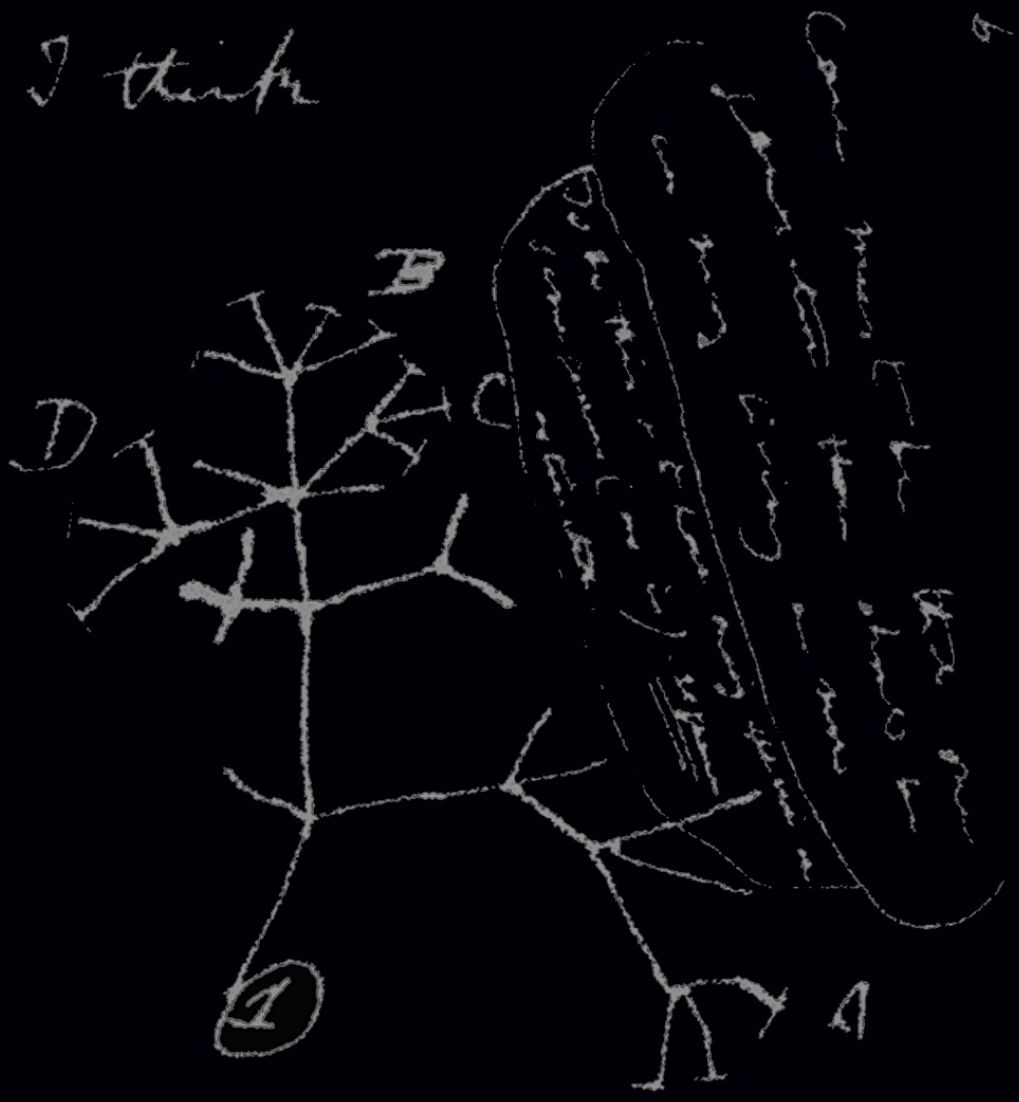
NoR HGT & LUCA



2nd NorHGT & LUCA conference
28 - 29 October 2014

Network of Researchers on Horizontal Gene Transfer & Last Universal Cellular Ancestor

NoR HGT & LUCA is a member of the Society of Biology

The routes of emergence of life from LUCA during the RNA and viral world

Preface

It is highly probable that life on Earth would be unlikely to exist if it were not for the process of horizontal gene transfer (HGT) and furthermore that, even if life had somehow managed to emerge, then it is almost certain that without HGT, its evolution on Earth would have been exceedingly slow. This is because genome modification, gene duplication and gene pool augmentation, as well as the acquisition of new genes is not necessarily possible in the absence of HGT. In short, HGT was a 'quick fix' method of acquiring new physiological, biochemical and other traits, which led to the emergence of such a vast diversity of life on Earth, primarily due to the fact that HGT occurs both ubiquitously and on a large scale in nature. So, what is HGT? A broad definition is that HGT can be construed as being a mechanism whereby there is a transfer of genetic material (either DNA or RNA) between donor and recipient cells, or there is active take-up of heterologous DNA from the environment by recipient competent cells, followed by recombination or replication. During HGT a recipient cell may receive many genes from many different individual donor cells within the same genus, as well as from different genera and even different phyla, viruses or the environment, thus potentially giving rise to a new strain or species of living entity – for example 10% of the genetic make-up of the different strains of lactic acid bacteria is due to phage mediated genetic diversity (Berger *et al*., 2007). However, this does not necessarily mean that all the received genes are always conserved - for example any cyanobacterium with dysfunctional photosynthetic apparatus can receive a functional gene from another cyanobacterium via the mechanism of transduction; but the acquired gene may not be propagated (Knoll 2003).

Nevertheless the major role of HGT is the introduction of biodiversity among living entities. The mechanisms of biodiversity include: conjugation (Lederberg Tatum, 1946), transduction (Kellenberger 1962), transformation (Griffith 1928), gene transfer agents (GTAs) (Hynes 2012) and membrane vesicle transfers (MVTs) (Gaudin *et al*., 2013) - although the latter two could technically be placed under the heading of transduction. It is believed that viruses are a major source of biodiversity on the Earth, for example it is estimated that there are over 10^{31} virus particles in the Earth's biosphere (Suttle 2007). These virions are involved in HGT on a universal scale (McDaniel, 2010), and have been controlling every niche and, invariably, every aspect of the biology of life on Earth ever since the advent of cellular life forms.

Equally important is the fact that Patrick Forterre (2005) has postulated that the evolutionary unrelated viruses were present during the LUCA era and that these were involved in the transfer of their genetic material (predominantly RNA initially) to the recipient competent LUCA cells, enabling HGT to play a vital role in the modification of LUCAs and their ultimate progression towards becoming the three domains of life; for example the DNA replication, transcriptional and translational may have their routes in viruses (Retroviruses) (Forterre 2013).

LUCAs were the progenitors of the three domains of life and also were purely (initially) RNA organisms which engaged in HGT between themselves on a universal

scale. That LUCAs were such predecessors is primarily because of the dual properties of RNA molecules, ie acting as repositories of genetic information and also as ribozymes. The evidence of the existence of LUCAs has been inferred from various comparative genomic studies and it is now certain that they were fairly complex organisms which possessed an RNA-based replication system and had rudimentary ribosomes for protein synthesis, but lacked double stranded DNA encoded genomes (Leipe *et al*., 1999). The continued evolution of rudimentary ribosomes in the form of transcriptional and translational apparatus meant that LUCA was able to make sophisticated proteins including enzymes ribonucleotide reductase and two thymidylate synthases (Leipe *et al*., 1999; Forterre 2006). These enzymes led to the formation of the DNA molecule, which had certain error minimisation properties when compared with RNA; thus DNA became a more stable keeper of the genetic code. Such high fidelity and error minimisation assisted the all-important mechanism of Darwinian evolution, which resulted in LUCA(s) evolving into the three domains of life which we recognise on Earth today.

The essence of this meeting is to untangle the genetics of LUCAs so that we can work steadily back to the origins of RNA and pre-RNA chemistry, investigating every avenue along the way. Such probing into the 'dark-age-of-LUCA', whilst being a long shot, is vital if we are ever truly to discover and understand the exact mechanisms of how life began. With the exceptionally high calibre of abstracts received and speakers who will be presenting their results and hypotheses, this second NoR HGT & LUCA conferences looks set to make further inroads into finding potential solutions to the unanswered questions of the evolution of LUCAs.

References

Berger, B., R. D. Pridmore *et al*., (2007). "Similarity and differences in the Lactobacillus acidophilus group identified by polyphasic analysis and comparative genomics." J. Bacteriology. **189**: 1311-1321. doi: 10.1128/JB

Knoll A. E. (2003). "Life on a young planet." Publisher: Princeton University Press. Pages: 16-31. ISBN: 0-691-12029-3

Lederberg, J, and E. L. Tatum (1946). "Gene Recombination in Escherichia Coli." Nature. **158**(4016): 558

Kellenberger, E. (1966). "The genetic control of the shape of a virus." Scientific America. 215: 32-39

Griffith, F. (1928). "The significance of eumococcal types." Journal of Hygiene **XXVII**(2): 8-159

Hynes, A. P., R. G. Mercer *et al*., (2012). "DNA packaging bias and differential expression of gene transfer agent genes within a population during production and release of the Rhodobacter capsulatus gene transfer agent, RcGTA." Molecular Microbiology. **85**: 314-325

Gaudin, M., Gauliard, E., Schouten, S., Houel-Renault, L., Lenormand, P., Marguet, E. and Forterre, P. Hyperthermophilic archaea produce membrane vesicles that can transfer DNA. Environ. Microbiol. Rep. **5**(2013a): 109-16

Suttle, C.A. (2007). "Marine viruses—major players in the global ecosystem." National Reviews Microbiology. **5**: 801–812

McDaniel, L. D., E. Young *et al*., (2010). "High frequency of horizontal gene transfer in the oceans." Science **330**: 50

Forterre P. (2005). “The two ages of the RNA world, and the transition to the DNA world: a story of viruses and cells.” Biochimie. **87**(9-10):793-803

Forterre P. Why are there so many diverse replication machineries? J Mol Biol. 2013, 425(23):4714-26

Leipe, D. D., L. Aravind et al., (1999). “Did DNA replication evolve twice independently?” Nucleic Acids Research **27**(17): 3389-3401

Forterre P. (2006). “Three RNA cells for ribosomal lineages and three DNA viruses to replicate their genomes: a hypothesis for the origin of cellular domain.” Proc Nat Acad Sci USA. **103**(10):3669-74

(Sohan Jheeta)

Publications Arising from the Meeting

As we had an exceptionally high calibre of abstracts, we have been offered the opportunity by the International Journal of Astrobiology (IJA) to produce a special issue entitled: 'The routes of emergence of life from LUCA during the RNA and viral world' on which I shall be acting as guest editor. This particular issue only becomes a realistic proposition if we submit a minimum of 10 papers with a deadline for the submission of 31st March 2015. I'd also like to point out that there will be no associated publication costs on this special issue, but please bear in mind that submissions would be subject to the usual peer review criteria. So, I should like to encourage all of you to submit either a results generated or review article and thus help put NoR HGT & LUCA of the map.

In relation to the IJA you can find general details at:
http://journals.cambridge.org/action/displayJournal?jid=IJA

And submission details at:
http://assets.cambridge.org/IJA/IJA_ifc.pdf

Organising Committee

Dr Sohan Jheeta (Independent Researcher, UK - sohan7@ntlworld.com)
Dr Martin Dominik (University of St Andrews, UK - md35@st-andrews.ac.uk)
Prof Prakash C Joshi (Rensselaer Polytechnic Institute, USA - joship2@rpi.edu)
Prof Nigel J Mason (The Open University, UK - Nigel.Mason@open.ac.uk)

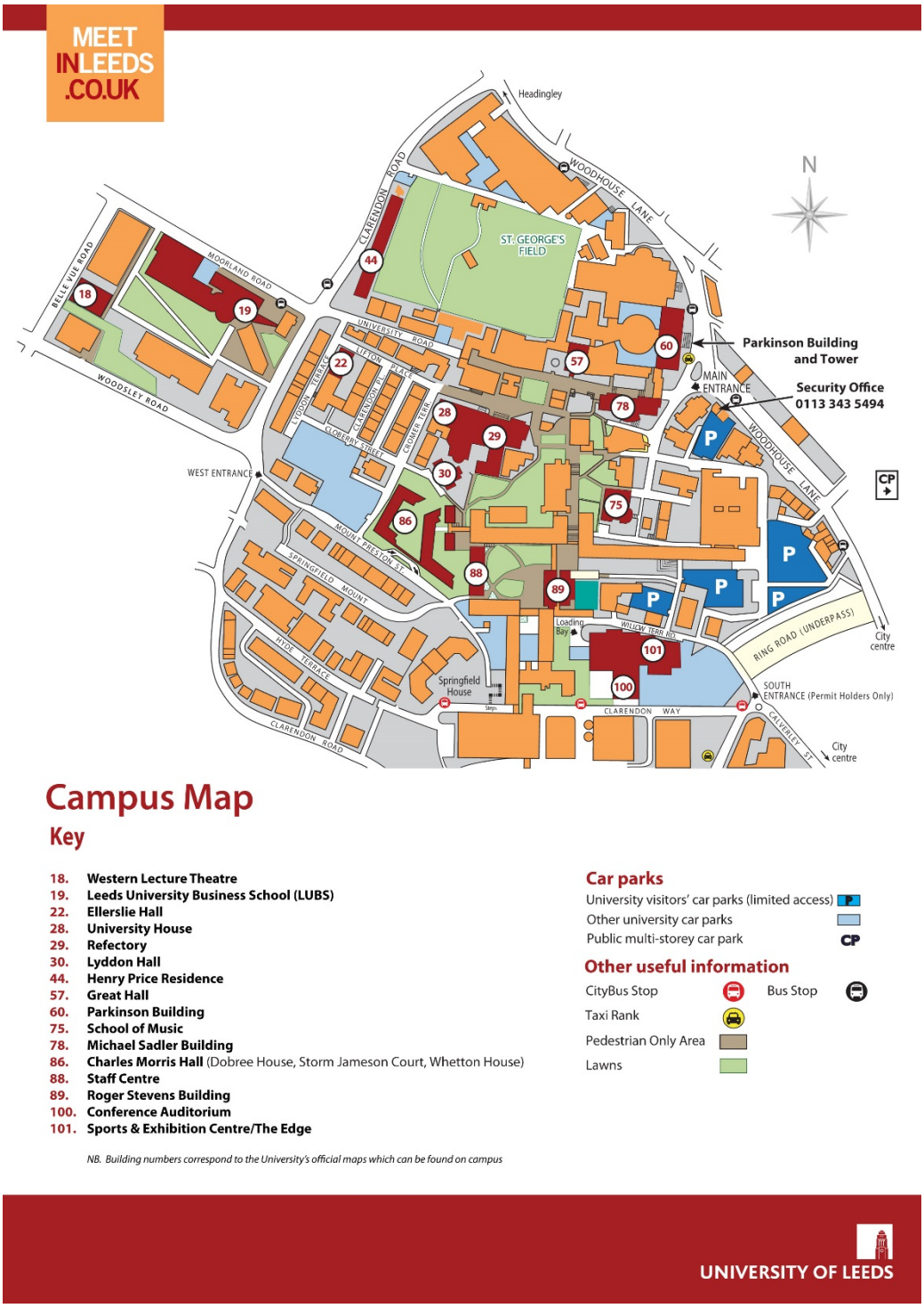
Conference photo

The end of October is generally not highly conducive for taking a good photo as it may be raining. So we will announce it nearer to the date after checking the weather forecast. One thing is for sure, it will be taken during a lunch break.

The Venue

The 2nd NoR HGT & LUCA conference will be held at:

2nd Floor, University House (position 28 on the map - next to the Student Union)
University of Leeds, Woodhouse Lane, Leeds, LS2 9JT



Campus Map

Key

18. **Western Lecture Theatre**
19. **Leeds University Business School (LUBS)**
22. **Ellerslie Hall**
28. **University House**
29. **Refectory**
30. **Lyddon Hall**
44. **Henry Price Residence**
57. **Great Hall**
60. **Parkinson Building**
75. **School of Music**
78. **Michael Sadler Building**
86. **Charles Morris Hall** (Dobree House, Storm Jameson Court, Whetton House)
88. **Staff Centre**
89. **Roger Stevens Building**
100. **Conference Auditorium**
101. **Sports & Exhibition Centre/The Edge**

NB. Building numbers correspond to the University's official maps which can be found on campus

Car parks

University visitors' car parks (limited access) P
Other university car parks
Public multi-storey car park CP

Other useful information

CityBus Stop
Bus Stop
Taxi Rank
Pedestrian Only Area
Lawns

UNIVERSITY OF LEEDS

	Schedule Day 1: Tuesday 28th October
08:15-08:45	**Arrival and registration**
08:45-08:50	**Prof John F Allen: University College London, UK** Welcome (5 min)
08:50-09:00	**Dr Sohan Jheeta** Motivation for the conference and directions for the day (10 min)
	Chair: Dr Martin Dominik: University of St Andrews, Scotland Theme: horizontal gene transfer connection
09:00-09:45	***Keynote speaker*: Prof Patrick Forterre, Institut Pasteur, Paris, France** LUCA and the different ages of early life evolution (from RNA cells and viruses to DNA viruses and cells) (30+15)
09:45-10:15	**Dr David Dulin, Clarendon Laboratory, Department of Physics, University of Oxford, UK** Highly parallelized single molecule magnetic tweezers assay (20+10)
10:15-10:45	**Dr Sukhvinder Gill, Institut de Génétique et Microbiologie, CNRS UMR 8621 Université Paris, France** Membrane vesicle (MVs) formation as a mechanism of cell to cell communication (20+10)
10:45-11:15	Coffee Break (30 min)
11:15-11:45	**Dr Søren Overballe-Petersen, Centre for GeoGenetics, University of Copenhagen, Denmark** Horizontal transfer of short and degraded DNA has evolutionary implications for microbes and eukaryotic sexual reproduction (20+10)
11:45-12:15	**Mr Amr Aswad, Department of Zoology, University of Oxford, UK** HGT Between Viruses: Perspectives from Paleovirology (20+10)
12:15-12:45	**Dr Eva Nowack, Heinrich Heine University Düsseldorf, Düsseldorf, Germany** The Evolution of a Photosynthetic Eukaryote - Paulinella chromatophora Replays the Tape (20+10)
12:45-13:45	**Lunch** (60 min)
	Chair: Prof John Allen Theme: continuing with the horizontal gene transfer connection
13:45-14:30	***Keynote speaker*: Prof David M. J. Lilley, CR-UK Nucleic Acid Structure Group, University of Dundee, UK** The crystal structure and a catalytic mechanism of the twister ribozyme (30+15)
14:30-15:00	**Prof Ernesto Di Mauro, Università di Roma "Sapienza", Italy** From formamide to RNA the prebiotic path is tenuous but continuous. (20+10)
15:00-15:30	**Dr Peter Sarkies, Department of Genetics, University of Cambridge, UK** Ancient and novel small RNA pathways compensate for the loss of piRNAs in multiple independent nematode lineages (20+10)
15:30-16:00	**Dr Alexander P Hynes, L'Université Laval, Quebec City, Canada** The non-contiguous 'genome' of RcGTA, the Rhodobacter capsulatus gene transfer agent (**20+10**)
16:00-16:30	Coffee break (30 min)
16:30-17:00	**Prof Tadej Kotnik, Dept. of Biomedical Engineering, University of Ljubljana, Slovenia** Lightning-triggered gene transfer (20+10)
17:00-17:30	**Dr Matthew Powner, University College London, UK** On the Origins of RNA (20+10)
17:30-18:00	**Dr Judit E Sponer, Institute of Biophysics, Academy of Sciences of the Czech Republic** The route from formamide to simple ribozymes: structures and mechanisms from advanced computational studies (20+10)
18:00-18.30	**Dr Chitvan Amin, Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, UK** Understanding structure-function relationship of RNA polymerases (20+10)
	Close of day 1: Relax, socialise and get ready for the conference dinner
20:00	**Drink and Conference dinner**

	Schedule Day 2: Wednesday 29th October
	Chair: Dr Sohan Jheeta Theme: pre-RNA and RNA chemistry
08:30-09:15	***Keynote speaker:*** **Prof Ramanarayanan Krishnamurthy, the Scripps Research Institute, La Jolla, USA** RNA: A product of chemical evolution? (30+15)
09:15-09:45	**Dr Phillipp Holliger, MRC Laboratory of Molecular Biology, University of Cambridge, UK** Towards RNA self-replication (20+10)
09:45-10:15	**Dr Omer Markovitch**, **School of Computing Science, Newcastle University, UK** Compositional Lipid Assemblies as Evolving Protocells (20+10)
10:15-10:45	**Dr Tamir Tuller, Laboratory of Computational Systems Biology, Tel Aviv University, Israel** The effect of codon usage bias on the success of horizontal gene transfer (20+10)
10:45-11:15	Coffee break (30 min)
11:15-11:45	**Prof Armen Mulkidjanian, University of Osnabrueck, Germany/A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Russia** Cell membranes as a precondition of spreading of the first RNA organisms (20+10)
11:45-12:15	**Dr Leonardo Sorci, Marche Polytechnic University, Ancona, Italy** Evolution of NAD biosynthetic pathway: from prebiotic synthesis to extant pathway diversification (20+10)
12:15-12:45	**Prof John Allen, University College London, UK** Redox and proton-motive homeostasis (20+10)
12:45-13:45	**Lunch break**
	Chair: Dr Sohan Jheeta/Dr Martin Dominik Theme: continuing with pre-RNA and RNA chemistry
13:45-14:30	***Keynote speaker:*** **Prof Tetsuya Yomo, University of Osaka, Japan** Constructive approach for the transition from non-living to living (30+15)
14:30-15:00	**Prof Savio Torres de Farias, Universidade Federal da Paraiba, João Pessoa, Brazil** tRNA core Hypothesis: A new model for origin of the biological system (20+10)
15:00-15:50	**Open Discussion**
15:50-16:00	**Close of meeting: Dr Sohan Jheeta**
16:00	**Close of day 2**

Submitted abstracts

DAY 1

Tuesday, time: 09:00-09:45

LUCA and the different ages of early life evolution (from RNA cells and viruses to DNA viruses and cells)

Keynote speaker: Patrick Forterre, Institut Pasteur, 25 rue du Docteur Roux, 75015, Paris
forterre@pasteur.fr

Abstract

Comparative genomic and molecular biology have shown that LUCA and its contemporaries were already rather complex organisms encoding sophisticated proteins and using the modern genetic code. They were also already infected by diverse groups of evolutionary unrelated viruses. From the molecular design of modern cells, it is possible to define several evolutionary steps from LUCA back toward the origin of life. The simplest kinds of organisms that we can confidently infer from this retro-diction are simple cells with RNA genomes (RNA-cells). These first RNA-cells had lipids and peptides but not *bona fide* proteins, since the ribosome was not yet present. Considering the complexity of RNA, these cells were probably not the first "living cells" that emerged from the prebiotic setting. However, there is presently no convincing scenario (to my knowledge) explaining the emergence of a primitive cellular metabolism able to produce ATP and other ribonucleotides to build the genomes of the first RNA-cells. Modern proteins originated through the progressive emergence of the ribosome, a giant RNA machine, opening a second age of the RNA world (1). During this age, proteins become more and more sophisticated, up to the emergence of ribonucleotide reductases, a prerequisite for the origin of DNA. During the same period, some RNA replicons became able to use proteins to disseminate from one cell to another, becoming the ancestors of modern viruses (2). This critical step initiates the arm race between capsid and ribosome encoding organisms that became a (the) major factor increasing the speed of life evolution (3). The emergence of viruses also increases the speed of protein production and diversification, as suggested by the fact that modern viruses are the major cradles of new proteins and biological functions (4). In that context, one can imagine that DNA first originated in the virosphere as a genome modification used by viruses to protect their genomes against cellular defences (5). The emergence and diversification of various DNA replication machineries in the ancient virosphere would explain why these machineries are more diverse today in the modern virosphere than in cellular organisms (6). Two versions (only) of this machinery are present today in the cellular world, one in Bacteria, the other in Archaea and Eukarya. This raises the possibility that LUCA still had a RNA genome and that DNA and DNA replication machineries were transferred from viruses to cells at least two times, once in the lineage leading from LUCA to Bacteria, a second time in the lineage leading from LUCA to the common ancestor of Archaea and Eukaryotes (other more complex scenarios are of course possible) (5-7). Reconstruction of the nucleotide and amino-acids composition of rRNA and proteins from LUCA and from the ancestors of Archaea and Bacteria has suggested that LUCA was a mesophile, but that the ancestors of Archaea and Bacteria were hyperthermophile (Archaea) or thermophiles (Bacteria) (8). This

suggests that LUCA is not specifically related to modern hyperthermophiles but that, nevertheless, adaptation to high temperature played a major role in the history of life, possibly explaining the origin of the "prokaryotic phenotype" by thermoreduction (9, 10). Some authors have criticized the above scenario suggesting that RNA is not sufficiently stable to support cellular life (11). Their major argument is that the largest RNA viruses presently known have a small genome (30 kb). I will argue that these viruses are not representative of ancient RNA-cells. A major project in synthetic biology could be to built (or try to) an RNA cell to in order to prove (or disprove) the validity of the RNA cell concept.

References

1. Forterre P., The two ages of the RNA world, and the transition to the DNA world: a story of viruses and cells. Biochimie. 2005, 87(9-10):793-803
2. Forterre P., Krupovic, M. The origin of virions and virocells: the escape hypothesis revisited, In G. Witzany (ed.), Viruses:Essential Agents of Life, DOI 10.1007/978-94-007-4899-6_3, Springer Science+Business Media Dordrecht , (2012*)*
3. Forterre P., Prangishvili D. The great billion-year war between ribosome- and capsid-encoding organisms (cells and viruses) as the major source of evolutionary novelties. Ann N Y Acad Sci. 2009, 1178:65-77
4. Forterre P., Prangishvili D. The major role of viruses in cellular evolution: facts and hypotheses. Curr Opin Virol. 2013, 3(5):558-65
5. Forterre P. The origin of DNA genomes and DNA replication proteins. Curr Opin Microbiol. 2002, 5(5):525-32
6. Forterre P. Why are there so many diverse replication machineries? J Mol Biol. 2013, 425(23):4714-26
7. Forterre P. Three RNA cells for ribosomal lineages and three DNA viruses to replicate their genomes: a hypothesis for the origin of cellular domain. Proc Natl Acad Sci U S A. 2006, 103(10):3669-74
8. Boussau B, Blanquart S, Necsulea A, Lartillot N, Gouy M. Parallel adaptations to high temperatures in the Archaean eon. Nature. 2008, 456(7224):942-5
9. Forterre P. The common ancestor of archaea and eukarya was not an archaeon Archaea. 2013; 372396. doi: 10.1155/2013/372396
10. Forterre P. Thermoreduction, a hypothesis for the origin of prokaryotes. C R Acad Sci III. 1995, 318(4):415-22
11. Takeuchi N, Hogeweg P, Koonin EV. On the origin of DNA genomes: evolution of the division of labor between template and catalyst in model replicator systems. PLoS Comput Biol. 2011 (3):e1002024

Tuesday, time: 09:45-10:15

Highly parallelized single molecule magnetic tweezers assay

David Dulin, Clarendon Laboratory, Department of Physics, University of Oxford, UK
david.dulin@physics.ox.ac.uk

Abstract

RNA viruses are responsible for many human pandemics, including hepatitis C, polio, influenza, and dengue fever. Due to their high mutation rates, RNA viruses evolve rapidly and are difficult to target with vaccines. On the molecular level, the dominant source of mutations is the error prone RNA-dependent RNA Polymerases (RdRPs) responsible for replicating the viral genomes. A high mutation rate increases evolvability, but also induces many deleterious mutations, and a delicate balance needs to be struck to ensure the pathogenicity of the viral population. Single point mutation in the active site of the RdRPs leads to a large change in the error rate, which is not compatible with the need of a precise error rate adjustment. The RdRPs, as well as the Reverse Transcriptases (RTs), have a highly conserved active site, which indicates a similar mechanism of nucleotide incorporation and error addition. Though these enzymes are particularly error prone, it is still a cha llenging problem to observe the dynamic of error incorporation, as such event happens only once in a thousand to once in ten thousands incorporated nucleotides. Stop-flow assays have been developed to look at error incorporation, but they are limited to the observation of a limited number of different nucleotides at a time. In order to circumvent this problem, we developed a highly parallelized single molecule magnetic tweezers assay that allows the observation of hundreds of RNA template, so possibly hundreds of polymerase activity, with few bases resolution and the four ribonucleotides triphosphate. The very large statistic acquired together with a bias free Bayesian analysis gave us the possibility to characterize the full elongation dynamic, and also error incorporation, of two RdRPs, the one of the dsRNA bacteriophage phi6 and the one of polio, a ssRNA human virus. We will present an unknown error incorporation mechanism conserved over the RdRPs of different virus family, and probably more largely conserved over the DNA polymerase A-family.

Tuesday, time: 10:15-10:45

Membrane vesicle (MVs) formation as a mechanism of cell to cell communication

Sukhvinder Gill, Marie Gaudin, Evelyne Marguet, Aurore Gorlas, Jacques Oberto and Patrick Forterre, Institut de Génétique et Microbiologie, CNRS UMR 8621 Université Paris, FRANCE
sukhi.gill@igmors.u-psud.fr

Abstract

Cells from the three domains of life produce extracellular membrane vesicles (MVs), suggesting that MV production is a fundamental aspect of cellular physiology. Membrane vesicles have been implicated in many aspects of cellular life in the three domains, including stress response, toxicity against competing strains, pathogenicity, detoxification and resistance against viral attack. These extracellular MVs represent an important mode of intercellular communication by serving as vehicles for transfer of DNA, RNA, proteins and lipids between cells.

Our group has previously shown that MVs produced by the hyperthermophilic archaeon Thermococcus kodakaraensis can be used as vehicles to transfer exogenous recombinant plasmid DNA from cell to cell (Gaudin et al., 2012). Furthermore, these MVs can be also associated to defective viral genomes (Gaudin et al., 2014). In Thermococcus species MVs are frequently secreted in clusters surrounded by the S-layer, producing either big protuberances (nanospheres) or tubular structures (nanotubes) (Marguet et al., 2013). These nanotubes can bridge neighbouring cells, forming cellular networks that could be used to expand the metabolic sphere around cells and/or to promote intercellular communication.

We now aim to create knock-out mutants of genes encoding proteins possibly involved in MV production and/or in fusion with recipient cells. We will focus on proteins that have already been identified in MVs and proteins with eukaryotic and/or bacterial homologues involved in vesicle formation and/or cell division.

References

1. Gaudin, M., Gauliard, E., Schouten, S., Houel-Renault, L., Lenormand, P., Marguet, E. & Forterre, P. . Hyperthermophilic archaea produce membrane vesicles that can transfer DNA. Environ. Microbiol. Rep. 5. 2013a, 109-16
2. Gaudin, M., Krupovic, M., Marguet, E., Gauliard, E., Cvirkaite-Krupovic, V., Le Cam, E., Oberto, J. & Forterre, P. Extracellular membrane vesicles harbouring viral genomes. Environ. Microbiol. 2014, April 16(4): 1167-75

Tuesday, time: 11:15-11:45

Horizontal transfer of short and degraded DNA has evolutionary implications for microbes and eukaryotic sexual reproduction

Søren Overballe-Petersen, Centre for GeoGenetics, University of Copenhagen, Øster Voldgade 5-7, 1350 Copenhagen K, Denmark
sopetersen@snm.ku.dk / soerenov@yahoo.dk

Abstract

Horizontal gene transfer of long DNA segments has already changed our view of bacterial evolution. Recently, we discovered that such processes may also occur with the massive amounts of short and damaged DNA in the environment, and even with truly ancient DNA. Although it presently remains unclear how often it takes place in nature, natural transformation of short and damaged DNA opens up the possibility for genetic exchange across distinct species in both time and space. Now we speculate on the potential evolutionary consequences of this phenomenon. We argue that it may challenge basic assumptions in evolutionary theory; that it may have distant origins in life's history; and that horizontal gene transfer should be viewed as an evolutionary strategy not only preceding but causally underpinning the evolution of sexual reproduction.

Tuesday, time: 11:45-12:15

HGT Between Viruses: Perspectives from Paleovirology

Amr Aswad and Aris Katzourakis, Department of Zoology, University of Oxford, UK
a.katzourakis@gmail.com

Abstract

Paleovirology is the study of endogenous viral elements (EVEs) - genomic features that result from the accidental integration of viral genetic material in the host germline. EVEs have predominantly been used as 'genetic fossils' that inform us about the evolution of the viruses, but recent studies have recognized the importance of a functional subset, co-opted to benefit the host. Conversely, there are host-derived genes in viruses, a phenomenon that has primarily been observed in large double stranded DNA viruses. Although exceptionally rare, gene flow between unrelated viruses also occurs. Our most recent work has demonstrated that virus-to-virus gene transfer can occur in a convergent evolutionary manner, demonstrating that a particular underlying evolutionary mechanism is at play. The principles of paleovirology can be used to study the horizontal gene transfer between viruses as well as between viruses and their hosts under the same evolutionary framework.

Tuesday, time: 12:15-13:15

The Evolution of a Photosynthetic Eukaryote - Paulinella chromatophora Replays the Tape

Eva Nowack, Emmy Noether Group "Microbial Symbiosis and Organelle Evolution",
Heinrich Heine University Düsseldorf, Düsseldorf, Germany
E.Nowack@uni-duesseldorf.de

Abstract

The endosymbiosis between a cyanobacterium and a heterotrophic protist >1 BYA has led to the evolution of photosynthetic eukaryotes. Today, complex interactions between the prokaryote-derived plastid and the eukaryotic nucleus/cytoplasm are key to fine tuning plastid growth, division, and metabolism in response to the state of the host cell and environmental factors. We are interested in understanding evolutionary processes that enable the merger of two physiologically and genetically different cells into a highly efficient chimeric organism. For this purpose we study the amoeba Paulinella chromatophora which harbors nascent photosynthetic organelles of cyanobacterial origin that are termed 'chromatophores'. The chromatophore genome is intermediate in size between that of a cyanobacterium and plastid. Reductive genome evolution resulted in the loss of biosynthetic capabilities (e.g. amino acids and various cofactors), clearly establishing a metabolic dependence of the chromatophore on its host. More than 30 genes that were lost from the chromatophore genome reside now in the nuclear genome of P. chromatophora. Most of these genes encode proteins involved in photosynthesis and light-acclimation. PsaE and PsaK are nuclear-encoded low molecular weight subunits of photosystem I. Biochemical studies have shown that PsaE and PsaK are trafficked from the amoebal cytoplasm, where they are synthesized, into chromatophores, where they assemble with chromatophore-encoded subunits into photosystem I. Our findings suggest that these proteins pass through the secretory pathway prior to entry into the chromatophore. Therewith, the host has gained a tool to directly modulate the photosynthetic apparatus. However, we expect that a lot more molecular factors are critical for successfully integrating host–endosymbiont metabolic and regulatory networks. Currently, we are exploring the nuclear genome of P. chromatophora to gain insight into how genetic repertoire evolves that allows the host to control and manipulate the chromatophore according to its needs.

Tuesday, time: 13:45-14:30

The crystal structure and a catalytic mechanism of the twister ribozyme

Keynote speaker: David M. J. Lilley, Yijin Liu and Timothy J. Wilson, CR-UK Nucleic Acid Structure Group, University of Dundee, Dundee DD1 5EH, UK
d.m.j.lilley@dundee.ac.uk http://www.dundee.ac.uk/biocentre/nasg/index.php

Abstract

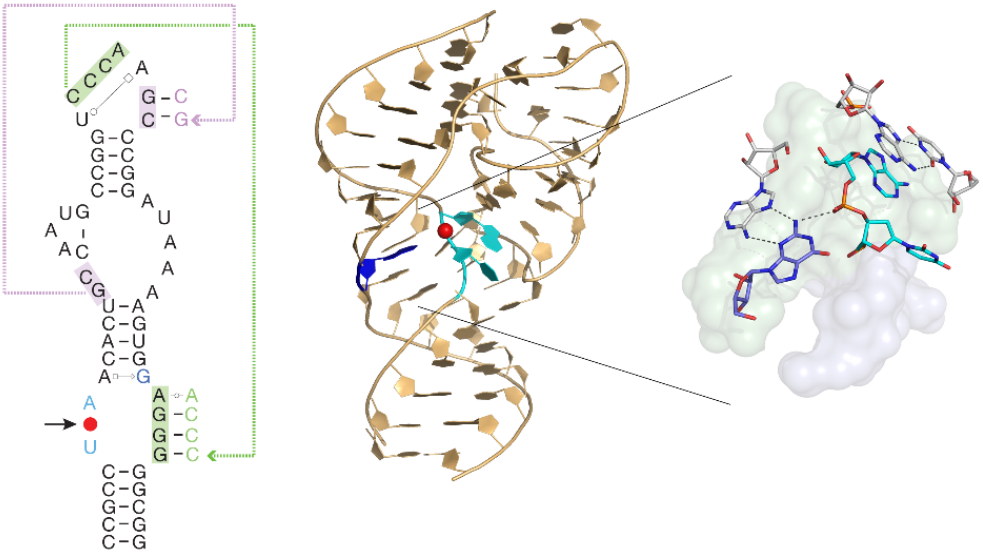
The nucleolytic ribozymes are a group of RNA species that undergo self-cleavage or ligation at a particular site. Originally discovered in plant pathogens, these were mostly involved in processing replication intermediates, and later found to be a means of regulating genetic expression in bacteria. However it has recently become clear that ribozyme sequences are in fact very widespread in many genomes, located within non-coding RNA sequences, and are strongly conserved and expressed inside the cell. Ribozymes of the HDV/CPEB3 and hammerhead families have been found in many eukaryotic species including human, and their location and expression indicate that they are functional *in vivo*.

The twister ribozyme, recently discovered in the Breaker laboratory, is another small nucleolytic ribozyme that is widely disseminated in the genomes of bacteria and eukarya. We have solved the crystal structure of this ribozyme from *Oryza sativa* at 2.3 Å resolution.

The RNA adopts a novel compact fold based on a unique reversed, double pseudoknot structure, with the scissile phosphate at its center. All highly-conserved nucleobases form key structural elements including a guanine nucleobase that has its Watson-Crick edge directed towards the scissile phosphate. The pH dependence of the cleavage rate is bell-shaped, consistent with general acid-base catalysis. We have structural and mechanistic evidence for the participation of guanine and adenine nucleobases in proton transfers to the participating O2' and O5' atoms in a mechanism with several novel features.

Reference

Y. Liu, T. J. Wilson, S. A. McPhee and D. M. J. Lilley Crystal structure and mechanistic investigation of the twister ribozyme Nature Chem. Biol. 10, 739-744 (2014).



Tuesday, time: 14:30-15:00

From formamide to RNA the prebiotic path is tenuous but continuous

Ernesto Di Mauro, Dipartimento di Biologia e Biotecnologie "Charles Darwin", Università "La Sapienza", P.le Aldo Moro, 500185 Roma, Italy
ernesto.dimauro@uniroma1.it

Abstract

Life is made of the intimate interaction of metabolism and genetics, both built around the chemistry of the most common elements found in the Universe (hydrogen, oxygen, nitrogen, carbon). The transmissible interaction of metabolic and genetic cycles results in hypercycles of organization and "de-organization" of chemical information, of living and non-living entities. The origin of life quest has long been split in several strands exemplified by the aphorisms "genetics-first" or "metabolism-first". Overstepping the opposition between these approaches by a unitary theoretical and experimental framework and, taking into account energetic, evolutionary, proto-metabolic and ur-environmental aspects, we propose a simple pathway leading to a complete prebiotic reactive system. Specifically, we analyze the synthetic reactions leading from the one-carbon atom compounds HCN and its hydrolyzed form, formamide (NH2COH) to prebiotically relevant compounds in the presence of catalysts. We observe the formation of all the extant biological nucleic bases of carboxylic and amino acids and also of condensing agents in the presence of tens of catalysts from terrestrial origin as well as from 12 meteorites. We also observe in the same chemical frame the formation of cyclic nucleotides and their spontaneous polymerization to oligonucleotides; their terminal ligation yielding longer polymers and a ribozyme activity causing the terminal transfer of nucleotides between in vitro abiotically generated oligomers. In vitro generated oligonucleotides thus automatically increase the chemical information of the system. This is not to say that from formamide, test-tube one can magically produce RNA. Numerous hurdles remain and the results are so far very partial: (i) extant nucleic bases - they can all be synthesized; (ii) nucleosides - observed formation of purine acyclonucleosides and of uridine; (iii) phosphorylation - observed formation of cyclic nucleotides from performed nucleosides; (iv) polymerization - characterized for 3', 5' cGMP and observed for 3', 5'cAMP. The path is only implicit, but looks to be a distinct possibility.

Nevertheless, these results would indicate that the spontaneous generation of proto-metabolic and proto-genetic systems would have required a less complex initial set-up. Rather, it probably came about as a result of the interplay between combinatorial chance and the thermodynamic necessity of the existing most abundant atoms.

Tuesday, time: 15:00-15:30

Ancient and novel small RNA pathways compensate for the loss of piRNAs in multiple independent nematode lineages

Peter Sarkies, Department of Genetics, University of Cambridge, UK
ps493@cam.ac.uk

Abstract

Small RNA pathways act at the front line of defence against transposable elements across the Eukaryota. In animals, Piwi interacting small RNAs (piRNAs) are a crucial arm of this defence. However, the evolutionary relationships piRNAs and other small RNA pathways targeting transposable elements are poorly resolved. To address this question we sequenced small RNAs from multiple, diverse nematode species, producing the first comprehensive analysis of how small RNA pathways evolve within a single phylum. Surprisingly, despite their prominence in C. elegans and closely related nematodes, piRNAs are absent in all other nematode lineages. We found that there are at least two evolutionarily distinct mechanisms that compensate for the absence of piRNAs, both involving RNA-dependent RNA polymerases (RdRPs). Whilst one pathway is unique to nematodes, the second involves RNA-directed DNA methylation, hitherto unknown in animals, and bears striking similarity to transposon-control mechanisms in fungi and plants. Our results highlight the rapid, context-dependent evolution of small RNA pathways and suggest piRNAs in animals may have replaced an ancient eukaryotic RNA-dependent RNA polymerase pathway to control transposable elements.

Tuesday, time: 15:30-16:00

The non-contiguous 'genome' of RcGTA, the Rhodobacter capsulatus gene transfer agent

Alexander P Hynes, Département de biochimie, de microbiologie et de bio-informatique as well as the Groupe de recherche en écologie buccale, Université Laval, Quebec City, Quebec, Canada
alexanderhynes@gmail.com

Abstract

Gene Transfer Agents (GTAs) are phage-like particles capable of transferring DNA from the producing strain in a process analogous to transduction. Their existence and continued maintenance in both Archaea and Bacteria raises interesting questions as to the origins of phages and evolutionary importance of horizontal gene transfer. The alphaproteobacterium Rhodobacter capsulatus produces the archetypal GTA, RcGTA. We sought to characterise of the contents of the RcGTA particles as well as the genes responsible for its production. We found, to our surprise, that the RcGTA particle appears to avoid packaging the genes required for its own production. Furthermore, we identified 9 additional genes, across 6 separate loci, consistently co-regulated with the RcGTA structural gene cluster. We disrupted several of these and identified, among others, a gene required for release of RcGTA by cell lysis – allowing us to establish an important 'cost' of RcGTA production. An investigation of the genomes of other bacteria carrying related GTA structural gene clusters revealed that most of these contained several of our additional 9 genes, suggesting a conserved, divided (non-contiguous) GTA 'genome'.

Tuesday, time: 16:30-17:00

Lightning-triggered gene transfer

Tadej Kotnik, Department of Biomedical Engineering, Faculty of Electrical Engineering, University of Ljubljana, Slovenia
tadej.kotnik@fe.uni-lj.si

Abstract

HGT research largely focuses on three well-recognized mechanisms – conjugation, competence, and transduction – yet it is unclear whether these account for all HGT. First, they are rather intricate products of evolution, and how HGT could have proceeded before their onset is unclear. Second, even among current prokaryotes, many are incapable of conjugative exchange, many possess no competence for uptake, while phages infect selectively and thus only transfer genes among restricted ranges of strains. Third, there is increasing evidence of HGT in eukaryotes, in which neither conjugation nor competence exists.

A tentative answer is offered by non-biochemical, yet natural mechanisms of destabilization of the membranes enveloping the genetic material: freeze-thaw cycles, abrasive action of gravel and sand, electroporation triggered by lightning strokes. Their contribution to natural DNA leakage, uptake, and transformation remains largely unexplored.

It is well-known that exposure to sufficiently strong electric fields increases lipid bilayer's permeability: water molecules penetrate the bilayer, causing reorientation of adjacent lipids, and the resulting metastable aqueous pores provide a transmembrane pathway for many molecules, including DNA. At the molecular level, pore formation is stochastic, but at the cellular level it can be considered deterministic, as for a fixed electric field amplitude and duration it is highly reproducible. This phenomenon, termed electroporation, occurs in pure bilayers and biological membranes regardless of their particular protein content; prokaryotes possessing a wall and/or a capsule merely require a stronger field.

Electroporation can be either reversible or irreversible. Reversible electroporation is among the most efficient techniques for artificial transformation (electrotransformation), and has to date been reported for species from at least 13 bacterial and 2 archaeal phyla. Irreversible electroporation causes DNA release (electroextraction), but less efficiently than standard procedures, and is thus rarely used in laboratory environment. Thus, electrotransformation is mostly performed by adding pre-isolated DNA to acceptor organisms before electric pulse delivery, yet it was also tried with electroextraction, and yields of over one transfer per million have been reported with a single pulse delivered into a mix of donor and acceptor organisms.

It is straightforward to show that conditions for electroporation-based DNA release, uptake, and transformation are also present in many natural habitats exposed to lightnings. Consider a lightning stroke with 30 kA peak current entering from the atmosphere into an aqueous habitat. Upon entry, current dissipates roughly radially,

and with increasing distance from its point of entry, the electric field it induces decreases continuously. In seawater, organisms are electroporated in a hemispherical volume of ~3 litres: irreversibly in the inner ~60 ml (DNA release only) and reversibly outwards (both DNA uptake and release). In freshwater habitats, about ~500 litres are electroporated, inner ~10 litres irreversibly.

There are typically over 10^8 microorganisms per litre of either seawater or freshwater. One transfer per million exposed organisms would thus yield over 300 and over 50000 transformants per stroke in seawater and freshwater, respectively. As lightning strikes these habitats over 10^9 times per year, and has been doing so for over 10^9 years, the number of evolutionarily significant occurrences of lightning-triggered HGT may well be non-negligible.

Tuesday, time: 17:00-17:30

Towards RNA self-replication

Philipp Holliger, MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Cambridge CB2 0QH, UK
ph1@mrc-lmb.cam.ac.uk

Abstract

A critical event in the origin of life is thought to have been the emergence of an RNA molecule capable of self-replication as well as mutation, and hence evolution towards ever more efficient replication. As this primordial replicase appears to be have been lost in time, we use synthetic biology to build modern-day "Doppelgangers" of the ancestral replicase to reconstruct and study their properties in an effort to learn more about life's first genetic system. I will discuss our progress in the engineering and evolution of RNA polymerase ribozymes as well as the potential role that structured media such as the eutectic phase of water ice may have played in the emergence of RNA self-replication.

Tuesday, time: 17:30-18:00

THE ROUTE FROM FORMAMIDE TO SIMPLE RIBOZYMES: STRUCTURES AND MECHANISMS FROM ADVANCED COMPUTATIONAL STUDIES

J. E. Sponer, J. Sponer, P. Stadlbauer and E. Di Mauro,
Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 612 65, Brno, Czech Republic and Fondazione "Istituto Pasteur-Fondazione Cenci-Bolognetti" c/o Dipartimento di Biologia e Biotecnologie "Charles Darwin", "Sapienza" Università di Roma, P.le Aldo Moro, 5, Rome 00185, Italy
e-mail: judit@ncbr.chemi.muni.cz and ernesto.dimauro@uniroma1.it

Abstract

The formamide-based synthesis of nucleic acid components offers a new alternative for the origin of informational polymers.1,2 This chemistry represents an elegant and continuous way from simple prebiotic precursors up to short catalytic oligonucleotides. Since this multistep synthesis proceeds in a very complex reaction mixture, it is very difficult to study its mechanism using purely experimental methods. Our presentation is aimed to illustrate that in such complicated cases computational chemistry might be instrumental to provide an atomic-level insight into the mechanistic details of the reactions.

In particular, we will present a mechanistic model for the spontaneous polymerization of 3',5'-cyclic nucleotides. This reaction is of paramount importance, since up-to-now this is the only chemistry, which leads to a selective formation of 3',5'-linked oligonucleotides. On the basis of high-level quantum chemical calculations we propose that formation of a stacked, ladder-like architecture may give rise to the experimentally observed chemistry. We will present a video simulation of the theoretically computed reaction pathway and provide an in-depth elucidation of all details of the mechanism (effect of cations, influence of dry-wet environment, etc.).

Further, using a combination of experiments and molecular dynamics simulations, we provide a mechanistic proposal for the emergence of a simple ribozyme-like catalytic activity in WC-complementary short oligonucleotide sequences. We propose that tetraloop-like overhang geometries are sufficiently stable to mediate transphosphorylation reactions leading to both ligation and catalytic cleavage. In addition, we will present a model for the most ancient catalytic oligonucleotides, which suggests that the catalytic activity of the first oligonucleotides could originate from their hydrolytic instability.

Finally, combining our experimental and theoretical findings we will formulate a unifying concept for the emergence of catalytically active oligonucleotides from 3',5'-cyclic nucleotides.

References

1) R. Saladino, C. Crestini, F. Ciciriello, S. Pino, G. Costanzo, E. Di Mauro, Res. Microbiol., 2009, 160, 441.

2) R. Saladino, G. Botta, S. Pino, G. Costanzo, E. Di Mauro, Chem. Soc. Rev., 2012, 41, 5526.
3) S. Pino, G. Costanzo, A. Giorgi, J. Šponer, J. E. Šponer, E. Di Mauro, Entropy, 2013, 15, 5362.

Tuesday, time: 18:00-18.30

Understanding structure-function relationship of RNA polymerases

Chitvan Amin, Department of Life Sciences, Faculty of Natural Sciences, Imperial College London, UK
c.bochiwal@imperial.ac.uk

Abstract

In bacteria, archaea and eukarya, transcription is carried out by DNA dependent RNA polymerases (RNAP), complex multi-subunit proteins whose structure is highly conserved in all the three domains of life. RNAPs carry out complex active site chemistry which requires a precisely orchestrated sequence of conformational changes of protein domains during the transcription cycle. Our lab specialises in using the combination of biochemical techniques, high throughput mutagenesis and Molecular dynamics to study the effect of single amino acid mutation on the structure-function relationship of the protein. Here I present my work on the mutagenesis of the nucleotide binding region present in the active site such as Bridge helix (BH), Trigger Loop (TL) and B-D domain. The mutagenesis and computational analysis results have provided a greater insight into the molecular mechanics at the active site, such as ratchet mechanism of Bridge helix (Weinzierl, 2010). My results provide further ev idence of unknown molecular interactions between the growing RNA chain and highly conserved residues in B-D-domain and TL. These key amino acids may carry an evolutionary-bias and perhaps were integral part of the simpler framework of the RNA polymerase in LUCA.

DAY 2

Wednesday, time: 08:30-09:15

RNA: A product of chemical evolution?

Keynote speaker: Ramanarayanan Krishnamurthy, the Scripps Research Institute,
10550 North Torrey Pines Road, La Jolla, CA 92037
rkrishna@scripps.edu

Abstract

The focus on RNA as a key player in origins of life research is exemplified by the RNAworld hypothesis. However, self-assembly of RNA from its constituents under potential prebiotic constraints has been a challenge. RNA's chemistry and biology, which are intertwined with the physicochemical properties of its chemical components in a neutral aqueous environment, are incompatible with plausible prebiotic environments. The question arises whether RNA could have appeared later, at a stage where both the chemical processes and the environment would have been more conducive for RNA's sustained origination and function.

Inspired by this question, we have been investigating structures that could be considered potentially natural alternatives to RNA. The results from these studies have implications not only for the structure-function relationship of RNA, but also for the consideration of RNA as a product of chemical evolution. In this context, the nature of the precursor(s) of RNA comes into question and raises the possibility that this optimal structure of RNA could have been selected from a library of structures (combination of various backbones, linkers, nucleobases and connectivities) based on its optimal function.

Wednesday, time: 09:15-09:45

On the Origins of RNA

Matthew Powner, University College London, UK
matthew.powner@ucl.ac.uk

Abstract

A plausible abiotic chemical route to the canonical nucleotides is a major goal in origins of life research. Some of our recent work to understand the intrinsic chemical requirements for nucleotide synthesis in water will be presented. Having previously reported a chemoselective, prebiotically plausible route to activated canonical pyrimidine ribonucleotides; we have recently demonstrated the first chemical steps towards a divergent pyrimidine and purine ribonucleotide synthesis.

Natural RNA is ribonucleotide polymer linked homogeneously by 5′-3′-phosphodiester backbone. However, ribonucleotides have a linkage ambiguity (5′-3′ vs. 5′-2′) at every phosphodieter bond. Ostensible this linkage heterogeneity requires must be addressed, in the absence of sophisticated enzymatic catalysis, for the effective abiotic synthesis of RNA. An acyl-transfer cascade, which is a potential solution to RNA linkage heterogeneity and the experimental demonstration of ribozyme catalysis in heterogeneous 5′-3′/5′-2′ RNA will be presented.

Wednesday, time: 09:45-10:15

Compositional Lipid Assemblies as Evolving Protocells

Omer Markovitch, School of Computing Sciences, University of Newcastle, Newcastle, UK
omer.markovitch@ncl.ac.uk

Abstract

Life is complex, and its origin is about how sufficient chemical complexity emerged to afford replication, which later gave rise to the last-universal-common-ancestor. A crucial aspect of replication and evolution is the faithful transmission of sufficient information to progeny. The graded autocatalysis replication domain (GARD) model, in the realm of the lipid world scenario, offers a possible route for such pursuit. In this framework, non-covalent assemblies of amphiphiles, such as lipid micelles or vesicles, can acquire adequate endogenous complexity, mediated by a set of catalyzed chemical reactions akin to metabolism. These assemblies store information in the form of non-random molecular composition. Our computer simulations show that GARD assemblies can transmit compositional information through catalyzed homeostatic growth followed by random fission, unlike the template-based replication of a polymeric strand such as DNA or RNA. Key in GARD dynamics are *composomes*, spontaneously-forming replication-prone compositional states. A group of composomes, gleaned by clustering, is termed *compotype*, and may be regarded as species in the framework of lipid world and GARD. Indeed, such GARD species were recently shown to display a significant measure of Darwinian evolution, and their populations are capable of displaying ecological dynamics, and obey the quasispecies formalism. Thus, the GARD suggests a path from random chemical environments ("primordial soup") to replicating and evolving protocellular structures.

References

Segre et al. (2000): Compositional genomes: Prebiotic information transfer in mutually catalytic noncovalent assemblies. P Natl Acad Sci USA 97 (8):4112-4117

Markovitch and Lancet (2012): Excess Mutual Catalysis Is Required for Effective Evolvability. Artif Life 18 (3):243-266

Markovitch O, Lancet D (2014): Multispecies population dynamics of prebiotic compositional assemblies. J. Theor. Biol. 357: 26-34

Gross R, Fouxon I, Lancet D and Markovitch O (2014): Quasispecies in population of compositional assemblies.

Wednesday, time: 10:15-10:45

The effect of codon usage bias on the success of horizontal gene transfer

Tamir Tuller, Laboratory of Computational Systems Biology, Tel Aviv University, Israel
tamirtul@post.tau.ac.il / tamirtul@gmail.com

Abstract

Horizontal gene transfer (HGT) is a central determinant of prokaryotic evolution. It was demonstrated that organisms with similar codon bias tend to be involved in more HGTs. Thus, dissimilarity in codon bias seems to be a central barrier to HGT. I will review the mechanisms by which codon bias contributes to the success of HGT. I will also discuss the possibility that frequent HGT contributes to codon bias similarity within a microbial community, and the ramifications of these associations.

Wednesday, time: 11:15-11:45

Cell membranes as a precondition of spreading of the first RNA organisms

Armen Y. Mulkidjanian, School of Physics, Osnabrueck University, 49069, Osnabrueck, Germany; School of Bioengineering and Bioinformatics, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Russia
amulkid@uos.de

Abstract

The recent years showed a great progress in reconstruction of chemical conditions under which spontaneous formation of nucleotides and their polymerization could take place.

It was demonstrated that nucleotides could spontaneously form and even join into RNA oligomers at high levels of formamide [1-5]. Owing to the high boiling temperature of simple amides of about 200ºC, formamide could selectively accumulate in evaporating environments, so that Benner and co-workers have suggested the synthesis of the first biomolecules in primordial deserts [4]. Even more conducive should have been anoxic geothermal fields over vapor-dominated geothermal systems; geothermal vapor is particularly enriched in organic molecules, phosphorous compounds, ammonia and catalytic transition metals [6,7]. However, while the first replicators may have emerged in specific amide-rich environments, the vast majority of fresh-water and marine basins at the primordial earth would have low levels of amides, if any. It would be argued that the emergence of membrane-encased, cell-like RNA organisms may have been driven by their foray into new, amide-depleted habitats.

References

1. Schoffstall, A. M. (1976) Prebiotic phosphorylation of nucleosides in formamide. Orig Life 7, 399-41
2. Saladino, R., Crestini, C., Costanzo, G., Negri, R., and Di Mauro, E. (2001) A possible prebiotic synthesis of purine, adenine, cytosine, and 4(3H)-pyrimidinone from formamide: implications for the origin of life. Bioorg Med Chem 9, 1249-1253
3. Powner, M. W., Gerland, B., and Sutherland, J. D. (2009) Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions. Nature 459, 239-242
4. Benner, S. A., Kim, H. J., and Carrigan, M. A. (2012) Asphalt, water, and the prebiotic synthesis of ribose, ribonucleosides, and RNA. Acc Chem Res 45, 2025-2034
5. Saladino, R., Botta, G., Pino, S., Costanzo, G., and Di Mauro, E. (2012) Genetics first or metabolism first? The formamide clue. Chem Soc Rev 41, 5526-5565
6. Mulkidjanian, A. Y., Bychkov, A. Y., Dibrova, D. V., Galperin, M. Y., and Koonin, E. V. (2012) Origin of first cells at terrestrial, anoxic geothermal fields. Proc Natl Acad Sci U S A 109, E821-830

7. Mulkidjanian, A. Y., Bychkov, A. Y., Dibrova, D. V., Galperin, M. Y., and Koonin, E. V. (2012) Open questions on the origin of life at anoxic geothermal fields. Orig Life Evol Biosph 42, 507-516.

Wednesday, time: 11:45-12:15

Evolution of NAD biosynthetic pathway: from prebiotic synthesis to extant pathway diversification

Leonardo Sorci, Marche Polytechnic University, Ancona, Italy
l.sorci@univpm.it

Abstract

Cofactors are ubiquitous, non-protein organic molecules required by many enzymes to catalyze reactions that are otherwise inefficient or impossible for typical amino acids. In cellular metabolism, the same cofactor is usually shared by enzymes with different substrate specificities; for example, Nicotinamide Adenine Dinucleotide (NAD) is used as electron carrier by several cellular dehydrogenases capable of oxidizing and reducing a large array of substrates. Evidence has also been provided that some cofactors, including NAD, behave as autocatalytic molecule, i.e. they are able to support their own synthesis. All these features speak in favor of an ancient metabolic history rooting into prebiotic chemistry. Different scenarios have been described as to the origin of nicotinamide coenzyme. It has been speculated that pyridine coenzymes might be fossils of an "RNA world" in which they were synthesized by ribozymes that used them as cofactors to perform redox reactions. A different scenario involves the "peptide world" where a population of stochastic polypeptides was produced by prebiotic chemistry, without the help of RNA. In such environment, prebiotic peptides might have served as catalysts for the formation of the first coenzyme-like molecules, giving rise to a "Peptide-Cofactor world" with improved catalytic efficiency. The polinucleotide or polipeptide-assisted NAD synthesis scenarios are in contrast with the "surface metabolism" theory which features an original hypothesis with a fully prebiotic pyridine nucleotide synthesis.

In modern metabolism, although the reactive portion of the coenzyme is just represented by the pyridine ring, the entire pyridine dinucleotide is the only form of the NAD(P) cofactor known to assist redox reactions. One line of my research focuses on evaluating the hypothesis that, in early evolutionary time of NAD synthesis, smaller portions of the pyridine dinucleotide might have served as ancestral pyridine redox coenzymes. Experimental conditions are presented in which both amidated and deamidated precursors of NAD as we know from current metabolism can be chemically reduced in vitro, yielding molecules with similar chemical and spectroscopic properties as the reduced form of NAD cofactor (NADH). Moreover, NAD-dependent oxidoreductases are found being capable of using NMN as a cofactor as well, although less efficiently. This evidence also speaks in favor of a primordial role of NMN as a coenzyme of proto-enzymes. The existence of pyridine mononucleotide as ancestral pyridine coenzyme is discussed in the context of the current evolutionary scenarios of nicotinamide coenzyme synthesis.

I will also describe early studies, including phylogenetic and comparative genome analyses, highlighting the impact of horizontal gene transfer events in the evolution of NAD biosynthetic pathway and proving how certain bacteriophages have retained some NAD genes to assist their propagation.

Wednesday, time: 12:15-12:45

Redox and proton-motive homeostasis

John Allen, University College London, UK
j.f.allen@ucl.ac.uk

Abstract

Two fundamental properties of life as we recognize it are homeostasis and vectorial redox chemistry. Redox homeostasis is defined as the maintenance of a constant electrochemical potential and ionic concentration gradient across a cellular boundary, despite fluctuations in the electrochemical potential of the external environment and despite changing identities and activities of electron donors and acceptors. I propose that redox homeostasis accompanied vectorial metabolism in the geochemical incubators of Earth's first living cells. Mechanisms securing pre-biotic redox homeostasis are considered. The transition to free-living cells depended on the proto-cells' ability to maintain a proton motive force that was regulated and sustained within limits set by it own chemical preconditions. The proton motive force remains today a common intermediate in transport and energy conversion, providing for metabolism, for active transport, and for synthesis of informational and structural macromolecules. Life became independent of geological sources of free energy with the origin of photosynthesis, which was initially anoxygenic. Free molecular oxygen came later, with the advent of oxygenic photosynthesis. Photosynthetic oxidation of water to yield atmospheric oxygen may have emerged from failure of a redox switch that maintains redox homeostasis in anoxygenic photosynthetic bacteria. The redox switch is predicted to function still in photosynthetic bacteria adapted to special environments where it selects between different anoxygenic photochemical reaction centres according to changing external redox conditions. Any exoplanet or moon with detectable atmospheric free oxygen is likely to have made these transitions independently of the emergence of life on Earth.

Wednesday, time: 13:45-14:30

Constructive approach for the transition from non-living to living

Keynote speaker: Tetsuya Yomo, University of Osaka, Japan
yomo@ist.osaka-u.ac.jp

Abstract

Although comprehensive understandings are still missing on the origin of bio-molecules, it is evident that polynucleotides, proteins and lipids assembled before reaching the last universal common ancestor. Is a simple assembly of the bio-molecules into micro-scaled compartments able to evolve a life-like complex network? We encapsulated RNA and other factors into lipid vesicles and emulsions, in which the information on the RNA was translated into RNA replicase, which in turn duplicated the original RNA. In a long-term passage experiment, the artificial cell model evolved two-order acceleration on its gene replication. Of interest was that the two short circuits, double-stranded RNA and short parasitic RNA, emerged at the beginning of the passage experiment, but were suppressed through the evolution.

Wednesday, time: 14:30-15:00

tRNA core Hypothesis: A new model for origin of the biological system

Savio Torres de Farias, Universidade Federal da Paraiba, Laboratório de Genética Evolutiva Paulo Leminsk, João Pessoa, Paraíba, Brazil
stfarias@yahoo.com.br

Abstract

The origin and evolution of biological systems encourages discussions in the various fields of science. This model proposes a scenario for the origin and evolution of biological systems, where molecules of proto-tRNAs began the process giving origin to the first proto- mRNAs and proto-rRNAs, and thus, gave origin to a primitive translation system, which acted as attractor for the organization of the system as a whole. Were reconstructed the ancestor sequence for all types of the tRNAs and this ancestor sequences were compared with the modern proteins and with the Peptidyl transferase center of the Ribosome. When the ancestor sequences of the tRNA were translated, we found similarities with the essential proteins in the energetic metabolism, translation, lipids metabolism, among other. With the Peptidyl transferase Center, we found a similarities of the 52% between this portion of the ribosome and the ancestor sequence of the tRNAs. This data suggested that the tRNAs were the molecule that organized the translation system and consequently, the biological system. In this work, we showed based in the recent data a new model for origin of the biological system.

Replaced in favour of Dr Judit E Sponer

Paradigm Shift Hypothesis: A case for RNA networks influencing life on Earth

Sohan Jheeta, NoR HGT & LUCA, 1 Scott Hall Crescent, Leeds LS7 3RB, UK
Sohan7@ntlworld.com

Abstract

In an historic context, genetic inheritance was thought to be carried by proteins, although this was never conclusively proven, nor is it likely to be. The breakthrough came in 1953 when Watson and Crick discovered that hereditary coding was actually contained in the series of bases, namely adenine, thymine, guanine and cytosine which are strung together to form a DNA molecule. This permanently imbedded the idea of the existence of DNA/protein life forms on Earth in the scientific community (Crick, 1968., Poole *et al*., 1998, Bada, 2004). Current literature and research would seem to 'rubber stamp' this position. How true is this credo, that all life forms are DNA/protein and not RNA/protein centred? Much has been written about RNA, beginning with the speculation by Gilbert (1986) of an RNA world, where RNA life forms may have inhabited the Earth and also that RNA may have dual properties - acting both as a genetic information carrier as well as a catalyst (ribozyme); this was eventually confirmed independently by both Altman (1989) and Cech (1981), joint Nobel Laureates in chemistry (1989). Further, Poole *et al*., (1998) hypothesised the presence of pre-RNA and, Lezcano and Forterre (1999) speculated the existence of an organism called the last universal common ancestor (LUCA). During the pre-RNA, RNA and LUCA eras, rudimentary proteins were also present and thus it was that the LUCA was an RNA/protein organism. There is ample evidence from various phylogenetic studies that there would have been a LUCA which was present at the dawn of the first emergence of the three domains of life, namely Archaea, Bacteria and Eukarya (Woose, 1990). However, the concept of the possibility of RNA/protein 'dominated' life forms remained consigned to the history of the LUCA epoch and the allure of DNA/protein became so entrenched in the scientific psyche that RNA/protein has never been considered as a serious contender for the controlling of life forms on Earth.

With the continued unearthing of new roles for both DNA and RNA – eg the activities of non-coding portions of these nucleic acids – it is now becoming clear that there are no such things as 'junk' nucleic acids; everything in the cell has a part to play and is sooner or later utilised or activated in one way or another. This is particularly true of RNA as the growing list of its functions, from acting as a simple co-enzyme to a macro-machine as in the case of ribosomes, clearly demonstrates. Moreover, studies on *Oxytricha trifallax*, show that RNA is still very much in control of that particular ciliate (Swart, 2013). In addition to highlighting the relevance of findings relating to RNA, this oral presentation will put a case for a paradigm shift in favour of cellular life forms being organised and controlled by RNA/protein.

References

1) Watson, J. D and F. H. C. Crick (1953). "A structure for deoxyribose nucleic acid." Nature. **171:** 737–738

2) Altman, S., M. F. Baer *et al*., (1989). "Catalysis by the RNA subunit of RNase P—a minireview." Gene. **82 (1):** 63–4. doi:10.1016/0378-1119(89)90030-9.
3) Cech, T. R., A. J. Zung *et al* (1981). "In vitro splicing of the ribosomal RNA precursor of Tetrahymena: Involvement of a guanosine nucleotide in the excision of the intervening sequence." Cell. **27:** 487–496.
4) Crick, F. H. C., (1968). "The origin of the genetic code." Journal of Molecular Biology. **38:**367-379
5) Pool M. A., D. C. Jaffares *et al*., (1998). "The path from the RNA world." Journal of Molecular Evolution **46:**1-17
6) Bada J. L. (2004). "How life began on Earth: a status report." Earth and Planetary Science Letters. **226:** 1-15
7) Gilbert, W. (1986). "The RNA world." Nature. **319:** 618
8) Lazcano A., P. Forterre (1999). "The molecular search for the last common ancestor." Journal of Molecular Evolution. **49:** 411-412
9) Woese C. R., O. Kandler *et al*., (1990). "Towards a natural system of organisms: proposal for domains Archaea, Bacteria and Eukarya." Proceedings of the National. Academy of Sciences. **87**: 4576-4579
10) Swart E. C., J. R. Bracht et al (2013). "The *Oxytricha trifallax* macronuclear genome: A complex eukaryotic genome with 16,000 tiny chromosomes." PLOSBiology. **11(1)**: e1001473

Additional Submitted abstracts

The Cell Survival Pathways of Ancient Unicellular (Amoebae; Choanoflagellates) and Multicellular (Amphioxus; Spongiae) Eukaryotes Were Conserved in Their Extant Descendants and Serve as Proto-oncogenes in Vertebrate Hosts

Joseph G. Sinkovics, Professor; Senior Scientific Medical Advisor, St. Joseph Hospital's Cancer Institute Affiliated with the H.L. Moffitt Comprehensive Cancer Center; Department of Molecular Medicine, The University of South Florida Morsani College of Medicine, Tampa, FL USA
Sinkovi.Joseph@baycare.org

To be presented at Symposia on Cancer Research "Illuminating Genomic Dark Matter" at The University of Texas M.D. Anderson Cancer Center, Houston TX on October 9-10 2014

Abstract 1

A morphological and biochemical comparison was carried out in various laboratories and libraries on the cell survival pathways (CSP) in use in stressed uni- and multicellular proto-vertebrate eukaryotic organisms, and the proto-oncogenic pathways of vertebrate, especially mammalian, hosts (prominently including Homo). Malignantly transformed cells of murine and human derivation were studied for various stages of stagnation (including autophagy and senescence) versus rapid replication, invasive amoeboid locomotion, trans-speciation from epithelial into mesenchymal configurations and vice versa. Immunological studies on concealment versus expression of native or endogenous retroviral antigens, and on the susceptibility versus resistance to cytolytic lymphocytes (with special attention to NK cells) were performed. Of unicellular eukaryotes, the kinetoplastid Trypanosoma, the amoeba Naegleria, and the schizonts of Theileria acted very similarly to cancer cells in parasitizing their hosts. The taxon of Dictyostelia amoebae is basal to Fungi and Metazoa. The cell cycle of *Dictyostelium discoideum* could overrule a retinoblastoma-like suppressor. Trypanosoma cells rapidly replicating in liquid media cap their telomeres after each cell division, thus escaping senescence and natural death. DNA complexes of the ciliates Oxytricha and Stylonychia assume G-4 quadruplex formations similarly to the oncogene c-myc in mammalian (human) cells. Descendant extant unicellular eukaryotes operate the systems spliceosomes, ribosomes and argonaute (the RISC, microRNA-induced gene-silencing complex) for transcriptional regulation of protein synthesis. The ancestral Hsp, (heat shock proteins) that appeared first in the thermostable archaea, *Archaeoglobus fulgidus*, are functional in all descendant eukaryotic cells for chaperoning vital (and viral) proteins. In tumor cells of vertebrate mammalian hosts, oncoproteins became the clients of Hsp. The ancient high mobility group (HMG), arginine methyltransferase (RMT), and Ki-67 proteins, and proliferating cell nuclear antigens, originally inducers of physiological DNA replication, became the drivers of cell divisions in malignantly transformed advanced mammalian cells. In the sea, cytoplasmic ATP-binding ABC-like proteins of toxic Symbiodinium xantella algae pump out toxins from the toxin-producer cells. Very similar ABC-proteins are used in cancer cells to gain chemotherapy resistance. The cnidarian sea anemone *Nematostella vectensis* delivers

β-catenin intranuclearly, and activates the tcfTCF/lefLEF pathways (way before the appearance of lymphocytes). This pathway functions as constitutive proto-oncogenes in vertebrate mammalian cells (colon cancer cells, etc). The cnidarian *ur*-miR-100 remains conserved in all vertebrates up to Homo. Members of the tax on Ciona (*C. intestinalis*) transform from swimming tadpoles into tubular organisms fixed to the bottom of the sea; they have diverged early from the lineages advancing toward true vertebrates (fish, reptilia, aves, mammals). The Ciona genome/proteome operates pathways for to-be proto-oncogenes (MAPK, E26/ETS, JAK/STAT, tcfTCF/lefLEF, Wnt with β-catenin, Hedgehog), and utilizes FGF-L-to-R circuitries. The alloreactive Botryllus colonies harbor the first ancestral NK cells. On dry land, the drivers of virulence factors of the plant-pathogene oomycete *Phytophthora infestans* are the MAPK/ERK, and PI3K/Ras CSP. Phytophthora cells perform gene and cell fusions to gain "hybrid vigor". Special attention is given to the proto-oncogene/oncoprotein complexes myb/Myb in the Trichomonas; pak/PAK in the choanoflagellates (that malignantly transforms mammalian cells upon transfection); and the circadian rhythm proteins CREB/ETS (cAmp-response elements; E26) of the fungus *Neurospora crassa*, showing up in the human tumor pinealoma, which receives light-stimuli from the retina. Back in the sea, the ancient genes achaete scute encoded the first nervous system in the protochordate amphioxus (represented by the *Branchiostoma floridae*). The human homolog protein ASH trans-speciates adenocarcinoma cells into neuroectodermal cells, and primarily encodes esthesioneuroblastomas of the olfactory ganglion. The homolog of the human B lymphocytes' Bruton kinase is functional in the Adriatic demosponge *Suberites domuncula*. In the human B lymphocyte, its loss-of-function mutation causes agammaglobulinemia, and its gain-of-function mutation results in malignant lymphomas (chronic lymphocytic leukemia; mantle cell lymphoma). Conclusion: The CSP encoded by the ancient RNA/DNA complex enabled the first protocells to establish life on Earth. The descendant extant RNA/DNA complex conserved this faculty, where-of it can individualize and re-immortalize some selected cells in highly differentiated multicellular hosts. These cells eventually kill their host and die within it. Under circumstances favorable to them (in tissue cultures; in xenografts), these cells prove their immortality. They so far failed to show if they can regenerate into a new organized community, or if they can live individually in nature(*).

Reference

*) Fully referenced and illustrated in JG Sinkovics: RNA/DNA & CANCER, Springer Verlag, 2014, in print

THE CELL SURVIVAL PATHWAYS HAVE BECOME THE PROTO-ONCOGENES

Joseph G. Sinkovics MD, Department of Molecular Medicine, The University of South Florida Morsani College of Medicine; St. Joseph's Hospital's Cancer Institute Affiliated with H.L. Moffitt Comprehensive Cancer Center, Tampa FL
Sinkovi.Joseph@baycare.org

Abstract 2

Aims

A morphological and biochemical comparison was conducted in various laboratories and libraries between the evolutionarily conserved "cell survival pathways" of extant eukaryotic cells and proto-oncogenes of malignantly transformed human and murine cells. Questions to be answered were if extant unicellular eukaryotes cap their telomeres after each cell divisions to escape senescence and to prolong life. If heat shock proteins (originating in Archaeoglobus fulgidus) chaperon life-line proteins. If high mobility group proteins bind nuclear DNA to induce accelerated mitoses. If arginine methyltransferases silence suppressor genes of cell divisions. If intranuclear Ki-67 proteins accumulate in rapidly replicating cells. If G4 DNA quadruplexes or fused genes formed in order to gain virulence. If cytoplasmic ATP-binding ABC-like proteins of toxic zooxanthella algae expel toxins from cancer cells for resistance to chemotherapy. The extant eukaryotic cells answered affirmatively to this inquiry.

Methods

Native murine and human tumor cells were observed for stagnant persistence versus rapid replication; induction of neovascularization; various microenvironmental changes including dissolution of matrix; antigenic concealment; endogenous retroviral expression; killing of, or succumbing to, attacking lymphocytes (with special attention to NK cells); mobilization of host lymphoid, myeloid, or monocyte-macrophage lineages; invasiveness by amoeboid locomotion; fusions with host lymphocytes and monocytes, or with genetically mutated other host cells; invasion of blood or lymph vessel cell walls; presence in the circulating blood or lymph; formation of metastases in lymph nodes or parenchymal organs. Murine and human malignantly transformed cells were maintained in long term cultures. Natural hybridoma formation was recognized in vivo and reproduced experimentally.

Results

The descendant extant unicellular eukaryotes operate spliceosomes, ribosomes and the argonaute organelles for pre-, trans-, and posttranscriptional regulation of protein synthesis by micro-siRNAs. Most cells retained functional mitochondria and/or chloroplasts. Trichomonas harbors a Myb gene related to a human counterpart. Giardia is driven by PI3K. The crawling amoeba uses the same cytoskeletal genes/proteins that drive the invasive cancer cell. Hydractinia possess an ancestral form of the human stem cell gene Oct, which can transform their epithelial cells into tumors. The Tetrahymena yields active ribozymes and displays tRNA/sRNA configurations similar to those of prostate cancer cells. The ciliates Oxytricha and

Stylonychia exhibit telomeric G4 DNA quadruplexes similar to those of the human proto-oncogene c-Myc. Trypanosoma replicating rapidly in liquid media re-cap their telomeres. The cnidaria Nematostella operates Wnt, Hedgehog, TCF and beta-catenin pathways in its ontogenesis and stress. The urochordate Ciona adds the Jak/STAT, TGF (with its opponent SMAD) and MAPK pathways and the natural killer homeobox protein (Nkx) with homology to thyroid transcription factor (TTF-1) of the mammalian genome.

Conclusions

The choanoflagellate Monosiga harbors the pak/Pak (p21-activated kinase), which transforms mouse or human cells upon its transfer. The cell cycle of Dictyostelia can overcome a retinoblastoma-like suppressor. The Adriatic demosponge Suberites domuncula expresses the ancestor of the leukemogenic human Bruton kinase proto-oncogene. The Branchiostoma amphioxus (B. floridae) harbors the first nervous system encoded by the achaete scute genome. In the human host, the achaete genes transspeciate cancer cells and primarily induce esthesioneuroblastoma of the olfactory ganglion. The circadian rhythm proteins of Neurospora crassa show up in the human pinealoma. By cell fusion, the proto-oncogene-loaded oomycete Phytophthora gaines "hybrid vigor". The elegant caenorhabditis has a lethal vulvar cancer. The clam Mya arenaria develops hemolymph cell leukemia. These, and the drosophila leukemia and brain tumor (glioma) express oncogenes deriving from cell survival pathways of their predecessors. Naegleria transspeciates itself; Theileria immortalizes bovine lymphoblasts. An ancient scenario opened up for Professor Preisz when he observed the invasion of his bacterial cultures with Pettenkoferia protozoa. There were no protozoa. LiCl in the media induced spheroplasts and a bizarre endless mutational cascade of the bacterial cells without killing them. The living cell, and its RNA/DNA complex within, will resist environmental provocations with a vigor of transformations, in order to secure its survival in any environment and in any form. In the multicellular host these transformations may redirect the individual cell to the life style of its original singular ancestors, which secured the survival of unicellular organisms over three billion years, in the largest biomass on Earth. The RNA/DNA complex preserved their inherent faculty to re-transform.

This abstract is to be presented at the Annual Scientific Conference of the Hungarian Medical Association of America in Sarasota FL on October 31 – November 7 2014. It will be printed in the Archives of the Hungarian Medical Association of America, issue October 2014. Abstracted from the volume Sinkovics JG: RNA/DNA & CANCER (Springer Verlag, 2014 in print)