microbial parasites in aquatic ecosystems

Télesphore SIME-NGANDO

UMR CNRS 6023, LMGE, Laboratoire Microorganismes: Génome & Environnement, Université Blaise Pascal (Clermont-Ferrand II), BP 80026, 63171 Aubière Cedex, France

Microbial parasites typically are characterized by their small size, short generation time, and high potential rates of reproduction, with simple life cycle occurring generally within a single host. They are diverse and ubiquitous in aquatic ecosystems, comprising viruses, prokaryotes and eukaryotes. This presentation focus on viruses from unexplored environments and on eukaryotic parasites of microbes, in a scientific context where technical and analytical advances are expected to continuously increase our knowledge of the reservoir of novel 'species', genes, and metabolic pathways, together with the related ecological potentials in aquatic ecosystems. The unexplored environments include the hypersaline tropical waters of Lake Retba (Senegal), and the 6,000 years old anoxic sediments of the unique meromictic Lake Pavin (French Massif Central) that preserves records of the geologic past exceptionally well. In these environments, we have recently unveiled viruses with particular morphotypes, typical of archaeal viruses. Concerning the unexplored eukaryotic parasites, they are known from recent surveys of the 18S ribosomal DNA and developments in sequencing technology and the use of SSU rRNA hypervariable tag sequencing, both of which have unveiled a large reservoir of unexpected diversity of small eukaryotes in both marine and freshwater ecosystems, suggesting significant ecological roles in ecosystem functions, and opening new perspectives in the context of aquatic food web dynamics. These microparasites are highly complex in terms of phylogenetic affiliation, belonging to diverse subgroups of the kingdoms Alveolata, Heterokonta, Fungi, and Rhizaria. Exciting future lines of environmental research on the unexplored viral and microbial parasites and their effects on the food web dynamics are thus expected in the decade to come.

Coccolithoviruses: Life and death after genome sequencing

Willie Wilson

(wwilson@bigelow.org)
Bigelow Laboratory for Ocean Sciences, 180 McKown Point Road, West Boothbay Harbor, Maine, 04575, US.

The virus genus Coccolithovirus (Cocco: derived from Greek *kokkis*, meaning berry or grain referring to their shape and Lith: from Greek *Lithos*, meaning stone) is a group of large, double stranded DNA viruses that infect the globally important marine coccolithophorid *Emiliania huxleyi*. The first observation of virus-like particles in *E. huxleyi* was reported back in 1974 though they are now known to be one of the causative agents of *E. huxleyi* bloom demise. We sequenced the 407,339 bp genome of one coccolithovirus and revealed that only 14% of the predicted genes confer any significant database homology. Here we will present data that shows how we use virus genomic information to help determine the ecological function of coccolithoviruses, helped by an overview of a mesocosm experiment carried out in June 2008. I will also present some methodological advances in analysis of single viruses and single infected cells sorted by flow cytometry. It is a real hotch potch of data but hopefully it will start to tell us something about this intriguing virus. **Future financial support in marine virus research needs to focus on methodological advances in analysis of specific virus-host systems *in situ*. Virus researchers needs to move away from making generalizations about the roles of viruses since almost every system has its own unique ecological niche.**

Coccolithoviruses: Drowning in data, thirsting for knowledge.

Mike Allen

Plymouth Marine Laboratory

The 21st century has witnessed a revolution in sequencing technology, it is now easier and cheaper than ever before to generate large volumes of sequence data. Yet, while we continue to accumulate new data at an increasingly rapid rate, we are failing to accumulate new knowledge and understanding of systems at anywhere near a similar rate. The coccolithovirus EhV-86 was sequenced in 2005. Since then at least 9 more coccolithoviruses have been sequenced, as well as a metagenomic library. But what have we really learnt in this time? Is it time we stopped sequencing and got back to basics in the lab?!?! How many more novel genes is it worth discovering before our focus shifts to characterising their protein products? For the good of scientific research, should we return our solexas and 454s to their manufacturers for disposal?!?

The Girus Gene Pool

Hiroyuki Ogata

Laboratory of Structural and Genomic Information, CNRS - UPR2589, Parc Scientifique de Luminy - 163 Avenue de Luminy - Case 934, FR- 13288, Marseille cedex 09, France
Email: Hiroyuki.Ogata@igs.cnrs-mrs.fr

Giruses are eukaryotic viruses with large genomes encoding several hundred genes. Most of their genes are not similar to host genes and many of them are lacking detectable similarity to any known sequences. In my talk, I will present several bioinformatics studies addressing the evolutionary dynamics of the girus genomes. Overall, our results reinforce the idea that giruses hold their own gene pool, which is only partially overlapping with the cellular gene pool. I also show that marine metagenomic data is an essential resource to shed light on the vastly under-explored girus gene pool. Finally, I will introduce a recently launched collaborative metagenomics program, Tara-Girus, running as an integrated part of the Tara-Oceans project.

Future lines of funding should focus on the (1) isolation of new giruses, (2) methodological development to enrich girus DNA from environmental samples and (3) bioinformatics development to integrate complex data being generated by high-throughput environmental microbiology.

Viruses viewed as obligatory symbionts instead of obligatory parasites

Annie Bézier, Vonnick Sibut, E. Herniou, C. Dupuy, Huguet, J-M Drezen

IRBI- Institut de Recherche sur la Biologie de l'Insecte
UMR CNRS 6035, Université François Rabelais-Tours

Polydnaviruses (PDVs) are associated with tens of thousands species of parasitic wasps that develop within the body of lepidopteran larvae. PDV particles, injected along with parasite eggs into the host body, act by manipulating host immune defences, development and physiology, thereby enabling wasp larvae to survive in a potentially harmful environment. Particle production does not occur in infected tissues of parasitized caterpillars, but is restricted to specialized cells of the wasp ovaries. The genome enclosed in the particles encodes factors used to manipulate the physiology of the parasitized host. We recently proved the viral nature of PDVs associated with braconid wasps by characterizing the genes involved in particles production. These genes reside permanently in the wasp chromosome(s) and are typical of nudiviruses (formerly know as non-occluded baculoviruses). PDVs associated with ichneumonid wasps originated from the integration of another type of virus and the nature of particles described in other groups of parasitoid wasps is still unknown (banchivirus, VLP). Symbiotic viruses may also be involved in the manipulation of plant physiology by gall-inducers. Finding new symbiotic associations is a new frontier in virology and requires the screening for viruses stably integrated in arthropod genomes and an in depth investigation of pathogenic viruses infecting arthropods using new generation sequencing approaches. These studies should be prioritized for future financial support because they can change the definition of viruses that may no longer be exclusively considered as obligatory parasites, but also as obligatory symbionts.

The virion proteome of *Cafeteria roenbergensis* virus reveals an ancient connection

Matthias G. Fischer[1], Isabelle Kelly[2], Leonard J. Foster[2] and Curtis A. Suttle[1,3,4]

Departments of [1]Microbiology & Immunology, [2]Biochemistry & Molecular Biology, [3]Botany, and [4]Earth & Ocean Sciences, University of British Columbia, Vancouver, V6T 1Z4, Canada

Cafeteria roenbergensis virus (CroV) is a giant marine virus with a 730 kbp DNA genome and 544 predicted protein-coding genes. CroV shares a third of its genes with *Acanthamoeba polyphaga* mimivirus. Although Mimivirus and CroV are found in different environments and infect hosts that belong to different eukaryotic supergroups, their infection cycles are remarkably similar and involve replication in large cytoplasmic virion factories. CroV and Mimivirus are also the only viruses known to have their own viral parasites, the virophages Mavirus and Sputnik. We have analyzed the virion proteome of CroV and compared it to that of Mimivirus. LC-MS/MS analysis revealed that the CroV particle is composed of at least 129 virus-encoded proteins, including structural proteins and predicted enzymes for transcription, DNA repair, redox reactions and protein modification; however, most of the packaged proteins were of unknown function. Homologs of 34 CroV virion proteins have been identified in the Mimivirus virion, revealing a core set of proteins that were presumably present in the virion of the last common ancestor of these viruses.

Our results corroborate the common ancestry of CroV and Mimivirus and suggest that packaging of transcription and redox proteins may be essential for the cytoplasmic mode of replication employed by giant viruses.

In order to elucidate the function and significance of the protein composition in giant viruses, biochemical and structural data of selected virion proteins are needed. Ultimately, further phagotrophic host organisms should be screened for giant viruses to enable a more thorough comparative analysis.

Prasinoviruses and their hosts as models for integrative marine eukaryotic microbial ecology

Nigel Grimsley, Rozenn Thomas, Camille Clerissi, Romain Blanc-Mathieu, Gwenael Piganeau, Eve Toulza,Yves Desdevises, Evelyne Derelle and Hervé Moreau

BIOM (Integrated Biology of Marine Organisms) Laboratory
UMR7232 CNRS / Arago / Observatoire Océanologique de Banyuls
University of Paris "Pierre et Marie Curie"
66650 Banyuls-sur-mer, France

The picoeukarotes *Bathycoccus*, *Micromonas* and *Ostreococcus* (genera within the Prasinophyceae) are distributed worldwide, commonly found in coastal waters, and are frequently attacked by large double-stranded DNA viruses. The complete genomes of several species of these unicellular algae and their viruses have now been analysed, providing useful models for investigating evolutionary and biological functionalities at the base of the green lineage. Comparisons of viral genomes revealed a surprisingly high level of sequence conservation, both for synteny and for orthologous genes, viral genomes showing a higher level of conservation than host genomes over the 3 genera, except for a large inversion in certain *O. lucimarinus* viruses and two very large proteins in *Bathycoccus* viruses. Certain amino acid synthesis pathways, not previously observed in viruses, were harboured by *Micromonas* and *Ostreococcus* viruses.

In laboratory cultures of wild-type hosts and viruses, diallel analyses of host-virus specificities revealed most viral isolates to be fairly species-specific, attacking mainly a few strains within one species. Susceptible cultured host strains could spontaneously acquire resistance to one specific viral strain during or following one cycle of infection of that strain. The definition of a species, both at the level of the host and the virus, is thus an important element for our comprehension of phytoplankton-virus interactions, but in the marine environment this is often limited to the availability of few environmental DNA sequences. Given the extent of protistan diversity and the unprecedented explosion of new sequence data becoming available, the choice of genetic markers, our understanding of how these represent eukaryotic diversity, and the mechanisms of host-species' resistance or susceptibility thus require further investigation.

Mining Tara Oceans data for giant viruses

Pascal Hingamp

Laboratory of Structural and Genomic Information, CNRS - UPR2589, Parc Scientifique de Luminy - 163 Avenue de Luminy - Case 934, FR- 13288, Marseille cedex 09, France
Email:pascal.hingamp@univmed.fr

The three year circumglobal Tara Oceans expedition is sampling plankton across a 1E4 fold size range, from phages to fish larvae. During 2010, a ship to land stream of imaging, cytometry and genomics data accompanied by biogeochemistry metadata has been set up. We will present an overview of preliminary genomic data analysis concentrating on large DNA viruses from Mediterrean and Indian Ocean legs, with a focus on taxonomic and functional descriptions as well as geographic distributions.

Our current experience with large scale biodiversity studies suggests funding is required to support the following strategic bottlenecks in girus biodiversity studies:

i) additional datasets and target DNA enrichment protocols are needed to overcome the dilution of giruses with similar sized more numerous bacteria

ii) additional girus reference genomes representing observed biodiversity are required to support the analysis of novel environmental data

iii) increased bioinformatics power is absolutely essential to cope with the current and future data volumes generated by ultra high throughput sequencers (both in terms of available HPC hardware such as clouds or grids, and in terms of supporting engineering manpower)

What can bacterial and archaeal genomes tell us about the diversity and evolution of viruses?

Mart Krupovic

Biologie Moléculaire du Gène chez les Extrêmophiles, Institut Pasteur, 25 rue du Dr Roux, 75015 Paris, France.

Evolutionary history of biological entities is recorded within their nucleic acid sequences and can be deciphered by thorough genomic analysis. Bacterial and archaeal DNA viruses often integrate their genomes into the host chromosome thereby becoming proviruses. A provirus, defective or not, represents a molecular record that a cell has been in contact with a particular virus. Therefore, identification and analysis of proviruses can advance our knowledge on viral diversity and evolution as well as the phylogenetic distribution of respective viruses. This approach is especially useful for studying underrepresented viral groups, i.e., those for which only few isolated representatives are available. During my talk I will provide several examples of how analysis of various proviruses has expanded our knowledge on viral diversity and evolution.
Numerous metagenomic analyses have revealed that viral diversity is still largely unexplored. Future research should therefore be directed towards isolation and detailed characterization of novel types of microbial viruses. This would allow us to comprehend the extent of the evolutionary dynamics in the virosphere and more fully appreciate the role that viruses play in the world we live in.

Viral Agents with Natural Genome Editing Competences

Guenther Witzany

Telos-Philosophische Praxis
Vogelsangstraße 18c
5111-Buermoos, Austria
Tel/Fax ++43 6274 6805
Email: Witzany@sbg.at

Background: Research of the last decades demonstrated that integration of viruses into host genomes is not a rare event but common use. Besides take-over and manipulation of cellular functions by viruses to replicate with often disease causing and even lethal consequences we know abundant examples in which viruses persistently colonize genetic host habitat without harming host. Interestingly persistent viral integration into host genome may lead to functional or not-functional viruses within genetic host habitat. An abundance of viral parts, i.e. “defectives”, co-adapt and serve as “effective” modular tools for cellular needs in gene regulatory processes.

Methods: The concept of “biocommunication and natural genome editing” investigates competent viral agent-driven generation and integration of meaningful nucleotide sequences into pre-existing genomic content arrangements, and the ability to (re-)combine and (re-)regulate them according to context-dependent (i.e. adaptational) purposes of the host organism. Natural genome editing additionally investigates RNA/RNP activities acting on RNA transcripts without altering DNA encoded genes.

Conclusion: Manfred Eigen introduced Noam Chomskys concept of “universal syntax” into molecular biology considering nucleic acid language as a reality not as a metaphorical description. But Manfred Eigen followed a rather rudimentary concept of code/language that is not coherent with current knowledge about natural codes/languages. This contribution will introduce currently known features of natural codes/languages to better understand and investigate natural genome editing by viral agents.

Witzany, G. (2010). Biocommunication and Natural Genome Editing. Dordrecht, Springer.
Witzany, G. (Ed) (2009). Natural Genetic Engineering and Natural Genome Editing. Ann NY Acad Sci 1178.

Metagenomic analysis of the viral communities of temperate freshwater lakes

Simon Roux, Francois Enault, Agnès Robin, Viviane Ravet, Sébastien Personnic, Sébastien Theil, Jonathan Colombet, Télesphore Sime-Ngando and Didier Debroas

Clermont Université, Université Blaise Pascal, Laboratoire "Microorganismes : Génome et Environnement", BP 10448, F-63000 CLERMONT-FERRAND
CNRS, UMR 6023, LMGE, F-63177 AUBIERE

Viruses are known to be the most abundant biological entities in aquatic environment, where they are (i) important top-down regulation factors for microbial communities, (ii) known to interfere in major biogeochemical cycles, and (iii) thought to be an important source of genetic diversity. Yet, the global viral diversity and distribution are still far from being understood, especially in freshwater ecosystems. Thus, we present here the results from the study of the first temperate freshwater viromes. Even with “large” size reads (400 bp), the major part of our viromes still showed no significant hit against sequences in public databases (i.e. ORFans). Taxonomic composition of the two viromes revealed a dominance of small single-stranded DNA viruses (Circoviruses and Microviruses), alongside with Caudoviruses. Thanks to the large read size and deep sequencing, a precise qualitative description of the diversity in these main viral families was assessed for the first time through direct phylogenetic analysis of specific marker genes. This analysis shed light on a great diversity, and retrieved previously unknown clades among single-stranded DNA viruses (Microviridae, Circoviridae, Nanoviridae) and double-stranded DNA viruses (Caudovirales).

These two viromes were also used in a global comparison including a set of previously published viromes which highlighted a significant genetic similarity between viral communities of related environments. Thus, viruses appear to be distributed worldwide, and are likely selected by the presence or absence of their hosts. Even with deep sequencing viromes, the viral gene pool present in our ecosystems is still not fully sampled. The viral world stands out as a fantastic reservoir of unknown genes, which remains partially sampled and understood.

This analysis of freshwater viromes pointed out that viral metagenome specificities require the development of new tools dedicated to their analysis. In this talk, we will finally introduce the Web-server dedicated to virome analysis we are currently developing, which will include the tools developed during this study.