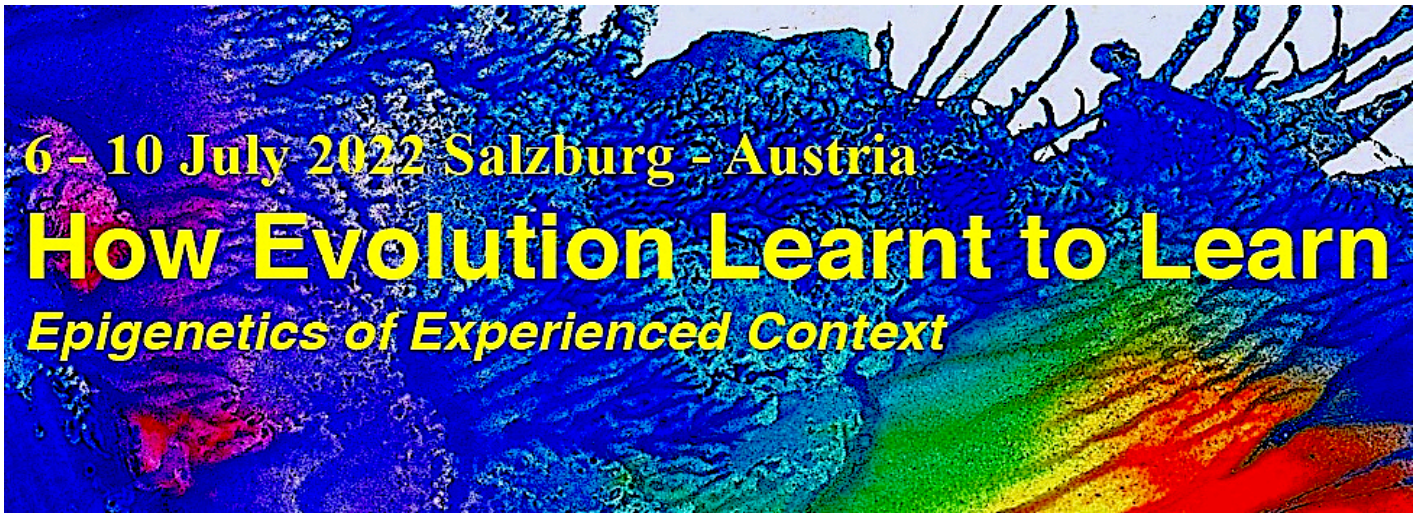

Programme

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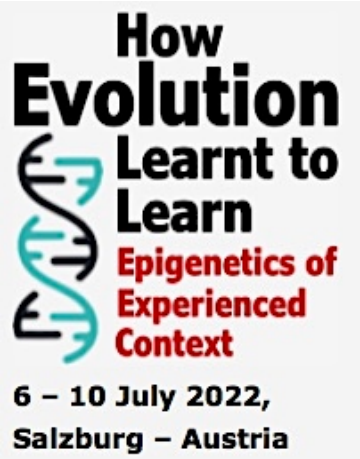
Abstracts



About

The regulatory system that works in development, morphology, cell fate and identity, physiology, genetic instructions, immunity, memory/learning, physical and mental disease depends on epigenetic marks. Genetic sequences of all organisms in all domains of life can be marked according to their environmental and social experiences. The communication of cells, persistent viruses and their defectives such as mobile genetic elements and RNA networks ensures both the transport of regulatory instructions and the reprogramming of these instructions.

With the emergence of epigenetic memory, organisms can fix historical and context- dependent impressive experiences. Evolution from now on learnt to learn. Learning means organisms can avoid reproduction of always the same. This is key to adaptation.

Epigenetic regulation emerges as a fine-tuned genome-wide network that can rapidly remodel and reprogram genetic content. Epigenetic switching outcompetes genetic mutations (error replications) during adaptation to changing lifeworld. Epigenetic markings can have both short-term and long-term functional effects such as soma to germline inheritance.

However, inheritance of acquired characteristics is only one of the many examples of the explanatory power of epigenetics. Behavioral epigenetics demonstrates the way in which environmental and social experiences produce individual differences in behaviour, cognition, personality, and mental health.

Goals

This symposium assembles experts from different fields to discuss a new paradigmatic understanding of how Evolution learnt to learn, i.e., epigenetic marking, transgenerational inheritance, cell fate and identity, morphology, physiology, genetic instructions, neuroepigenetic reprogramming, memory/ learning, physical and mental disease, immunity, and the roles of persistent viruses and their co-opted and exapted defectives such as non-coding RNA networks and mobile genetic elements.

organized by
Guenther Witzany

at
St. Virgil Conference Center
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Programme

Wednesday, July 6 2022

12:00 - 20.00	Registration at St. Virgil
19:45	Welcome drink and warm reception by the organizer

Thursday, July 7

8.30 Guenther Witzany
Organization Affairs and **Introduction**

9.00 – 9.30 **Eva Jablonka**
Epigenetic learning in the nervous system

9.30 – 10.00 **Jörg Bock**
Epigenetic programming of brain development and emotional behavior by early life stress: A transgenerational perspective

10.00 – 10.30 **Johannes Reul**
Epigenetic regulation of genomic corticosteroid receptor action in the brain in relation to stress coping

Coffee Break – Tea Time (15 minutes)

11.00 – 11.30 **Patrick McGowan**
The role of maternal factors in epigenetic programming of neurodevelopment

11.30 – 12.00 **Moshe Szyf**
How is trauma embedded in our genome? A possible role for DNA methylation

12.00 - 12.30 **Gianluca Ursini**
Genomic risk for schizophrenia and the environment in early life: insights on epigenetic plasticity

12.30 – 13.00 **Minoo Rassoulzadegan**
Progressive decline in the levels of six miRNAs from parents to children in autism

Lunch

Thursday, July 7

14.00 – 14.30 **Hermona Soreq**
Non-coding RNA controllers of acetylcholine signaling as body-brain communicators

14.30 – 15.00 **David Moore**
Evolving Learning: The Exaptation of Epigenetics as a Learning Mechanism

15.00 – 15.30 **Edi Barkai**
A biophysical mechanism for epigenetic inheritance of enhanced complex learning capabilities

Coffee Break – Tea Time (15 minutes)

15.50 – 16.30 **POSTER PRESENTATIONS**

16.30 – 17.00 **Shiv Grewal**
Transmitting epigenetic memory through modified histones

17.00 – 17.30 **Robert Feil**
Genomic imprinting, a stable inter-generational memory mechanism

17.30 – 18.00 **Özgür Bayram**
Epigenetic regulation of fungal secondary metabolite gene clusters: are we seeing the tip of the iceberg?

18.00 – 18.30 **Vadim Gladyshev**
Aging and Lifespan Control

Friday, July 8

8.30 Organization Affairs!

8.30 – 9.00 **Bojan Zagrovic**
Understanding the Physicochemical Language of Epigenetics: On the Interaction Preferences between Modified Nucleobases and Protein Residues

9.00 – 9.30 **Lars Jansen**
The nomadic behavior of the epigenetically inherited centromere

9.30 – 10.00 **Jason Brickner**
Interaction with the nuclear pore stimulates heritable histone H3 methylation and transcriptional memory

Coffee Break – Tea Time (15 minutes)

10.30 – 11.00 **Nelson Cabej**
On the origin and nature of the non-genetic information

11.00 – 11.30 **Richard Hunter**
Transposons as environmental stress detection modules, are eukaryotic genomes evolved to evolve?

11.30 – 12.00 **Erez Levanon**
From Mobile elements to RNA editing via dsRNA – a path for genomic novelty

12.00 – 12.30 **Gustavo Caetano-Anollés**
Entanglement: explaining novelty, recruitment and growth in biological systems

Lunch

Friday, July 8

13.30 – 14.00 **Giacomo Cavalli**
Epigenetic inheritance of chromatin states through cellular and organismal generations

14.00 – 14.30 **Ina Anreiter**
Epitranscriptomic regulation of behaviour: Individual differences and gene-environment interplay

14.30 – 15.00 **Eric Greer**
Epigenetics in unicellular to multicellular transition in Dictyostelium

Coffee Break – Tea Time (15 minutes)

15.30 – 16.00 **Mariusz Nowacki**
Evolutionary origins and impacts of genome architecture in ciliates

16.00 – 16.30 **Antónia Monteiro**
Odor preference learning and inheritance in Bicyclus anynana butterflies

16.30 – 17.00 **David Glanzman**
Role of retrotransposition in memory in Aplysia

17.00 – 17.30 **Bryan Cullen**
Epigenetic Silencing of unintegrated HIV-1 DNA

17.30 – 18.00 **John Mattick**
Climbing mount improbable: how did evolution solve the scaling and search problems

19.30 – 20.00 **Music Performance: Heidi Vereno, harp**

20.00 – 21.30 **Conference Diner**

Saturday, July 9

8.30 – 9.00 **Colin Logie**
On the nature of chromosome domain boundaries and their evolution

9.00 – 9.30 **Marla Sokolowski**
The foraging gene as a modifier of behavior: gene regulation, pleiotropy and plasticity

9.30 – 10.00 **Corrado Spadafora**
Sperm-mediated epigenetic evolution

Coffee Break – Tea Time (15 minutes)

10.30 – 11.00 **Germano Cecere**
Epigenetic maintenance of animal fertility by piRNAs in C. elegans

11.00 – 11.30 **Katalin Fejes-Toth**
Co-option of the germline piRNA pathway to regulate vertebrate neural crest specification

11.30 – 12.00 **Andreas Werner**
Natural antisense transcripts play different roles in soma and male germ cells

12.00 – 12.30 **Eörs Szathmáry**
Evolution in learning, learning in evolution

Lunch

Saturday, July 9

13.30 – 14.00 **Anton Petrov**
Use of pre-adaptations within the translational machinery during eukaryogenesis

14.00 – 14.30 **Jaques Demongeot**
A candidate RNA as a "proto-ribosome" at origin of life and its remnants in the present ribosomal factory

14.30 – 15.00 **Karin Mölling**
RT/RNase H reflecting evolution

Coffee Break – Tea Time (15 minutes)

15.30 – 16.00 **Sabine Müller**
Mobile genetic elements in the RNA world: How a small ribozyme supports RNA sequence variation

16.00 – 16.30 **Peter Unrau**
The modular evolution of an RNA polymerase ribozyme with promoter recognition and processivity

16.30 – 17.00 **Valerian Dolja**
Global metatranscriptome analysis reveals vast diversity of novel RNA viruses in bacteria and eukaryotes

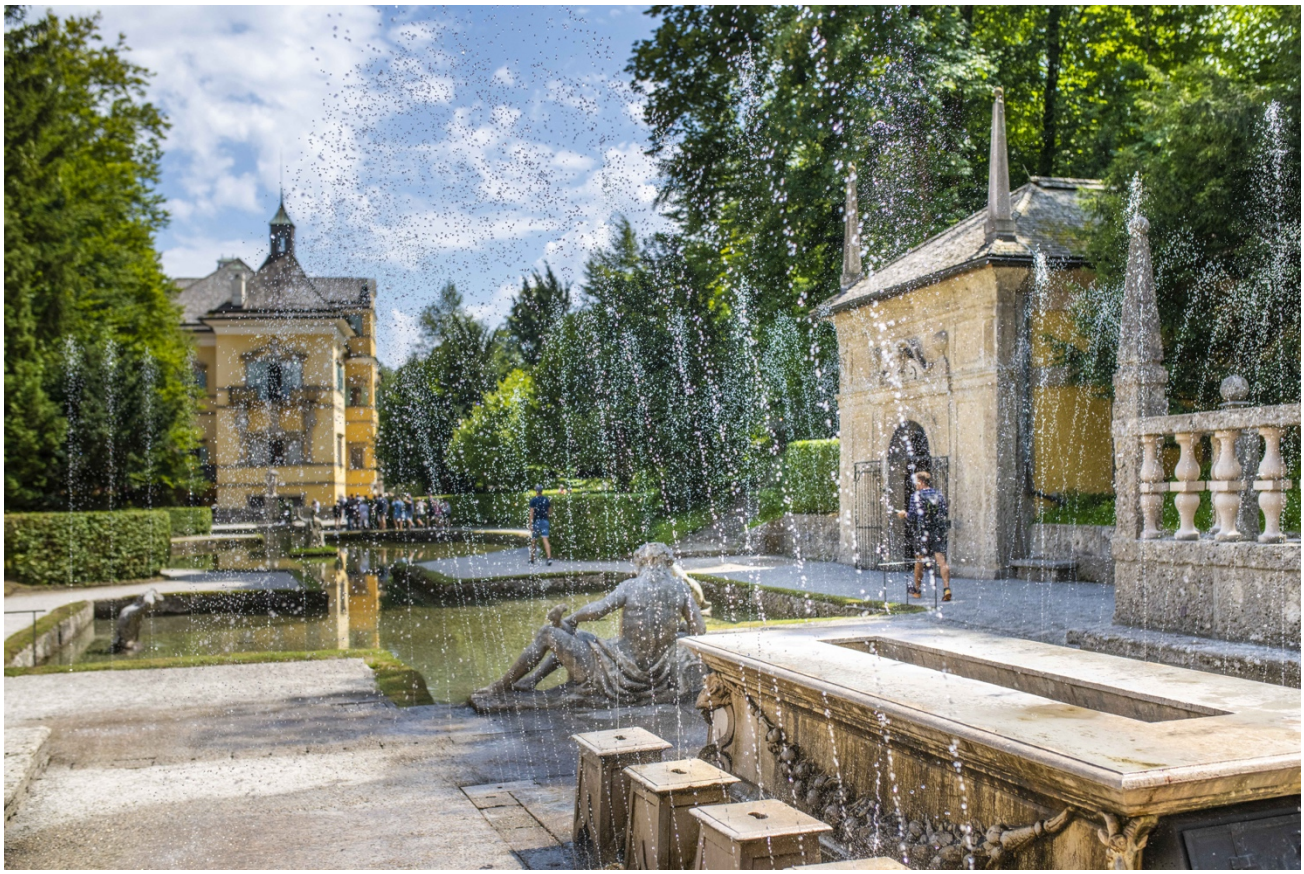
17.00 – 17.30 **Jordi Gómez**
Viruses as archaeological tools for uncovering archaic molecular relationships

Sunday, July 10

Sunday-Excursion half day (10.00 – 13.30)
to an extraordinary place near Salzburg: **Hellbrunn Palace & Trick Fountains**

(40 Euros including: transfer, guided tour and meals)

In 1612, only a few months after ascending the throne, Salzburg's Prince Archbishop Markus Sittikus von Hohenems commissioned a country residence to be built at the foot of the well-watered Hellbrunn Mountain. A lover of Italian art and culture, Markus Sittikus commissioned the famous Cathedral architect, Santino Solari, to design a "villa suburbana", a summer residence matching the elegance and spaciousness of the magnificent Italian architecture with which he was so obsessed. Within a relatively short period of time an architectural masterpiece was created just south of the city that remains one of the most magnificent Renaissance buildings north of the Alps: the Lustschloss ("pleasure palace") of Hellbrunn with its spacious park and its unique Wasserspiele (trick fountains).



ABSTRACTS

Talks
+
Posters

Epitranscriptomic regulation of behaviour: Individual differences and gene-environment interplay

Ina Anreiter
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Over the last two decades, epigenetic modifications have been shown to play important roles in modulating how the environment influences biological function. More recently, it has been discovered that epigenetic marks not only occur on DNA, but also on the RNA transcripts that are the intermediary product between genes and proteins (messenger RNA, mRNA). These modifications have been termed epitranscriptomics and have been shown to regulate the type and amount of protein produced through mRNA splicing, degradation, stabilization, and sub-cellular localization. Like epigenetic modifications, epitranscriptomic marks seem to have conserved biological functions across organisms, being involved stress response, cell differentiation and gametogenesis, food choice and reward, obesity and heart disease, brain development and cancer. The most common epitranscriptomic mark, m6A, plays an important role in sex determination and sex-specific phenotypes in *Drosophila*. We use *Drosophila* as a model organism to investigate the role of mRNA modifications, with a special focus on m6A, in brain development and behaviour. Our findings describe, for the first time, a sex-specific role for these modifications in several developmental and behavioural phenotypes.

A biophysical mechanism for epigenetic inheritance of enhanced complex learning capabilities

Edi Barkei
Department of Neurobiology, Faculty of Natural Sciences, University of Haifa, Israel;
ebarkai@research.haifa.ac.il

Acquisition of the ability to learn complex tasks, termed 'rule learning', is mediated by enhanced intrinsic neuronal excitability throughout the neuronal population in the relevant brain areas, which results from decreased conductance of slow potassium current(s).

Here we show that rats trained in complex tasks pass on trans-generationally superb learning capabilities. Such inheritance is also evident when only one of the parents (male or female) is trained, if the F1 generation is fostered by non-trained females and if the F2 or F3 generation is trained without any training of the F1 generation. Notably, offspring excel also in other, completely novel tasks.

At the cellular level, the biophysical properties of CA1 pyramidal neurons of trained rats' offspring differ significantly from neurons of controls' offspring. Their excitability is higher, as result of reduction in the slow potassium current(s), the very same change induced in the brains of the F0 rats only after they acquire the rule.

Thus, offspring excel in complex learning tasks since they are born with neurons that show the same biophysical change induced in parents' brains by training for rule learning.

Analysis of mRNA expression levels show that the hippocampi of trained offspring differs from the controls' offspring hippocampi in more than 500 genes. In particular, we found downregulation of genes that code for channels that suppress intrinsic excitability, and of genes that code for synaptic receptors.

We suggest that these changes create favourable set point for future increased plasticity, thereby granting trained rats' offspring superb learning capabilities.

Epigenetic regulation of fungal secondary metabolite gene clusters: are we seeing the tip of the iceberg?

Özgür Bayram 1, Koon Ho Wong 2, Özlem Sarikaya Bayram 1
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2 Faculty of Health Sciences, University of Macau, Macau SAR of China

Plants, bacteria and fungi have adapted mechanisms to produce bioactive compounds collectively named secondary metabolites. The latter two have grouped their genes into biosynthetic gene clusters (BGCs) responsible for production of secondary metabolites. One of the striking difference between bacterial and fungal BGCs is mode of regulation. Bacteria kept negative and positive regulators to control expression of BGCs. However, fungi lost the repressors and kept activators in BGCs due to the general repressive role of chromatin. Research on fungal chromatin with respect to production of secondary metabolites has intensified in last 15 years. Various components of chromatin modifying enzymes such as histone acetyltransferases, histone deacetylases, histone methyltransferases have been studied at genetic level. However, molecular mechanisms remain to be understood. In model filamentous fungus Aspergillus nidulans, we discovered a protein complex composed of the H3K4 histone demethylase KdmB, a cohesin acetyltransferase (EcoA), a histone deacetylase (RpdA) and a histone reader/E3 ligase protein (SntB). KERS is established from EcoA-KdmB and SntB-RpdA heterodimers in the nucleus. KERS is essential for the recruitment of the four epigenetic regulators to the pre-initiation complex at more than a thousand active core promoters, where the four subunits exert different transcriptional effects at different promoters. Interestingly, the four epigenetic regulators have a common positive effect on the regulatory genes of morphogenesis and biosynthesis of secondary metabolites, specialized biochemicals important for development and niche securement in filamentous fungi. The KERS complex provides the first mechanistic, chromatin-based understanding of how fungal reproductive development is connected with small molecule synthesis.

Epigenetic programming of brain development and emotional behavior by early life stress: A transgenerational perspective

Jörg Bock
Institute of Biology, Epigenetics and Structural Plasticity, Center for Behavioral Brain Sciences; Otto von Guericke University Magdeburg, Germany; joerg.bock@ovgu.de

Early Life Stress (ELS) critically influences brain development and behavioral functions and thus represents an important programming factor for mental health and disease at later life periods. Several studies, including our own, revealed that these (mal-)adaptive processes are mediated by epigenetic mechanisms resulting in altered gene expression patterns. Epigenetic modifications are also discussed to play a role in the transgenerational transmission of early adverse experiences. We established different animal models revealing that ELS exposure can interfere with brain development resulting in psychopathological symptoms but that in contrast and dependent on time point and duration of ELS also positive functional adaptations can occur, resulting in better stress coping and resilience against adversities later in life.

Our studies also reveal that the respective outcome of stress experiences is the result of a complex interaction between ELS and consecutive stress exposures at later life periods ("two-(or multiple) hit" concept). Our recent data show that these stress-induced adaptations are mediated via different epigenetic mechanisms (histone-modifications, DNA-methylation) and that stress-experiences are transmitted into the next generations, specifically with respect to modulatory and neurohormonal factors such as the dopaminergic, oxytocinergic and NPY-system. We focus on transmission via the maternal line and our recent results lead to the hypothesis that transgenerational transmission of ELS-induced adaptations is mediated by an interaction between "acquired" behavioural traits" and stable epigenetic marks in the germ line. Thus, ELS not only influences the stress-exposed individual but represents a transgenerational programming factor that defines an epigenetic predisposition for stress-reactivity in the following generations.

Interaction with the nuclear pore stimulates heritable histone H3 methylation and transcriptional memory

Jason Brickner
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j-brickner@northwestern.edu

For some inducible genes, the rate and molecular mechanism of transcriptional activation depends on the prior experiences of the cell. This phenomenon, called epigenetic transcriptional memory, accelerates reactivation and requires both changes in chromatin structure and recruitment of poised RNA Polymerase II (RNAPII) to the promoter. Memory of inositol starvation in budding yeast involves a positive feedback loop between transcription factor-dependent interaction with the nuclear pore complex and histone H3 lysine 4 dimethylation (H3K4me2). While H3K4me2 is essential for recruitment of RNAPII and faster reactivation, RNAPII is not required for H3K4me2. Unlike RNAPII-dependent H3K4me2 associated with transcription, RNAPII-independent H3K4me2 requires Nup100, SET3C, the Leo1 subunit of the Paf1 complex and, upon degradation of an essential transcription factor, is inherited through multiple cell cycles. The writer of this mark (COMPASS) physically interacts with the potential reader (SET3C), suggesting a molecular mechanism for the spreading and re-incorporation of H3K4me2 following DNA replication.

On the origin and nature of the non-genetic information

Nelson R. Cabej
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The discovery of several forms of inheritance that are not related to genetic information during the last decades led to the development of the concept of non-genetic information. While we know what the nongenetic information does, we currently lack solid knowledge about its origins, and we can't claim to understand the nature of non-genetic information without identifying its origins. Tracing back the causal chain upstream the end receivers (decoders) of the non-genetic information may lead us to its ultimate source. In this paper, I examine some typical cases of non-genetically determined phenomena, such as transgenerational phenotypic inheritance, epigenetic modifications of DNA and histones, predator-induced morphological changes, site-specific deployment of cytoplasmic determinants in gametes, alternative gene splicing, non-canonical mRNA translation, etc. Based on the empirical evidence presented herein, I propose for the first time a hypothesis on the origin of non-genetic information and the common source of its different forms.

Entanglement: explaining novelty, recruitment and growth in biological systems

Gustavo Caetano-Anollés

Department of Crop Sciences and C.R. Woese Institute for Genomic Biology, University of Illinois, Urbana, Illinois, USA; gca@illinois.edu

The rise of complexity in biology remains mysterious. Here we elaborate on a theory of entanglement that takes advantage of the dimensionality reduction offered by holographic principles. We propose that short and long-distance interactions and short-term and long-term functional effects are responsible for the increasingly granular and tangled structure of biological systems. Novelty generation, recruitment and growth are at the center of persistence. Without novelties, biological systems cannot adapt to changing environments. Without recruitment, novelties cannot spread and systems cannot grow and maintain identity through time. We define persistence within a framework of fluxes of matter-energy and information and signal processing in response to internal and external challenges. A 'triangle of persistence' describing reuse, innovation and stasis defines a useful polytope in a phase space of trade-offs between economy, flexibility and robustness. A biphasic (bow-tie) theory of module generation complements this frustrated dynamic. Finally, we explore the problem of identity and change unfolding in space and time at different timescales with metabolic networks, protein makeup, the functionome, the ribosome, and the rise of haplotypes of SARS-CoV-2 ‘variants of concern’ during the COVID-19 viral pandemic. We illustrate how the concept of temporal parts embraced by the perdurantist school provides a processual 4-dimensional ‘worm’ view of biology that is historical and atemporal. This view is made explicit with chronologies and evolving networks inferred with phylogenomic methodologies. Entanglement unfolds in a spatiotemporal landscape of communication and language in which systems become intelligent actors. It is here where evolution learnt to learn.

Epigenetic inheritance of chromatin states through cellular and organismal generations

Giacomo Cavalli
Institute of Human Genetics - CNRS and University of Montpellier, Montpellier, France;
giacomo.cavalli@igh.cnrs.fr

Transgenerational Epigenetic Inheritance (TEI) studies the transmission of alternative functional states through multiple generations in the presence of the same genomic DNA sequence. Our work showed that Polycomb Group (PcG) proteins convey TEI in *Drosophila*. PcG proteins form multimeric protein complexes that regulate chromatin via histone modifications activities and regulation of 3D chromosome architecture. We have previously described the 3D architecture of the genome and identified the Polycomb system as one of the fundamental folding and regulatory principles. We established stable and isogenic *Drosophila* epilines that carry alternative epialleles, defined by differential levels of the Polycomb-dependent H3K27me3 mark, by transiently enhancing 3D chromatin interactions. Once established, epialleles can be dominantly transmitted to naïve flies and induce paramutation. By forcing relocation of Polycomb binding sites in the 3D space of the nucleus, we can influence the efficiency of TEI. Furthermore, we recapitulated a 70-year old experiment by Conrad Waddington which induced an acquired wing vein character upon repeated exposure to heat stress. We are testing the epigenetic versus genetic basis of this type of inheritance. Finally, by transiently depleting a Polycomb protein in somatic cells, we induce a cell fate transformation that remains stable even upon restoring the normal protein levels. Our work sheds light on the mechanisms by which nuclear organization and PcG proteins contribute to phenotypic variability.

Acknowledgements
This work was supported by the European Research Council Advanced Investigator grant (3DEpi), by the "Fondation ARC pour la recherche sur le cancer", by the "Fondation pour la recherche médicale" and by the CNRS.

Epigenetic maintenance of animal fertility by piRNAs in *C. elegans*

Germano Cecere
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Eukaryotic genomes harbor invading transposable elements silenced by PIWI-interacting RNAs (piRNAs) to maintain genome integrity in animal germ cells and promote fertility. However, whether the maintenance of animal fertility is due to the conserved role of piRNAs in repressing repetitive elements (REs) remains unclear. I will present our results showing that the progressive loss of fertility in *Caenorhabditis elegans* lacking piRNAs is not caused by derepression of REs but is mediated by epigenetic silencing of all of the replicative histone genes. In the absence of piRNAs, downstream components of the piRNA pathway relocalize from germ granules and piRNA targets to histone mRNAs to synthesize antisense small RNAs (sRNAs) and induce transgenerational histone silencing. Removal of the downstream components of the piRNA pathway restores histone mRNA expression and fertility in piRNA mutants, and the inheritance of histone sRNAs in wild-type worms adversely affects their fertility for multiple generations.

In addition to piRNAs' role in promoting fertility by preventing misrouting of small RNAs on essential germline genes, piRNAs also directly promote fertility by regulating endogenous transcriptional programs during gametogenesis. We show that piRNAs trigger the transcriptional silencing of hundreds of spermatogenic genes during spermatogenesis to promote sperm differentiation and function. Thus, besides being a conserved small RNA-based mechanism to silence foreign genetic elements, piRNA regulation can also be co-opted to regulate endogenous gene expression programs required to maintain animal fertility.

Epigenetic Silencing of Unintegrated HIV-1 DNA

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The integration of the linear proviral DNA intermediate into the host cell genome is not only a defining feature of the retroviral life cycle but also essential for appreciable levels of proviral transcription and IN inhibitors are therefore widely used to treat human immunodeficiency virus 1 (HIV-1) infected patients. In the absence of IN function, unintegrated HIV-1 DNA is efficiently epigenetically silenced. Using an unbiased genetic screen, we identified all eight components of the host SMC5/6 complex as essential for this process. SMC5/6 first binds to chromatinized, unintegrated HIV-1 DNA and then triggers epigenetic silencing by inducing its SUMOylation. Inhibition of this SUMOylation step, either by point mutagenesis of the SMC5/6 component NSMCE2, a SUMO E3 ligase, or using the SUMOylation inhibitor TAK-981, blocks epigenetic silencing and rescues transcription from unintegrated HIV-1 DNA. Remarkably, T cells lacking a functional SMC5/6 complex can support a spreading HIV-1 infection in vitro in the absence of integrase function. We further demonstrate that loss of SUMOylation results in fewer latent HIV-1 infections in primary human T cells, thus arguing that the epigenetic silencing of integration competent HIV-1 proviruses by the SMC5/6 complex prior to integration can give rise to latent HIV-1 infections. These data identify the SMC5/6 complex as a host antiviral restriction factor that can recognize and epigenetically silence foreign DNA.

A candidate RNA as a "proto-ribosome" at origin of life and its remnants in the present ribosomal factory

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Univ. Grenoble Alpes, AGEIS, Grenoble, France; jacques.demongeot@yahoo.fr

The debate between the classic theories on the origin of life, DNA first or RNA first is not closed and the arguments for one or the other of these theories have recently fueled a debate in which the two have a high degree of likelihood. It therefore seems interesting to propose a third intermediate way, based on the existence of an RNA which may have existed before the later stages postulated by these theories and therefore be the missing link towards a common origin of them. We exhibit a candidate RNA existing in ring or hairpin form in the early stages of life, which could have acted as a "proto-ribosome".

Next, we show that remnants of this putative candidate RNA exist in molecules presently involved in the ribosomal factory, the concentrations of these remnants depending on the priority of these molecules within the translation process. The primordial structure represents the functional core of the translation and the remnants come during evolution from the addition of successive ribosomal functionalities rending the translation more efficient and secure.

Global metatranscriptome analysis reveals vast diversity of novel RNA viruses in bacteria and eukaryotes

Valerian V. Dolja[1,2] Valerian.Dolja@oregonstate.edu, Uri Neri[3], Yuri I. Wolf[2], Simon Roux[4], RNA Virus Discovery Consortium, Mart Krupovic[5], Nikos C. Kyprides[4], Eugene V. Koonin[2] and Uri Gopna[3] 1 Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA; 2 National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, USA; 3 The Shmunis School of Biomedicine and Cancer Research, Tel Aviv University, Tel Aviv 6997801, Israel; 4 Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA; 5 Institut Pasteur, Université de Paris, CNRS UMR6047, Archaeal Virology Unit, 75015 Paris, France

Mining 5,150 metatranscriptomes from diverse environments uncovered 2.5 million of contigs corresponding to a five-fold increase of RNA virus diversity. Extended RNA-dependent RNA polymerase (RdRP) phylogeny supports monophyly of the five established phyla, suggests the existence of two new phyla of bacteriophages, and reveals numerous novel virus taxa within the phyla. In particular, 18 new classes versus the 4 previously known in Pisuviricota, 20 new classes versus 3 previously known in Kitrinoviricota, and 18 new classes versus 6 previously known in Negarnaviricota were identified. It was found that a significant proportion of the viruses in the former two phyla utilize alternative genetic codes typical of ciliates and other protists. The dramatically expanded Lenarviricota phylum, consisting of bacterial and related eukaryotic viruses, now accounts for a third of the RNA virome diversity. Identification of CRISPR spacer matches and bacteriolytic proteins suggests that subsets of picobirnaviruses and partitiviruses, previously associated with eukaryotes, infect prokaryotic hosts. These results strongly suggest that drastic host shifts, known as horizontal virus transfer, between distantly related hosts, such as bacteria and eukaryotes, is a major route of RNA virus evolution. Multiple virus groups with pronounced genomic rearrangements, such as fission or fusion of genomic segments and rearrangement of the ORFs encoding polyproteins, were also identified. We found that the swapping of catalytic motifs (domain permutation) in RdRP is a common feature of several virus lineages. Gene content analysis revealed multiple domains previously not found in RNA viruses and implicated in virus-host interactions. This vast collection of new RNA virus genomes provides insights into RNA virus biology and evolution, and should become a key resource for RNA virology. In addition, vast expansion of the known RNA virosphere composed of the smallest replicators on this planet teaches us how RNA viruses learned to diversify and flourish in the experienced context of the ever changing biosphere.

Genomic imprinting, a stable inter-generational memory mechanism

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Genomic imprinting brings about mono-allelic gene expression, strictly depending on the parental origin of the allele. This dosage mechanism evolved in therian mammals and involves germline-acquired DNA methylation marks. At hundreds of loci these 'imprints' are maintained during preimplantation development. At only about twenty gene domains, parental imprints are maintained throughout development, in all the somatic lineages. The latter process is remarkably stable, involving specialized proteins and histone methylation. It is influenced by environmental factors, however, which may give aberrant imprinted gene expression. The developmental cycle of imprint establishment, imprint maintenance and imprint erasure is tightly controlled. This ensures that imprints are correctly re-established in the germ cells of each new generation, without occurrence of trans-generational inheritance. It is important to understand why this contrasts with the situation at other sequence elements, at which epigenetic regulation is less tight and polymorphic, in some cases leading to apparent epigenetic inheritance across generations.

The evolution of imprinting has given rise to an increasingly complex antagonism between the maternal and the paternal genomes in eutherians. Maternally and paternally expressed genes influence each-other at many different levels. Research by us and others shows that at several chromosomal domains imprinted long non-coding RNAs (lncRNAs) and miRNAs repress protein-coding genes expressed from the opposite parental chromosome. Remarkably, some imprinted miRNAs and lncRNAs have repressive effects on imprinted loci elsewhere in the genome as well, providing further antagonism and complexity.

Aging and Lifespan Control

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What is aging? When does it begin? How to control lifespan at the level of individual organisms and species? Can we rejuvenate organisms in addition to slowing down the aging process? There is no consensus on these questions, but recent developments in the field may allow to address these questions. In particular, DNA methylation of defined sets of CpG dinucleotides emerged as critical and precise biomarkers of aging and lifespan. Multi-variate machine learning models, known as epigenetic clocks, exploit quantitative changes in the methylome to predict the age and lifespan with reasonable accuracy. Additionally, the first epigenetic aging clock that works at the level of single cells has been developed. Together with advances in genomics and pan-mammal and within species longevity signatures, these tools support tracking and manipulation of the aging process. Moreover, these tools may also be used to assess the possibility of age reversal. Several types of rejuvenation have been described, including the recently discovered process of embryonic rejuvenation, culminating in ground zero, marking the beginning of organismal aging. Understanding these processes provides insights into the relationship between aging, development and lifespan control.

Role of retrotransposition in memory in Aplysia

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There is increasing evidence for functional interactions between epigenetic mechanisms and retrotransposons in the brain. Previously, we showed that long-term memory in Aplysia is regulated by epigenetic alterations, including histone acetylation and DNA methylation. Here, we investigated whether retrotransposition also plays a role in memory in Aplysia. To test this possibility, we examined the effect of inhibitors of reverse transcriptase (RT)—the enzyme that synthesizes DNA from RNA—on serotonin (5-HT)-induced facilitation of Aplysia sensorimotor synapses in dissociated cell culture. Five spaced pulses of 5-HT (5X5-HT training) induce facilitation of sensorimotor synapses that persists for ≥ 24 h (long-term facilitation or LTF). Treatment with either the nucleoside RT inhibitor lamivudine (10 μM) or the

Non-nucleoside RT inhibitor rilpivirine (10 μM) blocked LTF. Neither drug altered baseline sensorimotor synaptic transmission. These results implicate retrotransposition in long-term memory in Aplysia. In addition, we tested whether either RT inhibitor altered short-term facilitation (STF) of in vitro sensorimotor synapses due to a single, brief (2-min) application of 5-HT. Surprisingly, both lamivudine and rilpivirine impaired STF. The rapid time course of the effects of the RT inhibitors on STF appears inconsistent with disruption of retrotransposition; thus, RT may be critical for a retrotransposon-independent mechanism of synaptic plasticity. Taken together, our results imply that RT plays a necessary, heretofore unsuspected, role in both short-term and long-term memory in Aplysia.
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Viruses as archaeological tools for uncovering archaic molecular relationships

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The main hypothesis underlying our work is that viruses re-establish, at least partially, past molecular activities and relationships of the cell, which had been silenced, compartmentalized or repressed, but that have not been completely eliminated during evolution. The entry of a virus into the cell always implies a diverting alteration of some intracellular molecular relationships. Then our question to the molecules we are interested in is with which other molecules they align themselves, perhaps with viral expression products that mediate cellular disruption, or, on the contrary, to still maintain the cell's status quo. In this sense, viruses can be regarded as archaeological tools with which we can distinguish cellular activities that facilitate the success of the virus and that were not expressed in its absence. One example is the ability of aminoacyl-tRNA synthetases to charge an amino acid to the 3' genome of some plant RNA viruses. This modification unveiled the presence of a tRNA-like structure in their 3' genomic end, and has been related to the possibility of tagging the 3' ends of origin-of-life RNA replicons. Another example is the potential of RNA viruses to reproduce and evolve under conditions of high mutation rates, that may reflect the conditions of replication of ancient ribozyme polymerases. Other findings will also be discussed. The idea is that these latent activities are dangerous, but cellular evolution that overcomes viral infection increasingly learns to control those potentialities that it cannot eliminate. As a result, it will be better prepared to resist challenges of different natures: environmental, symbiotic and collaborative. But also to new viruses, and the learning cycle will continue.

Epigenetics in unicellular to multicellular transition in Dictyostelium

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The evolution of multicellularity is a critical event that remains incompletely understood. We use the social amoeba, Dictyostelium discoideum, one of the rare organisms that readily transits back and forth between both unicellular and multicellular stages, to examine the role of epigenetics in regulating multicellularity. While transitioning to multicellular states, patterns of H3K4 methylation and H3K27 acetylation significantly change. By combining transcriptomics, epigenomics, chromatin accessibility, and orthologous gene analyses with other unicellular and multicellular organisms, we identify 52 conserved genes, which are specifically accessible and expressed during multicellular states. We validated that four of these genes, including the H3K27 deacetylase hdaD, are necessary and that an SMC-like gene, smcl1, is sufficient for multicellularity in Dictyostelium. These results highlight the importance of epigenetics in reorganizing chromatin architecture to facilitate multicellularity in Dictyostelium discoideum and raise exciting possibilities about the role of epigenetics in the evolution of multicellularity more broadly.

Transmitting epigenetic memory through modified histones

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In multicellular organisms, the organization of the genome into distinct chromatin domains enforces appropriate gene expression patterns during development and underlies the ability of a single genome to give rise to a multitude of cell types. The assembly of repressive heterochromatin domains that can self-propagate and be epigenetically inherited across multiple cell divisions is crucial to prevent inappropriate gene expression. However, the key feature that determines self-propagation of heterochromatin remains undefined. Our recent work directly implicates methylated histones, specifically tri-methylation of histone H3 lysine 9 (H3K9me3), as carriers of epigenetic information that allow propagation of heterochromatin in a self-templating manner. Importantly, we find that epigenetic inheritance of heterochromatin requires maintaining a critical density threshold of H3K9me3 to effectively target the histone methyltransferase Clr4/Suv39h, which binds to and catalyzes additional H3K9 methylation ("read-write") to maintain heterochromatin. In addition to discussing our latest findings about how cells maintain the critical density of H3K9me3 and its associated Clr4/Suv39h methyltransferase to epigenetically propagate heterochromatin, I will highlight implications our work for transgenerational inheritance of epigenetic memory.

Transposons as environmental stress detection modules, are eukaryotic genomes evolved to evolve?

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While it is widely acknowledged that transposable elements (TE) have played a profound role in the evolution of both genomes and molecular epigenetics. Most thinking on the subject has focused on TEs as parasites which need to be controlled, rather than commensals providing adaptive advantages to the host. However, in the modern post-genomic it has become possible to think about what functions TEs might have both in organismal adaptation to environmental stress and to the evolution of adaptive traits at the population level. This is particularly true regarding the potential role of TEs as genome level environmental sensors, a result of the host stress sensing apparatus characteristic of the lytic viruses which represent the ancestors of many TEs. Many, if not most steroid response elements in the genome derive from TEs. Similarly, the majority of immediate early genes, the cell level machinery of genome-environment interaction, have a retroviral/TE origin. In at least one case we have identified, a TE is both regulated by stress hormones and regulates stress hormone receptors interactions with the genome in turn. This, and other evidence means that TEs represent an off the shelf inventory of environmentally sensitive switches and regulatory elements that allow genomes to evolve more rapidly and in a more directed fashion than the random accumulation of point mutations can allow. This suggests that complex genomes may not be simply passive marble for the forces of natural selection to sculpt, but have evolved to evolve in particular directions that may be more likely than not to be adaptive.

Epigenetic learning in the nervous system

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Epigenetic memory and epigenetic learning are ancient processes that are found in unicellular organisms and are essential for survival and reproduction. With the evolution of nervous system, new types of memory and learning, based on synaptic contact-wiring were added to intracellular, epigenetic memory and learning. I discuss the role of epigenetic encoding and memory in neurons and the interactions between epigenetic engrams and synaptic engrams during neural learning. I suggest that with the evolution of learning based on cognitive maps, new functions for epigenetic memory had evolved through an interplay between synaptic mapping, migrating regulatory RNAs and chromatin marks. I highlight the similarity and differences between this system of information processing and the genetic system, and argue that the computational possibilities that such organization enables during ontogenetic learning are expected to be as vast as those afforded by the genetic system during evolution.

The nomadic behavior of the epigenetically inherited centromere

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The centromere is a unique specialized chromatin domain responsible for driving chromosome segregation during mitosis and meiosis. In nature, human centromeres are assembled on satellite DNA but can occasionally vacate their canonical position and move to distal loci, so-called neocentromeres. This has led to the notion that the centromere protein complex is maintained epigenetically, largely independent of direct in cis DNA sequence information. Central to this mechanism are specialized nucleosomes featuring the histone H3 variant CENP-A. Using in vivo protein lifetime measurements, we show that the centromeric CENP-A generates an unusually stable chromatin structure, which is unique to these nucleosomes. Replication of CENP-A chromatin is tightly coupled to the cell cycle ensuring synchrony between cell division and centromere propagation. Moreover, we have recently developed methods to induce the formation of a human neocentromere that appeared and is maintained on non-centromeric DNA. I will discuss the emerging view that CENP-A chromatin constitutes a plastic epigenetic self-templated feedback system that drives centromere inheritance.

From Mobile elements to RNA editing Via dsRNA – a path for genomic novelty

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Mobile elements comprise a significant fraction of metazoan genomes. Accumulation of mobile elements is bound to produce multiple putative double-stranded RNA (dsRNA) structures within the transcriptome. These endogenous dsRNA structures resemble viral RNA and may trigger false activation of the innate immune response, leading to severe damage to the host cell. Adenosine to inosine (A-to-I) RNA editing is a common post-transcriptional modification, abundant within repetitive elements of all metazoans. It was recently shown that a key function of A-to-I RNA editing by ADAR1 is to suppress the immunogenic response by endogenous dsRNAs. In addition, RNA editing by adenosine deaminases changes the information encoded in the mRNA from its genomic blueprint. Editing of protein-coding sequences can introduce novel, functionally distinct protein isoforms and diversify the proteome.

We analyzed the transcriptomes of dozens of species across the Metazoa and identified a strong genomic selection against endogenous dsRNAs, resulting in their purification from the canonical transcriptome. This purifying selection is especially strong for long and nearly perfect dsRNAs. These are almost absent from mRNAs, but not pre-mRNAs, supporting the notion of selection due to cytoplasmic processes. The few long and nearly perfect structures found in human transcripts are weakly expressed and often heavily edited.

Purifying selection of long dsRNA is an important defense mechanism against false activation of innate immunity. This newly identified principle governs the integration of mobile elements into the genome, a major driving force of genome evolution. Furthermore, we find that most ADAR1 activity is not required to prevent an immune response to endogenous dsRNAs. Thus, we present a new detection approach by analyzing 9125 GTEx RNA-seq samples to produce a highly-accurate atlas of 1517 editing sites within the coding region and their editing levels across human tissues.

On the nature of chromosome domain boundaries and their evolution

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Human chromosomes occupy their own volume within the nucleus. This sub-micrometer inter-chromosome partitioning is achieved through enzymatic DNA looping activities, more precisely, loop extrusion activities. Moreover, loci of 100 kb to 1 Mbp of DNA have been shown to preferentially self-associate within topologically associating domains (TADs). This involves the CTCF protein that binds the CCCTC DNA sequence. The CTCF recognition site is asymmetric and therefore points to the right or to the left and it can block loop extrusion coming from the right or from the left, respectively.

We classified triplet CTCF site patterns into 'same', 'convergent', 'divergent' or 'convergent and divergent' patterns. Logically, convergent >< and divergent <> CTCF sites must alternate, while the two other classes can vary in abundance from 0 to 100%.

Evolution made TADs as follows; divergent CTCF sites code for TAD boundaries and are often located close to each other. They are flanked by convergent sites that often lie further from each other and that code for the interior of TADs. Indeed, two types of hexameric CTCF sequences are overrepresented in our genome: >>><<< and <<<>>>. The first represents the left and right sides of one TAD and the second represents a boundary lying between the right and the left sides of two neighboring TADs. Hence, the code for TAD boundaries and TAD interiors are represented by a CTCF site orientation grammar that exploits the mathematical obligation of <> pairs to alternate strictly with >< CTF site pairs along the length of chromosomes.

Climbing Mount Improbable: how did evolution solve the scaling and searching problems

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There are two major problems that evolution had to solve to enable the emergence of developmentally complex organisms, and to a lesser extent all other organisms.

The first is the quasi-exponential scaling of regulatory genes with gene number, which indicates that the proportion of the genome devoted to regulation must increase with organismal complexity, and be the limiting factor. Various strategies have been deployed, including subroutine modularity, spatial organization and decisional hierarchies, most of which are RNA-directed. In particular, evolution has writ large the principle of using RNA flexibly to guide generic protein effectors, as exemplified by the RNAi and CRISPR/Cas9 pathways, to construct a regulatory milieu of long noncoding RNAs that have extraordinary modularity and alternative splicing to generate a myriad of isoforms with varied cargoes and genomic target sites to control the epigenetic trajectories of development.

The second problem is searching. It is obvious that evolution cannot have proceeded by random search alone – the number of variables is too great. Moreover, the accepted evolutionary algorithm of "generate (variations) and test" slows to a crawl as the progeny number decreases and generation time increases, as has occurred in birds and mammals, which may have been ameliorated by controlled transposon mobilization and quality control during spermatogenesis. The limitation of random searches in complex systems was recognized two decades ago by Rodney Downey and Michael Fellows, who pointed out that in large complex systems random searches become computationally intractable because of the exponential increase in the possibilities. Downey and Fellows' suggested solution is to define the most productive subspace and optimal tactics to decrease the complexity of the search and increase the chances of productive outcomes, which they termed 'Parameterized Complexity'. The obvious candidates are non-random mutation and an interplay between genetic and epigenetic inheritance, via RNA-directed genome engineering and editing.

The role of maternal factors in epigenetic programming of neurodevelopment

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Early life events are potent determinants of vulnerability and resistance to stressors. In many species, including rodents, the mother is the primary mediator of behavioral and physiological responses to stress in offspring during development. Emerging evidence indicates that epigenetic modifications in the brain of developing offspring are associated with the effects of maternal stress as well as individual neural, physiological, and behavioral responses to adversity. I will discuss research in my lab focused on identifying the relevant genomic targets of maternal stressors that exert long-term 'programming' effects on stress responses. We have approached this question in several ways. First, we have studied ecologically important stressors applied during gestation. Second, we have investigated factors that co-occur or interact with maternal care, including variations in ambient temperature and offspring genotype, that influence neurodevelopment and later-life behavior. Third, we have explored direct exposure to maternal dietary stressors across the developmental period on offspring phenotype and genome-wide epigenetic modifications in offspring brain. Through these investigations we are exploring the significance of the maternal environment in neurodevelopment and the role of epigenetic mechanisms in important signaling pathways involved in susceptibility to stress-related illness.

RT/RNase H reflecting evolution

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The retroviral Reverse Transcriptase (RT) and its RNase H (RH) have been discovered 50 years ago. RT is the most frequently used and quoted molecule - RT-PCR! It was detected as enzyme for replication of retroviruses extending the Central Dogma to the RNA world. RT is ubiquitous including phages ("retrophage"), bacteria and eukaryotes, and can be traced back in evolution to endogenous retroviruses or retro-elements, LINEs, SINEs or Alu sequences in genomes and telomers. The RT is often associated with an RH removing RNA in hybrids, potentially derived from evolutionary precursors such as non-coding ribozymes, introns and retro-related elements. The RT/RH have orthologs PAZ/ PIWI causing gene silencing in plants (RISC), suggesting an evolutionary relationship between invading pathogens and cellular defense by similar molecular tool kits. Thus, immunity is a variation of infection. PIWI is essential for genome stability in germline cells controlling transposon mobility whereby PIWI´s knockout leads to infertility due to uncontrolled transposition. Thus both, innovation and destruction by retroelements require controls. piRNAs allow transgenerational inheritance. RH are rather unspecialized house-cleaning enzymes, recycling nucleic acid nutrients. RH is present throughout all domains of life. RH-folds are involved in prokaryotic CRISPR-Cas, eukaryotic V(D)J recombination, interferon systems, in splicing, R-loop and mononucleotide excision, DNA repair, genome stability, disease (AGS), and retrotransposon silencing. RHs comprise 152 families and are among the most ancient and abundant protein-folds with conserved DEDD. Retrovirus-related sequences are drivers of evolution which can induce dangers or benefits, such as cancer, placenta formation, immunity and allow reconstruction of evolutionary steps (doi:10.3389/fmicb.2019.00051).

Odor preference learning and inheritance in Bicyclus anynana butterflies

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Male butterflies use species-specific sex pheromones to court females whereas larvae use plant-specific odors to find their hosts. Given that each species usually has its own odor preferences, it is unclear how preferences for different odors begin to evolve. We are exploring the evolution of novel odor preferences through the process of odor learning. We have shown that adult B. anynana female butterflies can learn to prefer new male sex pheromone blends and pass on those learned preferences to their female offspring. Larvae can also learn to prefer new odors applied to their plant food, and pass on these learned preferences to their offspring. We are currently investigating the mechanism of odor preference learning within a generation by quantifying 1) changes in the volume of specific antennal lobe micropyles of adult brains, 1) changes in DNA methylation of the antennae, and 3) changes in gene expression in antennae, and brains, in naïve individuals and in those exposed to new odors. We are also exploring the mechanisms of odor preference transmission to the next generation by 4) performing hemolymph transfusions and examining changes in odor preferences in the same and in the next generation, and 5) testing whether odor preference learning leads to changes in gene expression in ovaries and spermatophores.

Evolving Learning: The Exaptation of Epigenetics as a Learning Mechanism

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Advantages accrued to single-celled organisms when they began to work together in colonies, and cooperation would have been improved in any colony containing cells all of which contained identical nucleic acids. To maintain organism-wide genetic homogeneity while permitting cellular differentiation, nature evolved epigenetic mechanisms that guide and constrain cellular specialization and thereby dramatically influence anatomical, physiological, and behavioral phenotypes. Although these mechanisms appear to have evolved to allow cellular differentiation during development, they have been exapted to facilitate various learning processes. For example, several lines of research suggest that epigenetic mechanisms can record environmental circumstances early in development, and that such epigenetic effects can alter an organism's future functioning – a form of physiological learning. In addition, researchers (e.g., Levenson & Sweatt, 2005) have proposed that the central nervous system might have co-opted epigenetic mechanisms for use in forming long-term memories, giving rise to behavioral learning (and in the long run, perhaps, to conscious learning). In this talk, I will consider how and why evolutionary processes ultimately yielded the developmental processes that produce the kinds of learning and memory that characterize complex animals.

Mobile genetic elements in the RNA world: How a small ribozyme supports RNA sequence variation

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In early life, i.e. the RNA world, the exchange of sequence segments between two RNAs is an intriguing evolutionary concept that allows new RNA molecules with novel functionality to emerge instead of always reproducing the same ones. Similarly, RNA splicing contributes to variation in genetic context and thus functional changes. It is unclear when introns and splicing appeared in the evolution of life, but the efficiency of self-splicing introns suggests an origin in the RNA world. Perhaps self-splicing introns, which are considered selfish genes in today's DNA world, were one of the first parasitic elements that inserted themselves into other RNA species to be replicated without regard to the overall effects on the host RNA. Nevertheless, RNA organisms may also have derived some benefit from such processes. As seen today in eukaryotes, alternative splicing can be used to rearrange segments of a gene to obtain different phenotypes from a likely restricted genotype. Furthermore, exon-exon shuffling between genes within an RNA or trans-splicing between genes of two different RNAs has an evolutionary effect that produces new combinations of genes, as metazoan radiation clearly shows. Self-splicing introns may have been useful for transferring genetic material between RNA populations through reverse-splicing mechanisms. In our recent RNA engineering projects, we have developed models of simple RNA-mediated RNA recombination and splicing. Based on the hairpin ribozyme (HPR) with its unique cleavage and binding properties, we have designed and characterized small functional RNAs with the required properties to support these processes. Recently, we have demonstrated a model of genetic transposition at the RNA level. Based on general hairpin ribozyme activity, a segment (the transposon) was spliced out of a parent RNA and reinserted at another site of that RNA. In the talk, I will discuss these scenarios in the context of evolution in the RNA world.

Evolutionary origins and impacts of genome architecture in ciliates

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Genome architecture is well diversified among eukaryotes in terms of size and content, with many being radically shaped by ancient and ongoing genome conflicts with transposable elements (e.g., the large transposon-rich genomes common among plants). In ciliates, a group of microbial eukaryotes with distinct somatic and germ-line genomes present in a single cell, the consequences of these genome conflicts are most apparent in their developmentally programmed genome rearrangements. This complicated developmental phenomenon has largely overshadowed and outpaced our understanding of how germ-line and somatic genome architectures have influenced the evolutionary dynamism and potential in these taxa. In this talk I highlight three central concepts: how the evolution of atypical ciliate germ-line genome architectures is linked to ancient genome conflicts; how the complex, epigenetically guided transformation of germline to soma during development can generate widespread genetic variation; and how these features, coupled with their unusual life cycle, have increased the rate of molecular evolution linked to genome architecture in these taxa.

Use of pre-adaptations within the translational machinery during eukaryogenesis

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A major transition in biology represents a change in the way that heritable information is stored and transmitted. Here we use evolutionary studies of the translational machinery to trace down the pathways for the major transitions that led to the origins of eukarya (formation of nucleus, cytoplasm, and mitochondria). Specifically, we demonstrate (Penev, GBE 2020) that the large ribosomal subunit (LSU) rRNA of two Asgard phyla, *Lokiarchaeota* and *Heimdallarchaeota*, considered to be the closest modern archaeal cell lineages to Eukarya, already contain several expansion segments (ES9 and ES39) – features that are characteristic to all eukaryotic ribosomes – thus bridging the gap in size between prokaryotic and eukaryotic LSU rRNAs. Similar observations are made upon comparison of the ribosomes of bacteria and mitochondria (Petrov, MBE 2019). Thus, all α-proteobacterial ribosomes contain a reduced LSU rRNA content compared to other bacteria in the regions that are to be patched by mitochondrial-specific ribosomal proteins during the later stages of mitochondriogenesis. Ablation and expansion of mt-rRNA generates metastable regions of mitoribosomes that require patching by pre-existing elements that may confer new functions. Patching of mito-ribosomal particles is further performed by a near-universal subset of mitoribosomal proteins which may have been recruited from a pool of pre-existing proteins as a result of gene transfer or gene duplication. The extent and type of modifications that can be made in different species are determined by the available structural toolkit. Thus, we demonstrate that there had been pre-existing adaptations to the translational machinery that were engaged in a cascade of events resulting in the major remodeling of the ribosomal particles during eukaryogenesis.

Progressive decline in the levels of six miRNAs from parents to children in autism

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The growing burden of a gradual increase in the births of children with autism has placed its diagnosis and research into molecular mechanisms at the center of the concerns of major laboratories around the world. We previously detected the decrease in the levels of six miRNAs (miR-19a-3p, miR-361-5p, miR-3613-3p, miR-150-5p, miR-126-3p, and miR-499a-5p) in parents and their children inherited at a lower level. Here, we suggest that the down-regulation of each of these six miRNAs inherited from parents contribute to the development of children with autism. We compare the distribution levels of these six miRNAs in each family between the autistic child and her or his sibling. We find that the distribution of the levels of all six miRNAs in the siblings (not diagnosed as autism) is not always higher than in the autistic child, but it is at varying levels. These data support a model where autistic behavior relies on low levels of all of these six miRNAs expressed in children potentially associated with autistic syndrome (ASD). The intimate connection of the levels of miRNAs with behavioral characteristics suggests possibilities for understanding the basic circuitry involved in the autism and thus advancing in a partial knowledge of brain functions. As a more immediate aim, the earlier detection of the child with autism would make it possible to alleviate the diagnose of the autism at an early age, so as to provide children as early as possible with an environment conducive to their development.

Epigenetic regulation of genomic corticosteroid receptor action in the brain in relation to stress coping

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Adaptation to stressful events requires gene expression changes in the brain, particularly in the limbic hippocampus. Our previous research has shown that stress-induced signaling mechanisms driving epigenetic modifications underpin the induction of immediate-early genes and the consolidation of behavioral adaptation including contextual memory formation. The epigenetic modifications, we focused on, were the dual histone mark, phosphorylation and acetylation of histone H3 (H3K9acS10p). Glucocorticoid hormones, secreted during stressful events, play a critical role in orchestrating adaptive responses to such challenges. They exert their effects via mineralocorticoid (MRs) and glucocorticoid receptors (GRs) in the hippocampus acting as transcription factors resulting in changes in gene expression. Recently, we conducted chromatin immunoprecipitation combined with sequencing (ChIPseq) and RNAseq studies to assess the genome-wide interaction of MR and GR with the hippocampal chromatin and their gene transcriptional consequences under baseline (non-stress) and acute stress conditions. These transcription factors were found to interact with neuroplasticity and ciliary genes underpinning neuronal functions like neuromorphology, long-term potentiation, neuronal differentiation and ciliogenesis, as well as learning and memory processes. H3K9acS10p ChIPseq conducted in parallel with the MR and GR ChIPseq analyses revealed a substantial overlap between the location of the epigenetic modification and the genomic binding of MR and GR. We found significant correlations between enrichment levels of MR and GR, and H3K9acS10p as well as between MR, GR, H3K9acS10p and gene transcriptional responses after acute stress. Therefore, we conclude that epigenetic changes play an important role in site-specific interaction of corticosteroid receptors with the hippocampal genome after stress.

The *foraging* gene as a modifier of behavior: gene regulation, pleiotropy and plasticity

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The *Drosophila melanogaster foraging* (*for*) gene, with its rover and sitter larval foraging variants, is an established behavior genetics model. Orthologues of the *foraging* gene also modulate the individual and social behavior of a wide range of species including the regulation of behavior in eusocial insects. In Drosophila, *foraging* modifies the expression of multiple traits, including feeding and foraging, stress tolerance, sleep, metabolism, aggression, escape responses, social behavior, and learning and memory. From a social context perspective, *Drosophila foraging* affects larval clustering during foraging under high larval densities, adult social behavior and social networks and social learning. We wondered how *foraging a*ccomplishes its behavioral pleiotropy at the molecular level. We found that *D. melanogaster foraging* has a complex modular genomic structure with four promoters, 21 transcripts and eight protein isoforms. The four promoter modules are differentially regulated during development and in a timescale, tissue and cell-type dependent manner. Genetic variation in the *foraging* gene interacts with some of *foraging*'s regulators. Several examples of this differential gene regulation include *foraging* related phenotypes regulated in the fly brain by G9a, a histone methyltransferase, and in the larval ventral chord by Pumilio a protein that regulates post-transcriptional gene expression. We provide a picture of the molecular basis of *foraging*'s pleiotropy and its contributions to behavioral plasticity. This data sets the scene for discussions of *foraging's* co-option in social behavior evolution.

Non-coding RNA controllers of acetylcholine signaling as body-brain communicators

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Acetylcholine (ACh) is the very first neurotransmitter known, but the full scope of its brain and body impact is still incompletely understood. To address this challenge, we study the RNA regulators of ACh signaling, aiming to assess the complexity of the mechanisms controlling cholinergic functioning in health and disease. In particular, we focus on the short non-coding RNA regulators, including microRNAs and more recently, the re-discovered family of transfer RNA fragments controlling ACh signaling. We investigate how these RNAs are inter-changed under acute states to affect ACh-mediated processes and inflammation in neurodegenerative diseases and in women and men**,** with a special focus on brain-related conditions including ischemic stroke and mental diseases**.** Our findings highlight the complex control over ACh synthesis, its activation of receptors and its enzymatic degradation under small RNAs interference which is adapted to address disease conditions; and may shed new light on both the brain regions and neuronal structures involved and the recent clinical big data reports demonstrating long-term impact of anti-cholinergic medications as risk factors of dementia in the elderly.

Sperm-mediated epigenetic evolution

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Epididymal spermatozoa from most animal species can spontaneously take up exogenous DNA or RNA molecules, either as naked nucleic acids or in cell-derived extracellular vesicles (ECVs), such as exosomes, and internalize them into nuclei. The heterogeneous cargo of ECVs predominantly contains small regulatory RNAs, such as microRNAs (miRNAs) and transfer RNA-derived small RNAs (tsRNAs). ECVs are released from somatic cells and tissues in the bloodstream, can cross the Weismann barrier, reach the epididymis, and therein are taken up by spermatozoa, which can deliver them to oocytes at fertilization, henceforth propagating in early embryos. These processes establish a transgenerational flow of regulatory RNAs potentially capable to reshape the early embryo transcriptional landscape, thus promoting the emergence of novel traits in the offspring. Importantly, environmental stressors to which sperm cells may be exposed, impact on the sperm RNA composition and can influence the offspring's phenotype. External stimuli are thus "converted" under the form of regulatory RNAs that drive the reshaping of the embryonic genome and promote developmental changes. These data suggest a mechanistic model in which early embryos provide a "permissive", change-prone environment where such crucial conversions occur. LINE-1 retrotransposons play pivotal roles in the process, as stress-responsive sources of regulatory RNAs and chromatin organizers. These data evoke a Lamarckian-type scenario and are reminiscent of the Darwinian pangenesis theory.

Evolution in learning, learning in evolution

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Both learning and evolution can produce adaptive solutions, sometimes metting very complex challenges. The idea that there can be important commonalities shared by these processes is not new. Skinner regarded operant condition as analogous to natural selection. Bateson regarded evolution as a cognitive process. Changeux and Edelman addressed aspects as brain dynamics from a selectionist point of view. Smolin and coworkers speak about an autodidactic Universe and cosmological natural selection. I aim to present the state-of-the-art of such investigations, mainly showing how much of evolution is best understood in terms of learning theory, and whether there can be bona fide evolutionary dynamics (beyond mere selection) in brain function.

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How is trauma embedded in our genome? A possible role for DNA methylation

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Introduction: Traumatic experience has long term consequences, which become manifest as Post-traumatic stress disorder (PTSD), an incapacitating trauma-related disorder, with no reliable therapy. DNA methylation is a covalent modification of DNA that is associated with cell type differentiation during embryonal development. Environmental and behavioral exposures alter DNA methylation patterns. Chronic diseases are associated with DNA methylation changes.
1st Objective: We tested the hypothesis that DNA methylation determines susceptibility to PTSD and that they mediate between exposure to trauma and PTSD.
Model: Rats exposed to cat urine which develop PTSD like behaviors.
Results: Using this model, distinct DNA methylation profiles of PTSD susceptibility and resilience in the nucleus accumbens (NAc) with overall hypomethylation of different genomic CG sites in susceptible animals. This hypomethylation in susceptible animals is correlated with reduction in expression levels of the DNA methyltransferase, DNMT3a. We also identified enrichment in the RAR activation and LXR/RXR activation pathways that regulate Retinoic Acid Receptor (RAR) Related Orphan Receptor A (RORA) activation. Intra-NAc injection of a lentiviral vector expressing either RORA or DNMT3a reversed PTSD suggesting causal role in PTSD. To translate our results into a potential pharmacological therapeutic strategy, we tested and found that combined treatment with the methyl donor SAM and retinoic acid reversed PTSD-like behaviors.
Conclusions: Our data support an epigenetic mechanism of PTSD susceptibility and a causal role for epigenetic alterations in mediating PTSD. This has implications on our understanding of how adverse experience impacts our genome and our life trajectories.

Co-option of the germline piRNA pathway to regulate vertebrate neural crest specification

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Across metazoa, Piwi proteins play a critical role in protection and maintenance of the germline genome by piRNA-mediated repression of transposable elements. In vertebrates, activity of Piwi proteins and the piRNA pathway was thought to be confined to the gonads. Our results reveal expression of Piwil1 in a vertebrate somatic cell type, the neural crest–a migratory embryonic stem cell population. We show that Piwil1 is expressed at low levels throughout the chicken neural tube, peaking in neural crest cells just prior to the specification event that allows them to undergo an epithelial-to-mesenchymal transition and migrate into the periphery. Importantly, we find that loss of Piwil1 impedes neural crest specification and emigration. Small RNA sequencing reveals somatic piRNAs with sequence signatures of an active ping pong loop. RNA-seq and functional experiments identify the transposon-derived gene ERNI as the target of Piwil1 in the neural crest. ERNI, in turn, suppresses *Sox2* levels to precisely control the timing of neural crest specification and epithelial-to-mesenchymal transition. Our data provide mechanistic insight into a novel function of the piRNA pathway as a regulator of somatic development in a vertebrate species and an example of co-option of a transposon-derived gene and its regulation into a gene regulatory network governing vertebrate development.
The modular evolution of an RNA polymerase ribozyme with promoter recognition and processivity

The modular evolution of an RNA polymerase ribozyme with promoter recognition and processivity

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Ancient RNA replicase ribozymes appear likely to have been responsible for both genomic replication and gene expression early in the evolution of life. But how might such complex RNA catalysts have evolved? DNA replication starts at a fixed origin and assembles highly processive DNA replication factories, one for each replication fork. DNA-dependent RNA polymerases control gene expression by using DNA promoters. After binding to a promoter such polymerases must rearrange into a form capable of processive elongation. The two-step processes used in DNA replication and RNA transcription are quite fundamental as molecular recognition requires binding, while elongation requires a conformation that can slide along a nucleic acid template.

We have selected an RNA polymerase ribozyme that can, just like a DNA-dependent RNA polymerase, use a sigma-like specificity primer to locate a promoter sequence. Once found this RNA enzyme rearranges into a topologically clamped form able to processively extend a single-stranded RNA template. The clamped polymerase stays associated with circular templates but falls off of short linear templates indicating that it can move around the template during polymerization.

This polymerase consists of three domains. The first domain was selected for its ability to ligate itself to an RNA primer by phosphodiester chemistry. The second domain was selected to take the core catalytic core of the first domain and enable NTP polymerization on a primed RNA template. The third, and most recently selected domain, confers promoter recognition and topological clamping. I will compare the evolution of this artificial ribozyme to that of other naturally existing ribozymes and suggest that such modular evolution was likely to have been common early in the evolution of life.

Keywords: Ribozyme polymerase, promoter, clamp based processivity, multidomain ribozymes

Genomic risk for schizophrenia and the environment in early life: insights on epigenetic plasticity

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The process of gaining knowledge, i.e., learning, is intrinsic to evolution as well as development, both increasing complexity, leading to systems able to handle higher amounts of information. Why? Managing higher loads of information is not a *condicio sine qua non* for survival, thus selection can't be the only answer. We will discuss our studies on genomic risk for schizophrenia, early-life events, placenta and brain genomics, which support a link between early development, epigenetic plasticity, learning, and risk for complex disorders. Our data indicate that epigenetic plasticity is essential in early life, in embryonic and extraembryonic tissues, because it can allow the organism to adapt to the fluctuations of the environment, increasing its possibility to survive and later reproduce. Thus, genome elements that allow and enhance plasticity are essential to life itself and development. The same elements are critical for learning and the acquisition of all the behavioral features held as integral part of a "healthy" human - although no human possesses them right away, at birth. The flip side is that they can also allow a single cell of the body to acquire mutations, eventually leading to cancer; indeed, cancer signaling pathways are crucial in early development. Finally, the same elements, also when defective, can be a factor in altering trajectories of brain development and patterns of brain activity, leading to disorders in the adult.

Natural antisense transcripts play different roles in soma and male germ cells

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Natural antisense transcripts (NATs) constitute a significant group of regulatory, long noncoding RNAs. They are prominently expressed in testis but are also detectable in other organs. NATs are transcribed at low levels and co-expressed with the related protein coding sense transcripts. We have investigated NATs from a genomic perspective in mouse testis and HEK293 cells and from a gene specific angle (*SLC34A/PFN3pseudogene*) in two human kidney cell lines (HKC-8 and HEK293).

Testis express a highly complex transcriptome including RNAs that potentially form double-stranded RNA (dsRNA). We isolated dsRNA from testis using the monoclonal J2 antibody and deep-sequenced the dsRNA-enriched samples. The genes that generate dsRNA are significantly expressed in isolated male germ cells with particular enrichment in pachytene spermatocytes. Moreover, dsRNA-associated genes associate with endogenous siRNAs and genes that form NATs.

The genome wide studies in testis contrast the results from cell lines where we induced sense and antisense transcription of a specific locus (*SLC34A1/PFN3pseudogene*) using epigenetic modifiers and CRISPR-Cas9. We found concordant sense and antisense transcription with both activation strategies. Furthermore, expression was paralleled by reduced sense promoter methylation and an increase in activating histone marks.

These findings underpin a hypothesis where NATs have different biological roles in soma and germ cells, respectively. Accordingly, NATs share the mechanistic principles established for lncRNAs in somatic cells -with the benefit of close proximity to a potential target gene. In germ cells, however, our findings suggest a different biological role for NATs that requires RNA complementarity, dsRNA formation and endo-siRNA processing.

Understanding the Physicochemical Language of Epigenetics: On the Interaction Preferences between Modified Nucleobases and Protein Residues

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Covalent modifications of nucleobases affect epigenetic regulation of gene expression and modulate mRNA stability. In order to study how nucleobase modifications affect their interactions with protein residues, we have derived the absolute binding free energies and analyzed the binding modalities between four key modified DNA/RNA nucleobases (hypoxanthine, 5-methylcytosine, 5-hydroxymethycytosine and N6-methyladenine) and all non-prolyl/non-glycyl protein side chains using molecular dynamics simulations and umbrella sampling in high- and low-dielectric solvents. Our analysis elucidates several context-dependent rules of the physicochemical language of epigenetics and provides a quantitative foundation for understanding, predicting and sculpting the interactions between key epigenetic marks and proteins at the atomistic level.

Posters

Unravelling the „inflammatory-chromatin" epigenetic code using Drosophila genetics

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Cells live in constantly changing environments and need at any time to be able to adapt. Depending on the stimulus, a fast and specific response must be engaged to preserve not only the cellular, but also the organism's integrity. In general, this response is governed by the expression of inducible genes, leading to the activation of signalling cascades. Inducible genes, such as immune response genes need to be i) shut down in normal conditions, ii) activated upon stimulation and iii) rapidly inactivated after the stimulus ends. This implies highly dynamic changes at the molecular level, which involve epigenetic mechanisms.

Our team use the fruit fly *drosophila melanogaster* as a model to study the molecular regulation of NF-κB innate immune response pathways. The discovery of the co-activator Akirin brought new insights in the regulation of these pathways (Goto *et al*., 2008). We showed that Akirin is implicated in the transcriptional activation of a subset of NF-κB target genes in both *Drosophila* and mammals through the recruitment of the chromatin remodeling complex SWI/SNF (Tartey *et al*., 2014). Also, these genes are characterized by acetylated histone 3 lysine 4 (H3K4ac) (Bonnay *et al*., 2014). To describe the dynamic of these epigenetic events, we combine genetic approaches and next-generation sequencing techniques such as CUT&RUN, MNase-seq and RNAseq.

Epigenetic information carriers in the human male germline

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Epigenetic erasure and resetting in gametogenesis and early embryogenesis is probably best studied for imprinting in mammals. Our knowledge about the "reprogramming" of other epigenetic information carriers or mechanisms, including small non-coding RNAs (sncRNAs) and residual histone and protamine (modifications), lags behind, especially in the context of their evolutionary potential.

My research focuses on epigenetic information carriers that persist during male germline reprogramming. I am determining the epigenetic states related to ncRNA and residual histones and protamine from sperm nuclei of fully differentiated spermatozoa (sperm heads). We assume that this best reflects the epigenetic status of the paternal pronucleus in the zygote.

First, I determined the overall profiles of sncRNAs using small RNAseq. Comparing datasets from testes, whole sperm, and sperm heads with respect to expressed YRNA orthologs, I found an enrichment of Y-RNA fragments (YsRNA) in sperm heads that is markedly different from the situation in oocytes. I am currently analysing the 5'- and 3'-YsRNA modifications and hypothesize that YsRNA are transcribed from source genes and inherited transgenerationally, representing a paternal epigenetic contribution to the zygote and its development.

Moreover, I analysed the genome-wide distribution of protamines and histone residues in sperm heads. Interestingly, another class of noncoding RNAs, lncRNAs, is preferentially bound by residual histones, possibly mediating dynamic transcription of lncRNAs in the sperm head as a future RNA payload for the zygote. These lncRNAs, their possible association with sperm head chromatin, and possible transgenerational inheritance are currently being explored.

The impact of metabolism on heterochromatin regulation

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Epigenome-modifying enzymes, for example histone methyl-transferases and acetylases among others, use metabolic intermediates as substrates and cofactors for chromatin modifications, thus potentially connecting nutritional fluctuations and cellular metabolism to chromatin state. Additionally, nutritional instabilities alter microbiota composition, which is indispensable in host's metabolism regulation. Yet, how nutritional oscillations and metabolites availability influence chromatin remains largely unknown.

In our group, we investigate how metabolites and microbiota regulate heterochromatin, the silenced portion of the eukaryotic genome, essential to preserve genetic stability and ensure cell-type specific transcription and identity. For this purpose, we use the model organism C. elegans, whose transparent body enables the screening of heterochromatin state changes using a well-established GFP-based reporter. In a targeted RNAi screen aimed at knocking down metabolic enzymes, we found that perturbing the one-carbon metabolism leads to a de-repression of the heterochromatic reporter. In a parallel screen, we took advantage of the exclusively bacterial diet of C. elegans that matches its microbiota, to feed the nematode with different bacteria. Intriguingly, we identified one bacterial strain that alters the expression of the reporter and we are currently characterizing it.

Ongoing experiments will determine which histone post-translational modifications are involved in the observed dietary/metabolic-mediated de-regulation of heterochromatin and will investigate the effects on global transcription.

With this work, we will gain insights on how chromatin processes and the metabolic state of the cell are connected, increasing our understanding of epigenetic alterations involved in metabolic disorders such as obesity and type 2 diabetes.

Chromatin contacts between two distant loci trigger Transgenerational Epigenetic Inheritance of a PRC2-dependent phenotype in Drosophila melanogaster

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Increasing evidence suggests that in some cases epigenetic modifications can persist across generations, potentially contributing to relatively stable phenotypic changes independent of DNA sequence. This process of Transgenerational Epigenetic Inheritance (TEI) is documented in a variety of organisms and can involve factors such as DNA methylation, histone modifications and non-coding RNAs. One case of TEI in Drosophila occurs at the transgenic Fab2L locus, in which heritable differences in PRC2-desposited H3K27me3 are triggered by a transient genetic perturbation: a single generation of heterozygosity at the homologous Fab7 locus located elsewhere in the genome. These changes lead to differences in eye colour which can be selected, resulting in either over- or under-expression of the mini-white gene that is stable for many generations. We show that the transient heterozygosity of the Fab7 locus leads to an increase in chromatin contacts between the transgenic Fab2L and endogenous Fab7 loci in the nucleus, potentially contributing to the observed epigenetic differences. To further investigate the role of these chromatin contacts, we artificially induce contact between the two loci using synthetic biology tools. We show that even in the absence of a genetic perturbation, this artificial induction of contacts is able to trigger TEI at the Fab2L locus. This demonstrates an essential role for chromatin contacts in the transgenerational inheritance of H3K27me3 at the Fab2L locus, and raises questions about their role in other instances of TEI.

Autoregulation as a potential pathway of miRNA: host-gene interaction conserved across species

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MicroRNA (miRNA) genes are either located in the extragenic space or within host genes, but intragenic miRNA::host gene interactions are largely enigmatic. Therefore, we investigated the location and co-regulation of all to date available miRNA sequences and their host genes across species in an unbiased computational approach.

Custom written Python and R pipelines were applied to classify miRNAs based on the location in the genome and 4 groups (intragenic, antisense, overlapping, extragenic) were defined. The Diana Tools microT-CDS v5 algorithm and an iterative randomized model (IRM) was used to predict and evaluate possible miRNA::mRNA interactions. Direct and indirect autoregulative functions were employed by means of integrated network analysis. The NOCICEPTRA tool was developed to explore miRNA::mRNA networks during sensory neuron development

The majority of miRNAs were located within intronic regions of protein-coding and non-coding genes and showed increased target probability as well as higher target prediction scores as compared to IRM (PMID: 31963421). This was associated with a higher number of miRNA recognition elements for the hosted miRNAs within their host genes. Strong indirect autoregulation of host genes was identified, and intragenic miRNAs appeared to interact with functionally related genes. Networks of genes harboring intragenic miRNAs were more susceptible to regulation and were identified during the differentiation and maturation of sensory neurons from induced pluripotent stem cells (iPSCs, PMID: 34486248).

Our unbiased approach suggests direct and indirect autoregulative mechanisms induced by intragenic miRNAs across species arguing for biological relevance in a broad spectrum of cell functionalities including neuron development and maturation.

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The epigenetic basis of size in the Florida carpenter ant

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Animal size is a defining characteristic across the animal kingdom and is exemplified in mammals by the dominant behavior of larger animals, leading to greater access to food resources and mating opportunities. Yet, perhaps nowhere else is size more important than in eusocial insects, where workers exhibit two distinct modalities of size that then determine their social roles within their colonies. In the carpenter ant species Camponotus floridanus, the larger major ants devote more time to the defense of the colony while the smaller minor workers are more involved in foraging. These two worker castes are entirely female and are genetically highly (~75%) related, suggesting that any developmental differences in size are mediated by nongenetic factors. In our work, we sought to determine the molecular basis of this differential development from apparently bipotent larvae. To do so, we collected larvae that span the entire potential worker size range and subjected them to whole-transcriptome, -DNA methylome, and -genome analyses. We reaffirm that genetic differences do not correlate with ant size and that, instead, major changes in global gene expression – including several key genes – associate with size differences in the ant larvae. We find a role for several well-known and novel microRNAs as well as changes in the DNA methylation levels of a substantial fraction of differentially expressed genes. Currently, we seek to understand the mechanisms by which DNA methylation is involved in gene regulation in C. floridanus and to determine whether experimentally modulating the levels of key transcripts and microRNAs could be sufficient to reprogram larvae to grow to larger and smaller sizes.

The remarkable journey of peptide substrates for the Major Histocompatibility Class I pathway: from full length proteins to DRiPs to spliced peptides and to translation of pre-spliced mRNAs

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Every cell continuously reports its condition to the immune system via surface presentation of the small pieces of self-proteins (8-10 amino acid-long), namely antigenic peptides (AP), on the Major Histocompatibility Class I (MHC-I) molecules. Both infection and neoplastic transformation change the repertoire of antigenic peptides and trigger presentation of non-self antigens, representing infectious agents or genomic alterations acquired during cell transformation. Recognition of non-self antigens by CD8+ T lymphocytes triggers an immune response and leads to the destruction of the non-self presenting cell. Antigenic peptides thus lie at the heart of the self-discrimination and the origin of AP substrates provides an interesting evolutionary aspect of self vs non-self development.

In this poster we present how the view on the origin of AP has evolved over the years and what is our current understanding of the 'source of self'. We will focus on the pre-spliced mRNA as a matrix for the synthesis of antigens during the pioneer round of translation taking place in the nucleus. This view for the first time proposes that synthesis of AP is mediated by an alternative mRNA translation event that is separate from the synthesis of full-length proteins and that fits with previous reports on a co-transcription/translation event in mammalian cells. It helps to explain why intronic peptides and peptides coming from the genomic viral-derived elements of the genome (eg. Alus) can be presented on the MHC-I and used for the self-discrimination. The production of class I antigens from the pre-mRNA supports the view on the viral origin of the immune system.

N-degron pathways in plants

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A protein´s amino-terminus (N-terminus) affects the half-live of the protein. N-recognins are Ubiquitin ligases that bind de-stabilizing N-termini (so-called N-degrons) and mediate ubiquitination and subsequent proteasomal degradation of the protein harboring the N-degron. Alternatively, autophagy receptors can act as N-recognins and promote vacuolar/lysosomal degradation of their substrates. An N-degron has three characteristics: 1.) an unstructured N-terminus, 2.) a Lysine spatially close to the N-terminus for ubiquitin attachment and 3.) a de-stabilizing N-terminal amino acid. There are two types of de-stabilizing amino acids, type 1: positively charged amino acids (Arg, Lys, His); type 2: bulky hydrophobic amino acids (aromatic: Phe, Tyr, Trp; and aliphatic: Leu, Ile).

Whereas yeast encodes only one N-recognin (UBR1), animals and plants encode several N-recognins with varying substrate preferences. Yeast UBR1 and mammalian UBR1 and UBR2 and plant PRT6 are sequelogs, but PRT6 lost the domain for binding type 2 residues. PRT1 is a plant-specific ubiquitin ligase that recognizes aromatic, but not aliphatic, first residues. The goal of this project is to identify the plant N-recognin targeting proteins with N-terminal Leu and Ile.

Based on mutant screens and inhibitor studies, we found that in Arabidopsis degradation of proteins with N-terminal Leu occurs both via the proteasome and to a minor degree via the vacuole. We present data on genes involved in Leu N-degron turnover. So far, we can only speculate why N-recognins in plants are more diverse and specialized than N-recognins in other eukaryotes. The existence of multiple degradation routes offers flexibility to a plant cell. While the PRT6-mediated pathway is central for sensing the concentration of oxygen and nitric oxide, and PRT1 has a role in biotic stress responses, the biological functions of the other pathways remain to be determined.