# Welcome!

# We wish you a beautiful and interesting stay in Salzburg

IMPRESSUM:
F.d.I.v.: ARGE GenConText: Günther Witzany, Erich Hamberger
Redaktion, Layout, Produktion: Günther Witzany;
Vogelsangstr. 18c, A-5111-Bürmoos
Fassung vom 17.6.2008

GenConText Research


Natural Genetic Engineering and Natural Genome Editing
3-6 July 2008. Salzburg-Austria

# Salzburg

(Austria)

# 3 – 6 July 2008

# Programme

and

# Abstracts

of

**Talks**

and

**Posterpresentation**

## organized by

Günther Witzany and Erich Hamberger

## in co-operation with

Schatzkammer Land Salzburg
Kulturelle Sonderprojekte

### Partner

Leopold Kohr Academy

## Sponsors

Bundesministerium für Bildung, Wissenschaft und Kultur

Universität Salzburg

Tecan Austria Gmbh

## Organizing Commitee

Guenther Witzany
Erich Hamberger
Peter Eckl
Nikolaus Bresgen
Hiltrud Oman
Pierre Madl

**Webdesign**
Konzeptionsbüro Mayr: http://www.taxham.at/
kama@taxham.net

# Programme

GenConText Research SYMPOSIUM Natural Genetic Engineering and Natural Genome Editing
3-6 July 2008. Salzburg-Austria

St. Virgil Conference Center
Ernst-Grein-Straße 14; A-5026 Salzburg, Austria
Tel: +43/662/65901-0 | Fax: +43/662/65901-509
E-Mail: office@virgil.at

## Wednesday, July 2, 2008

| | |
|---|---|
| 12:00 - 20.00 | Registration at St. Virgil |
| 18:45 | Welcome drink and warm reception by the co-operating partner Alfred Winter (Gouvernment of Land Salzburg) and Michael Breitenbach (Department of Cell Biology, University of Salzburg) |

# Thursday, July 3, 2008

**Morning** Chair: Hamberger

**Information Processing replaces Mechanics**

| | | |
|---|---|---|
| 09.00 – 09.15 | Prologue | Guenther Witzany:<br>*Motives to organize this Symposium* |
| 09.15 – 10.00 | Talk 1 | James Shapiro:<br>*Revisiting the Central Dogma in the 21st Century* |
| 10.00 – 10.45 | Talk 2 | Gertrudis Van de Vijver:<br>*No Genetics without Epigenetics? No Biology without Systems Biology? On the Meaning of a Relational Viewpoint for Epigenetics and Current Systems Biology* |
| 10.45 – 11.15 | **Coffee Break – Tea Time** | |
| 11.15 – 12.00 | Talk 3 | Guenther Witzany:<br>*Natural Genome Editing: why Editing needs Editors* |
| 12.00 – 12.45 | Talk 4 | Patrick Forterre:<br>*The Role of Viruses in the Origin and Evolution of DNA and Modern Cells* |

**Afternoon** Chair: Witzany

**Viral Infection-Driven Eukaryotic Evolution**

| | | |
|---|---|---|
| 14.30 – 15.15 | Talk 5 | Philip Bell:<br>*The Viral Eukaryogenesis Theory and Eukaryotic Evolution* |
| 15.15 – 16.00 | Talk 6 | Frantisek Baluska:<br>*Cell-Cell Channels and Cell-Cell Fusions in Multicellular Organisms: Viruses-Induced Manipulations with Impacts on Evolution?* |
| 16.00 – 16.30 | **Coffee Break – Tea Time** | |
| 16.30 – 17.15 | Talk 7 | Manfred Heinlein:<br>*TMV-infection: Spread of Viral RNA and of a Virus-Induced Systemic Recombination Signal* |
| 17.15 – 18.00 | Talk 8 | Amin Rustom:<br>*"Highway to Hell": Intercellular Spread of Viruses via Nanotubular Highways?* |

# Friday, July 4, 2008

**Morning** Chair: Hamberger

**Communal Evolution**

| | | |
|---|---|---|
| 09.00 – 09.45 | Talk 9 | Nigel Goldenfeld:<br>*What does the Genetic Code tell Us About Early Life?* |
| 09.45 – 10.30 | Talk 10 | Kalin Vetsigian:<br>*Coevolution between tRNA Expression Levels and Codon Usage Catalyzed the Optimization of the Genetic Code*. |
| 10.30 – 11.00 | **Coffee Break - Tea Time** | |
| 11.00 – 11.45 | Talk 11 | Frederick Arnaud:<br>*Symbiotic Relationship between Endogenous Retroviruses and their Host: Lessons from the Sheep* |
| 11.45 – 12.30 | Talk 12 | Eshel BenJacob:<br>*Rethinking the Genome – Hints from Bacteria Complexity* |

**Afternoon** Chair: Witzany

**Natural Genome Editing**

| | | |
|---|---|---|
| 14.00 – 14.45 | Talk 13 | Gil Ast:<br>*The Origin of Alternative Exons* |
| 14.45 – 15.30 | | Warm reception by the Austrian Federal Minister of Science and Research Dr. Johannes Hahn (Coffee Break – Tea Time) |
| 15.30 – 16.15 | Talk 14 | Alessandro Quattrone:<br>*The Intelligent Ribosome: Shaping of Proteomes by Translational Control* |
| 16.15 – 17.00 | Talk 15 | Juergen Brosius:<br>*Natural Genomic Lottery* |
| 17.00 – 17.45 | Talk 16 | King I. Jordan:<br>*Repetitive DNA and the Logic of Human Gene Regulation* |

**Evening**

| | |
|---|---|
| 18.30 – 19.00 | **Salzburg String Quartett** with W.A.Mozart performance |
| 19.00 - | **Conference Dinner (**by the local organizer commitee) |

# Saturday, July 5, 2008

**Morning** Chair: Hamberger

**Symbiotic Interactions**

09.00 – 09.45 Talk 17 Bruce Webb:
*The Evolution of Polydnavirus Segments*

09.45 – 10.30 Talk 18 Peter Gogarten:
*The Role of Gene Transfer in Innovation and Speciation*

10.30 – 11.00 **Coffee Break - Tea Time**

11.00 – 11.45 Talk 19 Nika Lovsin:
*APOBEC3 Cytidine Deaminases – Editors with Antiretroelement Activity*

11.45 – 12.30 Talk 20 Luis Villarreal:
*The Source of Self: Genetic Parasites and the Origin of Adaptive Immunity*

**Afternoon** Chair: Witzany

**Epigenetic Control**

14.00 – 14.45 Talk 21 Marcella Faria:
*The lack of RNAi and Transcriptional Modulation in T. cruzi – Where are the Epigenetic Controls in this Model?*

14.45 – 15.30 Talk 22 Ehud Lamm:
*Networks Connect Nature and Nurture*

15.30 – 16.00 **Coffee Break – Tea Time**

16.00 – 16.45 Talk 23 Shiv Grewal:
*Epigenetic Genome Control by RNAi and Transposon-derived Proteins*

16.45 – 17.30 Epilogue Erich Hamberger:
*Cultural Genetic Adaptation*

19.00-20.00 Open Space Discussion about Goals of this Symposium

# Titles of the Talks

Frederick **Arnaud**: Symbiotic Relationship between Endogenous Retroviruses and their Host: Lessons from the Sheep.

Gil **Ast**: The Origin of Alternative Exons

Frantisek **Baluska**: Cell-Cell Channels and Cell-Cell Fusions in Multicellular Organisms: Viruses-Induced Manipulations with Impacts on Evolution?

Philip **Bell**: The Viral Eukaryogenesis Theory and Eukaryotic Evolution

Eshel **Ben Jacob**: Rethinking the Genome – Hints from Bacteria Complexity

Juergen **Brosius**: Natural Genomic Lottery

Marcella **Faria**: The lack of RNAi and Transcriptional Modulation in T. cruzi – Where are the Epigetic Controls in this Model?

Patrick **Forterre**: The Role of Viruses in the Origin and Evolution of DNA and Modern Cells

Peter **Gogarten**: The Role of Gene Transfer in Innovation and Speciation

Nigel **Goldenfeld**: What does the Genetic Code tell us about Early Life?

Shiv **Grewal**: Epigenetic Genome Control by RNAi and Transposon-derived Proteins

Erich **Hamberger**: Cultural Genetic Adaptation

Manfred **Heinlein**: TMV-infection: Spread of Viral RNA and of a virus-induced Systemic Recombination Signal

I.King **Jordan**: Repetitive DNA and the Logic of Human Gene Regulation

Ehud **Lamm**: Networks connect Nature and Nurture

Nika **Lovsin**: APOBEC3 Cytidine Deaminases – Editors with Antiretroelement Activity

Alessandro **Quattrone**: The Intelligent Ribosome: Shaping of Proteomes by Translational Control

Amin **Rustom**: „Highway to hell": Intercellular Spread of Viruses via Nanotubular Highways?

James **Shapiro**: Revisiting the Central Dogma in the 21st Century

Gertrudis **Van de Vijver**: No Genetics without Epigenetics? No Biology without Systems Biology? On the Meaning of a Relational Viewpoint for Epigenetics and Current Systems Biology

**Kalin Vetsigian**: Coevolution between tRNA Expression Levels and Codon Usage catalyzed the Optimization of the Genetic code

Luis P. **Villarreal**: The Source of Self: Genetic Parasites and the Origin of Adaptive Immunity

Bruce **Webb**: The Evolution of Polydnavirus Segments

Guenther **Witzany**: Natural Genome Editing: why Editing needs Editors

# Titles of the Posterpresentations

Mahmoud M. **El Hefnawi**: Natural Genetic Engineering of Hepatitis C Virus NS5a for Immune System Counterattack

Peter **Gogarten**: Evolutionary Conservation of the Intron and Intein Insertion Sites

Peter **Gogarten**: Genomic Analyses of the *Thermotogales*: New Revelations on an Old Order.

Nika **Lovsin**: Editing keeps Genomic Editing in Check

D.M. **Mahishi**: A Model for Amino Avid Substitutions in Proteins as a Result of Natural Point Mutations and its Consequence on Molecular Evolutionary Trends

Jerica **Sabotič**: Genetic Heterogeneity in the Mycocypin Family of Fungal Cysteine Protease Inhibitors

Reinhard **Vlasak**: Evolution of Viral Hemagglutinin-Esterases

# ABSTRACTS
Of
# Talks

# Symbiotic Relationship between Endogenous Retroviruses and their Host: Lessons from the Sheep.

**F. Arnaud**[1], M. Caporale[1], M. Varela[1], M. Golder[1], M. Mura[1], Bernardo Chessa[2], A. Alberti[2], L. Murphy[1], A. Armezzani[1], T.E. Spencer[3] and M. Palmarini[1].
[1]INSTITUTE OF COMPARATIVE MEDICINE, UNIVERSITY OF GLASGOW VETERINARY SCHOOL, UK. [2]ISTITUTO DI PATOLOGIA SPECIALE E CLINICA MEDICA VETERINARIA, UNIVERSITY OF SASSARI, ITALY.
[3]DEPT. OF ANIMAL SCIENCE, TEXAS A&M UNIVERSITY, USA.
Email: f.arnaud@vet.gla.ac.uk, 464 Bearsden Road; Glasgow G61 1QH. Scotland (UK)

Endogenous retroviruses (ERVs) originate from ancient retroviral infections of the germline and are transmitted vertically from generation to generation. ERVs have coevolved with their host throughout evolution. The majority of ERVs are defective and replication incompetent. However, some ERVs have maintained some or all of their genes intact for million of years suggesting that they have provided a beneficial role to their host. A possible reason for the selection of ERVs is their ability to protect the host against incoming pathogenic exogenous retroviruses. We have studied the complex interaction between ERVs, exogenous pathogenic retroviruses and their host using the sheep as model system. Jaagsiekte sheep retrovirus (JSRV) is the causative agent of ovine pulmonary adenocarcinoma, a transmissible lung cancer of sheep. JSRV coexists with highly related endogenous retroviruses (enJSRVs) that colonise the sheep genome. *In vitro,* these enJSRVs are able to block JSRV at early and late steps of the retroviral cycle. Indeed, JSRV and enJSRVs use the same cellular receptor (Hyaluronidase 2) and enJSRVs envelope proteins can prevent JSRV entry by receptor competition. In addition, two enJSRV proviruses (enJS56A1 and enJSRV-20), which entered the host genome within the last 3 million y, acquired in two temporally distinct events a defective Gag polyprotein resulting in a transdominant phenotype able to block late replication steps of related exogenous retroviruses. Both proviruses with transdominant (protective) phenotypes became fixed in the host genome of the domestic sheep supporting the idea of their positive selection during or immediately before sheep domestication (9,000 y ago). Moreover, we identified 5 enJSRV proviruses with an intact genomic organization. One of these proviruses, enJSRV-26, is extremely rare. enJSRV-26 appears to have integrated in the sheep genome less than 200 y ago and interestingly escapes restriction induced by enJS56A1 and enJSRV-20. These data provide evidence that the invasion of the sheep genome by endogenous retroviruses of the JSRV/enJSRVs group is still ongoing and has not reached equilibrium. We also discovered that enJSRVs play a critical role in conceptus development and placental morphogenesis of sheep. Inhibition of enJSRVs Env expression *in utero* retarded blastocyst growth and elongation and inhibited trophoblast giant binucleate cell (BNC) differentiation culminating in loss of pregnancy. Therefore, sheep provide an exciting animal model to study the co-evolution between ERVs and their host throughout evolution.

**Keywords:** Virus–Host Coevolution, endogenous retroviruses, Viral Restriction and placental morphogenesis.

# The Origin of Alternative Exons

**Gil Ast**

Department of Human Molecular Genetics and Biochemistry,
Sackler Faculty of Medicine,
Tel-Aviv University, Ramat Aviv 69978, Israel.
E-mail: gilast@post.tau.ac.il

Alternative splicing enhances transcriptomic diversity and presumably leads to speciation and higher organism complexity, especially in mammals. Around 80% of human genes are alternatively spliced. However, it is unclear which of these products are biologically functional and which are non-functional products of inaccurate splicing. Thus understanding the changes in the genome that dictate fixation of beneficial alternative splicing or deleterious events (e.g., mutations leading to genetic disorders or cancer), or aberrant splicing events (noise in the system) are of great interest. There are three known origins of alternatively spliced exons: 1) exon shuffling, which is a form of gene duplication; 2) exonization of intronic sequences; and 3) change in the mode of splicing from constitutive to alternative splicing during evolution. I will talk about these processes and how they create genomic diversity.
Also, examination of the human transcriptome reveals the highest levels of RNA editing compared to all other organisms tested to date. This is indicative of extensive double-stranded RNA (dsRNA) formation within the human transcriptome. Most of the editing sites are located in the primate-specific transposon called *Alu*. About 480,000 *Alu*s are found in intronic sequences of annotated genes, implying extensive *Alu*-*Alu* dsRNA formation in mRNA precursors. I will also show the effect of this intronic dsRNA on splicing.

# Cell-Cell Channels and Cell-Cell Fusions in Multicellular Organisms: Viruses-Induced Manipulations with Impacts on Evolution?

**František Baluška**

IZMB, University of Bonn, Kirschallee 1, D-53175 Bonn, Germany
Email: baluska@uni-bonn.de

Due to endosymbiotic origin of organelles, eukaryotic cells represent, in fact, 'cells within cells'. Moreover, many eukaryotic cells are multinucleate due to mitotic divisions which are not followed by cytokinesis. As the next complexity feature, both prokaryotic and eukaryotic cells, at all levels of cellular complexities, show an inherent tendency to form cell-cell channels. The most obvious example is the plant 'supercell' where all the cells of the plant body are connected via plant-specific cell-cell channels known as plasmodesmata. Also fungal cells fuse together into supracellular mycelia, exchanging their motile nuclei. Recently, the first reports of similar cell-cell channels between animal cells have been published. In plants and insects, cell-cell channels are known to be induced by viruses for their spreading through tissues. In animals, similar scenario is emerging for some viruses too – they induces so-called viral synapses specialized for cell-cell spread of these viruses. Moreover, HIV-1 induces and uses nanotube-like cell-cell channels for their spread. Finally, cell-cell fusion during mammalian placenta formationis induced by protein, syncytin, of viral origin. It might be that cell-cell channels as well as cell-cell fusions scored in diverse multicellular organisms have evolutionary roots linked to viruses, indicating that these might represent active agents of the supracellular evolution.

**Literature**

Baluška F, Volkmann D, Barlow PW (2006) Cell-Cell Channels. Landes Bioscience - Springer Verlag

# The Viral Eukaryogenesis Theory and Eukaryotic Evolution

**Philip Bell**

Microbiogen Pty Ltd; Department of Biological Sciences,
Macquarie University, Sydney, New South Wales 2109, Australia, AU
Email: philip.bell@microbiogen.com

Understanding how the massive gulf between prokaryotic and eukaryotic cellular design evolved is a major scientific challenge. Distinctive features of the eukaryotic cell include nuclear membranes, linear chromosomes, telomeres, mRNA capping, nuclear pores, membrane fusion, mitochondria etc. The unique processes of sex, meiosis and mitosis, crucial in determining how eukaryotic genomes evolve, are particularly difficult to explain in the absence of obvious prokaryotic analogues.

The Viral Eukaryogenesis hypothesis seeks to explain eukaryotic origins by suggesting the first eukaryotic cell was a multi-member consortium consisting of a viral ancestor of the nucleus, an archaeal ancestor of the eukaryotic cytoplasm, and a bacterial ancestor of the mitochondria. Using only prokaryotes and their viruses, and invoking selective pressures observed in modern environments, the VE hypothesis provides an explanation for the origin of the eukaryotic cell, as well as sex and meiosis.

In the VE hypothesis, a cell wall-less archaeon and an alpha-proteobacterium established a syntrophic relationship where the bacterium converted organic material into Carbon dioxide and Hydrogen, which the archaeon used for methanogenesis. A DNA virus permanently lysogenised the archaeal syntroph forming the tripartite consortium that eventually evolved into the eukaryotic cell. Viral derived processes were crucial in the evolution of several features of the eukaryotic cell. In particular, the mechanisms used by the virus to replicate, control its copy number and segregate to daughter cells led to the evolution of the mitotic cycle. Furthermore, a combination of viral and prokaryotic modes of replication led to the evolution of sex and meiosis.

**Keywords:** Nucleus, sex, meiosis, mitosis, eukaryogenesis

# Rethinking the Genome – Hints from Bacteria Complexity

**Eshel Ben Jacob**

School of Physics and Astronomy Raymond &
Beverly Sackler Faculty of Exact Sciences Tel Aviv University, 69978 Tel Aviv, Israel
eshel@tamar.tau.ac.il

Bacteria lead rich social life in complex hierarchical communities. Collectively, they gather information from the environment, learn from past experience, and take decisions. The colony structures form as adaptive responses to laboratory-imposed stresses that mimic hostile environments faced in nature. They illustrate the strategies that bacteria have developed, strategies of cooperation through intricate communication capabilities, such as quorum sensing, chemotactic signaling and exchange of genetic information. Bacteria do not store genetically all the information required for generating the patterns for all possible environments. Instead, additional information is cooperatively generated as required for the colonial organization to proceed. Hence, new features can collectively emerge during self-organization from the intra-cellular level to the whole colony. I will discuss the interpretation that bacteria are able to sense the environment and thus have to poses internal information processing capabilities for extraction of latent information embedded in the complexity of their environment. I will then discuss our genome sequencing project of the Paenibacillus dendritiformis and Paenibacillus vortex bacteria and our "rethinking" of the concept of the genome.

# Natural Genomic Lottery

**Juergen Brosius**

Institute of Experimental Pathology, ZMBE,
University of Muenster, Von Esmarch-Str. 56, 48149 Muenster, Germany.
RNA.world@uni-muenster.de

Many multicellular organisms generate large amounts of superfluous DNA either by segmental duplications or, importantly, by the continuous conversion of RNA to DNA. One of the consequences is that almost every sequence in our genome, directly or indirectly, could be traced back to reverse transcription of an RNA molecule. Any cellular RNA from messenger RNA (mRNA) to non-protein coding RNA (npcRNA) to virus-related RNA can serve as template for retroposition. Most of these sequences are devoid of any function, evolve neutrally and after 100-200 million years, their origin is not discernible anymore. At any stage of decay, such extra sequences can be recruited (exapted) into a novel function, be it as regulatory region, npcRNA or as (part) of a protein coding region. This process often is gradual, involving additional events, such as point mutations and indels. In case of novel exon acquisition, for most part, an initial recruitment is slightly deleterious or neutral at best and often the event will not persist. Even if at one stage more or less beneficial, it could be lost again over time prior to subsequent speciations, or after speciations in certain lineages, or occasionally it could persist in all subsequent lineages. Examples will be given employing phylogenetic studies in mammals. Since everything depends on chance and selection, I personally prefer the term "natural genetic lottery" over "natural genetic engineering".

# The lack of RNAi and Transcriptional Modulation in T. cruzi – Where are the Epigenetic Controls in this Model?

**Marcella Faria**

Laboratório Especial de Toxinologia Aplicada CAT-CEPID, Instituto Butantan,
Av Vital Brazil 1500; CEP 05503-900 São Paulo-SP Brazil
Email: marcella@butantan.gov.br

RNA interference or RNAi is defined as the mechanism through which double-stranded RNA (dsRNA) triggers degradation of homologous transcripts. Besides providing an invaluable tool to downregulate gene expression in a variety of organisms, it is now evident that RNAi acts beyond the cytoplasm and is involved in a variety of gene silencing phenomena in the nucleus. In my presentation I will review the current status of RNAi in protozoan parasites of medical and veterinary importance throughout Africa, Asia and the Americas. RNAi was first discovered in Trypanosoma brucei, a species of the family Trypanosomatidae, and it rapidly became the method of choice to downregulate gene expression in these organisms, where it also plays a role in controlling retroposon transcript abundance. Surprisingly it became evident that other members of the same family of organisms, namely T. cruzi and Leishmania major, are RNAi-negative, probably due to the lack of Ago protein analogs in their genomes. Thus, as previously shown in fungi, protozoan parasites are genetically heterogeneous as far as the RNAi pathway is concerned. Since database mining predicts that very primitive organisms as Entamoeba histolytica and Giardia intestinalis have an RNAi pathway it is plausible to postulate that gene silencing by dsRNA appeared very early during evolution of the eukaryotic lineage and that its absence in T.cruzi and Leishmania came as secondary loss. We will discuss T. cruzi genome organization to propose that the lack of RNAi and promoters in these parasites is symptomatic of alternative epigenetic controls, orchestrated by parasite-host interactions.

# The Role of Viruses in the Origin and Evolution of DNA and Modern Cells

**Parick Forterre**

Institut Pasteur, 25 rue du Docteur roux, 75005, Paris and
Institut de Génétique et Microbiologie, University Paris-Sud, Orsay Cedex, France
forterre@pasteur.fr
patrick.forterre@igmors.u-psud.fr

Recent progress in comparative genomic and structural biology, together with the discovery of unusual viruses in Archaea and giant viruses in Eukarya, have rejuvenated tdiscussions on the nature and origin of viruses. Viruses are definitely living organisms that have played a major role in the evolution of modern life. Many scientists now believe that viruses even predated the last common ancestor of all extant cellular organisms (*the Last Universal Common Ancestor, LUCA*). Viruses are today the major component of the biosphere and cellular genomes are continuously visited by viruses/plasmids coming from a hidden viral reservoir of huge magnitude. Interestingly, known viruses (the tip of the iceberg) already exhibit a much greater diversity than cells in the nature of their genomic material and in their mechanisms of genome replication. Combining this observation with odd data from comparative genomics, this suggests that present cellular genomes and mechanisms of DNA replication could be a subset of those invented in a primordial virosphere of DNA viruses infecting RNA cells. If true, viruses could have played a major role in the transition from ancient cellular RNA genomes to the DNA genomes of modern cells (they might have « invented » DNA), and possibly in the establishment of the three cellular domains of life.

**Keywords:** virus origin, LUCA, DNA replication, early evolution

# The Role of Gene Transfer in Innovation and Speciation

**J. Peter Gogarten**[1], Gregory P Fournier[1], Kristen Swithers[1], Thane Papke[1], Olga Zhaxybayeva[2], Jinling Huang[3]

[1] Dept. of Molecular and Cell Biology, Univ. of Connecticut, Storrs, CT 06260-3125, USA
[2] Dept. of Biochemistry and Molecular Biology, Dalhousie University, Halifax, Canada
[3] Dept. of Biology, East Carolina University, Greenville, NC, USA
Email: gogarten@uconn.edu

Transfer of genes between divergent organisms facilitated the assembly of new metabolic capabilities (e.g., acetoclastic methanogenesis[1]) and the integration of intracellular symbionts (e.g., photosynthesis[2]). Gene transfer from now extinct lineages complicates tracing gene histories through the "tree/web of life". Furthermore, reconstructing organismal evolutionary histories is complicated by "highways of gene sharing" that may overwhelm phylogenetic signal from shared ancestry, if no precautions are taken. However, gene transfer between divergent organisms that provides an adaptive advantage, and that leads to a long-term integration of the transferred gene into the recipient, appears to be rare. Frequent gene transfers occur between closely related organisms and from the mobilome (genes that are only sometimes integrated into the organismal genome, but more frequently are part of molecular parasites, phages or plasmids) and the chromosomal genome. Many of these latter transfers may to be neutral or nearly neutral for the recipient, and as a result the transferred genes persist in the recipient lineage only over short periods of time[3].
In eukaryotes speciation is associated with erecting pre- and postmating hybridization barriers; in some prokaryotes frequent introgression that might reflect insipient speciation has been observed[4]. For pro- and eukaryotes, geographical isolation may represent the most important hybridization barrier, but in the case of prokaryotes susceptibility to phages and gene transfer agents might play an equally important role.

1. Fournier, G.P., and Gogarten, J.P. (2008) J Bacteriol 190, 1124-1127
2. Huang, J., and Gogarten, P. (2007) Genome Biol 8, R99
3. Gogarten, J.P., and Townsend, J.P. (2005) Nat Rev Microbiol 3, 679-687
4. Zhaxybayeva, O., et al. (2006) Genome Res 16, 1099-1108

**Keywords:** Intracellular Gene Transfer, Horizontal Gene Transfer, Bacterial Species, Extinction, Biochemical Pathways

# What does the Genetic Code tell us about Early Life?

**Nigel Goldenfeld**

Department of Physics,
University of Illinois at Urbana-Champaign
1110 West Green St Urbana IL 61801, USA
Email: nigel@uiuc.edu

Relics of early life, preceding even the last universal common ancestor of all life on Earth, are present in the structure of the modern day canonical genetic code. In this talk, I will draw attention to these relics, and discuss their interpretation from the perspective of the dynamical system that is evolution. I will argue that this viewpoint, and the quantitative, statistical dynamical calculations that it entails, suggest a natural scenario in which evolution exhibits three distinct dynamical regimes, differentiated respectively by the way in which information flow, genetic novelty and complexity emerge. Possible observational signatures of these predictions are discussed.

Reference: K. Vetsigian, C.R. Woese and Nigel Goldenfeld. Communal evolution of the genetic code. Proc. Natl. Acad. Sci. 103 , 10696-10701 (2006) .

# Epigenetic Genome Control by RNAi and Transposon-derived Proteins

**Shiv Grewal**

Laboratory of Biochemistry and Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA
Email: grewals@mail.nih.gov

Eukaryotic genomes are often replete with transposable elements (TEs) and their remnants. These elements referred to as "junk DNA" have driven the genome evolution in diverse ways. Due to the their proliferation and mutagenic potential, host genomes have evolved defense mechanisms against TEs including histone methylation and RNAi. However, these mechanisms have only minor roles in regulating *Tf2* long terminal repeats (LTRs) retrotransposons in the fission yeast *Schizosaccharomyces pombe*. We have uncovered a novel genome surveillance mechanism for retrotransposons by a family of transposase-derived CENP-B homologs. *S. pombe* CENP-Bs localize at and recruit class I and II histone deacetylases to silence *Tf2* retrotransposons. CENP-Bs also repress retrotransposon relics scattered throughout the *S. pombe* genome, and often located near gene promoters to exert influence on the expression of genes. Surprisingly, *Tf2* elements dispersed throughout the genome are clustered into "Tf" bodies, the organization of which depends on CENP-Bs that display network-like nuclear structures. CENP-B-mediated surveillance is proactive, capable of preventing an "extinct" Tf1 retrotransposon from reentering the host genome by blocking its homologous recombination with extant Tf2, and silences and immobilizes a Tf1 integrant that, remarkably, becomes sequestered into Tf bodies. These results reveal a likely ancient retrotransposon surveillance pathway important for host genome organization and maintenance of genomic integrity. Our recent progress in understanding the mechanisms of epigenetic genome control by RNAi and transposon-derived CENP-B proteins will be discussed.

# Cultural Genetic Adaptation

**Erich Hamberger**

c/o University of Salzburg
Department of Communication Science
Rudolfskai 42, 5020 Salzburg / Austria
Email: erich@hamberger.cc

More and more empirical data show the circumstance: genome in motion. There is strong evidence, that the genome is incorporated in a dynamic scenario, in a permanent process of reconfiguration, which is unalterable for its solid stabilty. Besides it becomes also increasingly clear, that living entities cannot be – totally – understood from the level of their (a-biotic) molecular representations.
In my contribution the congress subject "Natural Genetic Engineering and Natural Genome Editing" should be discussed from the perspective of a bio-communicative approach. The term "bio-communication" is used here for the thinking, that interaction-processes in living "systems" are – at last – not (only) stimulus-response-cycles based on natural laws, but also and first speech-analog "cultural"-acts; with all to this reason related conditions: e.g. inwardness, quasi-subjectivity, ability to differentiate between self, we, non-self and non-we, ability to speech-analog-(re-)actions, possibilty to react variable to attractions of the environment.
It should be argued a viewpoint, according to which all procedures of "natural genetic engineering and natural genome editing" (genome restructuring, genome-editing-competence et cetera) are also describable as "cultural-genetic-adaptation-events". In conclusion it should be pointed out with help of the well known biologist Sidney Brenner and the widely unknown Austrian philosopher Ferdinand Ebner, that it seems to be actual necessary, "to learn to understand not only the vocabulary of machine language, but also to observe what we call the grammar of a biological system" (Brenner 2002).
In a final argumentation step the presented conception should be discussed in regard to the connection of cancer, - the central topic of the following congress "Cancer and (Bio-) Communication" next year (2.-5. July 2009) at the same place.

**Keywords:** cultural genetic adaptation, bio-communication, cancer and communication,

# TMV-Infection: Spread of Viral RNA and of a virus-induced Systemic Recombination Signal

**Manfred Heinlein**

Institut de Biologie Moléculaire des Plantes (IBMP), CNRS UPR 2357, Strasbourg, France;
Botanical Institute, University of Basel, Switzerland
manfred.heinlein@ibmp-ulp.u-strasbg.fr; manfred.heinlein@unibas.ch

*Tobacco mosaic virus* (TMV) spreads cell-to-cell through plasmodesmata (PD), the cytoplasmic cell wall channels through which adjacent plant cells communicate. The spread of the viral RNA (vRNA) is independent of coat protein and, thus, occurs in a non-encapsidated form, likely through exploitation of the cellular RNA transport machinery. The spread of infection requires virus-encoded movement protein (MP), which modulates the size exclusion limit of PD and is proposed to form a complex with vRNA, thus mediating transport to and through PD. *In vivo* studies have shown that the spread of vRNA involves interactions of MP with PD, the ER, and microtubules. Further studies focus on the analysis of mobile, MP-associated particles that have been observed in cells at the leading front of infection and correlated with the spread of vRNA. To spread efficiently, the virus has to protect the vRNA against host defense responses. Interestingly, while the viral replicase acts as a suppressor of the RNA silencing pathway, the MP promotes the spread of virus-induced silencing signal. Given the manifold interactions of the virus with the plant cells, it is not surprising that infected cells show characteristic RNA expression profiles. However, in addition, TMV infection triggers the generation of a systemic signal that spreads ahead of infection to evoke responses in non-infected tissues. These responses include a systemic activation of recombination, which can result in an increased frequency of progeny plants with genetic and epigenetic changes. This latter phenomenon may be part of an adaptive measure to virus infection.

**Keywords:** TMV, plasmodesmata, RNA silencing, recombination

# Repetitive DNA and the Logic of Human Gene Regulation

**I. King Jordan**

Associate Professor
School of Biology
Georgia Institute of Technology
310 Ferst Drive
Atlanta, GA 30332-0230
404-385-2224
http://esbg.gatech.edu

The human genome is made up primarily of repetitive DNA elements, particularly interspersed repeats, or transposable elements, which make up close to half of the euchromatic sequence. In the last few years, it has become increasingly apparent that transposable element sequences exert a substantial influence on patterns of human gene regulation and expression through a variety of mechanisms. I will discuss some of many ways that transposable elements have contributed to the regulation of human genes including the generation of alternative promoters, the donation of transcription factor binding sites and the origination of microRNA genes. In addition to being extremely abundant, transposable element sequences are distributed non-randomly along chromosomes. Accordingly, the transposable element environment of individual genes (promoters) can vary greatly, and we are just beginning to appreciate how the repetitive DNA landscape affect human gene regulation on a larger scale, perhaps through epigenetic mechanisms. Finally, I will explore the relevance of transposable elements to changes in gene expression that are related to evolutionary divergence as well as the etiology of human cancers.

# Networks connect Nature and Nurture

**Ehud Lamm**

The Cohn Institute For the History and Philosophy of Science and Ideas, Tel Aviv University, Tel Aviv 69978, Israel
ehud.lamm@gmail.com

Epigenetic mechanisms can be activated by both environmental and genomic stress and lead to developmentally induced large-scale genomic repatterning events in a wide variety of species. Genome reorganization of this type and epigenetic inheritance, leading to transgenerationally extended plasticity, show that heredity can be developmentally constructed. I develop the argument that Goldschmidtian "systemic mutations" are the effects of structural genomic regulation mechanisms and may result from genomic reorganization following stress.

I argue that the key to unraveling the evolutionary mechanisms and consequences of these observations is understanding the dynamics of trans-generational and inter-organism regulatory networks. I show how this perspective helps problematize a set of parallel dualities: development-heredity; plasticity-evolvability; epigenetic-genetic; and the sharp distinction between "inside" and "outside" the spatial and temporal boundaries of the individual. The cases in which the boundary between the elements in these pairs is most sharp are, I argue, probably the result of selection. An important part of key evolutionary transitions was probably the introduction of mechanisms that sharpened the boundaries between the phenomena/processes paired above. The mechanisms that take part in network interactions between organisms are part of the internal "coping mechanisms" of genomes, and constrain subsequent evolution. Interactions between foreign genomes, typified by hybridization and host-virus interactions, probably played an important role in establishing some of the mechanisms operating in intra-genome organization and re-organization, and in the response to environmental and genomic stress, the latter being an important factor in hybridization and thus of central importance for understanding the web of life.

**Keywords:** genome organization, nature-nurture debate, Richard Goldschmidt, macroevolution, epigenetic inheritance.

# APOBEC3 cytidine deaminases – Editors with Antiretroelement Activity

Okeoma, C. M.1, **Nika Lovšin[2],** Matija B. Peterlin[3], Ross, S.R.1[¶]

[1] Department of Microbiology and Abramson Family Cancer Center, University of Pennsylvania, Philadelphia,
[2]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia,
[3]University of California, San Francisco, USA
Email: nika.lovsin@ijs.si

Several members of apolipoprotein B editing complex (APOBEC3) family of proteins exhibit a potent antiretroviral activity against lentiviruses, MLV, EIAV, HBV, endogenous retroviruses and retrotransposons. APOBEC3 proteins are cytidine deaminases and deaminate C to U in the minus strand of viral cDNA during reverse transcription causing hypermutations and subsequent degradation of viral DNA. Indeed, human APOBEC3G was first identified as an anti-viral factor in HIV infection. Whereas human genome contains 7 APOBEC3 genes, the presence of one A3 (mA3) gene in the mouse genome suggests that these genes have been under selection by exogenous and endogenous retroviruses during evolution. To gain insight into the physiological role of APOBEC3 proteins we generated APOBEC3 knock out mice. Using mA3 -/- knock-out mice, we found that mA3 partially restricts MMTV infection in mice (1). We demonstrated that mA3 and hA3G interact with the MMTV nucleocapsid in an RNA-dependent fashion and are packaged into progeny virions. As observed with the mutant HIVΔVif, mA3- and hA3G-containing MMTV virions had a dramatic reduction in titer in tissue culture cells. Importantly, A3-/- mice were more susceptible to MMTV infection and viral spread was more rapid and extensive in these mutant mice compared to their wild type (WT) littermates. Interestingly, there was no evidence of cytidine deamination of the MMTV genome in WT mice. These findings indicate that the mA3 provides partial protection to mice against MMTV infection and represent the first demonstration that A3 proteins function during retroviral infection in vivo.

1. Okeoma, C. M., Lovsin, N., Peterlin, B. M., and Ross, S. R. (2007). APOBEC3 inhibits mouse mammary tumour virus replication in vivo. Nature *445*, 927-930.

**Keywords:** MMTV virus, APOBEC3 deficient mouse, APOBEC3 proteins

# The Intelligent Ribosome: Shaping of Proteomes by Translational Control

**Alessandro Quattrone**

Director, Centre for Integrative Biology
University of Trento ; Via Sommarive, 14 38050 POVO – TRENTO, Italy
Email: alessandro.quattrone@unitn.it

The central dogma of molecular biology, the simplified frame which guided mechanistic investigation in the last century, has been associated in the last thirty years by the constant assumption that gene expression in eukaryotic cells is basically controlled at the transcriptional level, i.e., that cells are reprogrammed in the nucleus to perform new functions by transcription factors acting on the machinery which produces mRNAs, while mRNA export, localization and translation are largely default processes reproducing the changes impressed in transcription by cell signaling.
This view is now increasingly substituted by a scenario in which regulation of genome function largely depends on complex networks of signals acting post-transcriptionally on mRNAs, which shape the proteome by changing mRNA accessibility to translation. This layer of regulation, which seems to be partially independent of transcriptional networks, is controlled by the activity of RNA binding proteins and of a variety of classes of non-coding RNAs, by modification of the cytoplasmic fate of the targeted mRNAs. In this talk we will present experimental evidence showing how translational control of gene expression is a strong "pseudo-epigenetic" force which is gradually appearing as a major driver of such diverse physiological processes as the establishment of long term memory and the differentiation of stem cells, and of pathological derangements as the onset and progression of cancer.

# „Highway to hell“: Intercellular Spread of Viruses via Nanotubular Highways?

**Amin Rustom**

Interdisciplinary Center of Neuroscience (IZN),
Institute of Neurobiology, University of Heidelberg, INF 364,
Heidelberg 69120, Germany.
Email: amin.rustom@urz.uni-heidelberg.de

It is well known that many viruses encode for sets of highly specific proteins, facilitating the transfection and intercellular spread of viral progeny. In this context, it now becomes evident that defined viral proteins enhance the formation of so called tunneling nanotube (TNT) related membrane connections between mammalian cells and can be transferred along these channels from cell to cell. So far, the peculiar membrane tubes were shown to interconnect various cell types and facilitate important cellular functions, like e.g. the intercellular spread of a great variety of components - ranging from multidrug resistance proteins up to mitochondria - thereby enforcing reconsideration of previous conceptions of intercellular communication and the application of innovative technologies. Together, these observations suggest that preexisting intercellular communication pathways of mammalian cells can be amplified and exploited by viruses to facilitate and enhance their spread in tissue - a model, showing striking conceptual similarities to e.g. their misuse of plasmodesmata in the plant kingdom.

**Keywords:** "tunneling nanotube", "TNT", "membrane channel", "intercellular spread", "viral protein", "virus", "plasmodesmata"

# Revisiting the Central Dogma in the 21st Century –

**James A. Shapiro**

Department of Biochemistry and Molecular Biology
University of Chicago, IL, USA
Email: jsha@uchicago.edu

Since the elaboration of the central dogma of molecular biology, our understanding of cell function and genome action has benefited from many radical discoveries. The discoveries relate to interactive multimolecular execution of cell processes, the modular organization of macromolecules and genomes, the hierarchical operation of cellular control regimes, and the realization that genetic change fundamentally results from DNA biochemistry. These discoveries contradict atomistic pre-DNA ideas of genome organization and violate the central dogma at multiple points. In place of the earlier mechanistic understanding of genomics, molecular biology has led us to an informatic perspective on the role of the genome. The informatic viewpoint points towards the development of novel concepts about cellular cognition, molecular representations of physiological states, genome system architecture, and the algorithmic nature of genome expression and genome restructuring in evolution.

**Keywords:** Biological Theory, Evolutionary Theory, Genome System Architecture, Cognition, Informatics

# No Genetics without Epigenetics?
# No Biology without Systems Biology?
# On the Meaning of a Relational Viewpoint for Epigenetics and Current Systems Biology

**Gertrudis Van de Vijver**

Centre for Critical Philosophy
Blandijnberg 2 (room 212), 9000 Ghent, Belgium
Email: Gertrudis.VandeVijver@Ugent.be

Developments in epigenetics, epigenomics and systems biology, challenge the idea that living systems can be understood and explained on the basis of their basic constituent components alone. They challenge in particular the idea that there are identifiable constituent components that are as such causally relevant and explanatory, the onset of a causally linear chain that remains unchanged and is untouchable whatever goes beyond ("epi") it. This awareness of a "beyond" implies, on the one hand, the need to understand what precisely goes beyond the parts and in what sense this "beyond" constitutes the context or the perspective within which the parts can have a place and a meaning. In this regard, the question of context or perspective cannot be evaded without risking to reduce the significance of the "epi" to something epiphenomenal like a "spirit" or a "veneer". On the other hand, if the message of context is taken seriously, the relation with genetics itself needs to be clarified. In this regard, in a trivial (but not necessarily adequate) historical sense, it can be said that there is *no epigenetics without genetics*, that epigenetics presupposes genetics, that genetics comes first and epigenetics after or beyond it. But in a less trivial, conceptual sense, it can as well be said that there is *no genetics without epigenetics*. More boldly, it can be said that there cán be no genetics without epigenetics (no biology without systems biology), or even that there has never been a genetics without an epigenetics. At stake is the awareness of the meaningfulness of parts in function of a context, and this in two ways: on the one hand, a meaning is revealed of parts within a context, but on the other hand, the impossibility emerges to have parts without a context.

In this paper, we propose to reflect on the meaning of current epigenetic (epigenomic, systems biological) developments in biology, by considering them as instantiating, within the sciences, the idea of a contextual, and stratified, determination of living systems. We attempt to trace as clearly as possible the abstract core of the idea of parts and wholes that is at stake in this contextual determination. In doing so, we have found inspiration in Kant's account of living systems, for a number of precise reasons that we will explain. We argue that Kant's transcendental account can be relevantly actualised and extrapolated as a relational account of living systems, and will explore its ramifications starting from a multi-level viewpoint of living systems. More in particular, in order to understand what is "epi" about epigenetics or epigenomics (or what is "systemic" about current systems biology), it is important (i) to analyse the precise meaning of a determinative context or level, (ii) to make explicit the epistemological and ontological implications of a multi-level and relational viewpoint on causality.

# Coevolution between tRNA Expression Levels and Codon Usage Catalyzed the Optimization of the Genetic Code

**Kalin Vetsigian**

Department of Systems Biology,
Harvard Medical School, Boston, USA
Email: Kalin_Vetsigian@hms.harvard.edu

The genetic code is highly optimized (similar codons are assigned to similar amino acids), yet it seems unevolvable since codon reassignments disturb many protein sequences simultaneously. The extant variant genetic codes and their phylogeny demonstrate that the genetic code was somewhat evolvable after the emergence of the tree of life -- that is within the context of modern day translation. I explain how the implementation of translation as a competing community of different tRNA species spontaneously and generically leads to optimization of the genetic code. The coevolution between the tRNA species and their environment -- the codon usage -- catalyzes (over evolutionary time) code changes in a direction specified by selection at the cellular level. After the optimization of the universal genetic code, multistability in the coevolutionary dynamics leads to the emergence of codon bias diversity. Analogous coevolution exists for replication and transcription, offering an explanation of nucleotide composition bias diversity (GC content, skews).

**Keywords**: genetic code, optimality, frozen accident theory, coevolution

# The Source of Self: Genetic Parasites and the Origin of Adaptive Immunity

**Luis P. Villarreal**

Director Center for Virus Research,
University of California, Irvine, USA
Email: lpvillar@uci.edu

All living organisms must have systems of identity that differentiate self from non-self and communicate such membership. Such systems also constitute immune systems. Immune systems are generally thought to originate from natural selection from the host variation which increases host survival to counteracts both viral (genetic) and organismal parasites and pathogens. In contrast to this view, I have previously proposed that the stable colonization by genetic parasites can provide non-parental sources of immune systems which persist and counteract and control related genetic parasites. Although the presence of genetic parasites in the genomes of all living systems is well established, they have been dismissed as lacking phenotype and representing the product of selfish DNA. The origin of adaptive immunity in jawed vertebrates has been particularly enigmatic to understand since none of the needed systems of adaptive immunity are found jawless ancestors of vertebrates. In this review, I examine the origin of the adaptive immune system of vertebrates from a 'virus first' perspective. The T-cell receptor (Ig superfamily), the RAG1/2 mediated system of gene rearrangement, clonal selection of blood cells (lymphocytes) that kill non-self (virus infected) cells by apoptosis, and the origin of MHC gene loci involved in antigen processing are all examined for evidence of early viral involvement in their origin and evolution and compared to earlier immune systems. The origin of the adaptive immune system is especially correlated with a massive colonization with endogenous retroviruses (ERVs) of the chromovirus family, along with their related produces (LTRs, SINES). Indeed, compelling evidence indicates early and ongoing ERV involvement in the duplication events that led to the evolution of immune system complexity. Distinct waves of ERV colonization have continued to provide the major distinctions that shape the evolution of the primate and human MHC locus. I conclude that the origin of adaptive immunity can be best explained by the action of genetic parasites.

# The Evolution of Polydnavirus Segments

**Bruce Webb**, Stephanie Dickey, Julianne Deacutis

Department of Entomology
University of Kentucky
Lexington, KY 40546-0091; USA
Email: bawebb@uky.edu

Polydnaviruses are an unusual group of insect viruses that are obligate symbionts of some parasitic wasps. Polydnaviruses have defective life cycles in which the virus replicates in specialized cells of the oviduct, bud into the oviduct lumen and are transmitted to lepidopteran larvae when the wasp oviposits into the body cavity of a caterpillar. In the lepidopteran host, tte virus infects but does not replicate with virus expression required for survival and development of the wasp parasite.

This obligate mutualism has dramatic effects on the viral genome. The viral genome is segmented, contains several gene families and is largely non-coding. Viral genes are largely expressed only in lepidopteran larvae with genes involved in virus replication not encoded by the encapsidated viral genome. Sequence of five polydnavirus genomes documents these patterns exist in all 3 of the evolutionarily distinctive polydnavirus lineages. We focus on application of phylogenetic approaches to establish relationships among the various polydnavirus segments. We will also examine the evolutionary patterns among several of the polydnaviral gene families to explore the hypothesis that the biological entities we recognize as polydnaviruses have evolved to deliver a virulence gene set related to genes in the wasp genome (rather than virus). We also consider the hypothesis that the genes required for virus replication now reside in the wasp genome where they are expressed only to support virus replication.

# Natural Genome Editing: why Editing needs Editors

**Guenther Witzany**
Telos – Philosophische Praxis
Vogelsangstr. 18c, A-5111-Bürmoos, Austria
Email: witzany@sbg.at

The biocommunicative approach investigates both communication processes within and among cells, tissues, organs and organisms as sign-mediated interactions and nucleotide sequences as code, i.e. language-like text which follows in parallel three kinds of rules: combinatorial (syntactic), context-sensitive (pragmatic) and content-specific (semantic). From the biocommunicative perspective, natural editing of genetic text sequences need, similarly to signalling codes between organisms, biotic agents which are competent in rule-governed sign use. We use the term natural genome editing for competent agent-driven generation and integration of meaningful nucleotide sequences in pre-existent genomic content arrangements and the ability to (re-)combine and (re-)regulate them according to context-dependent (i.e. adaptational) purposes of the host organism. To read, write and re-write nucleotide sequences, editors competent in both editing techniques and target-recognition are necessary, if we assume the genetic code not to be a randomly-derived mixture of nucleotides.

**Keywords:** biocommunication, natural genome editing, DNA-storage medium, RNA agents

# ABSTRACTS
Of
# Posterpresentations

# Natural Genetic Engineering of Hepatitis C Virus NS5a for Immune System Counterattack

**Mahmoud M. El Hefnawi***and Suher Zada **

*Informatics and Systems Department, Division of Engineering Research Sciences, the National Research Centre, Cairo, Egypt,
** Biology Department, The American University in Cairo, Egypt
Email: mahef@hotmail.com

Natural genetic engineering is ubiquitous in viruses. For example, the HIV retrovirus uses the same strategy as other natural genome operators by attaching to genomic DNA. RNA viruses that cause chronic infection like Hepatitis C Virus (HCV) use their proteins to counteract the immune system pressures.
The non-structural 5A (NS5A) protein of HCV has been the subject of intensive research over the last decade for its many functions. We aim to shed new light into the importance of NS5a for HCV persistence and immune counteraction.
Our work has focused on 3 major tasks: domain assignment, 3D-structure prediction and functional motifs prediction as well as predicting protein protein interactions. This enabled us to discover new insights into the natural genetic engineering of this protein, as an important weapon for HCV to intercept the interferon signaling pathway, the general antiviral pathway (eIFII phosphorylation for general translation shut off),cellular immune interception (by binding to 2'5'OAS) and partial inhibition of the interferon antiviral response (through multi regulation of IL8).

Firstly, NS5a domain assignment was refined using the DOMAC server, and the last domain was divided into two sub domains using ProDom and SSEP servers.
Secondly, BLOCKS and PROSITE databases had been searched for different motifs all over the NS5a protein. Some of these motifs clarified why the v3 region mutations are most detrimental to therapy response determination and why the HCV infected patients showed higher levels of serum IL-8.
Signatures of PKC_PHOSPHO_SITE and two CKII phospho sites inside the v3 region that could possibly be involved in phosphorylation of PKC preventing it from activation of eIFII .We also found the interleukin 8B receptor (IL-8BR) and type 1 Interleukin 1(IL-1R) receptor precursor signatures from BLOCKS. Since the interleukin-8 promoter is transcriptionally activated by interleukin-1 (IL-1), we anticipate that NS5a induces higher levels of IL-8 which partially inhibits the Interferon antiviral response.

**Keywords:** Natural genetic engineering, Hepatitis C virus, Non –Structural 5a protein (NS5a) functions, In silico motif prediction , protein protein interactions, immune system counteraction , virus host interactions, domain separation

# Evolutionary Conservation of the Intron and Intein Insertion Sites

Kristen Swithers, Alireza Senejani and **J. Peter Gogarten**

Dept. of Molecular and Cell Biology,
Univ. of Connecticut, Storrs, CT 06260-3125, USA
Email: gogarten@uconn.edu

Inteins and introns are genetic elements that are removed from proteins and RNA after translation and transcription, respectively. Their origin, evolution, and their significance, are still debated; however, their distribution on the tree of life suggests that they have a long history and may have evolved strategies to resist elimination. Many inteins and group I introns encode homing endonucleases, which are endonucleases that recognize large12-40 base pair sites. These homing endonucleases provide mobility to the intein and group I intron. Group II introns are also mobile but use a reverse transcriptase mechanism. This mobility has allowed for these genetic elements to invade new target sites in inteinless or intronless alleles and has also granted them the title of parasitic genetic elements. In order to elucidate how these parasites are able to invade and resist loss from their host proteins we preformed comparative and statistical analyses of various host protein sequences. This revealed that inteins and group I introns appear at highly conserved sites within their host proteins and mapping of these insertion sites to the structure of their host proteins shows these elements are mostly present in the active site or at interfaces involved in subunit association. In contrast, group II introns and spliceosomal introns do not show a statistically significant preference for conservered sites. It appears form our analyses that inteins and group I introns may have evolved a different strategy than the group II introns to propagate and evade loss. Inteins and group I introns have taken advantage of site conservation which provides a safe haven to evade elimination and a means to invade new populations. If an element inserts into a functionally conserved site the removal of such element would have to be exact or else functionality of the protein will be lost. Moreover, targeting conserved sites would allow for invasion of a new inteinless or intronless allele and thus propagation through a new population. In contrast, group II introns have not taken advantage of this site conservation strategy and appear more randomly distributed.

**Keywords:** Intein, Intron, Homing, Molecular Parasites, Selfish Genes

# Genomic Analyses of the *Thermotogales*: New Revelations on an Old Order

Kristen S. Swithers, Pascal Lapierre, Kenneth M. Noll, **J. Peter Gogarten**

Dept. of Molecular and Cell Biology,
Univ. of Connecticut, Storrs, CT 06260-3125, USA
Email: gogarten@uconn.edu

Members of the *Thermotogales* are thermophilic anaerobes found in marine and fresh water geothermal environments. Recently seven genomes from this order have been completely sequenced, and analyses of these sequences indicate that horizontal gene transfer, internal gene duplications and transposable elements each have played major roles in shaping genome evolution among members of this order. Genome sequences are now available from species from across the phylogenetic spectrum of the *Thermotogales*; four from the genus *Thermotoga* and one each from *Fervidobacterium*, *Thermosipho* and *Petrotoga*. Genome sizes and GC contents range from 1.8 Mb to 2.2 Mb and from 34% to 46%, respectively. The observed differences in genome size may be due to internal duplications, which appear to be more frequent in the larger genomes. The seven genomes share 949 orthologous open reading frames and exhibit little synteny, with the exception of three *Thermotoga* species. These three genomes had three major inversion events, two of which were flanked by transposable elements. Our comparative analyses revealed that the *Thermotogales* genomes contain only 10% - 13% archaea-derived genes, lower than that estimated previously for *Thermotoga maritima*. These analyses have also pointed out that *Thermotoga lettingae* does not belong to the genus *Thermotoga.* Various genomic skews were used to identify the putative origin of replication. Interestingly, with the exception of the three syntenous genomes, each putative origin of replication is located in different positions and none of the putative origin of replications are located near the conventional *dnaA* gene.

**Keywords:** Thermotogales, gene transfer, origin of replication, genome evolution

# Editing keeps Genomic Editing in Check

**Nika Lovšin**

Faculty of Chemistry and Chemical Technology,
University of Ljubljana, Slovenia
Email: nika.lovsin@ijs.si

Retrotransposons comprise almost half of the human genome and are considered to be one of the major driving forces in the evolution of eukaryotic genomes. They have profoundly shaped the genomes via insertions, deletions and DNA rearrangements and are important agents in the evolution of genes as well as complex regulatory networks in the organism. However, when they integrate in the genes, they are harmful to their hosts. In mammals, several mechanisms have evolved to alleviate the threat of retroelements activity: transcriptional silencing via DNA methylation, posttranscriptional silencing via RNA interference (RNAi) and cytidine deamination via APOBEC3 family of proteins. APOBEC3 proteins are recently discovered family of cytidine deaminases that are capable of DNA and RNA editing. They were shown to inhibit replication of retroviruses and retroelements. Indeed, human APOBEC3A, APOBEC3B, APOBEC3F inhibit retrotransposition of human LINE-1, mouse IAP and MusD retrotransposons. The exact mechanism of their action is unclear. In our study, we examined whether mammalian APOBEC3 proteins inhibit retrotransposition of vertebrate LINE2 retrotransposons. To address this question, in vitro retrotransposition assay with marked LINE2 retrotransposon was performed in the presence or absence of APOBEC3 proteins. Indeed, several mammalian APOBEC3 were shown to inhibit retrotransposition of LINE2 retrotransposon suggesting that APOBEC3 proteins may represent a general mechanism against vertebrate retrotransposons.

**Keywords:** LINE2 retrotransposons, APOBEC3 proteins

# A Model for Amino Acid Substitutions in Proteins as a Result of Natural Point Mutations and its Consequence on Molecular Evolutionary Trends

**D. M. Mahishi**

Department of Applied Genetics,
Acharya Institute of Sciences, Cholanagar, RT Nagar Post,
Bangalore 560032, INDIA
Email: dmmahishi@yahoo.com

The amino acid composition in a protein molecule plays an important role in imparting the structural and functional uniqueness for a given protein. A novel model has been proposed in this paper which relates the occurrence of point mutations in DNA molecule with the consequent change in amino acid sequence in the protein. A comparison was made across the twenty amino acids and the natural mutational events in DNA molecule which has revealed a definite pattern for amino acid substitutions. Such a model is important in interpretation of the molecular evolution in phylogenetic comparisons.

**Keywords:** Mutation, Amino Acids, Substitutions, Protein, Evolution, Phylogenetics

# Genetic Heterogeneity in the Mycocypin Family of Fungal Cysteine Protease Inhibitors

**Jerica Sabotič**, Kristina Gruden, Jože Brzin

Department of Biotechnology,
Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia
Email: Jerica.Sabotic@ijs.si

Mycocypins are a new family of cysteine protease inhibitors unique to basidiomycetes. The first protein cysteine protease inhibitor characterized from mushrooms was clitocypin from *Clitocybe nebularis*. Clitocypin is encoded by a small gene family of closely related genes with more than 90% identity. Clitocypin is abundant in basidiocarps and is present in comparable amounts evenly throughout the basidiocarp. The proposed physiological role of clitocypin is protection against viruses or pathogenic and parasitic organisms. We have used the Yeast Protoarray technology for detecting protein-protein interactions with the *Saccharomyces cerevisiae* proteome as a model system to find potential binding targets of clitocypin. Yeast Protoarray revealed binding to proteins localized to the nucleus or nuclear membrane involved in tRNA processing, mRNA transport and ribosome assembly. Results suggest a potentially important role for clitocypin in cell physiology associated with RNA metabolism in addition to its role as a cysteine protease inhibitor. The second representative of the mycocypin family, macrocypin from *Macrolepiota procera*, is also encoded by a family of genes, composed of four exons and three short introns, however the heterogeneity is even higher compared to the clitocypin gene family. The deduced amino acid sequences of macrocypin sorted into four isoforms show that the sequence identity among isoforms is 80 – 86% and sequences belonging to one isoform share more than 95% sequence identity. Despite low sequence identity, clitocypin and macrocypin show similar biochemical properties and inhibitory spectra. Mycocypins are unlike any other cysteine protease inhibitors and are unique to basidiomycetes. So far only the genome of the mycorrhizal basidiomycete *Laccaria bicolor* has revealed clitocypin-like genes that share low sequence identity to clitocypin and macrocypin at the deduced amino acid sequence level. There are no similar sequences present in the completed and unfinished prokaryotic or other eukaryotic genomes therefore, the evolutionary origin of mycocypins is unknown.

**Keywords**: clitocypin; cysteine protease inhibitor; genetic heterogeneity; gene family; basidiomycetes

# Evolution of Viral Hemagglutinin-Esterases

**Reinhard Vlasak[1],** Sigrid Arming[1], Ulrike Vilas[1], and Raoul de Groot[2]

[1] *Glycobiology/Virology Research Group, Department of Cell Biology, University Salzburg, Billrothstr. 11, Austria, and*
[2] *Virology Section, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands*
Email: rvlasak@applied-biotech.at

The first and most critical step in virus replication is the binding to cellular receptors. Many viruses bind to glycans located on proteins or lipids at the plasma membrane. Often sialic acids serve as receptors. Sialic acids are a family of acidic C-9 sugars. This family consists of approximately 50 naturally occurring derivatives of 5-*N*-acetyl-neuraminic acid, which contain substitutions at carbons 4, 5, 7, 8, and 9. Because sialic acids are negatively charged at physiologic pH, and are exclusively found at the outermost end of carbohydrate chains, they represent ideal ligands for viral receptor-binding proteins. During their evolution, viruses with different genome organizations have acquired genes encoding hemagglutinin-esterases (HE), presumably by independent recombination events with host cell mRNAs. HE genes are found in orthomyxoviruses, which possess a segmented single-stranded negative sense RNA genome, and in corona- and toroviruses, which have a nonsegmented single-stranded positive sense RNA. Within virus families, the HE genes are often horizontally transferred during mixed infections. Especially in coronaviruses, which possess a second receptor-binding protein, the newly captured gene can rapidly evolve new receptor-binding specificities, e.g. the switch of recognition of 4-O to 9-O-acetylated sialic acids and vice versa. In all viruses tested so far, both the receptor-binding (hemagglutinin) and the receptor-destroying (esterase) domain exhibit strict specificities for either 9- or 4-O-acetylated sialic acids. Bovine toroviruses have evolved another substrate specificity towards double-O-acetylated sialic acids, which are the major sialic acids in the gastrointestinal tract of cattle.
Most recently we have identified the human gene for the enzyme which transfers O-acetyl groups to sialic acids. Its aminoterminal domain has a similar architecture as the viral HE proteins. They belong to a family of "SGNH hydrolases". We suggest that the human CasD1 gene or homologous genes in other species may represent the original ancestors of viral HE genes.

**Keywords:** receptor-binding protein, receptor-destroying enzyme, sialic acids, HE genes, SGNH hydrolases, influenza C virus, infectious salmon anemia virus, coronavirus, torovirus, RNA recombination, antigenic shift, reverse genetics

# Sunday, July 6, 2008

Sunday-Excursion whole day **8.00 a.m. – 17.30 p.m.**

to a Pinzgau Tour visiting the

**Wildkogel Peak** (one of the most exiting mountain panoramas of Austria)

**Leopold Kohr Academy,** (Neukirchen a.Großvenediger)

**Bramberg Heimat-Museum** (a folkloristic museum with outstanding examples of alpine mineralogy)

We would like to encourage you to participate at the continuing congress next year at the same place

# CANCER and COMMUNICATION

## The bio-communicative processing of tumors

Salzburg, **2 – 5 July 2009**