

Designed by Dr Sohan Jheeta

HORIZONTAL GENE TRANSFER (HGT) AND THE LAST COMMON UNIVERSAL ANCESTOR CONFERENCE, 5-6 SEPTEMBER 2013

Motivation for the Conference

Probably from its very beginnings, humankind has wondered about where we come from and whether there is life elsewhere in the Universe. Still, we do not have an answer to either of these questions, but we are now provided with unprecedented opportunities to explore and investigate that have given rise to the new scientific disciplines of synthetic biology, bioinformatics, systems biology and, and not forgetting astrobiology, aiming to finally solve this long-standing mystery of our very own existence.

Our knowledge of the diversity of life on Earth has increased significantly since the discovery of the domain of Archaea by Carl Woese in the mid 1970s; we now have a better understanding of the extreme conditions under which life could possibly have originated and evolved. Life has been found in the most inhospitable and extreme places imaginable on Earth. For example micro-organisms have been found in arid deserts, permafrost, within rock pores and between aggregate grains, hot springs, deep in the ocean in the vicinity of black smokers, in extreme alkalinity, acidity, in the vicinity of nuclear reactors and the outer most reaches of the Earth's atmosphere – there appear to be few regions within which life cannot exist.

How did life gain a foothold on Earth? Currently there are two hypotheses to explain this monumental event, namely metabolism or genetics first hypotheses. The 'metabolism first hypothesis' does not necessarily form part of our discussion at this meeting, since it can be taken as read that molecules which control the future outcomes of potential living entities have already been synthesised. Therefore, genetics is the main topic of this conference. Moreover, genetics being the controller of metabolisms, are preserved in all life forms. These manifestations of the 'controller' molecules can be observed in the unchanging biochemistry of life, even if the controller itself mutates, the molecules of life and the pathway in which they are involved, do not.

What were the initial controller molecules? There are three possibilities; either DNA or RNA, or could there have been another unknown precursor molecule – such as peptide nucleic acid (PNA)? We don't know much about PNA because it is part of the initial chemistry and as such is not preserved and is lost for all eternity; the best we can do is speculate on PNA's properties. Neither could it be DNA, because the hydroxyribose sugar in the DNA is not made *de novo* as it is made from an existing sugar molecule, ribose; similarly, DNA can be made from RNA if the uracil in RNA is replaced with thymine nucleobase. Some people believe DNA arose at or during the first branch of the phylogenetic tree of life and that it arose twice independently. That is, DNA emerged after the appearance of RNA, so it seems that RNA must have been dominant during the LUCA era. Some circumstantial evidence can be gleaned from the tree as well as from viruses present at the time of LUCA - in particular the RNA viruses. With this in mind, it is believed that RNAs were involved in HGT and thus hold clues to the understanding of genetics during the period of LUCA.

Moving on to the process of HGT, the mechanisms by which the transfers occurred are as follows: transduction, conjugation, transformation, gene transfer agents (GTA) and membrane vesicle transfer (MVT). It was these mechanisms which reorganised the genetics in the LUCA and ultimately led to the emergence of the three domains as represented by the phylogenetic tree. Moreover, the LUCA epoch was the time of unprecedented levels of gene transfer. In this meeting, a range of pertinent issues will be addressed which relate to the formation of the three main domains. Broadly, there will be discussions on the formation of RNA (Prakash Joshi), through to

making the LUCA an un-necessary explanatory tool for evolutionary theory (Michael Syvanen; Nigel Goldenfeld; Thomas Beatty). Others will highlight the mechanism of HGT (Matxalen Llosa; Duccio Medini; David Dryden). Redox chemistry and complexity feature in other presentations (Armen Mulkidjanian; John Allen; Addy Pross) and the origin of domains is a further theme (Filipa Sousa; Vinod Gupta; Sohan Jheeta). Finally the importance of the formation of membranes is the focus of the talk by Jonathan Lombard. More importantly, since this meeting is the first of its kind, it should, in this instance, serve as a platform for future conferences/workshops and will be a means to disseminate information relating to the period when it is promulgated that LUCA was the predominant potential living organism. In addition such studies may lead to the better understanding of the LUCA and it may bring to light new ways of viewing early pre-chemistry and chemistry of LUCA and the emergence of the three domains.

Formation of the Horizontal Gene Transfer Society

Currently this society is in embryonic stages and thus this conference is also seen as the platform for the launch of the Horizontal Gene Transfer Society (HGTS). It is hoped that all participants will support this endeavor and encourage like minded individuals to do the same. The mission statement of this new society will be *to advance the developments in this arena and to study and disseminate research information pertaining to the chemical evolution of the Last Universal Common Ancestor (LUCA), its emergence and its evolution to the point of the advent of the three domains of life.* We would like to hear your opinions and comments on the formation this new society in the open discussion at the end of the conference.

Sohan Jheeta Martin Dominik Nigel J Mason

International Scientific Committee Members

Prof Christopher P McKay	NASA Ames, Moffett Field, California, USA
Prof Michael Russell	Jet Propulsion Laboratory, Pasadena, California, USA
Prof Patrick Forterre	Institut Pasteur, Paris, France
Prof John F Allen	Queen Mary, University of London, UK
Prof Robert Pascal	Institut des Biomolécules Max Mousseron, Université Montpellier. France
Prof Eugene Koonin	NCBI, NLM, NIH, Bethesda, Maryland, USA
Prof Armen Mulkidjanian	University of Osnabruck, Germany
Prof Prakash Joshi	Rensselaer Polytechnic Institute, Troy, New York. USA
Prof Addy Pross	Ben-Gurion University of the Negev, Beersheba, Israel
Prof Andrew Pohorille	NASA Ames Research Centre, California, USA

Local Organising Committee

Dr Sohan Jheeta Dr Martin Dominik Prof Nigel J Mason OBE Mrs Beverley Bishop

Conference Group Photo

This will take place on the afternoon of the Friday, 6th September.

Publications Arising from the Meeting

Horizontal gene transfer is an important process in modern biological systems, resulting in the spread of resistance genes among pathogens, and even gene sets for metabolic processes. There is also good evidence for ancient horizontal gene transfer events, indicating that the evolutionary history of genes within genomes is best understood in terms of networks. This special issue invites contributions that consider the extent to which horizontal gene transfer contributed to the early evolution of life on Earth. These contributions will be published in a special issue on Horizontal Gene Transfer in the journal: Life, a new Swiss-based open access journal with a focus on origins, evolution and astrobiology (http://www.mdpi.com/journal/life/special_issues/life-extremophiles). The deadline for submission is 28th February 2014. Dr Anthony Poole from the University of Canterbury, New Zealand and Dr Sohan Jheeta are to be co-guest editors. Please bear in mind that submissions would be subject to the usual peer review criteria.

Dr Anthony Poole's email: anthony.poole@canterbury.ac.nz

Link for the special issue: <u>http://www.mdpi.com/journal/life/special_issues/gene-transfer</u>

Schedule day 1

	Date: Thursday 5 th September			
00.20 10.00				
09:30-10:00	Arrival and registration			
10:00-10:15	Prof Nigel J Mason: The Open University Welcome			
10:15-10:30	Dr Sohan Jheeta Motivation for the conference and directions for the day			
	Chair: Dr Martin Dominik: University of St Andrews, Scotland			
10.30-11:15	<i>Keynote speaker</i> : Prof Thomas Beatty, University of British Columbia, Canada Gene transfer agents in prokaryotes: viral progenitors, or revamped phages?			
11:15-11:45	Coffee break			
11:45-12:15	Prof Matrolon Llose University of Contohnia Spain			
11.45-12.15	Prof Matxalen Llosa, University of Cantabria, Spain Bacterial conjugation: a protein secretion system adapted to DNA transfer, or <i>vice versa</i> ?			
12:15-12:45	Dr Jonathan Lombard, National Evolutionary Synthesis Center (NESCent), USA A systematic phylogenomic investigation supports the presence of phospholipid membranes in the cenancestor			
12:45-14:30	Lunch			
12.10 11.50				
14:30-15:15	Chair: Prof Nigel J Mason			
	<i>Keynote speaker</i> : Prof Armen Mulkidjanian, University of Osnabrueck, Germany/ Lomonosov University, Moscow, Russia. Energetics of the first cells and the Last Universal Cellular Ancestor (LUCA)			
15:15-15:45	Prof John Allen, Queen Mary, University of London, UK Coupling between information processing and redox chemistry. Cellular co-location of energy transduction with genome function			
15:45-16:15	Dr David Dryden, University of Edinburgh, UK Restriction and antirestriction systems in bacteria: their impact on HGT and the LUCA			
16:15-16:35	Coffee break			
16:35-17:00	Dr Søren Overballe-Petersen, Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen Natural transformation of bacteria by fragmented, damaged and ancient DNA			
17:00-17:30	Prof Vinod Gupta, C.M.D. Post Graduate College, India Photosynergistic collaboration of non-linear process at mesoscopic level and emergence of a minimal protocell-like photoautotrophic supramolecular assembly "Jeewanu" in a simulated possible prebiotic atmosphere			
17:30	Close of day 1			
10.00				
19:30	Conference dinner			

Schedule day 2

	Date: Friday 6 th September		
00.15.00.20	Chair: Dr Martin Dominik		
09:15-09:30	Mr Nabor Lozada, Heinrich-Heine University of Düsseldorf, Germany Metabolic transformation via gene transfer at the origin of higher taxa in Achaea		
09:30-10:15	<i>Keynote speaker</i> : Prof Addy Pross, Ben Gurion University, Israel Seeking the evolutionary roots of horizontal gene transfer		
10.15-10.45	Prof Prakash Joshi, Rensselaer Polytechnic Institute, USA Prebiotic RNA synthesis by montmorillonite catalysis: significance of mineral salts		
10:45-11:15	Coffee break		
11:15-12:00	<i>Keynote speaker</i> : Prof Michael Syvanen, University of California, USA Why LUCA is no longer needed as part of evolutionary theory?		
12:00-12:30	Dr Filipa L Sousa, Institute of Molecular Evolution, Heinrich-Heine-Universität Düsseldorf, Germany Genomic evidences for the methanogenic origin of archaea		
12:30-13:00	Dr Duccio Medini, Novartis Vaccines Research Centre, Siena, Italy Length-modulation of horizontal gene transfer explains contradictory population structure in pathogenic bacteria		
13:00-14:30	Lunch		
14:30-15:15	Chair: Dr Sohan Jheeta Keynote speaker: Prof Nigel Goldenfeld, University of Illinois at Urbana-Champaign, USA How a "collective LUCA" drove the rapid evolution of early life: clues from the canonical genetic code		
15:15-16:15	Open Discussion Prof Nigel Goldenfeld Prof Michael Syvanen Prof Addy Pross Prof Armen Mulkidjanian What part did HGT play during the evolution of LUCA leading to the three domains?		
16:15-16:30	Dr Sohan Jheeta Where do we go from here?		
16:30	Close of day 2		

Submitted Abstracts

Thursday, time: 10:30-11:15

Gene transfer agents in prokaryotes: viral progenitors, or revamped phages?

Hao Ding, Cedric Brimacombe, Alexander Westbye, and <u>J. Thomas Beatty</u> Department of Microbiology & Immunology, the University of British Columbia, jbeatty@mail.ubc.ca

Abstract

Diverse prokaryotes engage in a poorly-understood horizontal gene transfer (HGT) process mediated by phage-like particles called gene transfer agents (GTAs) which: contain random double-stranded DNA segments of the host cell genome; the DNA molecules packaged are of length insufficient to encode all the structural proteins of the particle; are controlled by cellular regulatory proteins; function solely for mediating genetic exchange.

A model GTA (RcGTA) is produced by the purple non-sulfur bacterium *Rhodobacter capsulatus*, and is encoded by chromosomal genes. Some RcGTA structural genes in R. capsulatus are related to phage genes, but the cellular regulatory mechanisms that control RcGTA production indicate that RcGTA is more than just a defective prophage. RcGTA is produced maximally in the stationary phase of laboratory cultures, but it was not known what aspect of stationary phase induces the expression of RcGTA structural genes. We discovered several independent pathways that affect the production of extracellular RcGTA particles. One pathway involves the response-regulator CtrA, which is essential for transcription of RcGTA structural genes. A second pathway transmits a quorum-sensing signal, produced by a homoserine lactone synthase, which is needed for maximal induction of transcription of RcGTA genes. A third pathway requires the sensor-kinase homologue CckA for maturation of RcGTA particles and release from cells. The mechanism of RcGTA particle release from cells had been puzzling for decades. We found that RcGTA particles are released from cells by lysis, but that only a small percentage of wild type cells induce RcGTA gene expression and go on to essentially commit suicide. The decision for turn-on of RcGTA genes appears to be controlled by a stochastic process involving changes in gene transcription, as opposed to a genetic modification.

Genome sequences show that most prokaryotes contain prophage-like gene clusters, but possible relationships to GTAs were unclear. We found that RcGTA-like gene clusters are widespread but found exclusively within the alpha-proteobacteria. A phylogenetic tree comparing 16S rRNA and RcGTA gene homologue sequences indicates that these two types of gene have evolved in concert. It appears that a single RcGTA ancestor arose in the alpha-proteobacteria after divergence of this line from other prokaryotes. The similarity of 16S rRNA and RcGTA-like protein sequence trees in present-day species indicates that the transmission of RcGTA-like genes was largely vertical, as for most cellular genes, with little or no HGT. We suggest that GTAs have arisen independently in multiple prokaryotic lines, and that at least RcGTA arose long after the initial divergence of prokaryotes into ancestors of present-day species.

Thursday, time: 11:45-12:15

Bacterial conjugation: a protein secretion system adapted to DNA transfer, or vice versa?

Matxalen Llosa IBBTEC – University of Cantabria, Santander, Spain <u>matxalen.llosa@unican.es</u>

Abstract

I will provide some insights into the molecular mechanism of bacterial conjugation, a major mechanism for horizontal DNA transfer among bacteria, and beyond. The current models for conjugative DNA transfer propose that a protein related to DNA replicases nicks and covalently binds to the transferred DNA strand, and this nucleoprotein complex would be transported through a multiprotein complex known as Type IV secretion system (T4SS). A coupling protein (CP) is proposed to mediate substrate recognition and DNA transfer through the T4SS. We propose that bacterial conjugation arose by merging a rolling-circle replication system with a T4SS. However, T4SS which are involved only in protein secretion seem to have derived from DNA transfer machineries. Thus, the ancestral T4SS may have been lost during evolution. The CP may be a key factor in the evolution of these secretion systems, as it interacts with both the secreted substrate and the T4SS with different specificities.

Thursday, time: 12:15-12:45

A systematic phylogenomic investigation supports the presence of phospholipid membranes in the cenancestor

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Abstract

Cells from the three domains of life (Archaea, Bacteria, Eucarya) are surrounded by lipid membranes of which the main component are phospholipids. The universality of this characteristic could let us think that the last common ancestor of living organisms (the cenancestor) was already surrounded by (phospho)lipid membranes. However, phospholipids have very different compositions from one lineage to another. Bacteria and eukaryotes have sn-glycerol-3-phosphate backbones linked to fatty acids through ester bounds, whereas archaeal phospholipids are made up of the opposite enantiomer, sn-glycerol-1-phosphate, linked to isoprenoid chains by ether bounds. These striking dissimilarities are mirrored by different metabolic pathways to synthesize the phospholipid components. Thus, some hypotheses proposed that the cenancestor had no lipid membranes and that phospholipid biosyntheses independently evolved twice in their respective lineages. This triggered a fascinating and hot debate about the cenancestral boundaries. Nevertheless, all the confronting hypotheses were generally proposed from the perspective of current debates on the origins of life rather than from the comparison of modern organisms, which is necessary to any natural definition of the cenancestor. The first attempt to describe an enzymatic mechanism that might have been involved in phospholipid synthesis in the cenancestor concerned the synthesis of the glycerol-phosphate enantiomers. It was observed that the two unrelated dehydrogenases responsible for this function belong to respective universal families. Each family was shown to have at least one representative in the cenancestor, and the cenancestral enzymes appeared to be less specific than modern enzymes. Thus, although these less-specific machineries were likely not to be devoted to this single function, each cenancestral enzyme could have unspecifically synthesized one of the two glycerol-phosphate enantiomers.

This promising result encouraged us to carry out a systematic phylogenomic analysis on the other metabolic pathways involved in phospholipid biosynthesis in the three domains of life. Our results showed that: (1) One of the metabolic pathways that synthesizes isoprenoid precursors is ancestral to all the three domains of life and, therefore, its presence can be proposed in the cenancestor. (2) Most bacterial fatty acid synthesis (FAS) genes have homologues in archaeal genomes and, based on biochemical information available in the literature, archaea were proposed to synthesize fatty acids through an ACP-independent FAS pathway. Since the bacterial and the hypothetical archaeal FAS are homologous and the phylogenies of their genes could not exclude their presence in the respective ancestors of each domain of life, the ACP-independent FAS was proposed to have been present in the cenancestor. (3) The presence of some enzymes involved in phospholipid assemblage was also inferred in the cenancestor.

Consequently, phylogenomic analyses support the plausible enzymatic synthesis of phospholipids in the cenancestor. The products synthesized by the cenancestral enzymes were probably not specific of the phospholipid synthesis but common to several pathways in the metabolic network, suggesting that modern differences among domains of life originated through specialization from more promiscuous enzymatic precursors.

Thursday, time: 14:30-15:15

Energetics of the first cells and the Last Universal Cellular Ancestor (LUCA)

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Abstract

We have reconstructed the 'hatcheries' of the first cells by combining geochemical analysis with phylogenomic scrutiny of the inorganic ion requirements of universal components of modern cells [1]. These ubiquitous, and by inference primordial, proteins and functional systems show affinity to and functional requirement for K^+ , Zn^{2+} , Mn^{2+} , and phosphate. Thus, protocells must have evolved in habitats with a high K^+/Na^+ ratio and relatively high concentrations of Zn, Mn and phosphorous compounds. Geochemical reconstruction shows that the ionic composition conducive to the origin of cells could not have existed in marine settings but is compatible with emissions of vapor-dominated zones of inland geothermal systems. Under anoxic, CO_2 -dominated atmosphere, the elementary composition of pools of condensed vapor at anoxic geothermal fields would resemble the internal milieu of modern cells.

The scientists who address the origin of life problem from a purely chemical viewpoint argue that specific formation of of biologically relevant molecules, such as activated, cyclic ribonucleotides with a potential for polymerization, could take place in formamide-rich solutions, particularly under the action of UV light and in the presence of borate and phosphorous compounds [2, 3]. The exhalations of even modern geothermal fields contain high amounts of ammonia, phosphate, borate and hydrocarbons [1], so that anoxic geothermal fields should have been conducive for formation of simple amides and nitrogen-containing organic molecules, including activated nucleotides. Hence, the anoxic geothermal fields, which we identified as tentative cradles of life by using the top-down approach and phylogenomic analysis [1], could provide exactly those geochemical conditions that were suggested as most conducive for the emergence of life by the chemists who pursued the complementary bottom-up strategy [2, 3].

The absence of any enzymes related to autotrophy in the ubiquitous protein set suggests that the first cells were heterotrophs, i.e., their growth depended on the supply of abiotically produced organic compounds. These compounds could form in the process of hydrothermal alteration of ultramafic rocks [4]; vapor should have delivered such organic molecules to the surface. Metal sulfides, which should have precipitated at anoxic geothermal fields [1], could (photo)catalyze not only the formation of organic molecules but also their interactions with nitrogen-containing compounds [5], providing the first cells with food and building material.

Phylogenomic analyses of several key energy-converting enzymes, such as diverse ATPases and GTPases [1], rotary membrane ATP synthases [6-8], and quinol:cytochrome c oxidoreductases [9] indicate that the evolution of bioenergetics seems to have followed the sequestration of protocells from the environment [10]. The K⁺ -dependence of many ubiquitous ATPases and GTPases [1] indicates that they emerged in K⁺-rich environments. The development of still primitive, but sodium-impermeable membranes, supposedly, at the stage of the LUCA, could prompt the emergence of Na⁺- outpumps, including the rotary membrane ATPases [6-8]. After obtaining such enzymes, the first cells could survive in ample habitats with K⁺/Na⁺ < 1, which may have ultimately led to the invasion of the

ocean by life forms. The more structurally demanding proton-tight membranes, which could accommodate proton-specific pumps, appear to emerge later, independently in bacteria and archaea [7,8].

Acknowledgements The support from the *Deutscher Akademischer Austausch Dienst, Deutsche Forschungsgemeinschaft*, the Russian Foundation for Basic Research (10-04-91331) and the Russian Government (02.740.11.5228) is appreciated.

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- 2) S.A. Benner, H.J. Kim, M.A. Carrigan (2012) Asphalt, water, and the prebiotic synthesis of ribose, ribonucleosides, and RNA, J. Am- Chem. Sci. 45: 2025-34
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- 10) E.V. Koonin, A.Y. Mulkidjanian, Evolution of cell division: from shear mechanics to complex molecular machineries, *Cell*, 152 (2013) 942-944.

Thursday, time: 15:15-15:45

Coupling between information processing and redox chemistry. Cellular co-location of energy transduction with genome function

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Abstract

Common ancestors of eukaryotes. CoRR: Co-location for Redox Regulation of gene expression. I propose that bacterial redox regulatory systems survive, in modified form, in both mitochondria and chloroplasts, where they made possible adaptive radiation from the common ancestors of, respectively, all eukaryotes, and then plants and algae. Redox regulatory systems connect electron carriers with DNA promoter binding sites. They have been refined by natural selection in order to provide coupling between information processing and redox chemistry. This coupling is necessary for life, and was present in the Last Universal Common Ancestor (LUCA) of all life. In eukaryotes, bioenergetic genes and gene products are transmitted from cell to cell, and from generation to generation, in cytoplasm, giving non-Mendelian, uniparental inheritance of characters central to energy transduction in photosynthesis and respiration.

Common ancestors of photosynthesis. The redox switch hypothesis for the first cyanobacterium predicts the existence of an anoxygenic photosynthetic bacterium retaining genes for both type I (photosystem I) and type II (photosystem II) reaction centres. These genes are never expressed at the same time. Instead, they are switched on and off in order to provide a versatile and flexible metabolism that allows the bacterium to grow either with or without hydrogen sulphide as the electron donor for photosynthesis. I propose that this phototrophic prokaryote flourished prior to the origin of the cyanobacteria to which it gave rise, and that its two modes of metabolism produced the laminar structure of ancient stromatolites. Stromatolites are known which date from the mid-Archean, before the signatures of free oxygen as banded iron formations and the cessation of massindependent sulphur fractionation. The precursor of cyanobacteria gave rise to oxygenic photosynthesis. Expression of type I reaction centre genes in the presence of hydrogen sulphide was accompanied by silent type II reaction centre genes. Type II reaction centre genes were themselves induced under non-reducing conditions, when type I genes became repressed. Loss of redox regulatory control of gene expression allowed co-existence of type I and type II reaction centres, with complementary functions. In place of hydrogen sulphide, the type II centre, as photosystem II, oxidised manganese, and then water. Electron transfer from water to photosystem II and photosystem I liberated oxygen, changing Earth's biology and geochemistry irreversibly, and forever.

Thursday, time: 15:45-16:15

Restriction and antirestriction systems in bacteria: their impact on HGT and the LUCA

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Abstract

DNA restriction-modification systems represent one of the largest groups of enzymes known and are found in over 50% of bacterial genomes where they maintain methylation of specific target sequences of the host DNA and destroy invading DNA lacking the appropriate methylation pattern. They represent a formidable barrier to horizontal gene transfer. This barrier can be circumvented using antirestriction-modification methods encode by the mobile element. I will summarise the most recent results on RM and anti-RM and discuss their relevance to the LUCA.

Thursday, time: 16:35-17:00

Natural transformation of bacteria by fragmented, damaged and ancient DNA

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Abstract

Release of DNA, from living and dead organisms, maintains an environmental pool of extracellular DNA, some of which can be thousands of years old. The degrading DNA is fragmented and damaged. Such DNA is only recognized as microbial nutrients and is not considered as direct contributors to bacterial evolutionary processes.

This PhD-study shows natural transformation by very short DNA (\geq 20bp) and further by short damaged DNA of the most common damage types in degrading DNA. This is emphasized with successful natural transformation by 43,000-year-old DNA. We find that the DNA integrates through a novel and simple variant of natural transformation. On top, we illustrate with full-genome comparisons that the integration process has general relevance in extant bacteria.

Our findings reveal that the large environmental reservoir of short and damaged DNA retains capacity for natural transformation, even after thousands of years. This describes for the first time a process by which cells can acquire functional genetic signatures of the deeper past. Moreover, not only can old DNA revert microbes to past genotypes, but degrading DNA can also produce new variants and combinations of already functional sequences.

The identified novel pathway of natural transformation represents a basal evolutionary process that only requires growing cells that feed on oligonucleotides; a process that possibly is a primeval type of horizontal gene transfer. In extension, our results also provide mechanistic support to hypotheses of horizontal gene transfer playing an important role in the early evolution of life.

Thursday, time: 17:00-17:30

Photosynergistic collaboration of non-linear process at mesoscopic level and emergence of a minimal protocell-like photoautotrophic supramolecular assembly "Jeewanu" in a simulated possible prebiotic atmosphere

Vinod Kumar Gupta, Department of Zoology, C.M.D. Post Graduate College, Bilaspur, India Email: <u>vkgcmd@gmail.com</u>

Abstract

Sunlight exposed sterilsed aqueous mixture of ammonium molybdate, diammonium hydrogen phosphate, biological mineral and formaldehyde shows photochemical formation of biomimetic, self-sustaining protocell-like assembly Jeewanu. The Jeewanu mixture have been found to show abiogenesis of various compounds of biological interest viz. amino acids in free as well as in peptide combination, nucleic acid bases as purines as well as pyrimidines, sugars as ribose as well as de-oxyribose and phospholipids-like material in them. The presence of various enzyme-like activities viz. nitrogenase, phosphatase, esterase and ATP-ase –like activities have been detected in the Jeewanu mixture (1). The EPR spectra of Jeewanu showed the presence of ferredoxin-like material in the mixture (2).Jeewanu can catalyse photolytic decomposition of water utilizing sunlight as a source of energy. Hydrogen thus produce is utilized in photochemical fixation of nitrogen and carbon dioxide (3).

The cytological and histochemical investigations revealed that they multiply by budding, grow from within by actual synthesis of materials and show various metabolic activities in them. The optical and scanning probe microscopes (SCM, TEM, AFM & Confocal) microscopes have revealed that they are blueish in colour and spherical in shape. Their microscopic examination revealed that they have a doubled walled boundary for charge separation and an intricate internal structure. IR, NMR and X-crystallographic investigations of Jeewanu showed their polymeric, heterogeneous and amorphous nature. Ultrafast laser induced flash photolysis studies in Jeewanu mixture showed the formation of photoproducts on exposure to 10⁻⁹ to 10⁻²⁰ sec.

In prebiotic atmosphere possibly photosynergistic collaboration of non-linear processes at mesoscopic level established autocatalytic pathways on mineral surfaces by selforganization, led to emergence of supramolecular photoautotrophic assemblies similar to Jeewanu which might have given rise to earliest energy transducing systems on earth or elsewhere.

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Friday, time: 09:15-09:30

Metabolic transformation via gene transfer at the origin of higher taxa in Archaea

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Abstract

Lateral gene transfer (LGT) has played a major role during the evolution of microbial metabolism and ecological traits across the three domains of life. LGT has often been detected among organisms within one of the three domains. Recent reports detect massive LGT across domains; for example, a recent report shows evidence of a massive LGT (~1000 genes) event from Eubacteria to the common ancestor of the Halophiles in Archaea (Nelson-Sathi et al., 2012). This lateral evolutionary event transformed the anaerobic autotrophic lifestyle of ancient methanogens into the aerobic heterotrophic lifestyle of haloarchaea. While haloarchaea lost their metabolic capabilities for methanogenesis -i.e., the autotrophic production of methane from different substrates: acetate, and carbon dioxide-, their close relatives formate. hydrogen (Methanosarcinales, Methanobacteriales, Methanocellales, Methanococcales, Methanomicrobiales) have remained methanogenic. These findings revealed other interesting questions, why this metabolic transformation only affected to haloarchaea and not to other methanogens? Is this due to the lack of a more massive LGT from Eubacteria and/or secondary losses in Archaea? Or is this due to the presence of different massive LGT events between Eubacteria and the methanogen ancestor? We are trying to answer these questions by assessing the impact of LGT from Eubacteria into the Methanosarcinales group, and thus to contribute to the understanding of the genomic origin and evolution of methanogenesis from their niches on an early anoxic Earth to current habitats.

Reference

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Friday, time: 09:30-10:15

Seeking the evolutionary roots of horizontal gene transfer

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Abstract

By examining the reactivity patterns of replicating molecules and the networks they establish, systems chemistry is beginning to fill the existing void between regular (non-replicative) chemical systems and inherently complex biological systems, an endeavor that is beginning to shine new light on the chemical roots of the evolutionary process. In this talk we will discuss recent advances in the area and explore how such studies may offer new insights into the chemical origins of HGT, its relation to vertical transmission, and its role within a more general evolutionary context.

Friday, time: 10:15-10:45

Prebiotic RNA synthesis by montmorillonite catalysis: significance of mineral salts

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Abstract

The dual properties of RNA as an enzyme catalyst and its ability to store genetic information suggest that early life was based on RNA and DNA & protein evolved from it. We have demonstrated synthesis of long RNA oligomers by Na⁺-montmorillonite catalyzed reactions of 5'end activated mononucleotides (Joshi et al., 2009). The Na⁺-montmorillonite not only catalyzes the prebiotic synthesis of RNA but it also facilitates homochiral selection (Joshi et al., 2011, 2013). The montmorillonite-catalyzed reactions of 5'-phosphorimidazolide of adenosine were further investigated to study the effect of mineral salts. These reactions were found to be dependent on the nature of mineral salts present. While montmorillonite (pH 7) produced only dimers in water, addition of sodium chloride (1M) enhanced the chain length of oligomers to 10-mers as detected by HPLC. Magnesium chloride (0.075M) produced a similar effect but the presence of both sodium chloride and magnesium chloride did not produce any difference in the oligomer chain length. The effect of monovalant cations in RNA synthesis was of the following order: $Li^+ > Na^+ > K^+$. A similar effect was observed with the anions, enhancing catalysis in the following order: $Cl^2 > Br^2 >$ I. Inorganic salts that tend to salt out organic compounds from water and salts which show salt-in effects had no effect in the oligomerization reactions indicating that the montmorillonite-catalyzed RNA synthesis is not affected by hydrophobic or hydrophilic interactions. A 2.3 fold decrease in the yield of cyclic dimer was observed upon increasing the sodium chloride concentration from 0.2M to 2.0M. Inhibition of cyclic dimer formation is essential for increasing the yield of linear dimers as well as the overall chain length. The results of this study show that the presence of salts is essential in prebiotic RNA synthesis catalyzed by clay minerals.

This research has been supported by NASA Astrobiology Institute grant NNA09DA80A.

- 1. P. C. Joshi, M. F. Aldersley & J. P. Ferris (2013) Progress in Demonstrating Homochiral selection in Prebiotic RNA Synthesis. *Advances in Space Research* 51, 772-779.
- 2. P. C. Joshi, M. F. Aldersley & J. P. Ferris (2011) Homochiral selectivity in RNA synthesis on montmorillonite-catalyzed reactions of D, L-purine and pyrimidine nucleotides, *Origins of Life and Evolution of Biosphere*, <u>41</u>, 213-236.
- 3. P. C. Joshi, M. Aldersley, J. Delano & J. P. Ferris (2009) Mechanism of montmorillonite catalysis in the Formation of RNA oligomers. *Journal of the American Chemical Society* <u>131</u>, 13369 13374.

Friday, time: 11:15-12:00

Why LUCA is no longer needed as part of evolutionary theory?

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Abstract

I am going to make the argument that horizontal gene transfer obviates the need for a postulated Last Universal Common Ancestor (LUCA). This hypothesis is based on the assumption that widespread horizontal gene transfer occurred during the period of the emergence of the very earliest forms of life and has occurred at a frequency sufficient to account for those gene found in life's three kingdoms.

In 1985, I postulated in a paper titled "Cross-species Gene Transfer; Implications for a New Theory of Evolution" (1) that the theretofore obscure phenomena of horizontal gene transfer provided the most compelling explanation for the unity of the genetic code. I hypothesized that the genetic code is uniform due to the fact that any organism or group of organisms that did not share in a common code would be at an evolutionary disadvantage caused by the loss of the genetic novelty available through HGT.

Building on this concept, I am putting forth the theory that we can account for the biological unities such as the unity of the genetic code without having to postulate the existence of a single ancestor from which all life descended. In fact, there is some evidence that during the period when the modern genetic code evolved, there could have been multiple forms of life with multiple codon variations which we would likely recognize as belonging to the three kingdoms of today. As new more efficient codon usages arose, and even as new amino acids evolved, these would have been spread to other life forms via horizontal gene transfer. I do not expect to find specific evidence against the theory that any single ancestor gave rise to all of life due to the self-evident fact that science cannot prove negatives. Rather, after consideration of a process that includes HGT, we will be left with the situation in which a LUCA is no longer required to account for current biochemical unities. The principle of parsimony suggests that a last common ancestor postulate is not necessary.

The evidence that I will present begins with a number of universal genes that are found in the three kingdoms that are not just unusually highly conserved but whose topologies, when deduced from their sequences, strongly suggest that they evolved after Archaea, Bacteria and Eukaryotes had already diversified. In other words, these universal genes appear to be younger than the taxonomic groups in which they are found today (2, 3). These genes include those that are involved in the biosynthesis of arginine and tryptophan as well as their respective tRNA ligases. I have proposed, therefore, that the modern genetic code was not established until well after the major kingdoms had already differentiated. The common ancestry of arginine and tryptophan are most easily explained by a widespread occurrence of HGT that originated from some unknown founders. Of course, we can easily come up with scenarios whereby a LUCA still existed but that it had a simpler genetic code than the modern one. This scenario cannot be rejected. However, we should remember that the existence of LUCA was established in the mind of science because of the existence of the universal genetic code. If we accept the evidence I presented here, we can see that LUCA does not explain the universal distribution of arginine and tryptophan. Once we begin to realize that

something this complex can spread by HGT there we can further conjecture that the entire genetic code evolved via this process. This conclusion has been supported by computer simulations (4).

This is not to question the common ancestry of many of life's genes; sequence homologies overwhelming support that idea. Rather we can account for the biological unities such as the unity of the genetic code without having to postulate the existence of a single ancestor that possessed those genes from which all life descended.

- 1) Michael Syvanen. 1985. Cross-species Gene Transfer; Implications for a New Theory of Evolution. J. Theor. Biol. 112: 333-343
- 2) Michael Syvanen. 2002. Recent emergence of the modern genetic code: a proposal. Trends Genet. 18 (5): 245-8
- 3) Michael Syvanen. 2002. On the Occurrence of Horizontal Gene Transfer among an Arbitrarily Chosen Group of 26 Genes. J. Mol. Evol. 54:258-266. (Papers 1, 2 and 3 are available as pdfs as papers 1, 9 and 10 respectively at <u>http://www.vme.net/hgt/</u> and the material in this talk was summarized in paper 15)
- 4) Vetsigian K, Woese C, Goldenfeld N. 2006. Collective evolution and the genetic code. *Proc. Natl. Acad. Sci. USA* 103:10696–701

Friday, time: 12:00-12:30

Genomic evidences for the methanogenic origin of archaea

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Abstract

In spite of their metabolic diversity and ubiquitous distribution, Archaea remains the most obscure domain of life. This domain consists of 5 phyla whose ancestry is still highly debated. From a phylogenetic point of view, for instance, the root of Archaea closer to the nanoarchaea phylum, proposed by Waters et al [1], was challenged and attributed to a long-branch attraction artifact instead [2]. Moreover, some studies support a methanogenic (Euryarchaeota phylum) origin of this domain [3]. Independent of this and based on the chemical proprieties of the vents, Lane and Martin [4] put forward a methanogenic origin of Archaea having the Wood-Ljungdahl pathway as the universal carbon fixation pathway between the two prokaryotic domains.

Microbiologists agree that the nature of the carbon metabolism of the extinct primordial organisms is a critical question to understand the origins of life [5,6]. Central to core carbon metabolism is C1 chemistry involving folate and its structural analog methanopterin. Could it be that an imprint of early chemistry is preserved in the C1 metabolism of modern organisms? We won't know unless we look, and genomes harbour abundant information.

By studying the distribution and frequency of the enzymes for methanopterin and folate biosynthesis within sequenced genomes, we found a clear dichotomy between the archaeal and bacterial pathways. The genomic data provides evidence in favor of the view that methanogenesis is the ancestral state of physiology among archaea.

- 1) Waters E., Hohn MJ., et al (2003), The genome of Nanoarchaeum equitans: insights into early archaeal evolution and derived parasitism. Proc. Natl Acad. Sci. USA 100, 984–988
- 2) Brochier C., Gribaldo S., et al (2005), Nanoarchaea: representatives of a novel archaeal phylum or a fast-evolving euryarchaeal lineage related to Thermococcales? Genome Biol. 2005;6:R42. 10.1186/gb-2005-6-5-r42
- 3) Kelly S., Wickstead B., et al (2010), Archaeal phylogenomics provides evidence in support of a methanogenic origin of the Archaea and a thaumarchaeal origin for the eukaryotes. Proc. R. Soc. B rspb2010.1427
- 4) Lane N., Martin WF., (2012), The origin of membrane bioenergetics. Cell 151: 1406-1416
- 5) Fuchs, G., (2011), Alternative pathways of carbon dioxide fixation: insights into the early evolution of life? Annu Rev Microbiol. 65, 631-58
- 6) Liu Y., Beer LL., (2012), Methanogens: a window into ancient sulfur metabolism. Trends Microbiol 20, 251-258.

Friday, time: 12:30-13:00

Length-modulation of horizontal gene transfer explains contradictory population structure in pathogenic bacteria

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Abstract

In 1994 John Maynard Smith challenged the clonality of asexually reproducing organisms, proposing three paradigms for bacterial populations: clonal, panmictic and the intermediate, "epidemic" structure.

While little doubt remains today as to the role of horizontal gene transfer in breaking up clonal relationships, the intermediate paradigm –particularly frequent in pathogenic bacteria – is still debated.

Based on whole-genome sequencing, we show that *Neisseria meningitidis* (Nm) – a deadly human pathogen – is structured in phylogenetic clades that have acquired specific genomic tracts, with a potential impact on the commensal/virulence behavior.

We find gene-conversion events to be longer within clades than between clades, suggesting a DNA cleavage mechanism associated with the species phylogeny. We also identify 22 restriction modification systems, probably acquired by horizontal gene transfer from outside of the species/genus, whose distribution in the different strains coincides with the phylogenetic clade structure. We show with *in-silico* evolution experiments that the length-modulation of DNA exchange provided by restriction modification systems is sufficient to generate the observed population structure.

These findings have general implications for the emergence of lineage structure and virulence in recombining bacterial populations, and could provide an evolutionary framework for the population biology of other species showing contradictory population structure and dynamics.

Friday, time: 14:30-15:15

How a "collective LUCA" drove the rapid evolution of early life: clues from the canonical genetic code

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Abstract

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 --- the map between DNA sequence and amino acids that form proteins. The code is not random, as often assumed, but instead is now known to have certain error minimisation properties. How could such a code evolve, when it would seem that mutations to the code itself would cause the wrong proteins to be translated, thus killing the organism? Using digital life simulations, I show how a unique and optimal genetic code can emerge over evolutionary time, but only if horizontal gene transfer --- a network effect --- was a much stronger characteristic of early life than it is now. These results 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).

Professor Forterre regrets that he was unable to attend due to unforeseen changes of circumstance

LUCA, viruses and the two types of gene transfer

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Abstract

Comparative structural biology has shown that LUCA and its contemporaries were already infected by a great variety of viruses. Ancient genes present in modern organisms may have therefore either a cellular or a viral origin (if they have been transferred from ancient viruses to cells). It is usually consider that one of the major role of viruses is to facilitate the transfer of cellular genes between different cellular lineages. I will argue that this is a minor role. Viruses are mainly engineers of new genes (thus new biological functions) that can be transfer to cells after integration of viral genomes into cellular genomes. There is a lot of confusion in the literature between cellular and viral genes as well as between the two type of lateral gene transfer (LGT), LGT of cellular genes between cells and LGT of viral genes into cells. The latter can confuse phylogenetic reconstruction and produce networks instead of trees. I will present a scenario in which LUCA and its contemporaries were RNA cells infected by both RNA and DNA viruses, suggesting that modern DNA genomes have a viral origin.

- 1.
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- 2. Forterre, P. and Krupovic, M. (2012) The origin of virions and virocells : the escape hypothesis revisited, In G. Witzany (ed.), Viruses: Essential Agents of Life, Springer Science+Business Media Dordrecht
- 3. Forterre, P. (2012) Darwin's goldmine is still open : variation and selection run the world. Front Cell Infect Microbiol. 2, 106, doi: 10.3389/fcimb.2012.00106 (2012)

Replaced in favour of Dr Søren Overballe-Petersen Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen

Hypothetical consideration: tRNAs are the kings of the hill

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Abstract

The discovery that pentaribonucleotide (GUGGC) is able to carry out aminoacylation, transacylation and peptide synthesis raises the profile of RNA in relation to the major part it played in the origin of life. RNA molecules were present just prior to the emergence of the 'hypothetical' cell now commonly referred to as the 'last universal common ancestor' or LUCA. The LUCA is thought to be the predecessor of the three domains (Archaea, Bacteria and Eukarya) as we now know of them. In my presentation I will put forward a hypothesis that, of the three prominent RNA molecules (mRNA, rRNA and tRNA) used by life on Earth today, tRNA may have been the molecule which (1) acted as the carrier of genetic information, (2) was responsible for catalysing the early reactions, thus displaying a catalytic activity and (3) carried both an anticodon and an activated amino acid at the 3' end of the tRNA molecule (4) was involved in the formation of the earliest types of ribonucleoproteins, (5) took part in the formation of early 'rudimentary' ribosomes, and (6) that Lamarckian type of evolution was in operation.

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