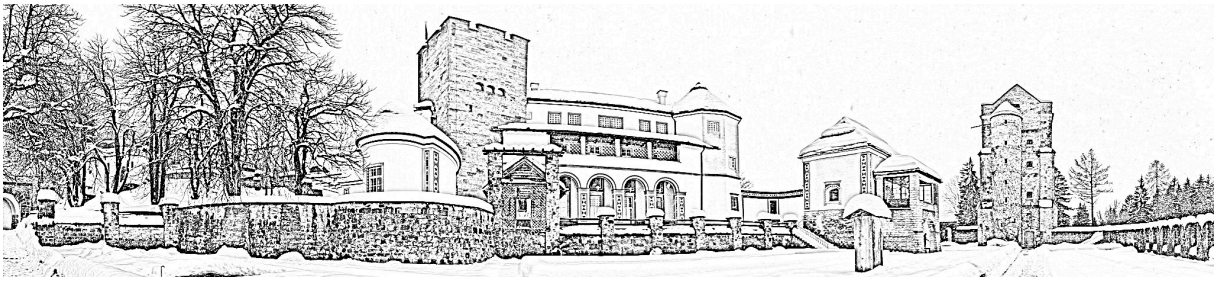

6th Ringberg symposium on Giant Virus Biology
– Abstract Book –

Keynote -Are there giant viruses?

Eugene V. Koonin

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The discovery of giant viruses two decades ago attracted broad attention of microbiologists, evolutionary biologists and even the broader public, triggering many fascinating evolutionary scenarios and hypotheses. In particular, it has been argued that giant viruses represented a distinct type of biological entities, being 'alive' and having evolved from cellular life forms, possibly, even a fourth domain of life, via the reductive evolution route. The observations that some of the giant viruses in the (current) phylum Nucleocytoviricota encoded multiple components of the universal translation machinery added fuel to these evolutionary scenarios. However, I will present multiple lines of evidence that, all their remarkable features notwithstanding, the giants of the virosphere are typical viruses that have evolved from smaller, simpler ones, on multiple occasions during the evolution of the two realms of viruses with dsDNA genome, Varidnaviria and Duplodnaviria. Within each of these realms, there is a continuum of genome sizes among clearly related viruses, from small ones, on the order of 10 Kb, to huge ones, up to more than 4 Mb. I will further submit that the divide between cellular life forms, on one side. and viruses (and other mobile genetic elements), on the other side, is unsurmountable, reflecting one of the central principles of biology, the distinction between reproducers and replicators, the two fundamentally different types of evolving biological entities.

Viruses and virus-like mobile genetic elements are ubiquitous parasites or symbionts of all cellular life forms and the most abundant biological entities on earth. The recent, unprecedented advances of comparative genomics, metagenomics and metatranscriptomics have led to the discovery of diverse novel groups of viruses and a rapid expansion of the chartered region of the virosphere. These discoveries provide for a vastly improved understanding of the evolutionary relationships within the virosphere. Arguably, we are approaching the point when the global architecture of the virus world can be outlined in its entirety, and the key evolutionary events in each of its domains can be reconstructed. I will present such an outline of the global organization of the virosphere and the corresponding megataxonomy, including 7 evolutionarily coherent realms of viruses, that has been recently approved by the International Committee on Taxonomy of Viruses, as well as additional candidate new realms. The expansion of the prokaryotic virosphere that now includes many groups of viruses, particularly, those with RNA genomes, previously thought to be eukaryote-specific, will be emphasized. I will further discuss the position of viruses within the wider space of replicators and the recent dramatic expansion of the "alternative virosphere" that includes viroids and diverse viroid-like viruses that seem to have evolved on multiple, independent occasions. Finally, I will present the current scenario for the origin of viruses based on the analysis of the evolutionary provenance of the key proteins involved in virion structure formation and viral genome replication.

Virus-Host Interactions

Lipid remodeling during host-giant virus interactions reveals infection and defense mechanisms

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Giant viruses profoundly remodel host metabolism to mediate successful infection, actively redirecting cellular resources toward viral replication. Through their large and diverse gene repertoires, including auxiliary metabolic genes (AMGs), giant viruses target key metabolic pathways, including energy production, carbohydrate metabolism, DNA replication, and lipid biosynthesis. However, despite growing genomic data, the function of most giant virus genes remains unknown, limiting our understanding of their metabolic capabilities and infection strategies. A major challenge is that viruses rarely encode complete biosynthetic pathways, instead relying on a combination of viral and host genes, which complicates the identification of pathway products and functional outcomes.

Lipids are central to cellular structure, signaling, and energy storage, and are increasingly recognized as critical mediators of virus-host interactions. They are also essential structural components of many giant viruses, as a large fraction contain at least one lipid membrane, often needed for viral infectivity and survival. Accordingly, giant viruses have been shown to extensively remodel the host lipidome to meet their energetic demands and to support membrane biogenesis, making lipid metabolism a key battleground in the evolution of viral infection and host defense strategies. To date, lipid remodeling has been documented in several host-giant virus systems, including *Gephyropapsa huxleyi*-EhV, *Micromonas pusilla*-MpV, and *Haptolina ericina*-CeV, though much of our current knowledge is derived from the *G. huxleyi*–EhV system, where the virus encodes an almost complete sphingolipid biosynthetic pathway.

In this talk, I will provide an overview of how lipid remodeling is studied in host-giant virus interactions, highlight emerging insights into viral infection and host defense strategies, and discuss the need to better resolve viral gene function within the context of host metabolic networks.

Evolutionarily conserved molecular grammar rules viral factories the hyperdiverse Nucleocytoviricota phylum

Hugo Bisio

Despite sharing fewer than 10 core genes, the hyperdiverse Nucleocytoviricota phylum (ranging from poxviruses to giant viruses) universally assembles viral factories (VFs) resembling biomolecular condensates. Regardless, it is unclear how these viruses achieve such a level of functional conservation without clear conserved genetic information. We demonstrate that the VFs produced by amoeba-infecting viruses have liquid-like properties and identify a conserved molecular grammar governing viral factory scaffold protein: charge-patterned intrinsically disordered regions that drive phase separation independently of sequence homology. This grammar predicts functional scaffold proteins across the 15 viral families, revealing evolutionary constraints invisible to sequence or structural analysis. Strikingly, VFs exhibit subcompartmentalization analogous to nuclei, segregating transcription and mRNA processing (inner condensates) from replication (interphase zones) and translation (host cytoplasm). Our work establishes phase separation as a fundamental organizational principle bridging extreme genomic diversity, explaining how biological complexity emerges without gene conservation. This grammar is likely also conserved in non-amoeba-infecting members of the phylum and thus may represent a primordial solution for organelle-like organization, with broad implications for antiviral targeting. In addition to discussing the already published work, I will touch base on the generation of a platform of recombinant mimivirus for genome wide screening of gene loss of function, proteome wide protein localization and the study of how viral factories mature during the infectious cycle.

A glimpse inside the viral factory

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Spatial separation of transcription and translation is a hallmark of eukaryotic cells. Recently, however, we have shown that Mimivirus viral factories achieve a comparable organization during late stages of infection, with striking structural and functional similarities to the eukaryotic nucleus. Within these factories, transcription occurs at discrete sites inside the compartment, while translation is restricted to its periphery. We have further shown that both of these sites are maintained by the recently described outer layer condensate of the Mimivirus factory. Investigating the viral factory throughout the entire infectious cycle has shown that around 10 hours post-infection, we observe structural transitions that appear to coincide with biophysical changes and a functional shift from DNA replication to viral particle assembly. These changes influence the spatio-temporal distribution of transcription and translation throughout Mimivirus infections, leading to a potential shift from no spatial separation to eukaryote-like spatial separation. Using a combination of imaging and molecular techniques, we investigate how architecture and fundamental function of the viral factory play a role in the spatial organization of key processes of viral infections and how the virus modulates this behaviour. This allows us to get insights into cytoplasmic viral factories on a cell biological and molecular level and provide a foundation for future studies of this unique viral structure.

Untangling complex host-virus relationships in an alpine lake

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Giant viruses are key regulators of global biogeochemical cycles, and they have profoundly influenced the evolution of eukaryotic genomes. Recent studies have found that some giant viruses co-infect their host with polinton-like viruses (PLVs), DNA viruses that integrate into eukaryotic genomes. Studying the relationship between giant viruses and their hosts is limited by the difficulty in culturing both the host and its virus in the lab. It is also challenging to understand the ecological role of viruses discovered via metagenomic studies without knowing their native hosts. Here, we use a novel, cultivation-free, single-cell RNA-seq approach to discover the native hosts of viruses in the alpine Gossenköllesee lake, which harbors unique viruses previously discovered using electron microscopy and metagenomic analysis. We identified hundreds of protist cells infected by giant viruses from diverse lineages, and, for the first time, identified co-expression of giant viruses and PLVs within the same host cells. We also found that a conserved eukaryotic anti-viral gene is upregulated in infected cells, suggesting that it may play a role in the response to viral infection. Our approach establishes scRNA-seq as a powerful tool for directly linking viruses from different lineages to their authentic hosts and for exploring complex interactions that cannot be understood solely through bulk analyses. This approach opens new opportunities to uncover the ecological and evolutionary roles of PLVs and giant viruses in the environment.

New Viruses

Sissivirus algeromassiliense: what could be the use of its huge 4 Mbp genome?

J. Andreani, P. Colson, B. La Scola

Aix-Marseille University

During the previous symposium in 2023, we presented the first data on a new giant virus that we named Sissivirus. This virus, which has an ovoid shape measuring approximately 800 × 600 nm, was isolated from a wastewater sample using the amoeba Stenamoeba massiliensis. Genome analysis revealed a nearly 4-megabase pair (Mbp) circular double-stranded DNA encoding 2,796 ORFs, placing it within the order Pimascovirales. The genomic analysis shows extreme complexity, with a substantial proportion of non-coding sequences (≈48%) and various types of repeats (33% of the genome) located both between and within genes.

These findings were supported by transcriptomic and proteomic data, which were challenging to obtain because the virus can only be grown in co-culture with Klebsiella aerogenes. Despite these constraints, we detected 398 proteins within the viral particles and gained insight into transcriptional kinetics. Moreover, Sissivirus was found to be isolated with a virophage possessing a 21,896 bp circular DNA genome, which we named Sissivirophage. It is the first isolated virophage known to infect a pimascovirus. Spatial transcriptomics was tentatively applied to investigate interactions between these two viruses and between them and their amoebal host at the single-cell level.

Deciphering the enigma of this trend toward genome gigantism in certain giant viruses—clearly driven by forces distinct from those affecting the megagenomoviruses described by Schulz et al., who also participated in the analysis of the Sissivirus genome—represents a new challenge in the study of giant viruses.

Naiavirus: an enveloped giant virus with a pleomorphic, flexible tail

Jonatas Santos Abrahão

Federal University of Minas Gerais, Brazil

Numerous studies have shown that viruses are present in a variety of environments on Earth, acting as drivers of biogeochemical cycles and powerful selective forces. Among them, giant viruses of amoebae have garnered attention from the scientific community due to their large particles and extensive genomes. Here, we describe the discovery of one of the largest tailed viruses in the known virosphere (averaging 1350 nm), named Naiavirus. This virus, isolated from a swamp biome in Brazil, has particles with a never-before-seen morphology and composition, and represents the first giant amoeba virus with an external envelope covering the capsid and extending over a flexible tail region. The Naiavirus genome, with nearly 1 million base pairs, reveals a unique set of genes, and does not resemble any other virus previously isolated so far.

More information at https://doi.org/10.1038/s41467-025-63463-6.

Egoviruses: a new order of giant viruses infecting unicellular eukaryotes in animal guts.

Morgan Gaia

Large and giant dsDNA viruses from the *Nucleocytoviricota* phylum are particularly diverse and collectively infect the entire eukaryotic diversity. They are divided into two major classes: *Megaviricetes*, which are especially abundant in aquatic environments where they predominantly infect protists, and *Pokkesviricetes*, which include asfuviruses and animal-infecting poxviruses. Until now, nucleocytoviruses had only been sporadically reported from animal digestive systems.

Here, we describe a new order within the Pokkesviricetes class, the Egovirales, comprising more than 200 reconstructed genomes. Egoviruses have linear genomes up to 360 kb and are predicted to form multilayered icosahedral capsids similar to those of asfuviruses, although phylogenetic inferences place them closer to poxviruses. Strikingly, they are found almost exclusively in the gut microbiomes of humans, livestock, and wild animals, where gene transfer signatures suggest they infect parasitic and symbiotic metamonads and ciliate alveolates.

The discovery of egoviruses reveals a previously hidden lineage of giant viruses in animal guts and expands our understanding of the ecology and evolution of Pokkesviricetes.

Bioprospecting for Nordic viruses of microbes

Lotta-Riina Sundberg, Juliana M Tatara, Victoria F Queiroz, Cindy J Given, Klara Stensvåg, Nicola Abrescia, Gabriel MF Almeida

Viruses are the most abundant organisms on Earth. The majority of the virosphere is represented by viruses of microbes, which can be divided into viruses of prokaryotes (bacteriophages) and viruses of unicellular eukaryotes. Genomic evidence points to a high diversity of viruses of microbes in the Northern hemisphere. Here I present results from a bioprospection effort for isolating Nordic viruses of microbes which started during 2019 in Central Finland and remains active until now in Northern Norway.

After screening over 400 samples we were able to obtain eleven viruses of *Acanthamoeba*, including Jyvaskylavirus, the first Finnish giant virus, and the northernmost isolates known to date from Norway. The Norwegian viruses comprise a unique cohort of viruses isolated above the Arctic circle. Our isolates show that marseilleviruses are common and diverse in the Boreal and Arctic environments, and that mimiviruses are also present. The results so far from our bioprospection effort increases the understanding of the Nordic virosphere and prove that unique viruses can be found in the North.

Our perspectives now are to explore these viruses as potential sources of bioproducts, and work on breaking the Acanthamoeba-bias of isolating giant viruses by combining a collection of rare samples and non-amoebal hosts for isolation.

GEVEs (giant endogenous viral elements)

A computational framework for detecting the dsDNA viral landscape in eukaryotic genomes

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Abstract: Genomic sequences of viral origin constitute a substantial fraction of many eukaryotic genomes, and the contribution of double-stranded (ds)DNA viruses to eukaryotic genome evolution is increasingly recognized yet remains largely unexplored. Here, we present a computational framework for identifying dsDNA viral regions (VRs) in eukaryotic genomes. This framework incorporates eleven eukaryotic viral groups and was applied to 37,254 genome assemblies, covering most sequenced eukaryotes across diverse lineages. We identified 781,111 VRs across 7,103 assemblies, with VRs accounting for up to 16% of individual genomes and reaching 12% in a human protozoan pathogen. These VRs revealed 174 class-level associations between viral and eukaryotic taxa, including 159 associations for which no experimentally isolated representative is available. The identified VRs provide evidence for putative viral lineages and imply the existence of novel viral clades. Overall, our study establishes a large-scale baseline for dsDNA viral elements embedded within diverse eukaryotic genomes and substantially expands the known dsDNA virosphere.

Endogenous Giant Viral Elements in a Polar Alga Show Dynamic Transcriptional Response to Abiotic Stress

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Giant viruses in the phylum *Nucleocytoviricota* possess exceptionally large genomes that encode hundreds of genes involved in replication, metabolism, and host manipulation. These viruses have emerged as major players in protist ecology and evolution. Recent studies reveal that their genomes are frequently endogenized in protists, contributing to structural innovation and functional novelty across diverse lineages. Here, we report widespread Giant Endogenous Viral Elements (GEVEs) in nine polar microalgae, revealing extensive viral integration. Most notably, *Chlamydomonas* sp. ICE-L, an Antarctic sea ice alga, harbors over 400 GEVE regions spanning more than 26 Megabase pairs - the most extensive giant viral endogenization recorded in any eukaryote. These insertions, derived from multiple *Nucleocytoviricota* lineages, encode >25,000 genes, including those associated with replication, chromatin remodeling, stress response, and transposable elements. Transcriptomic analyses show that nearly 40% of GEVE genes are actively expressed, with hundreds differentially regulated under UV radiation, salinity, and temperature stress. Co-expression network analysis reveals modular regulation patterns, which suggests functional integration of viral genes into host transcriptional networks. Additionally, phylogenetic analysis supports giant viruses as important mediators of horizontal gene transfer of key freeze-tolerance proteins such as ice-binding proteins (IBPs) in polar algae. Our findings position giant viral endogenization as a key driver of genome content, regulatory complexity, and environmental adaptation in polar algae.

Cell-type specific latency and reactivation of a giant virus

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Viral DNA integrated into eukaryotic genomes is widespread, yet how such endogenous viral elements (EVEs) interact with their hosts remains poorly understood. Moreover, while latency via genome integration is a important viral strategy, its molecular basis in multicellular eukaryotes has not been directly resolved. Here we identify a transcriptionally active EVE in the model brown alga *Ectocarpus*, closely related to *Ectocarpus siliculosus* virus-1, that is reactivated specifically in gametangia to produce infectious particles. Using single-nucleus RNA sequencing and RNA-FISH, we show that viral proliferation is spatially restricted by host gene expression programs, including the induction of defense-associated pathways, while viral activation depends on a defined subset of viral genes expressed only in reproductive cells. We further demonstrate that these viral loci are essential for initiating replication and driving hallmark symptoms, linking cell-type–specific host responses with viral gene requirements. Together, our results establish the first direct mechanism for cell-specific giant EVE reactivation in a multicellular eukaryote, revealing how genomic 'fossils' can become conditional, developmentally regulated viruses that shape host reproduction and defense.

Vertical inheritance and activation of latent integrated viruses in brown algae

Carole Duchêne, Rory J. Craig, Claudia Martinho, Rémy Luthringer, Ferran Agullo, Katharina Hipp, Pedro Escudeiro, Vikram Alva, Fabian B. Haas, Susana M. Coelho

Phaeoviruses, a Nucleocytoviricota lineage that infect brown algae, have a latent life cycle, with viral symptoms only appearing at the onset of sexual reproduction of the algae. Another key feature of these viruses is the persistence of viral symptoms across algal generations through meiosis and through fertilization. This complex life cycle and the discovery of Endogenous viral elements (EVEs) in host genomes suggested that the viral genome may integrate into the host and be transmitted vertically. Here, we demonstrate that EVEs in the brown alga *Ectocarpus* can reactivate and drive active viral infections. Using long-read sequencing and transcriptomics, we identify full-length, transcriptionally active giant viruses integrated within the host genome, and show that viral symptoms strictly correlate with the presence of active EVEs. By resolving the genomic integration sites, we propose a mechanism for phaeovirus integration, and the sequencing of actively replicating viruses led to new hypotheses on DNA replication and processing in the phaeoviral lineage. By demonstrating long-standing hypothesis using modern genetic tools, this work establishes a new model to explore latency, inheritance and the evolutionary impact of large dsDNA viruses.

Highlights of 45 Years of Chlorovirus Research

James L Van Etten

University of Nebraska-Lincoln

This talk will mention some of the highlights of studying the chloroviruses for the past 45 years. All of the enzymes I mention below have been cloned and characterized. 1. First viruses infecting a photosynthetic eukaryote to be plaque assayed. 2. They have a bacterial like infection process. 3. One chlorovirus was the largest virus genome to be sequenced for about 7 years. Now about 140 chlorovirus genomes have been sequenced. 4. Chloroviruses can serve as the sole food source for several protists. Thus, viruses need to be considered part of the food chain. 5. The viruses contain and release a chemotaxic agent(s) that attracts paramecia, which contain the virus host algae. The unusual polyamine homospermidine is part of the story. 6. They encode 5 enzymes involved in polyamine metabolism, including a homospermidine synthase. 7. The viruses have genomes with 5mC and 6mA nucleotides. 8. First viruses to encode DNA restriction/modification enzymes and the story is backwards, the viruses encode the enzymes, not the host, and it is in a eukaryotic system. 9. Some of the DNA restriction endonucleases cleave one strand of the DNA and so are DNA nicking enzymes. They do not cleave DNA smaller than about 20 nucleotides and so one can create DNA primers for amplifying anonymous DNA. 10. They encode the world's smallest potassium ion channel – 83 to 95 amino acids and there are currently over 60 publications on the K^+ channel. 11. They also encode aquaglyceroporin channels, and potassium and calcium transporters. 12. They encode the world's smallest vSET protein that dimetylates Lys 27 in histone 3. 13. They encode the world's smallest DNA topoisomerase II enzyme and it cleaves DNA ~40 times faster than the human enzyme. 14. They encode the first DNA ligase that works on RNA templates as well as DNA templates. 15. They encode a prolyl-4-hydroxylate but no hydroxylated proline containing proteins exist in the virus particle. 16. They encode the usual flavin dependent thymidylate synthetase Thy X, which had only been found in some bacteria and Dictyostelium. 17. They code as many as 6 cell wall degrading enzymes. 18. They encode most, if not all, of the machinery to synthesize the glycans attached to their major capsid proteins. 19. Some viruses encode hyaluronan and chitin synthetases. First time hyaluronan synthetase was found outside of vertebrates and a few pathogenic bacteria. 20. They are the largest virus to have their structure revealed at 3.5Å resolution. 21. Some of the viruses have 3 types of introns and inteins.

Virus Ecology

Synergy in giant viral-bacterial coinfection expedites algal bloom demise

Assaf Vardi

Weizmann Institute of Science, Israel

Algal blooms are hotspots for diverse microbial interactions affecting bloom dynamics. Viral infections often regulate the demise of these blooms, recycling more than 25% of oceanic fixed carbon. Although these blooms collapse synchronously, less than 30% of algal cells show active viral infection, suggesting additional mortality agents. By generating a synthetic community composed of alga, bacteria and viruses isolated from an algal bloom, we experimentally quantified their tripartite interaction dynamics. We discovered that bacteria significantly enhanced virus-mediated algal mortality. We quantified this interaction using two novel metrics: the Synergy Index (SI) measuring viral-bacterial interaction strength, and the Benefit Index (BI) assessing changes in bacterial fitness. Alteromonas bacteria were identified as drivers of viral-bacterial coinfection, with consistently high SI and BI. We deployed in situ chemotaxis assays and found that in algal blooms, Alteromonas displayed high chemoattraction towards the unique chemical seascape of infected cells and accumulated in particles derived from bloom demise. This viral-bacterial synergy represents a previously unrecognized mortality agent controlling bloom termination, modulating the balance between the carbon-recycling viral shunt and the carbon-exporting viral shuttle pathways, likely influencing global carbon cycling.

Narrowing down the search: Marine virus-host interactions in Antarctica

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Marine phytoplankton support Antarctic ecosystems and force the Southern Ocean carbon sink. However, climate-driven environmental shifts along the Antarctic Peninsula are altering phytoplankton communities, impacting food-web dynamics, carbon cycling, and host-virus interactions. Viral lysis is a critical process shaping phytoplankton mortality and carbon transfer through the microbial loop. Despite their ecological importance, detailed knowledge of virus-phytoplankton host interactions remains limited, particularly for the Southern Ocean.

We coupled flow cytometric sorting of diverse Antarctic phytoplankton populations with bulk metagenomic sequencing to link dsDNA viruses to their phytoplankton hosts across a seasonal cycle. We identified diverse *Nucleocytoviricota* (NCVs), virophages, and Polinton-like viruses (PLVs) associated with key phytoplankton taxa, including prasinophytes (*Micromonas*), haptophytes (*Phaeocystis*), bolidophytes (*Triparma*), cryptophytes (*Geminigera*), and dictyophytes (*Pedinellales*). Prasinoviruses and novel Group X PLVs controlled *Micromonas* populations, while Tethysviruses and PgVV-group PLVs were dominant viruses of *Phaeocystis antarctica*, coinciding with their seasonal declines. Moreover, we report the first marine cryptophyte-infecting allomimivirus and identify *Pedinellales* as the probable host of Organic Lake-like NCVs and virophages. Seasonal analysis revealed tight correlations between phytoplankton abundances, virus-host dynamics, and population-specific viral lysis rates, highlighting distinct roles of NCVs and smaller dsDNA viruses in bloom regulation and ecological succession. Our study provides a novel understanding of who infects whom, demonstrates the impact of these viruses on their phytoplankton hosts, and highlights interactions between NCVs and smaller viruses in coastal Antarctica. Integrating targeted metagenomics, flow cytometry, and viral lysis measurements provides critical insights into the dynamic and complex ecological roles of viruses under changing Antarctic conditions.

Revealing population heterogeneity during infection and recovery in Ostreococcus tauri with in situ Hybridization Chain Reaction

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Algae from diverse lineages develop resistance to viruses. The molecular mechanisms underlying this resistance remain largely unknown, despite their ecological importance. Ostreococcus (1 µm cell diameter green alga) and its prasinoviruses provide an ideal model to study algae-virus interactions due to their ease of culture, where resistant populations arise reliably after infection. Previous studies have indicated that virus-resistant Ostreococcus populations contained subpopulations of susceptible cells that produced infectious viruses, enabling host–virus coexistence (Yau et al. 2016, 2020). Population modelling suggested this dynamic was consistent with a spontaneous phenotypic switch between resistant and susceptible cells. Nonetheless, to gain insight into the mechanism of resistance requires following the transition of the Ostreococcus population from virus-susceptible to virus-resistant with a technique to quantify infected cells at single-cell resolution. To address these needs, fluorescence in situ hybridization based on the amplification mechanism of hybridization chain reaction (HCR) was adapted for the Ostreococcus–prasinovirus system. HCR is highly specific, as it uses split probes that eliminate off-target binding, and is highly sensitive, as the chain reaction amplifies the signal of target mRNA, allowing detection of low-abundance transcripts. In this study, we harnessed autofluorescence of chlorophyll a, as a label-free method to detect Ostreococcus, and HCR targeting the mRNA of the viral major capsid protein to measure the proportion of actively infected cells. HCR enabled the detection of active infection in as low as ~1% of the population, which was previously challenging to detect based solely on cell growth. Tracking the infection of Ostreococcus tauri by the prasinovirus OtV5 showed that almost immediately post infection, some cells were actively producing viral transcripts, indicating the tempo of infection between cells was not synchronous. Further, we revealed cryptic infections arose during recovery and stationary phases. Together, these data showed population heterogeneity was key to deciphering host–virus dynamics.

Investigating viral diversity in high altitude oligotrophic lakes

Tomas Alarcon Schumacher[1], Amir Fromm[2], Christopher Bellas[3], Ruben Sommaruga[3], Matthias G. Fischer[1]

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High-altitude lakes are extreme ecosystems featuring high levels of UV radiation, low temperatures, reduced atmospheric pressure, and limited nutrient input. Recent studies have opened new avenues into understanding the diversity and prevalence of giant viruses, belonging to the phylum *Nucleocytoviricota*, in these remote environments. Here, we expand the existing knowledge about the diversity of microbial communities and their associated giant viruses in high-altitude lake ecosystems. Our single-cell metagenomic approach revealed that members of the *Imitervirales* and *Asfuvirales* were the most abundant in the model lake Gössenkollesee and they infect an array of microbial hosts, ranging from algae to amoeba. Building on this framework, a recent multidisciplinary expedition to several high-altitude lakes strived to characterize the diversity and activity of their microbial communities and associated giant viruses. We uncovered that unicellular eukaryotes in high-altitude lakes presented smaller cell sizes and lower morphological complexity, reflecting the environmental pressures and trophic state of these lakes. Additional samples were preserved for metagenomics, single-virus particle imaging, and host isolation assays, and we expect that this expedition generates one of the most exhaustive high-elevation viral datasets to date and affords an unparalleled opportunity to resolve how giant viruses persist and interact with their hosts across these dramatic alpine ecosystems.

Virus Diversity

Diversity of the Chlorovirus Capsid

David D. Dunigan, Garry A. Duncan, Rodrigo A. L. Rodrigues, João Victor R. P. Carvalho, Irina V. Agarkova, Roger M. Carlson, James L. Van Etten

Virus particles are diverse in morphology, biochemical composition and functionality. The chlorovirus virion structure has been evaluated extensively over the past 20+ years with a current resolution of 3.5 Angstroms presenting a quasi-icosahedral symmetry with a unique vertex with a spike-like structure. The PBCV-1 virion contains 148 virus-encoded proteins, of which 30 combine to create the mature capsid. These structural proteins serve multiple purposes and contain various types of information including i) architectural scaffolding that forms a lattice to support the outer capsid shell, ii) outer capsid shell with multiple capsomers carrying virus-encoded glycans, iii) the unique spike structure found at a single vertex that is postulated to function as the host binding unit, iv) four types of fiber protein that form homo- or hetero-trimers associated with three unique types of capsomer that may function in attachment to either the replicative host and/or holobiont host, and v) six types of capsomer that are either homogenous or heterogenous consisting of three major capsid proteins. While the biophysical and biochemical composition of the chlorovirus virion is emerging, the full functionality is yet to be determined and little is known about the virion diversity in the genus. Recent genomic analyses of approximately 130 viral isolates from varying ecological settings and representing three subgenera of the chloroviruses offers a unique opportunity to explore the levels of conservation of structural proteins either within a species, between species of the same subgenus or between subgenera. This presentation will summarize i) the diversity of the capsid proteins in the context of the newly identified taxonomic ranks and ii) approximate the essential core genes for virion assembly.

A multilayered network reveals the centrality of newly discovered Nucleocytoviricota in wastewater treatment plant communities

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Metagenomic analysis of Danish wastewater treatment plants has revealed dozens of novel nucleocytoviruses (NCVs), virophages, and polinton-like viruses (PLVs), highlighting these engineered ecosystems as hotspots for viral diversity. To elucidate the complex ecological and evolutionary relationships between these entities, we developed a multilayered network approach that integrates signals of co-occurrence, gene sharing, integration events, and defensive mechanisms. This analysis demonstrated that NCVs act as central interaction facilitators, structuring the microbial community by elevating the connectivity of their associated hosts and symbionts. Finally, we present three of these interaction clusters, demonstrating an intricate web of relationships whose complexity and diversity likely represent a fundamental stabilising feature of microbial communities in dynamic environments such as wastewater treatment plants.

Figure 1: Graphical abstract.

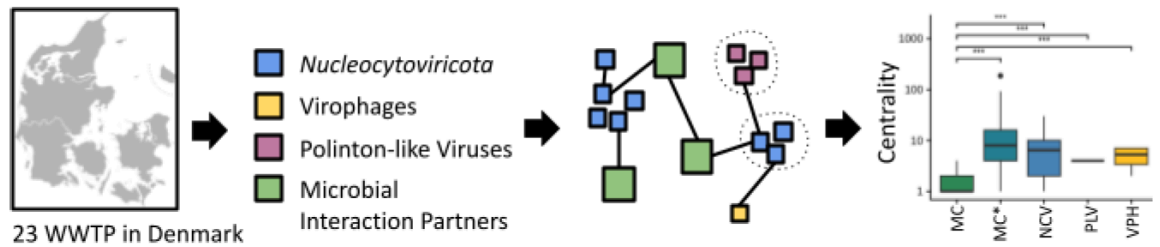

Figure 1: Graphical abstract.

Mirusviruses: genomic, metagenomic, and cell biological perspectives

John Archibald

Mirusviruses are enigmatic DNA viruses with a mixed evolutionary history – they have informational genes of Nucleocytoviricota ancestry and virion module genes most like those of herpesviruses. Mirusvirus genomes were discovered in marine metagenomic data but, despite their abundance and broad environmental distribution, their host range is unclear. The marine thraustochytrid protist *Aurantiochytrium limacinum* was recently found to possess two mirusvirus-like genomic elements, one a circular episome (AurliV-1) and the other (AurliV-2) a chromosomal integrant. Here we show that *A. limacinum* mirusvirus genes are expressed and virions are produced in all culture conditions examined, including standard growth medium. Of 67 AurliV-1-encoded proteins detected using proteomics, 45 were enriched under starvation conditions, including major capsid protein and triplex proteins. We also show that virions contain mainly AurliV-2 DNA, and transmission electron microscopy revealed ~140 nm virions in the nucleus, in cytoplasmic vesicles, between the plasma membrane and cell wall, and in the extracellular environment. These results establish *Aurantiochytrium* as the first model system for elucidating mirusvirus-host interactions and reframe the concept of persistent viral infection in microbial eukaryotes.

Widespread and intron-rich mirusviruses are predicted to reproduce in nuclei of unicellular eukaryotes

Tom Delmont

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Mirusviruses infect unicellular eukaryotes and are related to tailed bacteriophages and herpesviruses. We expanded the known diversity of mirusviruses by screening diverse metagenomic assemblies and characterizing more than 1,000 non-redundant environmental genomes. Mirusviricota comprises a highly diversified phylum of large and giant eukaryotic viruses. Critically, major Mirusviricota lineages lack essential genes encoding components of the replication and transcription machineries and, concomitantly, encompass numerous spliceosomal introns that are enriched in virion morphogenesis genes. These features point to multiple transitions from cytoplasmic to nuclear reproduction during mirusvirus evolution. Many mirusvirus introns encode diverse homing endonucleases, suggestive of a previously undescribed mechanism promoting the horizontal mobility of spliceosomal introns. Collectively, our data strongly suggest that nuclei of unicellular eukaryotes across marine and freshwater ecosystems worldwide are a major niche for replication of intron-rich mirusviruses.

Structural Virology

Ordering the virosphere – In memoriam Dennis Bamford

Mart Krupovic

Pasteur Institute, Paris, France

A genome delivery system in a marine giant virus revealed by cryo-electron tomography

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The storage and transfer of genetic materials are fundamental processes of living organisms. Viruses are parasitic entities, whose genetic processes are totally dependent on their hosts. Genetic materials of viruses are delivered from viral capsids to host cells at the beginning of their infection cycles. The discovery of giant viruses challenges the concept of viruses and raises intriguing questions regarding how to efficiently deliver megabase-sized viral genomes to the host cytoplasm. Here we report a putative genome delivery system adopted by *Prymnesium kappa* virus RF01 (PkV RF01), a giant virus with a 380 nm diameter capsid and 1.2-Mb long genome. We established a gentle concentration and purification method to generate high quality virus samples for cryo-electron tomography, and acquired and reconstructed 108 tomograms including 143 viral particles. Among the reconstructed PkV RF01 virions, we identified four groups of structural variants, i.e., intact virions containing genome and a tubular structure inside; virus particles with an internal tube but lacking genome; opening virus particles; and particles with extruded tubes. The tubes inside and outside their capsids are significantly different in their diameters: 73 nm inside *versus* 66 nm outside. Subtomogram averaging reveals a periodic helical architecture of the tube and shows pronounced conformational shifts inside versus outside the capsid. The tube may function as a syringe, through which the nucleoprotein filament is likely to be pumped into the host cell.

Electrostatic Interactions in the PBCV-1 Capsid

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The morphogenesis of giant viruses requires a coordinated interplay between membrane–protein and protein–protein interactions, yet the physicochemical principles governing these processes in Paramecium bursaria chlorella virus 1 (PBCV-1) remain insufficiently defined. Through two complementary studies, we show how electrostatic microenvironments regulate both the membrane recruitment of the major capsid protein (MCP) and the organization of MCP variants at critical architectural sites of the virion.

In the first study, integrating biochemical membrane-binding assays with multiscale electrostatic modeling, we demonstrate that the MCP selectively associates with anionic, physiologically relevant lipid bilayers enriched in phosphatidylglycerol and phosphatidylserine. This preference is strongly curvature-dependent, with maximal binding at vesicle sizes closely matching the native virion. Computational analyses further reveal that an amphipathic, membrane-proximal helix at the MCP terminus engages these anionic membranes through strong electrostatic complementarity, which is enhanced when negatively charged lipids are spatially clustered.

Recent near-atomic, non-icosahedrally averaged reconstructions of PBCV-1 confirm the existence of previously proposed hybrid MCP variants. Several of these, Type II, Type V, and the canonical Type I, are located around the unique five-fold vertex containing the portal complex. In the second study, molecular dynamics simulations and DelPhiForce analyses show that the Type V MCP generates substantially stronger long-range attractive electrostatic forces and forms a denser network of high-occupancy salt bridges with neighboring capsomers than Type I. These features indicate that Type V is structurally optimized to stabilize the vertex region and may help initiate or coordinate capsid assembly.

Collectively, these results establish electrostatic interaction as a central organizing force in giant virus assembly, governing both membrane engagement and capsid architecture.

Prasinovirus molecular architecture: insights into how the virion components informs function

Sheree Yau

Although virion composition and form are essential to understand viral function, very few marine large eukaryotic viruses have been structurally characterised beyond determining they are icosahedral particles. The main objective here was to reveal the molecular components and virion architecture of the most abundant marine algal viruses: the prasinoviruses. Our prasinovirus model (OtV) exclusively infects the marine algae Ostreococcus tauri, with high strain-specificity. In parallel, a collection of OtV isolates has revealed a region susceptible to genetic variations in the viral genome. These variations correlate with marked differences in the virus' host-strain range, suggesting these genes determine host specificity. Structural predictions of their encoded proteins correspond to long fibril-like structures, hypothesised to decorate the capsid surface. Transmission electron microscopy imaging of these OtVs, whose genomes encode up to 9 copies of capsid-like genes, uncovered unforeseen plasticity in their virion structures including protrusions and fibrils. Proteomic analysis identified 74 dominant virion-associated proteins. These including all predicted capsid proteins, the proteins associated with putative fibril-like structures, proteins associated with cellular adhesion and viral DNA condensation. This study provides the first insights into the protein candidates mediating virion formation and cell recognition.

Integrative Structural Biology of a T=219 Marine Giant Virus

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Viruses with capsids larger than 150 nm are considered "giant viruses" *(1)*. *Prymnesium kappa* virus RF02 (PkV-RF02) has a 583 kbp linear dsDNA genome and 200 nm diameter capsid *(2)*. Using mass spectrometry, we have identified 140 different proteins in purified PkV-RF02, with nearly 70% of them having unknown function *(3)*. We used cryo-electron microscopy (cryo-EM) single particle analysis to obtain high-resolution maps of ordered components of the PkV-RF02 icosahedral capsid, and cross-linking (XL) followed by mass spectrometry (MS) to identify protein-protein interactions (PPIs) present in both the icosahedral shell and non-icosahedral core.

Our ~3 Å resolution structure shows that PkV-RF02 has a triangulation number *pseudo*T=219, previously observed only for *Phaeocystis pouchetii* virus (PpV01) *(4)*. The PkV-RF02 capsid is composed by more than 20 different proteins and nearly 12,000 protein copies. PkV-RF02 has novel characteristics among giant viruses: a protein channel within the virion, an unusual penton protein organization, and a multilayered stabilization network reinforced by disulfide bridges and O-linked glycosylations on the surface.

Using XL-MS, we detected 561 PPIs happening between 112 different viral proteins. These PPIs define symmetry-mismatched features escaping localization by cryo-EM, as well as numerous PPIs within the PkV-RF02 non-icosahedral core, showing highly complex functional enzymes packaged in the viral particle: bacteriorhodopsin-like protein, disulfide isomerase or RNA polymerase complex, among others. This work shows a complete structural characterization of the capsid and core components of a giant virus combining cryo-EM and XL-MS, and is the first high-resolution structure of a *p*T=219 virus.

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Viromics

Prediction of eukaryotic host range for nucleocytoviruses and mirusviruses

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Genoscope

Aquatic unicellular eukaryotes play major roles in biogeochemical cycles and the ecology of plankton. They are infected by members of two diversified phyla of double-stranded DNA viruses: *Nucleocytoviricota* and *Mirusviricota*. Cultivation, single-cell genomics, and comparative genomics analyses (i.e., gene flux) can help determine or predict the host range of viruses. Here, we surveyed a large number of genomes corresponding to unicellular eukaryotes, nucleocytoviruses and mirusviruses that for many co-exist in the same ecosystems. We performed a global gene flux analysis and used this signal as a proxy to describe the global host range of nucleocytoviruses and mirusviruses in the context of their evolutionary history. As a whole, nucleocytoviruses displayed particularly strong signal with either Ochrophyta or Chlorophyta. In contrasts, mirusviruses displayed strong signal with either MAST, Cryptista or Haptista. Despite those contrasting trends, we identified many eukaryotic genomes with signal for both nucleocytoviruses and mirusviruses. In addition, we detected clear gene flux signal between lineages of the two viral phyla. Overall, our survey suggests that each lineage among the two viral phyla has a distinct ecological niche within plankton, but that many unicellular eukaryotes appear to be infected by both nucleocytoviruses and mirusviruses.

Strain-level differences of chlorarachniophyte-infecting giant viruses impact DNA methylation

Max-Emil Schön

Giant DNA viruses are ubiquitous among unicellular eukaryotes and occur in marine, freshwater, and terrestrial environments. Despite intense metagenomic data mining, their strain-level diversity remains largely unexplored. Here we introduce a model system comprising four isolates of a giant virus called ChlorV, which infects marine microalgae of the class Chlorarachniophyceae (Rhizaria) from station ALOHA, Hawai'i. The ChlorV genomes are 469 kbp to 493 kbp long and encode approximately 400 proteins, at least 106 of which are present n purified virions. Although the four viral genomes are highly syntenic, they differ by several insertions and deletions that often encode methyltransferases. Interestingly, we found that some of these methyltransferase genes correlated with specific DNA methylation patterns in the ChlorV strain Our study describes the first giant viruses infecting the eukaryotic supergroup Rhizaria and demonstrates how viral strain-level variation in gene content and epigenetic features may affect eco-evolutionary processes in marine microalgae.

Glycosylation in giant viruses: evolution, novelty, and activity

Lingie Meng

We investigated CAZyme genes within viral genomes from the GOEV database. Our results show that giant viruses are significantly enriched in glycosyltransferases compared to phages and bacteria (Figure 2), in terms of both gene density and diversity. These glycosyltransferase families display lineage-specific distribution patterns, for example, GT90 is predominantly found in pimascoviruses, while GT7 is predominantly found in mesomimiviruses. Ancestral state reconstruction reveals that specific gene gain events are linked to the appearence of several subfamily-level clades. For instance, GT7 was acquired by the ancestor of the highest diversified mesomimivirus lineage in the ocean, with phylogenetic evidence indicating an ancient origin of this event. Furthermore, we identified 864 GT-rich genomic islands across 587 viral genomes, which cluster into 45 groups often containing co-localized and co-expressed hypothetical genes.

Viral Biochemistry

Sialic acid modification attenuates viral infection in a cosmopolitan marine green alga

Ben Labbel

Weizmann Institute of Science

Viral infection in the ocean has a fundamental importance in driving the microbial ecology and biogeochemistry, yet little is known about the antiviral defense mechanisms. We uncover a glycan-based defense mechanism in the cosmopolitan green alga Ostreococcus tauri driven by cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH), which converts the sialic acid Neu5Ac to Neu5Gc. CMAH overexpression reduces virion adsorption, attenuates infection, and improves algal host survival. Resistant cells showed elevated CMAH expression and a marked shift toward Neu5Gc, demonstrating that O. tauri can tune its sialic-acid repertoire to reduce susceptibility to viral infection. Structural prediction and phylogeny analysis revealed functional conservation of the CMAH from algae to animals, in which it serves as a key regulator of glycan composition in the cell surface interface with diverse pathogens, while the human CMAH is mutated, likely by pathogen-driven selection. These findings establish hydroxylation-mediated sialic-acid remodeling as a conserved component of the host–pathogen arms race.

Applying metabolomic approaches to understand Pandoravirus massiliensis biochemistry

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The remarkable diversity of metabolic genes described in Giant Viruses (GVs) include enzymes involved in glycolysis, gluconeogenesis, TCA cycle, photosynthesis, and lipid β-oxidation. We have undertaken a metabolomics approach to understand the metabolism pathways of Pandoravirus massiliensis and their impacts on its amoeba host, Acanthamoeba castellanii. Liquid Chromatography/Mass Spectrometry (LCMS) of extracted lipids from purified P. massiliensis virions and A. castellanii, pre- and post-infection, revealed an abundance of phosphatidylcholine and phosphatidylethanolamine along with betaine lipid diacylglyceryltrimethylhomoserine, ceramides and triacylglycerols (TAGs). Furthermore, unusually large TAGs (>C20 fatty acids moieties) were observed to accumulate in amoeba membranes following viral replication and are not seen in amoeba pre-infection nor in purified virions. In contrast unique ceramides containing C25 long chain bases and >C20 fatty acids accumulate in pre-infection and purified virions but are not observed in amoeba membranes post-replication. Additionally, new classes of long chain fatty acids (LCFAs), including C30:2 and C30:3 LCFAs, were identified in a GV for the first time. Finally, detection of cellular metabolites in time course experiments over the course of P. massiliensis replication in A. castellanii using LC/MS positive and negative ion mode data showed good sensitivity and rich chromatography, with 2344 and 1466 metabolite peaks following control subtraction. 1984 peaks were annotated with MS2 data in positive ion mode, and 1006 in negative ion mode. In addition, 54 compounds attributable to purified virions were annotated, potentially providing insights into effected pathways. We are now employing molecular networking to investigate relationships of the large number of unknown compounds to annotated peaks, providing insight into chemical compound classes.

Chlorovisues major capsid protein glycosylation pattern

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Giant viruses (GVs) differ from regular viruses in many ways. With few exceptions, their physical size is above 200 µm and can be as large as 1 µm. The genome size is quite variable, ranging from 0.15 Mbp in Phaeoviruses (*Phycodnaviridae* family) to 2.5 Mbp in Pandora salinus (*Pandoraviridae* family). Despite these genomes' range in size, all of them encode genes with functions commonly not found in human pathogenic viruses, with a common trait being the presence of genes able to manipulate carbohydrates at a different level. For this reason, GVs are gaining interest in the field of Glycobiology. Giant dsDNA viruses are catalogued in different families, with some yet to be classified. This lecture will focus on the experimental data collected for Paramecium bursaria chlorella virus type 1 (PBCV-1), the prototype virus of the *Chlorovirus* genus (*Phycodnaviridae* family).

Chloroviruses are large (190 nm in diameter) icosahedral viruses with an internal lipid membrane, and genomes (290 to 370 kb) that encode up to 400 proteins [1]. The major capsid protein of PPBCV-1, Vp54, is N-glycosylated by a complex oligosaccharide (Figure 1) with four residues considered as conserved within this genus [2,3]. The identity of most of the glycosyltransferases involved in the synthesis of this complex glycan has been determined and these data will be reported along with those that recently have challenged the view of the conserved core region.

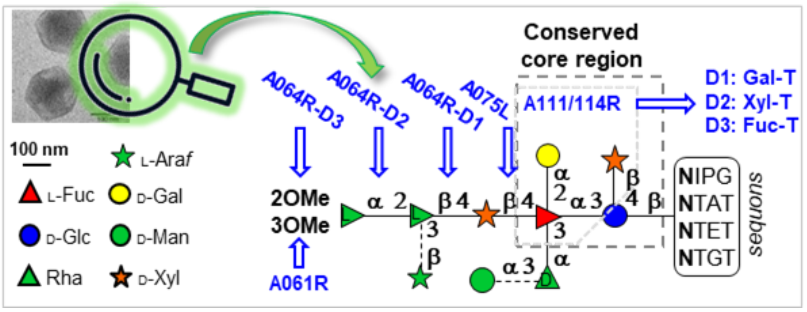

Figure 1.
N-glycan structure of PBCV-1 detailing in the grey dotted box, the four residues so far considered conserved in the chloroviruses, along with the sequons where the glycan is N-linked.
Blue text reports the glycosyltransferases whose activity has been determined so far.

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Cracking The Code Of Pyruvylation In Mimivirus: A Hidden Layer Of Viral Complexity

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Mimivirus, a giant virus from the Mimiviridae family, has challenged two major dogmas in glycobiology. First, it decorates the fibrils surrounding its icosahedral capsid with large polysaccharides, structures previously thought to be exclusive to cellular organisms1. Second, unlike typical viruses that hijack the host's glycosylation machinery, Mimivirus encodes its own glycan biosynthetic machinery with many of the genes within a cluster of 12 dedicated genes2.

Chemical analysis of its glycans has revealed that they are made up of bacterial and eukaryotic sugars, some of which are modified with non-carbohydrate appendages1. An interesting discovery was the identification of N-acetyl-glucosamine modified with a pyruvic acid linked as a ketal to the 4- and 6-hydroxyl functions, extending this modification from bacteria, yeasts, and algae3 to the viral world. Bioinformatic studies have identified L143 as a potential pyruvyl transferase. This study aimed to 1) biochemically characterise this enzyme; 2) understand the function in vivo of this sugar modification. L143 was cloned and expressed in E. coli as a recombinant protein with an N-terminal 6His-SUMO tag. Biochemical characterization revealed that L143 specifically modifies β-N-acetylglucosamine, but not its α-anomer or non-acetylated glucosamine, nor other sugars such as glucose, mannose, or N-acetylmannosamine. Importantly, like bacterial and eukaryotic enzymes, L143 does not act on nucleotide sugars (e.g., UDP-GlcNAc). Additionally, the enzyme activity is independent of cations, which aligns with the AlphaFold 3 prediction that the enzyme has a typical GT-B fold, known to be cation-independent.

Currently, we are investigating the activity of the viral enzyme at the level of disaccharide/tetrasaccharide precursors prior to the assembly of the polysaccharides. This study will identify the exact substrate of the pyruvilation reaction. To assess the functional role of this modification, we analysed glycan production in Mimivirus mutants lacking L143. The absence of L143 abolished the synthesis of the polysaccharide containing this sugar modification, suggesting its role in glycan polymerisation. Unexpectedly, this mutation also affected the production of the other polysaccharide that does not contain pyruvylation. These findings imply a functional connection between the two glycosylation pathways.

Overall, pyruvylation in Mimivirus appears to be more than a decorative sugar modification; it plays a central role in polysaccharide assembly, revealing that viral glycosylation can be unexpectedly complex.

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Virophages

The first virophage isolate infecting the subfamily *Klosneuvirinae*

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A Mimivirus-relative belonging to the order *Imitervirales*, infecting *Naegleria* spp. and named *Catovirus naegleriensis*, was described in 2024. Following the strategy previously developed for various *Acanthamoeba* species, we used *Naegleria jejunensis*, another species within the same genus, to isolate additional catoviruses. This approach enabled us to obtain several new isolates related to Catovirus.

In parallel, we co-isolated with one of these viruses a novel virophage, the first isolate in subfamily *Klosneuvirinae*. Its genome consists of an 18,703 bp circular double-stranded DNA. Preliminary annotation identified 18 ORFs, among which genes encoding a D5-primase helicase, a packaging ATPase, a cysteine protease, and a major capsid protein, as well as 13 ORFans based on searches against the NCBI non-redundant sequence database. Phylogenetic analyses indicate that this virophage represents a new lineage. Overall, this isolate expands our understanding of virophage diversity within the order *Imitervirales*.

Virophage integrase recognizes viral DNA via a terminal protein covalently linked to the genome

Anna Koslová

Virophages are small dsDNA viruses that parasitize lytic giant viruses of the phylum Nucleocytoviricota. Although endogenous sequences of virophages and related viruses have been found in many protist genomes, only a few viruses have been isolated in culture. The ability to integrate viral DNA into the host genome has so far been demonstrated experimentally only for the virophage mavirus, which provides a valuable model system to study the integration of DNA viruses in eukaryotes.

Mavirus encodes a retroviral integrase (MaV-INT), which is likely responsible for mediating genome integration. However, our data indicate that MaV-INT alone is, in contrast to retroviral integrases, insufficient to catalyze integration in vitro. We identified a terminal protein (TP) covalently attached to the 5′ end of the mavirus genome. Structural predictions suggest that TP interacts with MaV-INT, potentially positioning viral DNA close to the integrase active site. To test this hypothesis, we are producing recombinant proteins to directly analyze TP–integrase interactions and their role in DNA integration.

Our data point to a cooperative mechanism in which TP docks the viral DNA into the MaV-INT active site. This couples protein-primed DNA replication with integration, a process that has not been previously described. We hypothesize that this mechanism is not unique to the virophage mavirus but may also be employed by endogenous viral elements known as Polinton/Maverick. These elements, like mavirus, encode both a protein-primed polymerase and a retrovirus-like integrase.

Genetic manipulation of Sputnik virophage

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Virophages are dsDNA viruses that parasitize giant viruses. The first virophage, Sputnik, was isolated with a mimivirus and hijacked its replication machinery. Following the discovery, various virophages have been isolated or identified in metagenomic data, revealing their diversity and broad distribution in the environment. In contrast to advances in genomic analysis, the molecular mechanisms underlying the tripartite interactions between the host cell, giant virus, and virophage are largely unknown due to limited molecular biology tools for virophages. Here, we introduced a genomic manipulation system for Sputnik 3 virophage. In this system, the Sputnik 3 genome was constructed using the circular polymerase extension reaction (CPER). The constructed genome was then transfected into *Acanthamoeba castellanii* cells, followed by inoculation of Acanthamoeba polyphaga mimivirus. After passaging, we detected Sputnik 3 replication in the culture supernatant, which indicates the CPER-constructed genome recovers infectious Sputnik 3. We further manipulated the Sputnik 3 genome and introduced a premature stop codon or a synonymous mutation in the Sputnik major capsid protein gene. The construct in which a premature stop codon was introduced failed to recover the Sputnik 3. In contrast, with a synonymous mutation, the transfected genome recovered Sputnik 3. The recovered Sputnik 3 genome contains the designed synonymous mutation, which indicates that this system successfully manipulates the Sputnik genome.

Transcriptional competition governs the coinfection dynamics in a protist-virus-virophage system

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The interactions between giant viruses and their protist hosts play a key role in regulating microbial population dynamics, mortality rates, and marine ecosystem ecology. Virophages, small dsDNA viruses that parasitize giant viruses, can act as a defense mechanism during coinfection. We investigated the transcriptional dynamics of the giant virus CroV and its virophage mavirus in the marine protist *Cafeteria burkhardae*. CroV quickly dominated host mRNA, but mavirus gene expression negatively impacted CroV's late-stage genes through an apparent competition for transcriptional resources. Our study highlights the complex interactions between microbes and viruses, offering insights into how these interactions shape microbial communities and ecosystem processes.

Virus Evolution

Diverse evolutionary trajectories of giant viruses in amoeba hosts

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Giant viruses possess genomes and particles with sizes comparable to those of bacteria. These viruses, which primarily infect protists, are thought to have evolved through cycles of genome expansion and contraction, enabling them to adapt flexibly to changing environments and host pressures.

Using experimental evolution, we investigated how giant viruses adapt to different hosts by serially propagating three viruses in three distinct amoeba hosts. Remarkably, the outcomes varied widely across the virus-host combinations. Cytopathic effects either decreased, remained unchanged, or increased, depending on the virus. Viral replication success generally stayed the same or declined, except in one case where replication success improved after a few weeks of evolution. In this instance, we observed that viral infection altered host cell behaviour by attracting uninfected cells to infected ones, facilitating viral spread throughout the host population. Genome sequencing revealed several mutations potentially linked to the "bunch formation" phenotype observed in host cells. Ongoing investigations aim to determine whether these genomic changes actively drive bunch formation or represent secondary effects of infection.

Our findings highlight the rapid adaptability of giant viruses to novel hosts, likely driven by the genomic flexibility afforded by their large and complex genomes. This adaptability may confer a competitive advantage in environments with limited host availability, where each host encounter is critical. These results further underscore the evolutionary pressures favouring genome expansion in giant viruses.

How old are giant viruses?

Chuan Ku

Understanding the timeline of evolution is key to elucidating the origins of biological entities. While fossil records can be used for calibration in dating eukaryotes, viruses generally do not have (molecular) fossils, making it difficult to estimate their divergence times. Previous studies utilized gene trees to infer the time of origin for Nucleocytoviricota relative to eukaryotes, but the absolute time scale of giant virus (GV) evolution remains unknown. Here we developed an approach using ages of known host taxa to calibrate the maximum divergence times of GV clades and estimated the times of origins and diversifications within Nucleocytoviricota. We found that the last Nucleocytoviricota common ancestor (LNCA) existed much later than the last eukaryotic common ancestor (LECA) and dates back to ~700 million years ago. Therefore, the early evolution of Nucleocytoviricota coincided with that of animals in the Neoproterozoic era, which is characterized by a major oxygenation event on Earth. It was followed by extensive lineage diversifications and host shifts, including major transitions from amoebal to animal hosts that eventually led to the emergence of iridoviruses and African swine fever viruses. Our results suggest that GVs mainly evolved in a more aerobic environment similar to today, which agrees with previous observation that GV replication often requires mitochondrial energy. The time tree of GVs is also pivotal for further understanding their virus-host interactions through time and their functional roles in the history of Earth.

Tracing gene birth and expression dynamics in pandoraviruses

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With genomes reaching up to 2.5 Mb, pandoraviruses currently hold the record for the largest and most complex viral genomes, yet the evolutionary mechanisms underlying this remarkable expansion remain poorly understood. Several mechanisms are likely involved, including gene duplication, horizontal gene transfer, large genomic insertions, and the *de novo* creation of genes from non-coding regions. We isolated and sequenced several *Pandoravirus* strains which, together with previously published ones, provide a dataset of more than 26 complete (telomere-to-telomere) or nearly complete genomes. This enables us to better understand *Pandoravirus* genome evolution and phylogeny. In addition to comparative genomics, transcriptomic analysis of *Pandoravirus neocaledonia* gene expression revealed that recently created or acquired genes are expressed during the infectious cycle, although generally at lower levels than “older” ones. We also investigated the translation of *Pandoravirus* genes through ribosome profiling (Ribo-seq) and proteomics (MS/MS). These data, combined with RNA-seq expression profiles, indicate that *Pandoravirus* gene expression is subject to translational regulation during infection, in addition to transcriptional control. Furthermore, we determined the subcellular localization of proteins encoded by recently-created genes using fluorescence microscopy and identified their interacting partners to infer potential functional associations. Although their expression proved challenging, we identified protein partners for candidate genes using pull-down assays coupled with mass spectrometry. Altogether, this ongoing work highlights pandoraviruses not only as biologically unique entities but also as active players in evolutionary innovation.

Posters

Modeling Morphogenesis of (Sub)cellular Structures Generated by Protein Assemblies

Christoph Allolio

Cellular phenomena at the microscopic level are usually modeled via molecular simulations. These simulations have the advantage of giving an accurate description of the molecular interactions that give rise to biological phenomena. However, they are computationally expensive and hard to interpret. In particular, they are not capable of giving a satisfactory view of slow processes, such as viral budding or cell division. For this purpose, continuum models are preferable. These models can be validated by structural data (e.g. electron microscopy). However, they can also be directly parametrized by molecular simulations. I present such a "data-driven" model for large membrane deformations[1], e.g. induced by cell penetrating peptides[2] as well as a more reductive morphoelastic model for bacterial division[3]. As a newcomer to the field of giant viruses, I also show how coarse-grained simulations can help to decipher the molecular grammar of condensate-protein assembly at interfaces[4] and how hybrid continuum and molecular modeling can help elucidate the function of membrane-shaping protein assemblies.

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From algal genomics to virosphere: An unexpected detection of a nucleocytoviricot infecting the dictyochophyte Rhizochromulina marina

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The amoeboid alga *Rhizochromulina* marina was described in 1979 by D.J. Hibberd and Chrétiennot Dinet, and its authentic (type) strain is still available from the Culture Collection of Algae & Protozoa as CCAP 950/1. The alga belongs to the ochrophyte class Dictyochophyceae, a group that has recently attracted attention, especially regarding the plastid genome content and phylogenetic relationships. However, no genomic data were available for the type strain of R. marina. To resolve taxonomic questions and gain insights into algal biology, we obtained a draft genome assembly from a culture of strain CCAP 950/1. We retrieved from it a full plastid genome sequence, the analysis of which demonstrated that the previously sequenced alga referred to in the literature and databases as "*Rhizochromulina marina*" is very different from the authentic strain, indicating its misidentification. Unexpectedly, we also detected high-coverage sequences in the draft assembly corresponding to a virus belonging to the phylum Nucleocytoviricota (formerly known as NCLDV). We infer that the alga is infected by an actively replicating virus, a conclusion supported by transmission electron microscopy (TEM), which revealed putative viral particles within R. marina cells. Phylogenetic analyses of two marker sequences – family B DNA polymerase (PolB) and the major capsid protein (MCP) – showed that the virus belongs to the order Pandoravirales, specifically to the family denoted as PV_03. Notably, no named virus has yet been assigned to this group. Using Nanopore reads, we assembled the complete viral genome, which was found to be circular-mapping, consisting of 654,754 bp, and containing 583 open reading frames (ORFs). We aim to investigate this host-virus system further to better understand the interaction between the partners.

Giant virus-like particles with new morphologies from soil

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Large DNA viruses of the phylum Nucleocytoviricota infect diverse eukaryotic hosts from protists to humans, with profound consequences for aquatic and terrestrial ecosystems. While nucleocytoviruses are known to be highly diverse in metagenomes, knowledge of their capsid structures is restricted to a few characterized representatives. Here, we visualize giant virus-like particles (VLPs, diameter >0.2 µm) directly from the environment using transmission electron microscopy. We found that Harvard Forest soils contain a higher diversity of giant VLP morphotypes than all hitherto isolated giant viruses combined. These included VLPs with icosahedral capsid symmetry, ovoid shapes similar to pandoraviruses, and bacilliform shapes that may represent novel viruses. We discovered large isometric VLPs with structural modifications that had not been described before, including tubular appendages, modified vertices, tails, and capsids consisting of multiple layers or internal channels. Many giant VLPs were covered with fibers of varying lengths, thicknesses, densities, and terminal structures. These findings imply that giant viruses employ a much wider array of virion structures and mechanisms to interact with their host cells than is currently known. The forest soil was also rich in bacteriophage-like particles.

Giant Viruses And Their Potential Antimicrobial Peptides (AMPs)

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Giant viruses are part of the NCLDVs group, and they contain large genomes with many genes of unknown function [1, 2, 3], suggesting the potential to encode AMPs. Analysis of all publicly available giant-virus proteomes (up to February 2025) revealed substantial variation in predicted AMP abundance across families. For instance, Mimiviruses showed a positive correlation between genome size and the number of AMP candidates, whereas some other large genomes, such as Pandoraviruses, encoded comparatively few. A subset of predicted AMPs candidates was synthesized and tested against both gram-positive and gram-negative bacteria. One small peptide from *Acanthamoeba polyphaga* mimivirus exhibited antimicrobial activity at higher concentrations against most of the bacteria. Notably, this peptide derives from a small hypothetical protein that was part of the genetic material lost during the evolution of an axenic, in-vitro–adapted APMV lineage [4], which supports the role of these peptides to be used for competition. Curiously, this protein also possessed two other predicted AMPs. These findings suggest that giant viruses can encode functional AMPs and may engage in microbial competition within the host environment. Ongoing work will further test viral AMPs and characterize isolates from Arctic environments.

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Two novel giant viruses infecting chlorarachniophyte algae

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Giant viruses of the phylum *Nucleocytoviricota* are genetically diverse and ecologically relevant pathogens of various protists, including marine microalgae. Despite thousands of species described by metagenomics, few algae-virus systems have been isolated and are amenable to detailed characterization. Here, we report the discovery of two novel giant viruses infecting marine algae of the class Chlorarachniophyceae (Rhizaria). These viruses into two different families within the order *Imitervirales*, and each species is represented by four genetically and phenotypically distinct viral isolates. Viruses of the ChlorV group have 490 kbp long linear dsDNA genomes and are distantly related to the Cafeteria-infecting giant virus CroV of the family *Mimiviridae*. Viruses of the BigV group have 465 kbp long circular dsDNA genomes and represent the first isolates of a family that was so far populated solely by MAGs (IM13 cluster).

Deep annotation of a Ectocarpus sp. virus genome reveals crowned pentons and host-interaction candidates

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Despite being an extensively studied genus within Nucleocytoviricota, the functions of most genes in Phaeoviral genomes remain poorly understood. To tackle this, we developed a functional annotation framework that integrates multiple layers of evidence. Using a long-read genome assembly from a diploid Ectocarpus sp. line, our framework allowed for an exhaustive annotation of an endogenous viral element (EVE). As a case study, we identified and characterized the penton protein, and show that the modeled pentameric capsomer exhibits a crown-like insertion above the core jelly-roll fold, similar to the P1v1 penton variant of Paramecium bursaria chlorella virus 1 (PBCV-1), and the P1 penton of the Emiliania huxleyi virus 201 (EhV-201). We also found a (i) candidate trimeric spike/fiber with clear structural similarity to a bacteriophage tailspike, as well as a (ii) fibronectin type-III domain-containing protein, whose modeled homotrimer also shares hallmark structural features of tailspikes. Finally, we uncovered multiple putative outer-capsid proteins containing lectin- or fibronectin-like domains. Our work, provides a curated genomic foundation for future studies of Phaeovirus biology, and highlights virion components possibly involved in interactions with the host.

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How Condensates Change Morphology and Topology of Cellular Membranes

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Membrane-bound organelles have been considered the fundamental organizational compartments of cells for decades. However, more recent research has revealed that condensed biomolecular phases (aka membrane-less organelles, viral factories) play an additional intricate role in cells. Combining in vivo and in vitro experimentation with theoretical approaches allowed us to demonstrate that condensates and membrane interact in cells, which gives rise to novel capillarity-based force generation mechanisms that are implicated in fundamental physiological processes and might play a role during virus infection.

I will introduce capillarity and highlight how condensates drive morphogenesis of membrane-bound organelles by promoting membrane bending events[1,2] and also, by cutting membrane necks without ATP-consuming protein machineries like ESCRTs[3]. Examples include the formation of intraluminal vesicles in multivesicular bodies, of autophagosomes during autophagy and of protein storage vacuoles during plant embryogenesis. Our findings unveil previously unrecognized physiological roles of the condensate-membrane interplay[4,5], exemplifying how key compartments jointly contribute to intracellular organization.

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Annotating and discovering CAZymes with deep learning

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Carbohydrate-active enzymes (CAZymes) play a central role in carbohydrate metabolism and are essential for life. Their diversity in microbial communities significantly influences molecular and organismal interactions in diverse context. Despite their importance, there remains a lack of fast and accurate tools for CAZyme identification and substrate prediction. The CAZy database, a gold standard in the field, provides manually curated annotations but still relies heavily on expert curation. The current state-of-the-art tool, dbCAN, employs HMM-based domain detection for CAZyme annotation. However, its performance is limited by the quality of multiple sequence alignments (F1-score=0.848, Precision=0.870, Recall=0.857). In this study, we propose a deep learning framework for CAZyme identification and classification. Our approach integrates sequence embeddings derived from protein language models, convolutional neural networks (CNNs), and multilayer perceptron together. Our model achieves high accuracy in subfamily-level classification (F1-score=0.952; Precision=0.963; Recall=0.945), outperforming the conventional sequence similarity-based software dbCAN. Furthermore, our model can predict domain positions without explicit boundary information during training, indicating an intrinsic capability to learn biological structural features. We anticipate that this framework will become a powerful tool for CAZyme discovery, particularly in the context of large-scale metagenomic datasets.

A novel subgroup of Preplasmiviricota viruses endogenized in the nuclear genome of Paratrimastix pyriformis (Preaxostyla, Metamonada)

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The realm *Varidnaviria* comprises dsDNA characterized by double-jelly roll major capsid protein (DJR-MCP). Within this realm, the kingdom *Bamfordvirae* includes two principal phyla: *Nucleocytoviricota* and *Preplasmiviricota*. The former encompasses the nucleocytoplasmic large DNA viruses (NCLDVs), including many "giant viruses" of eukaryotes. Preplasmiviricots represent smaller DJR-MCP viruses, with the eukaryotic members groups in the proposed subphylum *Polisuviricotina*, which unites polintons (Polintoviruses), polinton-like viruses (PLVs), virophages (*Virophaviricetes*), and vertebrate adenoviruses. Except for adenoviruses, polisuviricotines frequently integrate into host genomes as endogenous viral elements (EVEs) with PLVs being widespread as EVEs across eukaryotes, yet only a few representatives have experimentally verified virions or giant virus-dependent lifestyles. Here we report EVEs that expand the known PLV diversity. Our analysis of the nuclear genome of the metamonad *Paratrimastix pyriformis* revealed the presence of DJR-MCP-encoding EVEs that define a new subgroup of PLV-type viruses when assessed by the features of their MCP. Specifically, our clustering analysis of DJR-MCP sequences, covering representatives of all previously described groups of PLVs, virophages, nucleocytoviricots (including mriyaviruses), together with the MCPs from the *P. pyriformis* EVEs grouped the latter into a separate cluster, distinct even from the previously defined PLV group found in other metamonad genomes (MMN). While most of the EVEs in the new group seem to be degraded, disrupted by inserted transposable elements, or incomplete due to fragmented genome assembly, we did reconstruct putative complete sequences of several representatives, allowing us to evaluate their gene repertoire. The *P. pyriformis* EVEs do expectedly encode other components of the capsid biogenesis module (minor capsid protein, packaging ATPase, adenain-like protease), but they vary a lot in their complement of proteins involved in DNA replication and integration. Most notably, while some encode the protein-primed family B DNA polymerase (pPolB) common in polintons and PLVs, a different subset instead exhibits a different type of PolB polymerase, which belongs among RNA/DNA-primed PolBs of cellular organisms, nucleocytoviricots, and two subgroups of the *Duplodnaviria* realm (herpsesviruses and mirusviruses), and forms a novel independent lineage of these polymerases in our phylogenetic tree. Strikingly, this polymerase exhibits a C-terminal extension contain a domain shared with the herpesviral protein UL9, a superfamily II helicase that initiations herpesviral DNA replication by binding the origin of replication. Furthermore, a stand-alone (unfused) version of this domain, which to our knowledge has not been reported from previously characterized PLVs, occurs also in some of the *P. pyriformis* EVEs exhibiting pPolB. Combined, our analyses have uncovered the existence of a unique subgroup of DJR-MCP viruses that further blurs the boundaries between established major viral taxa.

Demystifying African Swine Fever Virus

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Until recently, African Swine Fever Virus (ASFV), a major, economically important pathogen of domestic pigs, remained the only animal-infecting viral species in the order Asfuvirales (asfarviruses), whereas all other identified asfarviruses were associated with protist hosts. Thus, the origin of ASFV remained enigmatic, suggesting the possibility of a recent transfer from a protist to a mammalian host. However, the discovery of an asfarvirus infecting abalone suggested that a hidden diversity of animal asfarviruses might have been overlooked. Comprehensive search for homologs of asfarvirus genes associated (integrated or co-sequenced) with eukaryotic genomes revealed over 100 eukaryotic species previously not reported to host asfarviruses, and 124 eukaryotic contigs were positively identified as containing endogenous 'fossils' of asfarvirus genomes. The putative hosts of asfarviruses showed a wide distribution among both animals and protists. Fossils of ASFV relatives with family *Asfarviridae* were identified mostly in diverse animals, although not in vertebrates, whereas more distant asfarviruses were found in association with various protists and fungi. These findings suggest that evolution of asfarviruses included a 'jump' from protist or fungal hosts to invertebrates, and a subsequent transfer to swine via ingestion of invertebrates, likely, snails.

Species-specific Host Response During Naegleriavirus Infection: A Viral Dead End

Flo Panhölzl, Patrick Arthofer, Vincent Delafont, Alban Hay, Anouk Willemsen & Matthias Horn

The discovery of the giant virus Catovirus naegleriensis (Naegleriavirus, NiV), isolated in our laboratory in co-culture with the amoebic flagellate Naegleria clarki, expanded the known host range of giant viruses to include the class Heterolobosea, supergroup Excavata. We have elucidated the replication cycle and virion ultrastructure of NiV, as well as its genomic potential and virion proteome. The icosahedral capsid, with a diameter of approximately 500 nm, harbors a 1.16 Mbp dsDNA genome encoding 1000 predicted genes. NiV is the fourth isolate of the Mimiviridae subfamily Klosneuvirinae and, like its relatives, contains a large number of translation genes but no tRNAs. It has acquired genes encoding heat shock proteins and apoptosis-inhibiting factors, presumably for interaction with its host Naegleria. The genus Naegleria is known for the notorious human pathogen N. fowleri, which causes primary amoebic meningoencephalitis, a rare but almost always fatal disease. NiV infection was lethal for all Naegleria species tested, including N. fowleri.

We have observed that NiV infection of N. koreanum leads to a drastic reduction in viable host cells, but not to virus replication. Confocal laser scanning and transmission electron microscopy as well as transcriptome data show that N. koreanum is able to prevent the development of a virus factory in its cytoplasm. In addition, the cells show signs of autophagic processes and controlled cell death. We hypothesize that N. koreanum prevents the spread of the virus in the population by impairing the formation of a viral factory and/or inducing cell death upon infection, thereby conferring resistance at the population level.

This study will provide detailed insights into this phenomenon down to the molecular level and contribute to deepening our understanding of the complex interactions between NiV and its hosts and expanding the known ecological landscape of giant viruses in general.