DNA habitats and its RNA inhabitants

3 - 5 July 2014 Salzburg - Austria

Viruses, Mobile Genetic Elements, Viroids, Introns, Ribozymes and other RNAgents

Program

+

Book of Abstracts

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Kulturelle Sonderprojekte Land Salzburg

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KULTUR STADT : SALZBURG

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Abstracts

Talks

and

Posterpresentation

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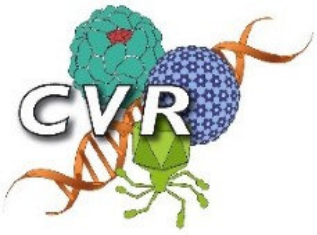
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Program

DNA habitats and its **RNA** inhabitants

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Viruses, Mobile Genetic Elements, Viroids, Introns, Ribozymes and other RNAgents

St. Virgil Conference Center
Ernst-Grein-Straße 14; A-5026 Salzburg, Austria
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Wednesday, July 2, 2014

12:00 - 20.00	Registration at St. Virgil
18:45	Welcome drink and warm reception by the organizer and partners

Thursday, July 3, 2014

8:45 Organisation Affairs !

9:00 - 9:30 Guenther Witzany
Introduction: Natural Codes do not code themselves

9:30 – 10.00 Eugene Koonin
Giant viruses and domains of life

10:00 – 10:30 Luis Villarreal
Viral consortia: A social force for ancient and recent life

Coffee Break – Tea Time (20 minutes)

11:00 – 11:30 David Prangishvili
Viruses from the dawn of life

11:30 – 12:00 Mart Krupovic
Evolutionary continuum between small RNA and DNA viruses

12:00-12:30 Valerian V. Dolja
Capsid-less and non-infectious viruses are integral to the Virus World

Lunch

13:45 – 14:30 Ricardo Flores
Viroids: filling the lower size niche for RNA genomes

14:30 – 15:00 Aare Abroi
Impact of (RNA) viruses on the genesis of cellular protein domain repertoire

Coffee Break – Tea Time (20 minutes)

15:20 – 16:20 POSTER Presentations

16:30 – 17:00 Sabine Mueller
Engineering of ribozymes with useful activities in the ancient RNA world

17:00 – 17:30 Gustavo Caetano-Anólles
Untangling the cellular origin of viruses

17:30 – 18:00 Eörs Szathmáry
Dynamical questions of the early RNA world

Dinner

Friday, July 4, 2014

8:45 Organisation Affairs !

9:00 – 9:30 Lennart Randau
Small RNA genes mediate genome rearrangement events in Archaea

9:30 – 10:00 Matti Jalasvuori
Patterns in genomic chaos: bacterial cells as vehicles of war in genetic struggle for existence

10:00 – 10:30 Mariusz Nowacki
RNA-mediated genome sculpting in ciliates

Coffee Break – Tea Time (20 minutes)

10:50 – 11:30 John Mattick
RNA at the epicentre of the evolution and development of complex organisms

11:30 – 12:00 Marilyn Roossinck
Persistent viruses in plants and fungi: molecular fossils

12:00 – 12:30 Eric A. Miska
Social RNA: update from invertebrates

Lunch

13:45 – 14:30 Robert Gifford
Endogenous retroviruses and the generation of genetic diversity in mammals

14:30 – 15:00 Frederick Arnaud
The evolutionary interplay between endogenous and exogenous sheep retroviruses and their host

Coffee Break – Tea Time (20 minutes)

15:20 – 16:20 POSTER Presentations

16:30 – 17:00 Keizo Tomonaga
Bornavirus infection: a unique life style of an animal RNA virus in the DNA habitat

17:00 – 17:30 Erez Levanon
DNA and RNA editing of mammalian retrotransposons

17:30 – 18:00 Joan Curcio
Host co-factors of retrotransposon RNA localization to nucleocapsid assembly sites

18:50 Congress Dinner with Music Performance

Saturday, July 5, 2014

8:45 Organisation Affairs !

9:00– 9:30 Karin Moelling
Reverse Transcriptase and RNase H

9:30 – 10:00 Juergen Brosius
3.8 billion years of RNA

10:00 – 10:30 Kevin Weeks
Towards an RNA structure of everything

Coffee Break – Tea Time (20 minutes)

10:50 – 11:30 Eric Westhof
Constraints and Limits due to Base Tautomerism in Recognition Fidelity

11:30 – 12:00 Peter Unrau
A bright, high affinity, RNA aptamer-dye complex suitable for in vivo RNA tracking

12:00 – 12:30 Andreas Werner
Endo-siRNAs from natural antisense transcripts

Lunch

13:45 – 14:30 Steinar Johansen
Role of self–splicing introns beyond RNA splicing

14:30 – 15:00 Harald Brüssow
Modulation of gut microbiota by oral bacteriophages and nutritional interventions: insights from human intervention trials

Coffee Break – Tea Time (20 minutes)

15:20 – 16: 20 POSTER Presentations

16:30 – 17:00 Corrado Spadafora
Retroelements in embryonic development, tumorigenesis and evolution

17:00 – 17:30 Ravindra Singh
A unique RNA structure that paved the way for the treatment of a leading genetic disease

17:70 – 18:00 Minoo Rassoulzadegan
RNA mediated heredity of an acquired pathology

Dinner

Sunday, 6th July

Sunday-Excursion half day to an extraordinary place near Salzburg: Hellbrunn
(30 Euros including: transfer, guided tour and meals)

Detailed Programm will be published after receipt of the abstracts (June 2014)

Aare Abroi

Impact of (RNA) viruses on the genesis of cellular protein domain repertoire.

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During the last ~10 years a very high abundance and genetic diversity of viruses in all biotopes has been recognized. Viruses encode virosphere specific (**Vsp**) protein domains at different levels. For example ~790 PfamA domains are found only in viruses. In spite of the fact that the fraction of structurally characterized viral proteins is lower compared to the cellular ones also Vsp structural domains exist at SCOP superfamily (~70 superfamilies) as well as fold level. Looking by the functional classes of viruses in all RNA viruses (dsRNA viruses, -ssRNA and +ssRNA) the fraction of Vsp domains is very high both in PfamA domains and SCOP domains. So, the RNA viruses have a remarkable fraction of protein domains (and also functional novelty) not found in cellular organisms. During the last ~5 years V2H gene transfer even for RNA viruses has been proven. Thus, RNA viruses are a source of new and novel protein domains for cellular proteins.

In addition to their own constituents viruses may transfer DNA, RNA, proteins and other biomolecules of cellular origin. In the case of high viral titre in the organism it is a significant amount of biologically active material. When studying cellular nucleic acid in viral particles the purity of the samples is crucial (for example with respect of exosomes). We carefully examined the purity of virus preparation to conclude on the presence of non-viral RNA in viral particles. “Out of Koch postulate” biological role of viruses will be discussed.

Frederick Arnaud[1,2], M. Caporale[2], M. Varela[2], M. Golder[2], M. Mura[2], Bernardo Chessa[3], A. Alberti[3], L. Murphy[2], A. Armezzani[2], T.E. Spencer[4] and M. Palmarini[2]

Interplays between Retroviruses and their Host during evolution: Lessons from the Sheep

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Endogenous retroviruses (ERVs) originate from ancient retroviral infections of the germ line and are transmitted vertically from generation to generation. Nowadays, the majority of ERVs are defective; however some of them have maintained part or all of their genes intact for millions of years, suggesting that they have provided a beneficial role to their host. We have studied the complex interplay between ERVs, exogenous pathogenic retroviruses and their host using sheep as model system. Jaagsiekte sheep retrovirus (JSRV) is the causative agent of ovine pulmonary adenocarcinoma and coexists with highly related endogenous retroviruses (enJSRVs) that colonized the sheep genome throughout evolution. Interestingly, we discovered that enJSRVs play a critical role in conceptus development and placental morphogenesis of sheep. In addition, two enJSRV proviruses (enJS56A1 and enJSRV-20), which entered the host genome within the last 3 million years, acquired a defective protein in two temporally distinct events resulting in a "protective" phenotype able to block JSRV late replication steps. These two proviruses became fixed in the genome of domestic sheep supporting the idea of their positive selection during, or immediately before, sheep domestication (9,000 years ago). Interestingly, we also identified a recent provirus (< 200 years old) with an intact genomic organization that escapes the restriction induced by enJS56A1 and enJSRV-20. These data provide evidence that the invasion of the sheep genome by endogenous retroviruses is still ongoing and has not reached an equilibrium yet. Therefore, sheep provide an exciting model to study the co-evolution between ERVs and their host.

Keywords: virus–host co-evolution, endogenous retroviruses, viral restriction and placental morphogenesis.

Juergen Brosius

3.8 billion years of RNA

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RNA was one of the first, if not the primordial macromolecule to solidly establish what we define as "life" on this planet. Interestingly, many evolutionary principles observable in extant cells, must already have existed in a cell that relied solely on RNA as its carrier of hereditary material, as well as constituting the major catalytical componentry. After several transitions from the RNA world to the RNP world or extant cells, RNA functions were gradually replaced by proteins and DNA respectively. However, RNA did not become obsolete or marginalized as messenger RNA. Instead, novel molecules continue to evolve. For example, over the past few years, many non-protein coding RNAs were discovered and their functions elucidated. Cytoplasmic BC1 RNA or small nucleolar RNA snorD116/HBII-85, for example, arose in a common ancestor of a mammalian order or the taxa of placental mammals, respectively. Their deletion in mouse models leads to distinct phenotypes, that can serve as animal models for genetic disease, such as the neurodevelopmental disease Prader-Willi-Syndrome (PWS) in case of SnorD116.

Harald Brüssow

A clinical trial with phages underlines the importance of commensal gut microbiota in childhood E. coli diarrhea

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Some bacterial pathogens have developed such a level of resistance that antibiotics are not any longer of clinical use. This is the case in children with Escherichia coli diarrhea, a major cause of childhood mortality in developing countries. Since E. coli and its phages belong to the best-investigated biological systems, we decided to explore the therapeutic potential of orally applied coliphages in children hospitalized with E. coli diarrhea. We conducted a randomized controlled clinical trial (RCT) at the world's leading diarrhea research hospital in Dhaka / Bangladesh. Oral-fecal transit of phage was observed, but we did not observe substantial in vivo replication of the oral phage or a decrease of intestinal E. coli pathogens nor an effect on quantitative diarrhea parameter. Even in microbiologically confirmed cases of E. coli diarrhea, E. coli did not dominate the gut microbiota as measured by high throughput sequencing of bacterial ribosomal DNA. However, we observed a marked dysbiosis of commensal gut bacteria in the acute phase of diarrhea which resolved with the recovery of the patients. Ratios of two particular gut commensals correlated better with diarrhea development than pathogenic E. coli. The data underline how few we know about phage-E. coli interaction in its natural niche and that re-establishing the normal gut commensal equilibrium may be as important as eliminating pathogens for resolution of bacterial diarrhea.

Gustavo Caetano-Anollés, Arshan Nasir and Kyung Mo Kim

Untangling the origin of viruses and their impact on evolution of cells

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While the genetic entities of the virosphere are more abundant and diverse than those of the cellular ribosphere of our planet, their significance remains neglected. Previous explanatory frameworks described viruses as founders of cellular life, as parasitic reductive products of cells, or as escapees of modern genomes. Each of these frameworks endows viruses with distinct molecular, cellular, dynamic and emergent properties. Here we attempt to dissect the origin of viruses, understand their evolution, and measure their impact on cellular life. We focus on the structure of protein and nucleic acid evolutionary modules that are highly conserved. We use information in thousands of genomes of cellular organisms and viruses with all known replicon types to build phylogenies describing the evolution of proteomes and protein domain components and RNA molecules and their structural parts. A truly universal 'tree of life' suggests viruses constitute a 'fourth supergroup' along with the three domains of life. Trees of protein domain structures uncover timelines that indicate viruses have evolved via massive and primordial reductive evolutionary processes. Three lines of evidence support the massive transfer of genetic information from viruses to cells: (i) early structures shared with viruses are significantly overrepresented in cellular organisms; (ii) virus-specific structures reveal viruses do not simply spread novelty but foster it; and (iii) viruses integrate into cellular genomes at high frequency altering cellular make-up. Finally, survey and retrodiction suggest viruses originated from and coexisted with primordial cells. They became parasitic once virions and diversified cellular life took over the planet.

M. Joan Curcio

Host co-factors of retrotransposon RNA localization to nucleocapsid assembly sites

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As drivers of genome remodeling, retrotransposons are central players in evolution, adaptation, tumorigenesis and aging. The S. cerevisiae Ty1 retrotransposon belongs to a family of retrotransposons that are progenitors of infectious retroviruses. Ty1 RNA and its capsid, Gag, assemble into nucleocapsid particles in a cytoplasmic focus known as a retrosome. We have shown that retrosomes are sites of localized translation of Ty1 RNA and translocation of nascent Gag to the ER lumen. Following retrotranslocation to the cytoplasm, Gag binds translating Ty1 RNA to initiate nucleocapsid assembly (Doh, Lutz and Curcio (2014) PLoS Genet. 10(3): e1004219). Other than being membrane-associated, the location of the retrosome is not known. Using multi-dimensional time-lapse microscopy, we have found that the retrosome is associated with the spindle pole body (SPB), the nuclear membrane-embedded microtubule organizing center in yeast. SPB duplication occurs at the end of the G1 phase of the cell cycle and is required for progression into S phase. The retrosome associates specifically with the daughter SPB that remains in the mother cell. We propose that this association controls the timing of Ty1 cDNA integration during replication fork pausing at Pol III-transcribed genes. In a strain harboring a specific mutant allele of RPL7A, Ty1 RNA is mislocalized and retrosomes do not form. Remarkably, the efficiency of retrotransposition is unaltered in this mutant, but Ty1 cDNA integration upstream of Pol III genes is substantially reduced. Thus, nucleocapsid assembly in concert with SPB duplication may be required for the target site selectivity of Ty1 integration.

Valerian V. Dolja[1] and Eugene V. Koonin[2]

Capsidless and noninfectious viruses are integral to the Virus World

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Capsidless virus-like agents with RNA genomes of variable size and diverse architectures share only the gene encoding the RNA-dependent RNA polymerase (RdRp). Phylogenetic analysis of RdRps suggests independent evolution of virus-like agents from distinct lineages of positive-strand RNA virus ancestors that have lost their capsid along with transmissibility via extracellular routes. Most of the capsidless RNA agents are classified as viruses including the *Narnaviridae, Hypoviridae* and *Endornaviridae* families, whereas a few are considered RNA replicons. Intriguingly, narnaviruses of the genus *Mitovirus* reproduce within fungal mitochondria and show evolutionary affinity to RNA bacteriophages, implying their potential origin from viruses of mitochondrial endosymbionts of the ancestral eukaryote. In contrast, fungal hypoviruses and plant/fungal endornaviruses most likely emerged via reductive evolution of Picornavirus-like and Alphavirus-like ancestors, respectively.

Another class of non-infectious virus-like agents, known as retrotransposons includes *Metaviridae* (Gypsy/Ty3-like elements) and *Pseudoviridae* (Copia/Ty1-like elements). The genomes of these agents are archived as DNA copies integrated in host chromosomes. However, expression of these genomes yields RNA copies that are not only reverse transcribed into transposable DNA, but are also encapsidated into virus-like particles. The pseudoviruses are widely represented in plants, as well as in some fungi and invertebrates. The metaviruses pepper the genomes of most if not all studied lineages of eukaryotes. This global distribution and phylogenetic analysis of metavirus reverse transcriptases supports their ancestral relationship with vertebrate-infecting *Retroviridae* and plant-infecting *Caulimoviridae*. Thus, tight evolutionary links between viruses and virus-like non-infectious agents, either capsidless or encapsidated, necessitate their inclusion within a unified virus world.

Ricardo Flores

Viroids: filling the lower size niche for RNA genomes

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In all extant cells DNA and proteins store and express the genetic information, respectively, with RNA apparently playing an ancillary connecting role. Yet, the situation changes upon descending within the biological scale, with many examples of viruses having RNA, instead of DNA, as their genetic material. Besides storing genetic information RNA can express it, as illustrated by the discovery of an increasing number of catalytic RNAs (ribozymes). This dual ability has served as the foundation for the proposal of an RNA World that preceded the emergence of DNA and proteins. If such a world ever existed, the possibility of finding its survivors in our present world does not seem a farfetched hypothesis. Indeed, viroids display unique properties consistent with those predicted for the first minimal replicons that might have evolved in the RNA World. These properties include: i) small size and high G+C content to cope with error-prone replication, compact folding to enhance survival, and circularity to achieve complete replication without recurring to genomic tags, ii) remnants of structural periodicity suggesting modular assembly into enlarged genomes, iii) lack of protein-coding ability in accordance with a ribosome-free environment, iv) replication in at least some of them mediated by ribozymes, the key signature of the RNA World and, v) plausible evolutionary pathways for the transition from the RNA World to a world with proteins and DNA, where the primordial viroids lost some of their competences and turned into the plant parasites found infecting plants today.

Robert J. Gifford

Endogenous retroviruses and the generation of genetic diversity in mammals

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Retroviruses are RNA viruses that cause chronic, persistent infections and have circulated in mammals for over 100 million years. Retroviral infection of germline cells can lead to integrated retroviral sequences being inherited as host alleles called "endogenous" retroviruses (ERVs). ERVs can proliferate within the germline through a variety of mechanisms, including viral replication and retrotransposition in cis or trans. They have been identified in the genomes of all jawed vertebrates, and typically constitute ~10% of mammalian genomes. This talk will explore the coevolution of mammals and retroviruses, examining (i) how the distribution and diversity of mammalian retroviruses has changed over time, (ii) the molecular mechanisms and evolutionary selection pressures have driven the proliferation of specific ERV lineages in mammals, and (iii) what can be predicted about the potential functional roles of ERV loci based on their sequences and/or genomic locations.

Matti Jalasvuori

Patterns in genomic chaos: bacterial cells as vehicles of war in genetic struggle for existence

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Prokaryotic biosphere is a vastly diverse system in various respects. Any given bacterial or archaeal cell may harbour in different combinations viruses, plasmids, transposons, and other genetic elements along with their cellular chromosome(s). These agents interact in complex environments in various ways causing multitude of phenotypic effects on their host cells. In this presentation, I explore the possibility to 'dissect' bacterial (or archaeal) cell in order to simplify the diversity into components that make bacterial evolution easier to approach. The cell itself is separated from all the genetic replicators that use the cell vehicle for preservation and propagation of the genetic information. I introduce the possibility to classify groups of different replicators according to their horizontal movement potential between cells and according to their effects on the fitness of their present host cells. The classification can be used to improve the means by which we approach general evolutionary tendencies in microbial communities. Namely, for example, we can better grasp the reasons behind the dissemination of antibiotic resistance genes among (pathogenic) bacteria. Overall, any given biosphere comprising prokaryotic cell vehicles and genetic replicators may naturally evolve to have various types of horizontally moving replicators.

Steinar D. Johansen

Molecular domestication and speciation of RNA self-splicing introns

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Group I introns represent a distinct class of RNA self-splicing introns with an ancient origin, a unique molecular splicing mechanism, and a well-defined RNA core structure [1]. Group I introns are widespread but sporadically distributed in nature. They are relatively common in nuclear ribosomal DNA of eukaryotic microorganisms, and in mitochondrial genomes of non-bilateral animals. A current view is that group I introns are selfish mobile genetic elements that perform splicing at the RNA level. We study function of group I introns beyond splicing and mobility. Our experimental approaches include RNA biochemistry, structural RNA assessments, molecular evolution, and RNA-Seq. Two intron model systems are investigated: A) Obligatory group I introns found in mitochondrial genomes of sea anemones and corals [2]. These giant introns (up to 20 kb in length) possess unusual structural features and splicing pathway, and recent results indicate regulatory roles in mitochondria due to host adaptation. B) Twin-ribozyme group I introns found in nuclear genomes of eukaryotic microorganisms [1]. These introns consist of a regular group I splicing ribozyme and a lariat capping (LC) ribozyme. The LC-ribozymes have evolved from ancestral group I ribozymes due to loss of selection pressure for self-splicing. The LC-ribozymes now constitute a new ribozyme family that perform mRNA capping and harbour a 3D structure that departs significantly from that of group I intron RNAs [3].

Recent refs:
[1]. Hedberg & Johansen (2013). Nuclear group I introns in self-splicing and beyond. Mob DNA, 4:17
[2]. Emblem et al. (2014). Sea anemones possess dynamic mitogenome structures. Mol Phylogenet Evol. 75: 184-193.
[3]. Meyer et al. (2014). Speciation of a group I intron into a lariat capping ribozyme. PNAS, 111: in press.

Eugene V. Koonin[1], Natalya Yutin[1], Mart Krupovic[2]

Giant viruses and domains of life

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Eukaryotic viruses with large double-stranded DNA genomes that at least partially reproduce in the cytoplasm of infected cells form a large class that apparently evolved from a single virus ancestor and is known as Nucleocytoplasmic Large DNA Viruses (NCLDV) or the proposed order Megavirales. Among the NLCDV, there are three groups of giant viruses with genomes exceeding 500 kb, namely Mimiviruses, Pithoviruses, and Pandoraviruses that hold the current record of viral genome size, 2.4 Mb. Phylogenetic analysis of conserved, ancestral NLCDV genes clearly shows that these three groups of giant viruses do not share a common origin. Maximum likelihood reconstruction of gene gain and loss events during the evolution of the NCLDV indicates that each of these groups of giant viruses evolved from viruses with substantially smaller and simpler gene repertoires. Initial phylogenetic analysis of universal genes, such as translation system components, encoded by some giant viruses, in particular Mimiviruses, has led to the hypothesis that giant viruses descend from a fourth, probably extinct domain of cellular life. The results of comprehensive phylogenomic analysis of giant viruses refute the fourth domain hypothesis and instead indicate that the universal genes have been independently acquired by different giant viruses from their eukaryotic hosts. Rather than deriving giant viruses and their smaller kin from hypothetical extinct domains of cellular life forms, comparative genomics suggests a unified scenario that links these viruses to bacteriophages, eukaryotic transposons, cytoplasmic plasmids and host genes.

Mart Krupovic

Evolutionary continuum between small RNA and DNA viruses

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Viruses with single-stranded (ss) DNA genomes infect hosts from all three domains of life and are present in all conceivable environments. Many new ssDNA viruses have been recently isolated, including those infecting algae, fungi, insects and even hyperthermophilic archaea. In parallel, culture-independent metagenomic approaches have illuminated the tremendous genetic diversity of these viruses, yielding valuable insights into their evolution. One of the significant recent advances in the field was the understanding of the mechanisms governing the evolution of ssDNA virus genomes. These viruses exhibit nucleotide substitution frequencies similar to those characteristic to RNA viruses and high rates of genetic recombination which has been pushed to the extreme in some of the viral groups. In particular, it has been recently found that ssDNA viruses can recombine not only with other DNA viruses but are also engaged in gene exchange with viruses carrying RNA genomes. During my talk, I will present several examples of such gene exchange between RNA and DNA viruses. I will integrate the available knowledge to propose a scenario under which certain groups of ssDNA viruses (including Geminiviridae, Circoviridae, Parvoviridae and Microviridae) have originated from plasmids via acquisition of jelly-roll capsid protein genes from ssRNA viruses. This scenario places structurally related viruses with DNA and RNA genomes into an evolutionary continuum and highlights general evolutionary trends in the virosphere.

Erez Levanon

DNA and RNA editing of retrotransposons accelerates mammalian genome evolution

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Retrotransposons had an important role in genome evolution, including the formation of new genes and promoters and the rewiring of gene networks. However, it is unclear how such a repertoire of functions emerged from a relatively limited number of source sequences.

Endogenous and powerful means of creating inner genomic diversity are known to exist: (1) RNA editing that leads to alteration of one nucleotide into another, (mainly A-to-I); (2) DNA editing that changes the DNA"s content by shifting C-into-U.

We have performed a genome-wide mapping of RNA and DNA editing of retroelements in various genomes using a combination of computational and genomic approaches. We found that, in human, virtually all adenosines within Alu repeats that form double-stranded RNA undergo A-to-I editing, although most sites exhibit editing at only low levels (<1%). Based on bioinformatic analyses and deep targeted sequencing, we estimate that there are over 100 million human Alu RNA editing sites, located in the majority of human genes.

In addition, we find numerous pairs of retrotransposons containing long clusters of G-to-A mutations that cannot be attributed to random mutagenesis. These clusters, which we find across different mammalian genomes and retrotransposon families, are the hallmark of APOBEC3 activity, a potent antiretroviral protein family with cytidine deamination function. As DNA editing simultaneously generates a large number of mutations, each affected element begins its evolutionary trajectory from a unique starting point, thereby increasing the probability of developing a novel function.

This explosion of genomic variety can have dramatic effect on diverse biological processes, such as brain complexity, cancer and evolution acceleration.

John S. Mattick

RNA at the epicenter of human evolution, development and cognition

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The genetic programming of complex organisms has been misunderstood for the past 50 years, because of the assumption that most genes encode proteins. This assumption has its roots in the mechanically-focused biochemical zeitgeist that has dominated molecular biology since its inception, and has led to many incorrect subsidiary assumptions, notably those concerning the structure of gene regulation and the non-functionality of transposable elements. Surprisingly, the number and repertoire of proteins in animals is relatively stable, despite vast differences in developmental and cognitive complexity. Despite assertions that there are combinatorial interactions between 'transcription factors' and other regulatory proteins (and therefore a presumed factorial scaling of the decisional possibilities), it appears that the amount of required regulatory information increases approximately quadratically with increasing complexity in all functionally integrated systems. Consistent with this, the vast majority of the human genome (and indeed all genomes) is differentially transcribed, in very precise patterns, and the evidence suggests that, far from being composed of evolutionary debris, most introns and intergenic sequences encode regulatory RNAs, many if not most appear of which to be involved in the 4-dimensional organization of chromosomes and the guidance of chromatin-modifying complexes to their sites of action, as a feed-forward control system that oversees the epigenetic trajectories of differentiation and development. Moreover, it seems that plasticity has been introduced and superimposed on this system, via RNA editing and modification, RNA-directed DNA 'repair', and mobilization of transposable elements, to underpin cognitive adaption and optimize evolutionary searchability. Evolution has learnt how to learn.

Eric Miska

Transgenerational epigenetic inheritance and RNAe

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Since August Weismann (1834-1914) formulated the distinction between innate and acquired characteristics at the end of the 19th century, the debate relating to the inheritance of acquired traits has raised many controversies in the scientific community. Following convincing arguments against (*e.g.* William Bateson) this debate was then set aside by the majority of the scientific community. However, a number of epigenetic phenomena involving RNA, histone modification or DNA methylation in many organisms have renewed interest in this area. Transgenerational effects likely have wide-ranging implications for human health, biological adaptation and evolution, however their mechanism and biology remain poorly understood. We recently demonstrated that a germline nuclear small RNA/chromatin pathway can maintain epi-allelic inheritance for many generations in *C. elegans*. This is a first in animals. We named this phenomenon RNA-induced epigenetic silencing (RNAe). We are currently further characterizing the mechanism of RNAe. In addition, we are testing the hypothesis that RNAe provides a transgenerational memory of the environment ("Lamarckism"). We are currently exploring related phenomena in mice. We are also working towards establishing iPS cells differentiating into germ cells as a model to study the mechanism of transgenerational epigenetic inheritance.

Karin Moelling, Felix Broecker

Reverse Transcriptase and RNase H

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Viruses activate antiviral defense mechanisms in their hosts which resemble properties of the viruses suggesting close coevolution. The composition of Retroviruses, comprising *gag*, *pol* (Reverse Transcriptase (RT), RNase H, integrase, protease) and *env* genes, resemble components of the siRNA machinery RISC with Argonaute comprising PAZ and PIWI domains (1). In mammalian cells the siRNA machinery is still active but dominated by the Interferon system (2). Most antiviral defense systems involve RNase H-like enzymes for destruction of invading nucleic acids such as Argonaute, Dicer in siRNA, CRISPR/Cas9 in bacterial DNA defense, transposases, RAG1,2 in mammalian protein defense with the V(D)J rearrangement of IgGs and the distantly related spliceosome component Prp8. The recently identified TtArgonaute involved in dsDNA defense appears to also belong to the family of RNases H. A major hallmark is a triad of conserved Mg-coordinating DDE or H amino acids and highly conserved structures with little other sequence homology. The cleavage specificities vary and can in part be reproduced *in vitro* by choice of ions (1). Other functions can be imposed by fusion proteins (3,4), including RNA primer removal in DNA synthesis and nucleotide excision as in the human disease AGS. An antiviral defense against HBV includes also a potential family member APE1. Integrases can also exert defense mechanisms. Evolutionary aspects of the RT/RNase H will be discussed. Questions arise about the abundance and role of RTs in bacteria. Furthermore the rare occurrence of retroviroids, retrophages, pararetroviruses is worth discussing as links between the RNA and DNA worlds.

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Sabine Müller

Engineering of ribozymes with useful activities in the ancient RNA world

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Evidence has been growing that an RNA World did exist before DNA- and protein-based life. In this regard, a long-standing research goal has been to develop functional RNAs capable of catalyzing numerous chemical reactions. Much effort has been made in finding RNA enzymes that catalyze the replication of RNA molecules, including the enzyme itself. This is far from being resolved, although recent experimental findings provide some reason for optimism. Apart from replication, other functionalities that contribute to higher genetic complexity and extended functional space are of high relevance to RNA world scenarios. We have engineered a variety of hairpin ribozyme descendants possessing activity for RNA recombination, circularization and oligomerization. Some of our designed catalysts can be regulated by external co-factors such as for example flavine mononucleotide (FMN). Furthermore, we have been interested in self-aminoacylation of RNA as a possible path towards transition from the RNA world to modern life. RNAs capable of self-aminoacylation might have had a selection advantage, for example by gaining additional functionality from the attached amino acid. Such aminoacyl-RNAs later could have served as starting material for the synthesis of peptides, suggesting that the conjugated amino acid was no longer used for any kind of activity, but provided as activated building block for the synthesis of polypeptides as known in modern biochemistry. In contrast to previous studies, where activated amino acids were used for aminoacylation, we succeeded in developing an aminoacyl-RNA synthetase ribozyme that accepts a "naked" amino acid for 3'-terminal RNA aminoacylation.

Mariusz Nowacki

RNA-guided genome editing in ciliates

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Maternally deposited RNAs exhibit a whole variety of functions in eukaryotes, from regulating gene expression to assuring genome integrity. Although present in a variety of organisms, maternally inherited characters are especially prominent in ciliated protozoa, where parental non-coding RNA molecules instruct whole-genome reorganization. This includes removal of nearly all non-coding DNA and transposable elements and producing extremely gene-rich somatic genomes. Different RNA-mediated mechanisms are involved in the programming of DNA elimination in ciliates. In *Paramecium*, a trans-generational genome comparison process employs a distinct class of germline small RNAs (scnRNAs) that are compared against the maternal genome to select the germline-specific subset of scnRNAs that subsequently target DNA elimination in the progeny genome. Later a second class of sRNAs (iesRNAs) is produced and used *in situ* in the developing nucleus and ensures complete DNA removal. In other ciliates, like *Oxytricha*, maternal non-coding RNAs are used as templates for sorting and reordering of hundreds of thousands of genomic DNA pieces, in addition to regulating chromosome copy number in the developing nucleus. Genome remodeling in ciliates has uncovered many new and fundamental discoveries in RNA biology and explored the biological limits of DNA processing in eukaryotes. Their extremely exaggerated genome plasticity and diversity of non-coding RNA pathways make ciliates compelling models for studying phenomena that relate to genome integrity and emphasize the power of RNA molecules to sculpt genomic information in eukaryotic cells.

David Prangishvili

Viruses from the dawn of life

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The three-domain tree, comprising Bacteria, Archaea, and Eukarya, is an essential framework for reconstructing scenarios of early evolution of cellular life. In my talk I will highlight the importance of this framework for tracing evolutionary history of viruses. Specifically, I will try to feature what we have learned about the origin and evolution of viral world from the studies on viruses which infect Archaea and their comparisons with viruses of Bacteria and Eukarya. Such comparisons lead to the hypothesis on the existence of primordial pool of viral genes, including those for viral capsid proteins, which predated the divergence between the ancestors of the three cellular domains. The astounding morphological diversity of DNA viruses of Archaea, which includes and far exceeds the morphological diversity observed in bacterial viruses, could have originated from the ancient virosphere. It appears to reflect the primordial variety of solutions for packaging and delivery of viral genetic material. The preservation of the ancestral viral morphotypes specifically in Archaea is likely to be due to the rudimentary nature of cell walls of these hosts, in many cases assembled from surface-layer proteins. Following this line of thought, the bacterial virosphere appears to be dominated with those ancestral virus types that have developed means to penetrate through the complex, peptidoglycan-containing bacterial cell wall; the inability to surmount this barrier may have been an evolutionary impasse for many ancestral virus morphotypes, which, however, remained associated with Archaea.

Lennart Randau

Small RNA genes mediate genome rearrangement events in Archaea

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The small RNA profiling of hyperthermophilic archaeal model organisms via RNA-Seq methodologies revealed unusual RNA processing pathways that include the trans-splicing and editing of transfer RNAs. Transfer RNA genes can be fragmented into two or even three transcriptional units and the nucleotide sequences of tRNA genes can be exchanged at the RNA level. These processing events hint at a strong evolutionary pressure that promotes seemingly unnecessary differences between tRNA and tDNA information. We hypothesize that tRNA gene fragmentation evolved as a cellular defense measure against virus integration at tRNA gene target sites. Accordingly, the abundance and activity of CRISPR RNA-guided antiviral defense systems was found to be increased in hyperthermophilic organisms. A hypothesis is presented that details possible reasons for the accelerated co-evolution of virus/host relationships in extreme environments. Finally, we observed a striking number of small C/D box sRNAs that guide ribosomal RNA modifications in hyperthermophiles and identified their genes in genome regions that suggest recent rearrangement events. The striking transcriptional plasticity of these small RNA genes is discussed.

Minoo Rassoulzadegan

RNA-mediated heredity of paramutation and acquired phenotype in the mouse

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Our laboratory reported three instances of RNA induced hereditary variations in the mouse. Increased transcriptional activities of the Cdk9 and Sox9 genes result in heart hypertrophy and gigantism, respectively, and a decreased expression of Kit, in fur colour variation and diabetes. Designated 'paramutations' by analogy with the known hereditary epigenetic variants in plants, they are induced by small RNAs homologous in sequence to the transcript, either the cognate microRNAs or oligoribonucleotides, injected in fertilized eggs. Sperm RNA, also active in the microinjection assay, appears as the likely transgenerational signal responsible for paternal transmission.

The genuine interest for the phenomenon of paramutation led often to ask questions about the molecular mechanisms of the change in gene expression. We could not so far cover all aspects of this complex issue in *in vivo* mouse models by classical genetic tools, we concentrate on the initial period of establishment in the very early embryo, leading to the entirely new concept of a control by methylation of the stability of a small noncoding RNA and thus of their signaling ability.

Finally, we are asking whether one could consider the same mode of transgenerational determination for complex disease with transgenerational epigenetic variations as well characterized in "diet-induced" mammals models. We now report the transgenerational transfer of metabolic pathologies- ie obesity and type II diabetes- by injection in naive embryos of testis RNA prepared from obese and diabetic males raised on high fat diet and demonstrate that sperm RNA act as a vector of paternal inheritance.

Marilyn Roossinck

Persistent Viruses in Plants and Fungi--Molecular Fossils?

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Plants and fungi are often infected with small RNA viruses that have a persistent lifestyle: they have strict vertical transmission; they do not move between host cells; and they have very long associations with their hosts, perhaps thousands of years. In a few cases persistent viruses are known to provide essential functions for their hosts, but in most cases their functions are unknown. Their lifestyles imply mutualism. In some cases portions of persistent viruses are found in plant and fungal genomes, and they are often expressed, again implying important functions. To date no plants with integrated persistent virus sequences have been found with a cytoplasmic form of the virus, although the data is limited and far from conclusive. An attractive hypothesis is that once the virus becomes integrated the plant or fungus no longer needs to carry it as a cytoplasmic element. The viruses may be remnants of an older system of genetic expression that lacked a DNA stage and relied on RNA elements for functional genes.

Natalia N. Singh, **Ravindra N. Singh**

A unique RNA structure as target for the treatment of a leading genetic disease

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Humans carry two copies of *Survival Motor Neuron* gene: *SMN1* and *SMN2*. Loss of *SMN1* coupled with the skipping of *SMN2* exon 7 causes spinal muscular atrophy (SMA), a leading genetic disease associated with infant mortality. We previously reported an intronic splicing silencer (ISS-N1) that has emerged as the leading target for an antisense oligonucleotide (ASO)-mediated splicing correction in SMA. The strong negative effect of ISS-N1 is exerted in part through two hnRNP A1 binding sites spanning from the 11^{th} to 24^{th} positions of *SMN2* intron 7. The first residue of ISS-N1 occupies the 10^{th} intronic position (^{10}C) that we have recently shown to be locked in a unique RNA structure facilitated by a long-distance interaction (LDI). We have termed this structure as ISTL1 (abbreviation for "internal stem formed by LDI 1") in which two stems are separated by 279 nucleotides. Using site-specific mutations and chemical structure probing we confirmed the formation and significance of ISTL1. Located inside the deep intronic region, the 3′ strand of ISTL1 falls within an inhibitory region that we term ISS-N2. We demonstrate that an ASO-mediated sequestration of ISS-N2 fully corrects *SMN2* exon 7 splicing and restores high levels of SMN in SMA patient cells. These results underscore the therapeutic potential of regulatory information trapped in secondary and high-order RNA structures of a human intron. Our findings also demonstrate that an ASO-based approach could be employed to favourably remodel the spliceosomal introns that occupy a vast portion of human genome.

Corrado Spadafora

A Reverse Transcriptase-dependent mechanism is active in embryo development and in tumorigenesis

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LINE-1 elements make up the most abundant retrotransposon family in mammalian genomes. Full-length members encode a reverse transcriptase (RT) required for their own mobilization, as well as that of non-autonomous Alu/SINE retrotrasposons. LINE-1 expression is high in embryos and cancer cells while being repressed in normal differentiated cells.

We have found that LINE-1 and SINE copy number progressively increases in stages of preimplantation development and during tumor progression. RT inhibition irreversibly arrests early embryo development (2- and 4-cell stages) and reduces cancer cell proliferation, promotes their differentiation and antagonizes tumor growth in animal models.

RT inhibition causes a global reprogramming of expression of coding genes, microRNAs (miRNAs) and ultra conserved regions (UCRs). The latter two are enriched in Alu sequences, often organized as pairs of inverted repeats. Density gradient centrifugation assays reveal Alu- and LINE-1-containing RNA:DNA hybrid structures in nucleic acids from cancer but not normal cells. Remarkably, hybrid molecules fail to form in tumor cells treated with RT inhibitors under the same conditions that block cell proliferation and reprogram gene expression profiles.

These results suggest that the highly expressed RT in cancer cells reverse-transcribes retroelement-derived RNA precursors, generating RNA:DNA hybrids that impair the formation of double-stranded RNAs and the subsequent production of regulatory miRNAs, with an ensuing impact on global gene expression. RT inhibition restores the 'normal' miRNA profile. We therefore propose that LINE1-RT drives a novel regulatory mechanism, required during preimplantation development and, when erroneously reactivated in adult life, responsible for the transformed state of cells in tumorigenesis

Eörs Szathmáry

The dynamics of the RNA world: Insights and challenges

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The problem of the origin of life is not only one of structure but also that of dynamics. Ever since the seminal result of Manfred Eigen in 1971 showing that early template replication suffers from an error threshold, research has tackled the issue of how early genomes could have been dynamically stable without highly evolved mechanisms such as accurate replication and chromosomes. I review the theory of the origin, maintenance and enhancement of the RNA world as an evolving population of dynamical systems. The roles of sequence space and population structure have been thoroughly investigated. The simplest form of population structure is limited diffusion on a surface. This mechanism can ensure the coexistence of competing ribozymes contributing to surface metabolism as well as the spread of efficient replicases despite the parasite problem. Once there are protocells there is no need for internal hypercyclic organization, however. Finally I review two crucial adaptations that enhanced the RNA world: chromosomes and enzymatic metabolism. Interestingly, these two have been presumably coevolutionarily linked because protocells harbouring unlinked, competing ribozymes are better off if the ribozymes remain inefficient but generalists. The appearance of chromosomes alleviated intragenomic conflict and was an enabling constraint for the emergence of specific and efficient enzymes.

Keizo Tomonaga

Bornavirus infection: a unique life style of an animal RNA virus in the DNA habitat

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Bornaviruses, a nonsegmented negative-strand RNA virus, are unique among animal RNA viruses in that they not only replicate in the cell nucleus, but establish a persistent infection. Recently we found that Borna disease virus (BDV), a mammalian bornavirus, closely associated with the cellular chromosome to ensure an intranuclear persistent infection. The viral ribonucleoprotein interacts directly with the host chromosome throughout the cell cycle, using core histones as the docking platform to chromatin. More interestingly, bornavirus has been shown to integrate a DNA genome copy into the host chromosomal DNA. We also discovered that bornaviruses have left their own endogenous elements, called endogenous bornavirus-like elements (EBLs), in the genomes of many animal species, including humans, as a fossil record of ancient infection events. Despite millions of years of evolution as endogenous viruses, EBLs still conserve relatively long open reading frames and are transcribed into the RNA in the host cells. Therefore, it is intriguing to investigate whether EBLs have acquired new functions and affected host genome evolution. In this talk I will present a unique life style of bornavirus in the DNA habitat of infected cells, providing new insights into the evolutionary relationship between RNA viruses and hosts.

Peter Unrau

RNA Mango aptamer-fluorophore: a bright, high affinity, complex for RNA labeling and tracking

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Since RNA lacks strong intrinsic fluorescence, it has proven challenging to track RNA molecules in real time. To address this problem and to allow the purification of fluorescently tagged RNA complexes, we have selected a high affinity RNA aptamer called RNA Mango. This aptamer binds a series of thiazole orange (fluorophore) derivatives with nanomolar affinity, increasing fluorophore fluorescence by up to 1,100-fold. Visualization of RNA Mango by single-molecule fluorescence microscopy, together with injection and imaging of RNA Mango-fluorophore complex in C. elegans gonads demonstrates the potential of our RNA-based system for live-cell RNA imaging. By inserting RNA Mango into a stem loop of the bacterial 6S RNA and biotinylating the fluorophore, we demonstrate that the aptamer can be used to fluorescently label and purify biologically important RNAs simultaneously. The high affinity and fluorescent properties of RNA Mango are therefore expected to simplify the study of biological RNA complexes.

Luis P. Villarreal

Viral consortia: A social force for ancient and recent life

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Historically, lytic viruses were thought to kill the host 'winner'. But persisting viruses/defectives can also promote host group survival, especially in a ubiquitous virosphere. In 1991 Yarmalinsky et al. discovered the addiction modules of P1 phage which employed opposing toxic (lytic) and protective functions to stabilize persistence. Subsequently I proposed that lytic/persisting virus provide addiction modules that also mediate group identity that typically involve cryptic viral mixtures. In the prokaryotes, DNA viruses predominate as either chronic (Archaea) or integrated proviruses (Bacteria) which I argue provide host group identity. But in the RNA world and in Eukaryotes, a distinct RNA virus-host relationship exist. Retroviurses and retroposons are major contributors of Eukaryotic genomes. Eukaryotic complexity now seems to be mostly mediated by regulation involving RNA (from retroposons). I then consider the emergence of the mammalian placenta as an examplar of mixed retroviral mediated network emergence. Retroviruses evolve via quasispecies, which contain cooperating, minority and even opposing RNA types. Quasispecies can also demonstrate group preclusion. Stem-loop RNA domains mediate retro (and other RNA) viral regulation/identity (found in LTRs). If stem-loop RNAs are ancestral to viroid/virus regulatory/identity functions, we can consider the RNA (ribozyme) world scenario from the perspective of addiction modules and cooperating subfunctional agents that must establish group identity. Such an RNA collective resembles a 'gang' but requires the simultaneous emergence of endonuclease, ligase, cooperative catalysis, group identity and history 'markers' (RNAs). I call such a collective a 'gangen' (pathway to gang) which has acquired interlinked features of a living system.

Kevin Weeks

Towards an RNA structure of everything

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The overarching vision of our research program is to use chemical principles to create information-rich chemical microscopes for examining the structure of RNA molecules. We seek to create experimentally concise, informationally rich, and ultimately transformative approaches for understanding the role of RNA structure in biology. Recent work focused on our efforts to develop highly accurate models of large viral RNAs at both the secondary structure and tertiary structure levels will be emphasized.

Andreas Werner

Endo-siRNAs from natural antisense transcripts

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The non-protein coding output of the genome in animals is overwhelming and the debate what is "functional" and what is not amounts to a religious controversy. The questions about functionality and biological roles also concern natural antisense transcripts (NATs); arguably the first long noncoding RNAs to come under scientific scrutiny.

In human and mice >50% of all transcribed loci show evidence of bi-directional transcription and potentially produce NATs. These are defined as fully processed, mRNA like transcripts that originate from the opposite strand of protein coding genes. Complementarity in exons implies that –upon co-expression of sense- and antisense transcripts- long RNA hybrids can form. Experimental evidence suggests that the hybrids indeed represent the starting structure for further processing steps. The synthesis of NATs is development- and tissue dependent with testis being the main site of NATs production.

The expression of NATs has gene specific as well as genomic implications. The over-expression of various gene specific NATs leads to disease and developmental malformations. The molecular mechanisms which trigger the aberrant phenotypes are diverse; examples include RNA interference and the masking of RNA-RNA or RNA-protein interactions by NATs.

At genomic scale, NATs are linked to monoallelic gene expression and transcriptional gene silencing. Furthermore, NATs potentially contribute to a signature of low abundant endo-siRNAs in somatic cells. In the germ line, NATs are hypothesized to enhance evolutionary fitness via an endo-siRNA based mRNA quality control mechanism in conjunction with transposon driven genome shuffling.

Eric Westhof

Base Pair Isostericity and Tautomerism in Molecular Recognition

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The beautifully organized regularity of DNA sequences is concealing a tremendous potential of three-dimensional structures, not only of amino acid sequences after translation, but also of RNA transcripts after transcription. After translation, the four nucleic acid bases are translated into the 22 amino acids. But, after transcription, only minor chemical changes are introduced into single-stranded RNAs. Thus, within the linear DNA habitat lurks an inhabitant that spontaneously forms complex folds. The architectures of RNA are primarily promoted by the use of non-Watson-Crick pairs. The base pairs with at least two hydrogen bonds are classified in twelve families, with the Watson-Crick family one of them. Some of the base pairs are isosteric between them, meaning that the distances between the C1' carbon atoms are very similar. The isostericity of Watson-Crick pairs forms the basis of RNA helices and of the resulting RNA secondary structure. Accurate recognition of Watson-Crick base pairs is a necessity during replication, transcription, and translational decoding. Polymerases and ribosomes exploit isostericity for recognition of Watson-Crick pairs on their minor groove edges. Although nucleic acids have a preference for one tautomer form, guaranteeing fidelity in their hydrogen bonding potential, base pairs observed in crystal structures of polymerases and ribosomes are best explained by base tautomerism, leading to the formation of base pairs with Watson-Crick-like geometries. These pairs with Watson-Crick-like geometries cannot be distinguished from the minor groove edge. These observations set limits to geometric selection in molecular recognition of Watson-Crick pairs for fidelity in replication and translation processes.

Guenther Witzany

Natural Codes do not code themselves

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There are few examples of scientific knowledge which lasted more than one hundred years. Empirically in nearly all cases the dynamic change in mainstream paradigms follows the historical structure of scientific revolutions: A main concept is dominating mainstream theoretical thoughts until empirical data increases that does not fit into the core assumptions. Then this paradigm is refuted and a new one takes it place with the benefit that this new one can integrate all the available data more coherently then the previous one. This means objective science is relative to the dynamics of research results and its interpretation by the scientific community. What remains as absolute value within this historical review is: without language the scientific community could not exchange arguments, thoughts, ideas, paradigms, research and its results. Empirically it is not possible to share any convictions or knowledge perspectives without formulating sentences, although some metaphysical concepts propose mysterious fields of energy or mind that transport informational content. Prior to artificial languages of scientific disciplines language users have to learn linguistic and communicative competences in social interactions within real life contexts. To get clear about the foundations of scientific knowledge research had to get clear about nature of languages. This contribution will give a short overview about the historical development and its results being relevant for biological theories about life and its basic agents viruses and RNA consortia.

POSTER

Presentations

Anna K. Crater, Emad Manni, **Sirinart Ananvoranich**

Utilization of inherent miRNAs in functional analyses of Toxoplasma gondii genes

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MicroRNAs (miRNAs) are part of crucial cellular mechanisms allowing for the regulation of gene expression. In Toxoplasma gondii, an intracellular obligate parasite of phylum Apicomplexa, a post-transcriptional gene silencing activity can be invoked using double-stranded RNAs of various sizes. Using a dual luciferase reporter system, we initially examined and evaluated the ability of endogenous Toxoplasma miRNAs (Tg-miRs), their mimics and inhibitors to alter the level of gene expression. Renilla luciferase (Rnluc) transcript was engineered to carry independent binding sites of two abundance species, namely Tg-miR-60a and Tg-miR-4a. The most abundant Tg-miR-60a is expressed ~2.4 times more than Tg-miR-4a. Interestingly their mode of silencing actions appeared different. While the Tg-miR-60a family induced transcript degradation, the Tg-miR-4a family suppressed translation.

Here a genetic system was developed to direct the most abundant Tg-miR-60a for loss-of-function analyses in Toxoplasma. Firstly to understand the significance of Tg-miR-60a in controlling gene expression, Tg-miR-60a targets were predicted and analyzed. Ubiquitin-like protease (Ulp1) was identified as the most promising target. Monitoring the steady state level of Ulp1mRNA, along with corresponding reporter system, the Ulp1expression could be altered using Tg-miR-60a inhibitor. As a proof of concept, we showed that when the binding sites of Tg-miR-60a were introduced into Toxoplasma transcripts, the silencing effect of inherit Tg-miR-60a could thus be directed and controlled to allow for loss-of-function analyses.

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Gene Regulation Mediated by Ancient Retroviral Elements in the Human Genome

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About 50% of the human genome is composed of retrovirus-related sequences; retroelements (REs), grouped into short- and long interspersed elements (SINEs, LINEs) and human endogenous retroviruses (HERVs).[1] The recent ENCODE project demonstrated that many REs attract transcription factors and are transcribed by host RNA polymerases.[2] Despite the vast number of ~3,000,000 REs per haploid genome, functional relevance of their activity on host gene regulation has only been attributed to some representative REs. Here we characterized a HERV family designated HERV-K(HML-10) on the genome-wide level, identifying 66 HERV-K(HML-10)-related sequences.[3] The infectious progenitor retrovirus invaded the primate genome ~35 million years ago. Today, HERV-K(HML-10) is either present as full-length proviruses with two flanking retroviral promoters, long terminal repeats (LTRs), or as solitary LTRs resulting from homologous recombination. These elements showed a distinct non-random genomic distribution, and a preference towards antisense orientation of those located within gene introns, indicating purifying selection and possible functional relevance. We demonstrate that about half of the LTRs have remained active promoters until today. These LTRs may exert regulatory functions on neighboring or encompassing genes by providing regulatory antisense transcripts. One of these LTR-dependent regulatory transcripts originated from an active LTR within an intron of the death-associated protein 3 (DAP3) gene involved in apoptosis. Knockdown of the LTR-dependent antisense transcript in human cells caused an increase in DAP3 mRNA expression levels and thereby promoted cell death. We conclude that retrovirus-derived sequences in the human genome can influence cellular morphology and function and may serve as drivers of evolution.

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Francesco Catania[1]* and Michael Lynch[2]

Networks, competition, trade-offs, and the evolution of genes in eukaryotes

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BACKGROUND

The instructions for the synthesis of proteins are stored within genes in sequences called exons. In eukaryotes, genes contain additional DNA sequences, called spliceosomal introns, which neither inform nor regulate the assemblage of amino acid chains. Why do eukaryotic genes have introns, and where do introns come from are still open questions in Biology.

RESULTS

Here, we revisit a proposed mechanism for the origin of spliceosomal introns, the *intronization* of exonic sequences [1], and discuss some ramifications that result from the application of the model to a scenario that considers (*i*) the extensive network of interactions between mRNA-associated processes and (*ii*) the antagonistic relationships between molecules mediating the co-transcriptional processes of mRNA splicing and mRNA 3′-end formation.

CONCLUSIONS

The simple and logical connections that arise from this exercise [2] suggest that a number of gene properties may be the byproduct of structural molecular constraints rather than adaptive selection.

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Carolyn J. Decker and Roy Parker

Analysis of Double-Stranded RNA from Microbial Communities Identifies Double-Stranded RNA Virus-like Elements

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Double-stranded RNA (dsRNA) can function as genetic information and may have served as genomic material before the existence of DNA-based life. Recent results indicate that microbial populations have a rich biodiversity although the full spectrum of genetic elements contributing to this diversity is unknown. Using a method we developed to purify dsRNA we are investigating the diversity of dsRNA in microbial populations. We detect large dsRNAs in multiple microbial communities. Analysis of dsRNA purified from microbes from one of these communities, a reclaimed water wetland, revealed that many dsRNA sequences match the metagenomic DNA sequences obtained from the same microbial population. The presence of these sequences suggests that microbes contain detectable dsRNA species encoded in their DNA such as sense-antisense transcripts. More interestingly, approximately 30% of the dsRNA sequences were not present in the corresponding DNA pool. These “dsRNA unique” sequences are strongly biased toward encoding novel or divergent proteins. Of these “dsRNA unique” sequences, only a small percentage share similarity to known viruses a large fraction assemble into novel RNA-virus-like contigs, and the remaining fraction has an unexplained origin. These results have uncovered dsRNA virus-like elements and underscore that dsRNA potentially represents an additional reservoir of genetic information in microbial populations.

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CRISPR-Cas function at conditions of self-targeting

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CRISPR-Cas systems were found in many bacteria and archaea and can serve for protection from DNA/RNA invaders through specific recognition of foreign DNA and its subsequent degradation by Cas proteins machinery. Protection (also referred to as CRISPR interference) is mediated by recognition of foreign DNA through complementary interactions mediated by short CRISPR RNA (crRNA) transcribed from CRISPR cassette, followed by its degradation. A CRISPR cassette contains identical repeats separated by variable-sequence spacers, some of which are complementary to foreign DNA. Spacers are acquired into CRISPR cassette in a poorly understood process called CRISPR adaptation. In current work we used Escherichia coli strains with chromosomal cas genes fused to inducible promoters and CRISPR cassette containing a spacer sequence complementary to E. coli genome, a situation which should cause self-targeting and, in the case of partial match between CRISPR spacer and targeted genome site, increased adaptation from bacterial DNA. Analysis of cell morphology of cells undergoing self-targeting was performed followed by PCR analysis of new spacer acquisition. Together, these data reveal new information about the consequences of CRISPR interference and adaptation.

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Genomic screens for regulatory ncRNAs targeting the ribosome in *Trypanosoma brucei*

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The pathogen *Trypanosoma brucei* responsible for sleeping sickness has to challenge tremendous environment changes, e.g. switch from the bloodstream form in mammalian hosts to the mid gut form present in tsetse flies. However, there is no evidence for differential regulation of RNA Pol II transcription. Instead, constitutive transcription appears to occur. This observation indicates that protein levels have to be conducted by post-transcriptional mechanisms.

It has been shown that non-protein coding RNAs (ncRNAs) are crucial in regulatory networks (e.g. chromosome remodelling; RNA polymerase activity; mRNA turnover; etc.), but all of the recently discovered ncRNAs involved in translation regulation target the mRNA rather than the ribosome. This is unexpected, since the ribosome has a central role during gene expression and due to the assumption that the primordial translation system most likely received direct regulatory input from small molecules including ncRNA cofactors.

In our lab, however, it has been discovered that ncRNAs are able to directly bind to the ribosome, therefore influencing the translation rate in *Haloferax volcanii* and *Saccharomyces cerevisiae*.

In order to extend this idea of ribosome-binding ncRNAs in mammalian parasites, we want to investigate this mechanism in *T. brucei*. Accordingly, we are performing a genomic screen for small ribosome-associated RNAs followed by functional analyses of possible ncRNA candidates.

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Characterization of Piwi proteins involved in *Paramecium* development

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In ciliates, Piwi-bound small RNAs are involved in massive DNA elimination that takes place during sexual development. During this process extensive genome-wide DNA rearrangement takes place in order to eliminate a bulk of non-coding DNA and transposable elements from the somatic genome. According to the "RNA scanning" model, small RNA-guide homology-dependent, trans-nuclear comparison of the somatic and germ line genome. This way the parental cell provides the progeny in all the information required to identify genomic regions that have to be eliminated. In *Paramecium*, Ptiwi01/09 are expressed during early development and are believed to bind small RNAs, which are involved in the scanning process (scnRNAs). Besides Ptiwi01/09 there are 12 other Piwi proteins in the *Paramecium* genome, several of them expressed exclusively during development. Recently in our lab, the presence of a second class of small RNAs – iesRNAs –produced from the excised portion of the genome and required for complete DNA elimination was detected, suggesting that the whole developmental process may be much more complex than initially thought.

The aim of this research is to characterize other developmentally upregulated Piwi proteins in *Paramecium*, their binding partners (sRNAs and proteins), and their precise roles during Paramecium development. The presence of a large number of Piwi proteins and multiple classes of small RNAs (differences in length, 5' and 3' sequence bias, expression time and localization) makes *Paramecium* an interesting model organism to study the diversified ensemble of RNA interference-related pathways co-existing and possibly cooperating at different stages of the cell cycle.

Tanja Gesell

SISSI in shape. From Simulating to Analyzing and Visualizing Site- Specific Interactions of RNAs in transcriptomes and genomes

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SISSI is a framework for SImulating Site-Specific Interactions along phylogenetic trees; it mimics sequence evolution under structural constraints in a unifying framework including arbitrary complex models of sequence evolution. The framework then feeds into a definition of a Phylogenetic Structure (PS) which consists of three aspects: the substitution matrix, a neighbourhood system and the phylogenetic tree. SISSI proved its true value in the field of non-coding RNAs. Beside its application to phylogenetic inference, simulated data sets with dependencies can be used to test structure analysis methods, both for inter- and intramolecular interactions. SISSI has proven useful to calculate a phylogenetic tree and site-specific rates under a dinucleotide model at a genomic scale. A follow-up application of SISSI's framework are adequate null hypotheses for RNA gene prediction. SISSIz is a variant of a thermodynamic structure-based RNA gene-finding program that is not biased by the dinucleotide content. Recently, an updated version of SISSIz was included in a pipeline providing an extensive set of functional transcriptomic annotations. At the moment, experimental RNA probing data such as Selective 2 -hydroxyl acylation analyzed by primer extension (SHAPE) chemistry will be integrated into SISSI and SISSIz. A multiscale visualization tool that allows for interactive explorations of the ncRNA candidates and their genomic regions includes additional information. This will further reduce the false positive rates of genetic screens and assist researchers in selecting, filtering and cataloging ncRNAs candidates toward their regulative networks and structure-function relationships.

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Signal nature of RNA viruses: the cell unit put in check by its own original molecular structure. A proposal within the view of life of Nietzsche's philosophy of power.

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Since they were first discovered, experts have still not reached an agreement whether viruses are by-products of cell evolution or living organisms that pre-date cells. Continuation of this debate requires a paradigm shift. Prebiotic theories imply that different cell-based functionalities may have arisen from different RNAs that were able to both cooperate and amplify themselves as well as to compete and suppress themselves. When the cell translates its genome into DNA, these mutually incompatible activities of RNA and those RNA activities that may prove to be toxic in the new DNA habitat must be very closely controlled if they cannot be eliminated. Our hypothesis is that the entry of an RNA virus into the cell would favour different molecular cross-talks, thereby permitting those risky RNA elements to inter-relate in a different manner, restoring suppressed molecular degrees of freedom, favouring pre-existing interactive affinities/incompabilities and opening up gaps in the cellular unit, with all these processes being opposed by cell control mechanisms. Thus, in this new paradigm the fight is more intracellular than between the virus and the cell, with the RNA virus informing the healthy cell RNA habitants that other states, the so-called "pathological states", are possible.

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RNA-dependent RNA polymerases in *Dictyostelium discoideum* and their influence on retrotransposon silencing

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DIRS-1 is the founding member of a poorly characterized class of retrotransposable elements that contain inverse long terminal repeats and tyrosine recombinase instead of DDE-type integrase enzymes. In *Dictyostelium discoideum*, DIRS-1 forms clusters that adopt the function of centromeres, rendering tight retrotransposition control critical to maintaining chromosome integrity. We report that in deletion strains of the RNA-dependent RNA polymerase (RdRP) RrpC, full length and shorter DIRS-1 mRNAs are strongly enriched. Shorter versions of a hitherto unknown long non-coding RNA in DIRS-1 antisense orientation are also enriched in *rrpC*– strains. Concurrent with the accumulation of long transcripts, the vast majority of small (21mer) DIRS-1 RNAs vanish in *rrpC*– strains. RNASeq reveals an asymmetric distribution of the DIRS-1 small RNAs, both along DIRS-1 and with respect to sense and antisense orientation. We show that RrpC is required for post-transcriptional DIRS-1 silencing, and also for the spreading of RNA silencing signals. Finally, DIRS-1 mis-regulation in the absence of RrpC leads to retrotransposon mobilization. In summary, our data reveals RrpC as a key player in the silencing of centromeric retrotransposon DIRS-1. RrpC acts at the post-transcriptional level and is involved in the spreading of RNA silencing signals, both in the 5' and 3' directions. We currently set up a genetically traceable version of DIRS-1 to study details of the retrantransposition. Additionally, we will present our data on the influence of RdRPs on the mobility of another retrotransposon, Skipper.

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Regulation of *Negr1* mRNA expression by its bidirectional lncRNA and miR-203

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Regulatory non-coding RNAs such as long non-coding RNAs and microRNAs are emerging as crucial determinants of central nervous system development and function. Neuronal growth regulator 1 (*Negr1*) is a cell adhesion molecule implicated in neurite outgrowth during neuronal development. Precise expression of *Negr1* is crucial for proper central nervous system development, hence in this study, we aim to elucidate the non-coding RNA molecules involved in the regulation of *Negr1*.

Bioinformatics analyses of the *Negr1* gene locus and mRNA identified a potential long non-coding RNA (NR_040400) bidirectional to the mouse *Negr1* gene. Several microRNAs, including miR-203, were also predicted to target the 3' untranslated region of the *Negr1* mRNA. Expression patterns of the *Negr1* mRNA and its associated non-coding RNAs during the maturation of murine primary cortical neuronal cultures were determined by microarray profiling. Functional studies were performed in these cultures to determine the regulatory role of NR_040400 and miR-203 on *Negr1* expression. Knockdown of NR_040400 resulted in significant downregulation of *Negr1* mRNA expression with a reduction in neurite outgrowth whereas miR-203 suppression increased *Negr1* mRNA expression and promoted neurite outgrowth. Conversely, miR-203 over-expression resulted in down-regulation of *Negr1* mRNA expression and decreased neurite outgrowth.

Hence, we found that expression of *Negr1* mRNA is enhanced by its bidirectional long non-coding RNA, NR_040400, and fine-tuned by miR-203. These findings highlight the importance of the regulatory non-coding RNAs in modulating the expression of the coding gene, *Negr1* for precise neuronal development.

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Dusan Kunec and Nikolaus Osterrieder

Analysis of codon pair bias of viruses shows that different forces shape codon pair preferences in viruses and their hosts

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The encoding of amino acids is biased as some synonymous codons are used more often than others. Similarly, certain neighboring synonymous codon pairs are present in protein coding sequences significantly more or less frequently than would be expected (codon pair bias, CPB). CPB was found in every species and can be fundamentally dissimilar between species. Although the biological significance and molecular mechanisms that shape CPB are largely unknown, it is linked to decoding accuracy and translation speed, as utilization of underrepresented codon pairs causes inefficient protein translation and can be used for attenuation of viruses. We analyzed CPB in protein coding genes of eukaryotic hosts (chicken, human) and select viruses to identify rules that govern selection of synonymous codon pairs in viruses. We found that encoding of genes of large DNA viruses, including herpesvirus and poxviruses, is not determined by the same forces that shape codon context in their hosts, since the CPB of viral genes was completely independent of the CPB preferences of the host. In contrast, all analyzed RNA viruses (including influenza A virus and poliovirus) show modest dependence on CPB of their host, and viral genes contain more underrepresented codon pairs than those of the host. Thus, despite the fact that viruses utilize translation machinery of their host, codon pair preferences in viral genes do not closely reproduce those of their hosts. It appears that ability of viruses to control the cell allows them to posses genes that are less efficiently translated than those of their hosts.

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What could virus derived small RNAs tell us about the population of plant RNA virus?

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RNA virus populations are one of the fastest evolving biological systems. They exist as a cloud of diverse sequences (quasispecies), which can have several biological implications [1]. Using next generation sequencing (NGS), variability of viral populations have been investigated in-depth for few important human and animal viruses, but little research has been conducted on plant viruses. The focus of our research is *Potato virus Y* (PVY), a single stranded (ss)-RNA virus. Sequences corresponding to ss-RNA viruses infecting plants constitute at least three distinct, but interconnected pools: (I) ss-RNA molecules packed in viral particles, (II) double stranded (ds)-RNA molecules formed during replication and (III) virus derived small interfering RNAs (vsiRNAs) [2]. We hypothesized that sequence diversity between these pools is similar but could slightly differ due to the errors or genetic bottlenecks introduced during viral cycle. Illumina deep sequencing of two different pools of viral sequences was employed: vsiRNAs [3] and ss-RNA isolated from purified viral particles [4]. The data was analysed to search for variants present in each of the two pools. The results show that variability of small RNAs is a good reflection of a "real" structure of PVY population (inferred from viral particles). This indicates sequence-independent targeting of RNAi mechanism towards invading PVY sequences in plants. Nevertheless, some differences were observed between the two sequence pools - small RNAs showing higher level of variation. Further experiments would be needed to elucidate their exact origin. Observed inter-host differences in viral diversity will enable better understanding of small-scale viral evolution processes.

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Intron recognition in eukaryotes: lessons from *Drosophila melanogaster*.

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Eukaryotic genes contain intervening noncoding sequences called introns. Introns are removed during transcription, in a complex process known as splicing which is guided by the spliceosome. Intron definition and exon definition are the two mechanisms conventionally employed to describe how the spliceosome recognize introns. In the intron definition model, the spliceosome recognizes an intron by targeting it. As such, the intron itself is the unit of recognition. In contrast, the exon is the unit of recognition in the exon definition model. Recently, a new model for spliceosomal intron recognition has been proposed, U1-definition (Catania and Lynch, BioEssays, 2013). If verified, this model would unify exon- and intron definition. U1-definition is based on the hypothesis that gene expression and architecture in eukaryotes are influenced by antagonistic interactions between splicing factors and cleavage/polyadenylation factors that compete for access to overlapping or neighboring binding sites. The strength and outcome of these interactions are expected to change along the mRNA transcript. Here we examine some of this model's predictions using Drosophila melanogaster as case study.

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Molecular variability of Potato spindle tuber viroid in ornamental plants in Croatia

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Viroids of the genus Pospiviroid are able to induce diseases in a wide range of host plants including important crop species. Although occasional disease outbreaks of Potato spindle tuber viroid (PSTVd) and closely related pospiviroids have been reported in potato and tomato, recent studies found an increase in number of latent infections in ornamental solanaceous species. In order to verify the presence of PSTVd in Croatia, a survey was conducted between 2009 and 2013. A total of 222 samples belonging to five ornamental species and two solanaceous crops were analyzed. Samples were collected from nurseries and consisted of eight randomly upper leaves taken from individual plants. Total RNA was extracted from symptomless leaf tissue and utilized as template in one-step reverse transcription - polymerase chain reaction (RT-PCR). The full length genome, corresponding to the viroid size (360 bp) was obtained using the PSTVd-32/ PSTVd-33 primer pair. Eleven plants belonging to two different species (Solanum jasminodes and Lycianthes rantonnetii) were found positive in this assay. The amplicons were extracted from agarose gel, cloned and sequenced. The Basic Local Alignment Search Tool (BLAST) confirmed their respective viroid identity and phylogentic relationships were evaluated using neighbour joining (NJ) implemented through MEGA 6.0. Although infected ornamentals do not show obvious symptoms of viroid infection, they pose a persistent danger of spreading infection by vegetative propagation material. Therefore, it is extremely important to use certified seed, apply sanitary measures and conduct regular inspection monitoring to prevent the spread this quarantine pathogen in the future.

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Small RNAs and riboregulators involved in legume resistance to biotic and abiotic stress

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Legumes such as *Medicago tTruncatula* and pea (*Pisum sativum*) have been selected as plant species to study the response to drought and *Fusarium* stress through transcriptional activation of several classes of small RNAs (microRNAs, siRNAs, tasiRNAs), long non-protein-coding RNAs (antisense RNAs, chromatin modulators), and mRNAs coding for key signaling enzymes (hormone fine tuning). Libraries of clones have been constructed and NGS data are under evaluation.
On one side, a Systems Biology approach is required to the understanding of stress signalling networks. In a comparative study, cloning of orthologous of resistance genes in pea is essential to monitor their activation or regulation. To this aim, Transcriptomic studies and Phenotyping analyses will provide data to be put in relation to drought stress responses. A functional analysis may support the generation of new varieties.

FPVII EU project Partners: Improving the resistance of legume crops to combined abiotic and biotic stress. Co-ordinated by FERA, UK. WP leader Martin Crespi, Institut de Sciences du Vegetal, CNRS, Paris.

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Understanding without reading: analogue encoding of physicochemical properties of proteins in their cognate messenger RNA

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Being related by the genetic code, messenger RNAs (mRNAs) and cognate proteins are polymers with mutually interdependent compositions, which further implies the possibility of a direct connection between their general physicochemical properties. How efficiently do different characteristics of mRNA coding regions reflect the features of cognate proteins and is it possible for the cell to obtain information about proteins from their mRNAs without first reading them on the ribosome? We address these issues in a theoretical proteome-wide analysis and show that average protein hydrophobicity, calculated from either sequences or 3D structures, can be encoded in an analogue fashion by many different mRNA sequence properties with the only constraint being that pyrimidine and purine bases be clearly distinguishable on average. Moreover, average characteristics of mRNA sequences allow for a reasonable discrimination between human proteins with different cellular localization and, in particular, cytosolic and membrane proteins, even in the absence of topogenic signal-based mechanisms. We discuss our findings in the context of protein and mRNA localization and propose that this cellular process may be partly determined by basic physicochemical rationales and interdependencies between the two biomolecules.

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Reverse Transcription Driven Uncoating of Mature HIV Capsids

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Until recently it was a commonly accepted notion that reverse transcription (RTion) in retroviruses, including HIV, takes place within the cytoplasm of the infected cell after uncoating of the mature capsid. However, accumulating evidence suggests that the RTion process happens largely within the mature capsid core, which protects the viral genome from host factors and maintains high local concentrations of the essential viral proteins within the RTion complex. In this work, we consider the problem of mature HIV capsid uncoating driven by polymerization of double stranded (ds) viral DNA during RTion. The millimolar concentrations of nucleocapsid protein (NC) contained within the capsid drives aggregation of both single-stranded (ss) gRNA and dsDNA, provided the capsid is intact. Flexible gRNA is aggregated by NC into a ribonucleoprotein complex occupying only a small fraction of the capsid volume. While the self-volume of the full-length pro-viral DNA (~104 bp) is the same as of its diploid gRNA genome, the dsDNA is very rigid, and gets condensed by NC into a large toroidal globule. We estimate that the weak dsDNA self-attraction induced by NC can lead to the size of the dsDNA globule similar to the size of the capsid. We predict very low value of mature capsid stability parameter for which it can be uncoated by pro-viral DNA. We describe the experiments in progress that measure the capsid stability, the strength of NC-induced dsDNA self-attraction, and the structure of NC-induced dsDNA globule. We also discuss the current in vivo evidence for the relationship between the RTion and mature capsid uncoating in HIV.

Aditi Singh

Unraveling the role of DNA methylation during programmed genome rearrangement and DNA elimination in *Paramecium tetraurelia*

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Genome rearrangement and DNA elimination is an elementary process that occurs during each sexual cycle of all the ciliates. For example, in Paramecium tetraurelia, two different types of nuclei (micronuclei and macronuclei) acquiring different genomic architecture and functions are formed from single zygotic nuclei. Genome rearrangement occurs during the macronuclear development via trans-nuclear comparison between maternal and zygotic genome mediated by small RNAs and the whole process is called 'RNA scanning'. During the process small RNAs called scnRNAs help in the excision of most of the non-genic DNA including transposons, minisatellites and internal eliminated sequences (IESs) in a homology dependent manner. So far very little is known about the mechanism used by the scnRNAs to distinguish between the wanted and unwanted DNA sequences and further to bring down the machinery for the proper excision. Here we report that nucleotide modification; in particular cytosine methylation is one of the essential marks that is used by the excision machinery during genome rearrangement.

Angelika Voronova, Viktorija Belevich, Dainis Rungis

Expression of retrotransposon-like sequences in the complex Scots pine genome under stress conditions.

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Induced transcription of retrotransposon-like sequences in the Scots pine (Pinus sylvestris L.) genome in response to heat stress, insect infestation (pine woolly aphid (Pineus pini L.)) as well as to treatment with abscisic acid and salicylic acid was characterized. Non-specific iPBS primers complementary to conservative retrotransposon tRNA binding sites were used. Differentially expressed fragments were sequenced, analysed and compared to various sequence databases. The majority of the identified fragments were Class I transposable elements, as well as chimeric transcripts. Fragments differed from each other in structure (TE pol, LTR, LTR-LTR, TE pol-chloroplast) and in domain representation (AP, RT, GAG). Identified fragment expression levels were determined by real-time PCR and characterized by high Ct values. A significant increase in expression was observed for chimeric fragments containing chloroplast-like sequences, which can be explained by the homology of parts of the sequence to the chloroplast genome or/and representation of highly similar regions of homology in multiple copies in the P.sylvestris genome. For one of these transcripts, expression of the Cereba retrotransposon-like part increased 45-73 fold after insect infestation and 5.62 fold after heat stress when compared to normal conditions. Expression of the chloroplast-like part of the fragment increased by 6888-9373 fold after insect attack and 255 fold after heat stress. These types of transcripts could take part in regulatory processes involving RNA interference mechanisms. The results are consistent with other published studies on mobile genetic elements and related transcribed sequences as well as with gymnosperm structural genomic studies.

Bojan Zagrovic, Anton A. Polyansky, Mario Hlevnjak, Matea Hajnic, Juan Osorio Iregui & Anita de Ruiter

Protein-RNA interactions and the origin of the genetic code

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In spite of 50 years of effort, the origin of the universal genetic code remains poorly understood. Among different theories, the stereochemical hypothesis proposes that the code evolved as a consequence of direct interactions between amino acids and appropriate bases. If indeed true, such physicochemical foundation of the mRNA-protein relationship could potentially also lead to novel principles of RNA-protein interactions in general. Inspired by this promise, we have recently examined the relationship between the physicochemical properties of mRNAs and their cognate proteins at the proteome level. Using 1) experimentally and computationally derived interaction propensity scales capturing the behavior of amino acids in aqueous solutions of nucleobase analogs, 2) computationally derived binding free energies between individual nucleobases and aminoacid sidechain analogs in different solvents, and 3) knowledge-based interaction preferences of amino acids for different nucleobases, we have revealed a statistically significant matching between the composition of mRNA coding sequences and the base-binding preferences of their cognate protein sequences. Overall, our results redefine the stereo-chemical hypothesis concerning the origin of the genetic code and provide evidence of direct templating of proteins from mRNAs before the development of ribosomal decoding. Moreover, our findings support the possibility of direct complementary interactions between mRNAs and cognate proteins even in present-day cells, especially if both are unstructured, with implications extending to all facets of nucleic acid/protein biology.

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Imaging of an RNA-splicing process by PNA labels

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"Seeing is believing": imaging bio molecules is a very exciting and common technique to track e.g. binding events or folding pathways. Proteins can be easily labelled, either directly by attaching fluorophores to the protein *via* functional groups, or by co-expressing the protein of interest with other self-fluorescent proteins. More challenging is the labelling procedure of large nucleic acids (> 200 bp) at specific regions, due to a lack of specific binding sites in the sequence. Here the use of complementary DNA oligonucleotides bearing the fluorophores is the usual procedure. However, we are applying a new labelling strategy for RNA by using hybridization probes with an unnatural backbone. Peptide nucleic acids (PNAs) are an excellent alternative to DNA oligonucleotides, since they are highly specific and affine to RNA and less prone to enzymatic degradation. The RNA system we are focusing on is the group II intron ai5γ originated from *Saccharomyces cerevisiae*, which folds in a defined three dimensional structure and actively induces its self-cleavage reaction during RNA maturation. This so-called splicing process is investigated by bulk FRET (Förster Resonance Energy Transfer) measurements and native fluorescent gel studies using PNA labels. Thereby, two PNAs carrying the relevant FRET dyes, either Cy3 or Cy5, are hybridized to the intron flanking regions. FRET occurs when the intron is self-spliced as the exons are ligated and the two fluorophrores attached to the PNA come nearby. With regard towards *in vivo* studies of the splicing mechanism, this approved labeling strategy is very promising.