MICROBIOLOGY SOCIETY

FOCUSED MEETING 2016

Molecular Biology of Archaea 5

POSTER ABSTRACT BOOK

1-3 AUGUST

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE, LONDON, UK

Posters abstracts
Table of contents:

Session: Genomes and evolution
Presentations: Monday 1 August 19:00-20:00

Session: DNA replication, chromosomes and cell cycle
Presentations: Tuesday 2 August 13:00 – 14:00

Session: Transcription and translation, CRISPR-Cas and genome editing
Presentation: Tuesday 2 August 18:00 – 19:00

Session: Molecular systems and central metabolism
Presentations: Wednesday 3 August 13:00 – 14:00

Session: Genomes and evolution
Presentations: Monday 1 August 19:00-20:00

P1

Phylogenetic analysis reveales a novel and extremely diverse archaeal community in the vlasa hot spring, Velingrad, Bulgaria

Margarita Disheva[1], Ivanka Boyadzhieva[1], Dimitrina Lyutskanova[1], Nadja Radchenkova[1], Nicoleta Boteva[1], Nils-Kåre Birkeland[2], Margarita Kambourova[1]
[1]Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, [2]Department of Biology and Centre for Geobiology, University of Bergen, Bergen, Norway

A culture independent approach based on the analysis of nearly complete archaeal 16S rRNA genes revealed an unusually high archaeal diversity in one of the hottest Bulgarian geothermal springs, Vlasa, Velingrad (86°C). Archaeal clones were referred to 30 OTUs. They were affiliated with both archaeal kingdoms, Euryarchaeota and the provisional Proteoarchaeota, with a predomination of proteoarchaeal clones. Representatives of five among seven currently provisional proteorchaeal phyla were identified in the sample - Crenarchaeota, Thaumarchaeota, Aigarchaeota, Korarchaeota, and Geoarchaeota. All sequenced clones showed closest similarity to uncultured representatives from geothermal niches and some of them formed separate branches. More than two third of the retrieved sequences were >3 % different from their closest matches, suggesting the presence of representatives of novel species, and even genera in this niche. Four of them differ from the closest relatives with more than 15%, the border for new phyla. More than one third of them are unclassifiable, as the sequence similarity to the closest classified relative in databases is lower than 80%. Low similarity with cultured archaeal representatives and low match to environmental 16S rRNA clones are unique features for this thermophilic niche, indicating that the archaeal diversity is still underexplored. The sequences retrieved from the Vlasa hot spring demonstrate that this environment harbors one of the richest archaeal communities found and contribute to expanding ourknowledge of archaeal diversity in geothermal environments.

P2

Highly rearranged Thermococcales chromosomes remain strictly organized.

Matteo COSSU, Violette DA CUNHA, Claire TOFFANO-NIOCHE, Patrick FORTERRE, Jacques OBERTO
Institute of Integrative Cell Biology, Orsay, France

The Thermococcales order constitutes one of the largest Archaeal genomic dataset available to date and amounts to more than 20 completely sequenced species. This phylogenetically deeply rooted clade is composed of three hyperthermophilic and anaerobic genera: *Pyrococcus*, *Thermococcus* and *Palaeococcus*. The shuffled nature of *Pyrococcus* chromosomes was observed years ago and attributed to high levels of genetic recombination. The development of new bioinformatics synteny tools and the recent availability of additional closely related *Thermococcus* genomes permitted to identify genetic inversions between tRNA genes and related sequences as the major phenomenon causing genomic rearrangements. The characterization and genome sequencing of a new species, *Thermococcus nautili*, allowed the identification of a plasmid-encoded enzymatic activity capable of catalyzing such inversions. We are presently dissecting this recombination mechanism *in vitro*. The apparent lack of chromosomal organization due to recombination antagonizes fast Thermococcales cell division cycle. We took advantage of Thermococcales sequence redundancy to explore their genomic landscape, chromosome organization and protein content with our tools. Computation of the core genome uncovered a number of essential gene clusters with a remarkably stable chromosomal position across species, in sharp contrast with the scrambled nature of their genome. The active chromosomal reorganization of numerous genes acquired by horizontal transfer, mainly from mobile elements, could explain this phenomenon.

P3

Lokiarchaea are not the ancestors of eukaryotes

Patrick FORTERRE, Morgan GAIA, Violette DA CUNHA
Institut Pasteur, PARIS, France

In a recent Nature article, Spang and colleagues published a universal tree in which Eukarya are sister group of Loki 3, both branching within a clade formed by Loki 1 and Loki 2. If this is correct, it means that we (Eukarya) are a subgroup of Lokiarchaea, why not? This is one more of many phylogenies published these last years in which Eukarya indeed branch within Archaea. However, there are two major problems that confuse these phylogenies. Firstly, in most of them, the authors have not removed fast evolving proteins (FEP) from their dataset. They argue that the sophisticated method of tree reconstruction now available prevent FEP to induce artefact. However, this is not true, as recently shown by Philippe and co-workers (see Figure 2 in ref. 1). Even the most sophisticated method cannot presently resolve this problem when the outgroup has a long branch, as it is the case for Bacteria in the universal tree. Secondly, in the most recent trees, the authors include genomes reconstructed *in silico* from metagenomic data. This is damaging because “dark matter” genomes often encode FEP and contain contaminating sequences. We have reanalyzed the Loki dataset and started studying all universal proteins taking into account proper principles of phylogenetic analyses and using insertion/deletions to identify contaminations and FEP. We conclude that Lokiarchaea are not the ancestors of eukaryotes and that the classical Woese tree of life is probably correct.

1. Gouy et al. Philos Trans R Soc Lond B Biol Sci. doi: 10.1098/rstb.2014.0329.

P4

Evolution of gene complement and gene order in archaea

Yuri Wolf, Kira Makarova, Eugene Koonin
NCBI/NLM/NIH, Bethesda, MD, USA

In the course of evolution organisms gain and lose genes and rearrange their genomes. We use a well-curated dataset of archaeal orthologous genes (arCOGs) to study the dynamics of the genome-level evolution over the long time scale (on the order of 1-2 Ga) using a novel approach, complementary to the previous analyses. Modeling of evolution of gene complement and gene order suggests that there exists at least two distinct classes of genes; one with a modest variance of replacement rates (a continuum, covering the previously identified “core” and “shell”), another consisting of genes with extremely high replacement of rates (“cloud” of singletons). Gene shuffling is shown to be essential to account for the observed divergence of gene order. It occurs with rate approximately 3 times higher than that of gene replacement. The model suggests that a single property of a gene (previously identified as “gene status”) is sufficient to describe both the conservation of genes themselves and the conservation of gene order.

P5

Replication-related constraints on the evolution of archaeal genome structure

Jelena Repar, Tobias Warnecke
MRC Clinical Sciences Centre, Imperial College London, London, UK

Prokaryotic genomes are generally organized in a way that enables fast DNA replication and cell division. This genome organization includes features such as positioning highly expressed genes close to the origin of replication and placing essential genes on the leading strand. To what extent these organizational principles constrain short- and long-term evolution of genome structure in archaea, however, remains largely unexplored. Here, we characterize the distribution of inversions and synteny breakpoints in various archaeal clades. We observe widespread non-random breakpoint and inversion patterns in both euryarchaea and crenarchaea, with a tendency towards inversions that are symmetric around the origin of replication. Our findings are consistent with ongoing selection for the conservation of local gene neighbourhoods around the origin of replication, and for maintenance of distance relationships around the origin of replication. Notably, we find similar patterns in genomes with single and multiple origins of replication such as *Sulfolobus*, where principles of genome organization and evolution apply to individual origin territories.

P6

Evolutionary Conservation of Sulfolobus islandicus Essential Genes

Alex Phillips, Changyi Zhang, Rachel Whitaker
University of Illinois Urbana-Champaign, Urbana, Illinois, USA

Combining forward genetic and comparative genomic analyses allows for a more detailed model of cellular evolution. For the recently defined Archaeal TACK superphylum, which recent analyses have suggested has a close relationship with the Eukaryotic domain, a shortage of available genomes and genetic systems has prevented such a model from being explored in detail. This study combines transposon mutagenesis in Sulfolobus islandicus, a thermoacidophilic archaeal model system, with an exhaustive comparative genomics analysis to elucidate the conservation of essential traits across all three domains of life from a novel perspective. With ~ 500 essential gene candidates bidirectionally BLAST searched across over 150 genomes, we aim to better characterize the essential functions in our model system as well as other systems within the TACK superphylum. In addition, a large portion of total candidates remain uncharacterized and have no hits outside of the Sulfolobales, potentially offering insight into the unique adaptations that define this group's ecological niche. With these combined results we aim to shed light on the evolution of the ubiquitous and poorly characterized TACK superphylum, complex life, and Sulfolobales.

P7

Genome assembly of hot ammonia-oxidizing archaea: a glimpse into the evolutionary success of Thaumarchaeota.

Sophie S Abby, Claudia Rossel, Romana Bittner, Michaela Stieglmeier, Melina Kerou, Christa Schleper
Archaea Biology and Ecogenomics Division, Dep. of Ecogenomics and Systems Biology, University of Vienna, Vienna, Austria

Not available

P8

Looking into LUCA's cofactors

Natalia Mrnjavac, , Madeline C. Weiss, Filipa L. Sousa, Sinje Neukirchen, Mayo Roettger, Shijulal Nelson-Sathi, William F. Martin

Institution: Institute for Molecular Evolution, Heinrich Heine University, Düsseldorf, North Rhine-Westphalia, Germany

The last universal common ancestor (LUCA) is a biological entity thought to have existed sometime between the origin of life and the divergence of the bacterial and archaeal domains (Williams and Embley, 2015; Nisbet and Sleep, 2001). After identifying candidate protein families in LUCA by phylogenetic analysis, we focused on the protein cofactors it used in order to try to elucidate its biology and relation to the environment where it originated. According to our findings LUCA's proteome encompassed enzymes involved in the biosynthesis of coenzyme F_{420}, the molybdenum and iron-molybdenum cofactors, tetrapyrroles, pterins, thiamine, glutathione and coenzyme M. The most commonly required small molecule for LUCA's reactions and processes was certainly ATP. However, proteins requiring transition metal-based cofactors were present at a comparably high extent (iron-sulfur and nickel-iron-sulfur clusters, molybdenum, iron-molybdenum and tungsten molybdopterins, corrinoids, hemes and active site iron, ferredoxin as electron carrier). The need for environmentally supplied transition metal catalysts is also mirrored in the number of metal transporters present, reflecting an environment rich in these metals. *S*-adenosylmethionine dependent reactions, especially of the radical type, were not unusual, and selenium had also already found its place in the make-up of selenoproteins.

References:

E.G. Nisbet, N.H. Sleep, The habitat and nature of early life, Nature 409 (2001) 1083–1091

T.A. Williams, T.M. Embley, Changing ideas about eukaryotic origins, Philos. Trans. R. Soc. Lond., B. 370 (2015) 20140318

P9

LUCA's informational core

Madeline C. Weiss, Natalia Mrnjavac, Filipa L. Sousa, Sinje Neukirchen, Mayo Roettger, Shijulal Nelson-Sathi, William F. Martin
Institute for Molecular Evolution, Heinrich-Heine University, Düsseldorf, North Rhine-Westphalia, Germany

Central to the studies of early evolution and the origin of life is the concept of a last common ancestor, or Luca and its genomic content. To date, most comparative genomic analysis of Luca's gene content considered Luca as the last common ancestor of bacteria, archaea and eukaryotes (Woese et al 1990). However, recent findings show that eukaryotic ribosomes branch within archaea (Williams, et al. 2013, Spang et al 2015, Raymann et al 2015), and in the more modern "two domain" trees, Luca is the last common ancestor of prokaryotes.
In here we wish to investigate the nature of the original ancestor, specifically which genes it contained as inferred from the gene collection present in extant genomes. Based on stringent phylogenetic criteria, the gene families shared between 134 archaeal and 1847 bacterial organisms were investigated. Besides evidence for genes coding for ribosome biogenesis within Luca's informational core, of special interest are the 8 rRNA nucleoside modifications protein families found, most of which, coding for SAM-dependent nucleoside methylations. This suggests the antiquity of methyl-based biochemistry at the start of Life.

References:

Woese, C.R., Kandler, O., and Wheelis, M.L. (1990). Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc. Natl. Acad. Sci. U.S.A. *87*, 4576–4579.

Williams, T.A., Foster, P.G., Cox, C.J., and Embley, T.M. (2013). An archaeal origin of eukaryotes supports only two primary domains of life. Nature. *504*, 231–236.

Spang, A., Saw, J.H., Jørgensen, S.L., Zaremba-Niedzwiedzka, K., Martijn, J., Lind, A.E., van Eijk, R., Schleper, C., Guy, L., and Ettema, T.J.G. (2015). Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature. *521*, 173–179.

Raymann, K., Brochier-Armanet, C., and Gribaldo, S. (2015). The two-domain tree of life is linked to a new root for the Archaea. Proc. Natl. Acad. Sci. U.S.A. *112*, 6670–6675.

P10

Does size matter?
Structure, function and evolution of the universal family Sua5/TsaC

Adeline Pichard-Kostuch[1-2], Wenhua Zhang[2], Dominique Liger [2], Marie-Claire Daugeron[1], Ludovic Perrochia[1], Patrick Forterre[1], Bruno Collinet[2], Herman van Tilbeurgh[2], Tamara Basta[1]

[1] University of Paris Sud, Institut de Biologie Intégrative de la Cellule, Département de Microbiologie, Orsay, France
[2] University of Paris Sud, Institut de Biologie Intégrative de la Cellule, Département de Biochimie, Biophysique et Biologie structurale Orsay, France

All living organisms, archaea, bacteria and eukarya, descend from a Last Universal Common Ancestor (LUCA). We are studying two universal proteins (present in all living beings), which are involved, together with other accessory proteins, in the synthesis of a modified base, called t^6A, present in all tRNAs that read codons ANN. This modification is essential for a correct reading of the genetic code and its lack is deleterious for cells.
The Sua5/TsaC universal family of proteins catalyzes the formation of the Threonyl-Carbamoyl-AMP (TCA), the necessary intermediate for the biosynthesis of t^6A. While TsaC is a single domain protein, Sua5 protein is composed of a catalytic TsaC-like domain connected to a smaller globular domain of unknown function via a flexible linker. The two variants are interchangeable *in vivo* and *in vitro.* Moreover, their phylogenetic distribution across the three domains of life is discontinuous which makes the evolutionary history of this family puzzling.
We are studying the Sua5/TsaC proteins with aim to discover the role of the supplementary domain in the Sua5 protein and retrace their evolutionary history from LUCA to present day. To this end, we have recently resolved the structure of Sua5 from the hyperthermophilic archaeon *Pyrococcus abyssi* including, for the first time, the linker. Structure guided mutagenesis and *in vivo* complementation experiments indicate that the non-catalytic domain and the linker of Sua5 are both essential for activity.
Using sequence analyses we identified signature residues positioned in the catalytic cleft that distinguish Sua5 from TsaC proteins. One exception to this rule is the Sua5 protein of the archaeon *Archeoglobus profundus.* We hypothesize that these Sua5 protein is in the process of evolving from one form to another; “shortening” or “lengthening”. The study of these proteins will help discriminate between these two evolutionary scenarios.

Session: DNA replication, chromosomes and cell cycle
Presentations: Tuesday 2 August 13:00 – 14:00

P1
Regulation of Polyploidy in *Haloferax volcanii*

Katharina Ludt, Jörg Soppa
Goethe University, Frankfurt, Germany
The ploidy level of six haloarchaeal species from three haloarchaeal genera was quantified. All of them were polyploid, including three species that were freshly isolated from 40 million years old salt samples[1]. Various evolutionary advantages have been discussed, and several advantages have been addressed experimentally, including desiccation survival and growth in the absence of external phosphate[2]. The ploidy level is highly regulated in a growth phase-dependent manner and in response to environmental conditions. Two of the three replication origins, oriC1 and oriC2, of the major chromosome of *Haloferax volcanii* were chosen for an analysis of the molecular details of copy number regulation. In a first approach, we attempted to delete the central origin regions (origin repeats and *orc* gene) as well as adjacent genes upstream and downstream. While both central regions could be deleted, the genes adjacent to oriC1 turned out to be essential. In a second approach, Haloarchaeal Artificial Chromosomes (HACs) were constructed from a suicide plasmid and various regions central to or around oriC1 and oriC2. In summary, the results revealed that oriC1 is much stronger than oriC2 and that the genes adjacent to the origins highly influence the copy number, both *in cis* to a central origin as well as *in trans* on another replicon.
[1] Jaakkola et al. (2014). PLoS ONE 9:e110533.
[2] Zerulla et al., (2014). PLoS ONE 9:e94819.

P2

Using Natural Isolates to Investigate Patterns of Gene Exchange and Recombination in Halophilic Archaea

Neta Altman, Uri Gophna
Tel Aviv University, Tel Aviv, Israel

Haloferax volcanii, a moderate halophilic archaeon was first isolated from the Dead Sea. However, different *H. volcanii* strains can also be isolated from the Mediterranean coastline, by sampling tidal pools that have high evaporation. Using this approach we have successfully obtained a diverse collection of *H. volcanii* isolates from several beaches that are genetically distinct.
The *polB* gene, an essential gene coding for DNA polymerase, is a known target for intein invasion and this gene may or may not contain this selfish genetic element in different isolates. The ability of Haloferax cells to undergo horizontal gene transfer, probably mediated by cell-cell fusion (mating) is believed to be a pre-requisite for intein invasion. Among the natural isolates, two different intein-free genotypes were identified, as well as seven distinct intein-containing genotypes. A phylogenetic reconstruction of extein sequences revealed that inteins have recently invaded intein-less sites in this population
We used genetically manipulated natural isolates and an intein cured laboratory strain to establish the genetic relationship between the intein invasion and recombination around the intein locus and were able to demonstrate a strong linkage between the *polB* and *trpA* genes despite the fact that these loci are over 60 Kb apart.
The presence of the HEN/intein promoted recombination when intein-positive and intein-negative cells were mated. Increased recombination due to HEN activity contributes not only to intein dissemination but also to variation at the population level, since recombination tracts during repair extend substantially from the homing site

P3

Identification of DNA methylations in the hyperthermophilic crenarchaeon *Sulfolobus acidocaldarius*

Mohea Couturier[1], Erik Pelve[2], Ann-Christin Lindås[1]
[1]Stockholm University Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm, Sweden, [2]Uppsala University Department of Ecology and Genetics - Limnology, Uppsala, Sweden

DNA methylation is the most common epigenetic modifications observed in the genomic DNA of prokaryotes and eukaryotes. DNA methyltransferases (MTases) transfer a methyl group from S-adenosyl-L-methionine to specific nucleobases, generating N6-methyladenine (m6A), N4-methylcytosine (m4C) or 5-methylcytosine (m5C) at specific sequences.

Most of the eukaryotic MTases belong to the m5C class participating in the control of gene expression and developmental regulation while the three modified bases are found in prokaryotes. In restriction-modification systems, prokaryotic MTases are associated with a cognate restriction endonuclease eliminating the invading DNA. Moreover, prokaryotic orphan MTases are involved in different processes such as regulating the initiation of DNA replication or the maintenance of the genome integrity.

We studied the methylome of *Sulfolobus acidocaldarius*. The strategy was to perform genome resequencing to identify DNA modifications occurring in exponentially growing cells by using bisulfite treatment and single-molecule real-time (SMRT) sequencing. The presence of the restriction-modification system was confirmed by SMRT sequencing and, surprisingly, the presence of m5C and m6A was revealed on the same motif by both methods. Then, we aim to investigate, using synchronized cells, if these methylations could be involved in the regulation of *S. acidocaldarius*' cell cycle.

P4

The impact of archaeal histones on gene regulation in a naïve bacterial system.

Maria Rojec, Tobias Warnecke
Imperial College London, London, UK

Archaea display a highly diverse complement of chromatin proteins: in some species the genome is associated with a set of DNA-binding proteins reminiscent of bacterial nucleoid-associated proteins, other species encode variable numbers of histones that are structurally homologous to eukaryotic core histones. Consequently, Archaea are interesting model systems to investigate how different gene regulation strategies have arisen over the course of evolution. In order to investigate the transition from a permissive bacterial-like transcription logic to a repressive eukaryotic-like state, and to understand whether histones are required for this transition, we introduced the two histone proteins from the archaeon *Methanothermus fervidus* into a naïve bacterial system (*E. coli*). Using MNase-seq, we map genome-wide nucleosomal occupancy in *E. coli* in both exponential and stationary phase and show that chromatin formation in the recombinant strains is consistent with a histone multimerisation model. We further dissect binding preferences, explore the functional context of binding and correlate binding patterns with gene expression changes obtained by RNA-seq. In short, we describe the global impact of histones on gene expression regulation in a naïve bacterial system.

P5

A novel DNA repair pathway found in achaeal order *Thermococcales*

Miyako Shiraishi[1], Sonoko Ishino[1], Yuriko Egashira[1], Shinichi Kiyonari[1], Takeshi Yamagami[1], Isaac Cann[2], Yoshizumi Ishino[1]

[1]Kyushu University, Fukuoka, Japan, [2]University of Illinois at Urbana-Champaign, IL, USA

DNA is always under threat of change or loss of the genetic information. One of the predominant DNA damages is base deamination. Deamination of cytosine, adenine, and guanine gives rise to uracil (U), hypoxanthine (Hx), and xanthine (X), respectively. If these bases remain in DNA, they lead to point mutations during DNA replication. To maintain genome integrity, all living organisms have DNA repair systems. Organisms that thrive under extreme environments are especially expected to have developed some efficient repair systems, since high temperature and ionizing radiation promote DNA damages. Recently, we detected a novel endonuclease activity that cleaves the 5' side of Hx from the cell extracts of *Pyrococcus furiosus*, and identified the corresponding gene. The endonuclease, designated as Endonuclease Q (EndoQ), recognized Hx, U, X, and AP site. EndoQ is conserved in *Thermococcales*, and some methanogens in Archaea. Furthermore, the EndoQ activity was enhanced by the cognate proliferating nuclear antigen (PCNA), which plays an essential role in DNA metabolism. These findings provide us with clues to elucidate the mechanism underlying a unique DNA repair system in Archaea. We will discuss DNA repair systems of damaged bases in Archaea based on distribution of the related genes in the archaeal genomes.

P6

Characterizing the methylome of *Haloferax volcanii* through gene deletion

Matthew Ouellette, Andrea Makkay, R. Thane Pape
University of Connecticut, Storrs, CT, USA

DNA methylation has a significant role in protecting cells from invading foreign DNA in prokaryotes, and also contributes to genome regulation. This process is carried out by DNA methyltransferases, which commonly function in tandem with restriction endonucleases and together are called restriction-modification (RM) systems. RM systems are well-characterized in Bacteria. However, little is known about these systems in Archaea. To gain insight into archaeal DNA methylation we used the model haloarchaeon *Haloferax volcanii*. We deleted all the predicted RM genes in *Hfx. volcanii* strain H26. We then used single molecule real-time (SMRT) sequencing to analyze the genomic methylation patterns (methylome) of H26 and our deletion mutants. Our results indicate that there are two motifs which are modified in the *Hfx. volcanii* genome: $C^{m4}TAG$ and $GCA^{m6}BN_6VTGC$. Analysis of our deletion mutants revealed that the m4C motif was removed from the methylome when the putative Type II CTAG methyltransferase gene *HVO_0794* was deleted. Deletion of putative Type I RM operon *rmeRMS* and Type IV restriction endonuclease gene *HVO_A0074* eliminated detection of the m6A motif. The roles of Type IIG genes *HVO_A0006* and *HVO_A0237* are less clear. Deletion of all putative RM system genes resulted in the abolition of all detected modified motifs. Understanding DNA methylation will shed light on many aspects of archaeal biology possibly including horizontal gene transfer, gene regulation, mutation rates, viral infections, and DNA replication.

P7

Gene regulation and genome compaction by archaeal histones HMfA and HMfB from *Methanothermus fervidus*

Bram Henneman, Ramon van der Valk, Remus Dame
Leiden University, Leiden, The Netherlands

To organize their genomes, archaea express histone proteins that are homologous to their eukaryotic counterparts H3 and H4. Eukaryotic histones are believed to originate from archaeal histones; this is underlined by the notion that histones from the recently discovered phylum Lokiarchaeota seem to be intermediates between archaea and eukaryotes, as are the organisms themselves. Unlike eukaryotic histones, which predominantly wrap DNA around an octameric histone core, archaeal histones seem to be able to bind to DNA as dimer, tetramer or larger multimer. Some archaea express multiple histones, which allows formation of heterocomplexes that may add a level of complexity to archaeal genome organization. Here, we examined the histones HMfA and HMfB from *Methanothermus fervidus*. Our data indicate that tetramers are formed on high-affinity sites on the genome and that higher concentrations allow for highly cooperative aspecific genome compaction. We speculate that aspecific binding is important for genome organization, whereas specific binding may have a more regulatory function. HMfA and HMfB have equal affinities for an aspecific substrate, but the affinities of HMfA and HMfB for a high-affinity site are very different, opening up the possibility of growth phase specific regulation via these proteins.

P8

Chromosome Architecture and DNA Replication in *Haloferax volcanii*

Hannah Marriott, Thorsten Allers
University of Nottingham, Nottingham, UK

Haloferax volcanii is used to study DNA replication and repair, it is unique amongst cellular organisms in that it can thrive without replication origins.
There are four replication origins on the main circular chromosome (including the integrated megaplasmid pHV4) and one each on the megaplasmids pHV1 and pHV3. Origins are required to initiate replication but *H. volcanii* has been found to grow 7% faster when all chromosomal origins are deleted. This has led to the suggestion of an alternative form of DNA replication by recombination-dependent replication (RDR).

The chromosome architecture of *H. volcanii* can be manipulated via the forced integration of pHV3 onto the main chromosome. When combined with the deletion of the pHV1 megaplasmid, this generates a strain with only one chromosome. Deleting all the origins of replication from this single chromosome could lead to even faster growth, and simplify the investigation of DNA replication with and without origins.

The link between chromosome architecture in *H. volcanii* and how this relates to DNA replication, origins of replication and RDR, will help us to understand how life is possible without origins.

P9

Biochemical and global analysis of *M. jannaschii* histones A3 and Mj1647

Sapir Ofer[1], Katherine Smollett[1], Algirdas Toleikis[2], Justin Molloy[2], Nick Kent[3], Finn Werner[1]
[1]University College London, London, UK, [2]The Francis Crick Institute Mill Hill Laboratory, London, UK, [3]School of Biosciences, University of Cardiff, Cardiff, UK

Not available

Session: Transcription and translation, CRISPR-Cas and genome editing
Presentation: Tuesday 2 August 18:00 – 19:00

P1

Development and Characterization of a Salt-Tolerant Luciferase to Investigate Gene Regulation in Haloarchaea

Christopher Davis, Brian Robertson
Middle Tennessee State University, Murfreesboro, TN, USA

Understanding gene regulation in archaea may reveal a unique mechanism for biological timekeeping, or provide further insight of timing systems similar to those in cyanobacteria and eukaryotes. Archaea also play a pivotal role in other developing research areas, including the CRISPR-Cas system. Haloarchaea, especially Haloferax volcanii, are of particular interest for genetics research due to the wide array of tools and procedures available.

The eukaryotic luciferase from fireflies and click beetles emits light when the protein catalyses the oxidation of a bioluminescent substrate luciferin. The light emission is an immediately measurable indication of enzymatic activity that can be coupled to regulation of transcription for a specified gene without the negative effects of GFP and other reporters.

Haloarchaeal enzymes have evolved several characteristics that promote proper protein folding and function at high salt concentrations, and mesophilic proteins that lack these characteristics often precipitate or fold improperly when expressed in halophilic archaea. However, proteins that acquire salt-stabilizing characteristics, either by human design or random mutation, can lead to variants that function in haloarchaea. It is our aim to synthesize a salt tolerant luciferase for haloarchaea using laboratory directed evolution in order to study possible time keeping genes, the CRISPR-Cas system, and gene regulation in a continuous culture environment. The mutations developed will then be characterized in order to identify changes in the protein leading to improved haline environment stability.

P2

Characterization of the role of phosphorylation of the TetR-like transcription factor SaFadR in *Sulfolobus acidocaldarius*

Hassan Ramadan Maklad[1], Karin Valegård[2], Ann-Christin Lindås[3], Eveline Peeters[1]
[1]Vrije Universiteit Brussel, Brussels, Belgium, [2]Uppsala University, Uppsala, Sweden, [3]Stockholm University, Stockholm, Sweden

The TetR-like transcription factor SaFadR (encoded by the gene *saci_1107)* in the hyperthermoacidophilic crenarchaeon *S. acidocaldarius*, which is involved in the regulation of fatty acid metabolism, was previously found to be phosphorylated *in vivo*.
As little is known about phosphorylation as a signal transduction mechanism of transcription factors in archaea, this makes it an interesting subject to unravel the effect of phosphorylation on protein structure and function. The aim of this work is to characterize post-translational phosphorylation of SaFadR and its effect on the physiological role.

Crystal structure analysis of the SaFadR protein revealed the phosphorylation of tyrosine 133, located in the ligand-binding pocket, on one of the two subunits. An *in vitro* phosphorylation assay revealed that SaFadR was specifically phosphorylated by a eukaryotic type-like kinase encoded by the gene *Saci_1193.* The binding of acyl-CoA to SaFadR ligand binding pocket causes the disruption of SaFadR-DNA complexes. Protein-DNA mobility shift assays with phosphomimetic mutants of SaFadR revealed that the phosphorylation of tyrosine 133 causes an inhibitory effect on the ligand binding.

P3

Transcriptional regulation of fatty acid metabolism in Sulfolobus acidocaldarius

David Sybers[1], Liesbeth Lemmens[1], Xiaoxiao Zhou[2], Lu Shen[2], Christopher Bräsen[2], Bettina Siebers[2], Eveline Peeters[1]

[1]Vrije Universiteit Brussel, Brussels, Belgium, [2]Universität Duisburg-Essen, Essen, Germany

One of the most distinctive characteristics of archaea are their membrane lipids, which contain isoprenoids linked by an ether bond to a glycerol moiety instead of the ester-linked fatty acids that are present in bacteria and eukaryotes. Despite this absence of fatty acids in the lipids, many archaeal genomes contain genes that are predicted to be involved in fatty acid metabolism. Sulfolobus acidocaldarius, a hyperthermoacidophilic crenarchaeon, contains a cluster of genes encoding enzymes involved in fatty acid and lipid metabolism and two transcriptional regulators, a TetR member (SaFadR) and a CopG member. Growth tests suggests that S. acidocaldarius can utilize fatty acids as a carbon and energy source. One of the regulators, a TetR family member named SaFadR, was characterized in this research. Using qRT-PCR comparing a SaFadR deletion strain with the wild type strain, we show that a lipase operon, a beta-oxidation operon are repressed by the regulator. In presence of long-chain acyl-CoA molecules, the ability of SaFadR to bind to its targets is disrupted in vitro, probably leading to derepression of the targets. These results indicate that this gene cluster is involved in fatty acid degradation.

P4

Biochemical Analysis of a putative CRISPR adaptation cassette comprising fused Cas1-4, Cas2 and DNA polymerase I in the euryarchaeon *Methanosaeta Harundinacea.*

Emily Walker, Edward. L. Bolt
University Of Nottingham, Nottingham, Nottinghamshire, UK

Methanosaeta Harundinacea (M. Ha) is an acetate utilizing methanogen isolated from a wastewater treatment plant in Beijing. It contains a type III like CRISPR- cas prokaryotic defence system that includes a cluster of genes encoding a fused Cas1-4 fusion and Cas2 proteins, juxtaposed to a putative DNA polymerase I, an unusual arrangement in Archea. These three genes are separated spatially from the other *cas* genes of the system by the CRISPR locus. We are using protein biochemistry to investigate if M. Ha Cas1-4, Cas2 and PolI can be reconstituted into a self-contained DNA capture and integration pathway for CRISPR adaptation. Here we report purification of M. Ha Cas1-4, Cas2 and PolI proteins and analysis of their substrate and sequence specificities for both nucleic acid binding and cleavage, and polymerase primer extension and gap filling reactions. Our experiments allow comparison with current models for CRISPR adaptation, and we aim to elucidate how CRISPR adaptation proteins work together with non-Cas host proteins such as PolI. This includes whether CRISPR adaptation in M. Ha is comparable to other CRISPR-Cas systems or is more alike to ancient casposons. These experiments will also help shed light both on the role of Cas4 within CRISPR-Cas systems.

P5

TFS2 – rescuing stalled transcription elongation complexes by promoting NTP misincorporation

Alice Carty, Thomas Fouqueau, Finn Werner
Institute of Structural and Molecular Biology, London, UK

Transcription elongation of RNA polymerase (RNAP) is inherently discontinuous and liable to pausing and arrest. Stalled RNAP can become locked into a catalytically inactive conformation, which is detrimental to transcription. Transcript cleavage factors are vital components that are able to relieve arrest; this activity is conserved across all domains of life. The archaeal cleavage factor TFS is able to resolve stalled elongation complexes by stimulating the endogenous endonucleolytic activity of RNAP, TFS also aids proof reading and increases transcription fidelity. Crenarchaeota typically encode between 2 and 4 paralogues of TFS. Here we show that the previously uncharacterized Sulfolobus solfataricus TFS2 has pleiotropic effects, it modulates pre-initiation complex formation, abortive initiation and early transcription elongation. Interestingly, TFS2 relieves stalled RNAP elongation complexes by increasing read-through at a dramatically lowered fidelity - an alternative mechanism to the canonical factor TFS1. A genetic analysis revealed TFS1 not to be essential for Sulfolobus viability, although the knock out causes a slow growth phenotype.

P6

Mechanisms of transcriptional repression by TFS4

Thomas Fouqueau[1], Fabian Blombach[1], Adam Belsom[2], Alan Cheung[1], Juri Rappsilber[2], Finn Werner[1]
[1]University College London, London, UK, [2]Wellcome Trust Centre for Cell Biology, Edinburgh, UK

During transcription elongation, RNA polymerase (RNAP) frequently pauses and moves in a retrograde direction ('backtracking'). Backtracking displaces the 3'-end of nascent RNA from the active site, which is subsequently extruded through the secondary channel, resulting in a stalled RNAP. The transcription factor TFS is involved in reactivation of stalled RNAP in archaea. TFS inserts its C-terminal Zn-ribbon (C-ribbon) through the secondary channel to reach the RNAP active site. TFS thereby stimulates the endonucleolytic cleavage of the RNA to generate a new 3'-end, allowing transcription elongation to resume. TFS is evolutionarily related to both transcription factor TFIIS and to RNAPII subunit RPB9 in Eukaryotes. Crenarchaeota encode multiple TFS paralogues (up to four paralogues in *Sulfolobus solfataricus*) making Sulfolobus an interesting model organism to study multiplication and functional diversification of *tfs*-like genes. Here we show that TFS1 (the canonical TFS in *S. solfataricus*) stimulates RNA cleavage and transcription processivity, whereas TFS4 strongly represses transcription initiation and elongation. We identified that the inhibitory mechanism depends on insertion of TFS4-C-ribbon into RNAP secondary channel. Finally, we show that swapping of TFS4-C-ribbon into *S. acidocaldarius* TFS1 causes severe growth inhibition of cells.

P7

Defining the role of TFEα/β in archaeal transcription

Fabian Blombach[1], Zoja Nagurnaja[1], Mark Zhang[1], Darya Ausiannikava[2], Kostas Thalassinos[1], Thorsten Allers[2], Finn Werner[1]
[1]University College London, London, UK, [2]University of Nottingham, Nottingham, UK

We recently identified the first dimeric variant of the basal transcription factor TFE termed TFEα/β in the crenarchaeon *Sulfolobus solfataricus*. TFEα/β encompasses two metal centres: a zinc ribbon and a cubane iron-sulfur cluster in in the α- and β-subunit, respectively. The genes encoding the TFEα and TFEβ subunits are not strictly conserved in archaea and variants without the Zn ribbon and iron sulphur cluster appear to have evolved in some archaea. We show that the euryarchaeaon *Haloferax volcanii* possesses an unusual dimeric TFEα/βvariant that lacks a canonical zinc ribbon domain and the iron-sulfur cluster suggesting that dimeric TFE variants are widespread in archaea.

In vitro experiments suggest that *Sulfolobus* TFEα/β functions as a basal transcription factor simulating transcription from all promoters to varying extent. TFEβ is depleted upon starvation and oxidative stress. Here we address some of the outstanding questions regarding TFE function: Does TFEα/β function as a basal transcription factor *in vivo*, is there are regulatory component in TFE function? We conducted chromatin immuno-precipitation in combination with high-throughput sequencing (ChIP-Seq) to generate genome-wide occupancy profiles of all basal components of the *Sulfolobus solfataricus* transcription initiation machinery and first data will be presented.

P8

The regulatory roles of two small non-coding RNAs in *Haloferax volcanii*

Jana Kliemt, Katharina Jaschinski, Julia Babski, Jörg Soppa
Goethe University, Frankfurt, Germany

The existence of small non-coding regulatory RNAs (sRNAs) in *Haloferax volcanii* was shown some time ago[1], a recent dRNA-Seq study unraveled the existence of about 2800 non-coding RNAs in *H. volcanii* (Babski et al., in revision). Generation and phenotyping of sRNA gene deletion mutants revealed their importance for many different biological processes, from stress adaptation to swarming[1]. Two examples were chosen for detailed characterization.

An *in silico* analysis predicted the 3'-UTR of an ABC transporter with annotated phosphate-specificity as a possible target for $sRNA_{132}$. The levels of the $sRNA_{132}$ and the ABC transporter mRNA were highly enhanced at low phosphate concentrations, and the mRNA level was much lower in the $sRNA_{132}$ deletion mutant, indicating a positive regulatory role of the sRNA. The importance of the $sRNA_{132}$ and the ABC transporter at low phosphate concentrations was verified using competitive growth experiments. Further targets of $sRNA_{132}$ were identified by microarray analysis. Characterization of the sRNA-mRNA interaction is under way.

A putative binding site for $sRNA_{63}$ was found within the ORF of a flagellin gene. The *fla* mRNA level is much higher in the $sRNA_{63}$ deletion mutant than in the wild-type, indicating a negative role of the sRNA on flagella production. And indeed, the swarming velocity of the sRNA deletion mutant is much higher than that of the wild-type. A biotinylated form of $sRNA_{63}$ was generated by *in vitro* transcription, and co-affinity purification of mRNAs and proteins that interact with $sRNA_{63}$ is currently under way.

[1] Jaschinski et al. (2014) PLoS ONE 9:e90763

P9

Random mutagenesis of the hyperthermophilic Thermococcale *Pyrococcus furiosus* using *in vitro* mariner transposition and natural transformation

Natalia Guschinskaya[1], Romain Brunel[2], Maxime Tourte[1], Philippe Oger[1], Xavier Charpentier[2]
[1]CNRS UMR5240 Microbiologie, adaptation et pathogénie, Villeurbanne, France, [2]Inserm U1111 Centre international de recherche en infectiologie, Villeurbanne, France

Transposition is a powerful tool in genetic analysis and has been widely used to identify essential genes and functionally annotate genomes. However, transposon-mediated mutagenesis has only been successfully applied to a limited number of archaeal species and has never been reported in Thermococcales. Here, we report random insertion mutagenesis in the hyperthermophilic Thermococcale *Pyrococcus furiosus*. The strategy takes advantage of the natural transformability of derivatives of the *P. furiosus* COM1 strain and of *in vitro* Mariner-based transposition. Natural transformation of a single transposition reaction routinely generates several thousands transformants. Southern-blot analysis and sequencing indicate that the obtained mutants contain a single and random genomic insertion. Polyploidy has been reported in Thermococcales and *P. furiosus* is suspected of being polyploid. Yet, mutants homozygous for transposon insertions are readily isolated on the first selection. Other mutants appeared heterozygous and two rounds of isolation on selective medium were sufficient to obtain gene conversion. This transposition mutagenesis strategy may be applied to other species capable natural transformation and will greatly facilitate functional exploration of the Thermococcales genomes.

P10

Studying life adaptation to high hydrostatic pressure environments: implication of sulphur and hydrogen metabolism revealed through genetic manipulations in the piezo-hyperthermophilic archeon *Thermococcus barophilus*

Tiphaine Birien[1], Marion Gardette[1], Yann Moalic[1], Mohamed Jebbar[1]
[1]Université de Bretagne occidentale, Brest, France, [2]CNRS, Brest, France, [3]Ifremer, Brest, France

In this study, physiological adaptations to HHP were investigated after genetic deletion of hydrogenase gene clusters in *T. barophilus*. Indeed, previous transcriptomic experiments have highlighted the over-expression of genes encoding hydrogenases under supra-optimal HHP conditions. Here, we aimed to refine the precise role of each of these enzymes in adaptation to HHP by taken advantage of the recent genetic system available for this archeon. Homologous recombination was used to delete two cytoplasmic sulfhydrogenases, (SHI and SHII), as well as two membrane bound hydrogenases (HYD and CAB). Notably, these constructions are proofs for the efficiency of the new counter-selection system based on the toxic adenine analogue, 6-methylpurine (6MP) instead of the 5-fluoroorotic acid (5-FOA), inverting the rate of false positives pop-out clones from 80% to 10%.

The first phenotypic studies of mutant strains under HHP conditions clearly demonstrate the impact of hydrogenases on culture growth. However some discrepancies exist between the different mutant strains, notably the strain ΔSHII can't grow at 70MPa without sulphur. Measures of hydrogenases activities, transcriptional compensations and metabolite production will be analyze and should provide new insights on energetic metabolism control in archaea subject to HHP.

P11

sRNA-mediated Regulation of Stress Response in Arid-adapted *Haloarchaea*

Diego Gelsinger, Jocelyne DiRuggiero
Johns Hopkins University, Baltimore, Maryland, USA

Small RNAs (sRNAs) are ubiquitously found in the three domains of life playing large-scale roles in gene regulation. sRNAs have been discovered to be abundant in haloarchaea but their functional roles still remain poorly understood. Our lab studies microorganisms inhabiting halite (NaCl) rocks from the Atacama Desert, Chile. We found that *Haloarchaea*, including *Haloferax* species, dominated these halite communities. In deserts, hypersalinity, intense solar radiation, and desiccation generate high levels of oxidative stress. My goal is to determine the regulation of stress response by sRNAs in *Haloarchaea* from these environments. I have identified differentially expressed sRNAs using small RNA-seq in response to oxidative stress in *Haloferax volcanii*. The approach constituted exposing the archaea to H_2O_2 or ionizing radiation to cause oxidative stress and profiling transcriptome changes. Both intergenic and antisense sRNAs were discovered, with the former being up-regulated and the latter down-regulated during oxidative stress, suggesting that these sRNAs can regulate expression positively and negatively. Antisense sRNAs were predicted to overlap both 5' and 3' UTRs of mRNAs demonstrating a hybrid system between Eukarya (3' UTR) and Bacteria (5' UTR) silencing systems. An RNA-binding motif was present in all intergenic RNAs showing potential interaction with other RNA transcripts or proteins. With identified oxidative stress response-specific sRNAs, future work includes determining their functions in *Haloarchaea* using reverse molecular genetics and ribosome profiling. This study will be one of the first to link oxidative stress response to sRNA-mediated regulation at the molecular level in Archaea.

P12

Towards the identification of archaeal β-CASP ribonuclease and Ski2-like helicase complexes in *P. abyssi*

D.K. Phung1, P.S. Langendijk-Genevaux1, Y. Quentin1, P.F. Pluchon, A.J. Carpousis1, G. Fichant1, D. Flament2, B. Clouet-d'Orval1
1 CNRS, UMR 5100 LMGM Université de Toulouse,Toulouse
2 CNRS, UMR 6197- LM2E-IUEM, Université de Bretagne Occidentale, Plouzané

RNA processing, which involves ribonucleases and ancillary enzymes such as RNA helicases, has a pivotal role in living cells and constitutes a crucial step in the regulation of gene expression. Numerous signaling pathways have been described in Eukarya and Bacteria in which RNA processing regulates gene expression. This process has been overlooked in Archaea and the mechanism of archaeal mRNA maturation and decay are far from being understood.
Our group addresses the molecular mechanism of RNA metabolism in Archaea involving β-CASP ribonucleases. Recently, we published phylogenomic and experimental work demonstrating that archaeal β-CASP proteins, aCFSF1 and aRNase J, are highly conserved ribonucleases in Archaea1–3. The β-CASP enzymes form a subfamily within the metallo-β-lactamase enzymes superfamily that has emerged as central to DNA and RNA metabolism over the last decade4. Archaeal aCPSF1, an ortholog of the eukaryal transcription termination factor CPSF73, is ubiquitous in Archaea suggesting an essential conserved function. Archaeal aRNase J, an ortholog of the bacterial ribonuclease RNase J, is conserved through a major phylum of the Archaea, the Euryarchaeota.

These findings suggest that the role of these enzymes in RNA processing can be reminiscent of ancient functions that had arisen early in evolution. We now want to focus on understanding the physiological role of aCPSF1 and aRNase J, the two major phylogenetically conserved archaeal β-CASP ribonucleases in the Thermococcales with the hyperthermophilic euryarchaeal *Pyrococcus abyssi* as model.

By analogy to eukaryal CPSF73 and bacterial RNase J, which are part of multiprotein complexes, clues to the function of the archaeal β-CASP homologs might come from the identification of archaeal multiprotein complex(es) containing aCPSF1 and aRNase J orthologs. Using *P. abyssi* cell extracts and recombinant aCPSF1 or aRNase J as bait, we performed affinity purification assays. Remarkably, we have found that both aCPSF1 and aRNase J are a part of protein interaction networks that include each other. In addition, these networks have proteins in common with the network of a putative Ski2-like RNA helicase that is a part of aRNase J network. Noteworthy, putative partner functions reveal involvement in central physiological processes such as RNA metabolism. In parallel, fractionation of *P. abyssi* whole cell extracts in sucrose gradient shows that aRNase J and the Ski2-like RNA helicase are present in high sedimentation fractions with ribosomal sub-units.

We also demonstrate a direct interaction of aRNase J with aSki2B by co-purification affinity chromatography experiment. These results suggest that aRNase J and a putative RNA helicase may be a part of the multienzyme complexes. To our knowledge, our results are the first experimental indications of interacting network containing ribonucleases and RNA helicase-like proteins in Archaea. Remarkably, aRNase J is an orthologue of the bacterial RNase J and aSki2B is an orthologue of the eukaryotic Ski2 family proteins. This shows that Archaea might possess a composite RNA processing system sharing both eukaryal and bacterial features. This highlights the advantage of an archaeal model to gain further mechanistic and evolutionary information of fundamental processes across the three domains of life.

1. Clouet-d'Orval, B., Rinaldi, D., Quentin, Y. & Carpousis, A. J. Euryarchaeal beta-CASP proteins with homology to bacterial RNase J Have 5'- to 3'-exoribonuclease activity. J. Biol. Chem. ***285,*** *17574–17583 (2010).*
2. Phung, D. K. et al. Archaeal β-CASP ribonucleases of the aCPSF1 family are orthologs of the eukaryal CPSF-73 factor. Nucleic Acids Res. ***41,*** *1091–1103 (2013).*
3. Clouet-d'Orval, B., Phung, D. K., Langendijk-Genevaux, P. S. & Quentin, Y. Universal RNA-degrading enzymes in Archaea: Prevalence, activities and functions of β-CASP ribonucleases. Biochimie ***118,*** *278–285 (2015).*
4. Dominski, Z., Carpousis, A. J. & Clouet-d'Orval, B. Emergence of the β-CASP ribonucleases: highly conserved and ubiquitous metallo-enzymes involved in messenger RNA maturation and degradation. Biochim. Biophys. Acta ***1829,*** *532–551 (2013).*

Session: Molecular systems and central metabolism
Presentations: Wednesday 3 August 13:00 – 14:00

P1
Nucleotide dependent FlaI-FlaH interaction is essential for the assembly and rotation of the Archaellum

Paushali Chaudhury[1], Tomasz Neiner[1], Edoardo D Imprima[2], Ankan Banerjee[3], Sophia Reindl[4], Abhrajyoti Ghosh[1], Andrew S Arvai[4], Deryck J Mills[2], Chris van der Does[1], John A Tainer[4], Janet Vonck[2], Sonja-Verena Albers[1]
[1]Albert-Ludwigs-University, Freiburg, Germany, [2]Max Planck Institute of Biophysics, Frankfurt, Germany, [3]Philipps Universität Marburg, Marburg, Germany, [4]Lawrence Berkeley National Laboratory, California, USA

Motility is a common phenomenon among many prokaryotes which assists them in chemotactic behavior and survival in unfavorable environments. The archaeal motility structure, the archaellum, is a unique nano-machine responsible for rapid and directed movement, which shares structural homology with bacterial type IV pili, while its functionality resembles the bacterial flagellum. The quality of being distinct from other motility structure lies in the fact that the archaellum is composed of only seven proteins in Sulfolobus acidocaldarius which are all indispensable for the assembly and function of the archaellum.

The motor complex of the archaellum comprises of the monotopic membrane protein FlaX, the bi-functional ATPase FlaI, the RecA family protein FlaH and the polytopic membrane protein FlaJ. FlaX forms a 30nm ring structure that acts as a scaffold protein for FlaI and FlaH. Although FlaH binds ATP with high affinity it is unable to hydrolyze it. Moreover, ATP binding to FlaH is essential for its interaction with FlaI and for archaellum assembly and motility. FlaH interacts at the C-terminus of FlaX and single particle analysis revealed monomeric FlaH assemble into a second ring inside the FlaX ring in vitro. Taken together, these data suggest a central structural role for FlaH through its regulatory function on the motor ATPase FlaI.

P2

ArnS, a kinase involved in starvation-induced archaellum expression

M Florencia Haurat[1], Ana Sofia Figueiredo[2], Lena Hoffmann[1], Lingling Li[1], Katharina Herr[1], Amanda Wilson[3], Morgan Beeby[3], Jörg Schaber[2], Sonja-Verena Albers[1]
[1]University of Freiburg, Freiburg, Germany, [2]Otto von Guericke University, Magdeburg, Germany, [3]Imperial College London, London, UK

Living organisms have evolved complex motility organelles that allow them to leave inhospitable environments and to conquer more prosperous habitats. The organisms sense environmental stimuli and transmit this integrated information to the motility machineries in order to direct migration. The motility organelles are complex surface appendages that require a tight, precise and hierarchical regulation of their expression. In the crenearchaeon Sulfolobus acidocaldarius, the swimming motility organelle (archaellum) biosynthesis is regulated by archaellum regulatory network proteins that differentially regulate archaellum subunit expression in a phosphorylation dependent manner. Although some components are known, the regulatory cascade triggered when the cells sense starvation is poorly understood. In this work, employing in vivo experiments and mathematical models, we describe, for the first time in archaea, the dynamics of key components involved in the archaellum activation. In addition, we identify the starvation-induced Ser/Thr protein kinase ArnS (Saci_1181) which is located in close proximity to the archaellum operon. Deletion of arnS results in reduced motility, though the archaellum is properly assembled. Therefore, our results indicate that ArnS is responsible that each component is expressed at specific and defined time points and levels to ensure that the archaellum works as a precision machinery.

P3

Structural and biophysical study of the proteasome regulatory complex from *P. horikoshii.*

Ziad Ibrahim[1], Matteo Colombo[1], Gaelle Hogrel[2], Didier Flament[2], Michael Hartlein[3], Guy Schoehn[1], Eric Girard[1], Frank Gabel[1], Bruno Franzetti[1]

[1]CNRS, Université Grenoble Alpes, Commissariat à l'Energie Atomique, Institut de Biologie Structurale F-38044 Grenoble, France, Grenoble, France, [2]Ifremer, UMR6197, Laboratoire de Microbiologie des Environnements Extrêmes Plouzané, France, Brest, France, [3]Institut Laue-Langevin, 71 Avenue des Martyrs, F-38000 Grenoble, France., Grenoble, France

The Rpt proteins are AAA+ ATPases forming a hexameric complex at the base of the 19S proteasome regulatory particle. They catalyze the energy-dependent unfolding of the target proteins. The proteasome activating nucleotidase (PAN) is the archaeal counterpart of the eukaryotic Rpt subunits. Compared to the more complex eukaryotic system it displays a homogeneous composition and a high stability in vitro. We previously showed that in Archaeal cell extracts, most of the PAN complexes are not assembled with the 20S proteasome (Chamieh *et al*. 2008. Biochem. J). In this work, *in vivo* studies revealed that a N-ter truncated form of the PAN complex is predominantly accumulated in Thermococcales. A monodisperse population of PAN hexamers was purified. We report a single particle Cryo-EM reconstruction of the functional assembled PAN hexamer at 15 Å resolution. The crystal structure of the ATPase C-terminal domain was determined and solution SAXS data were collected on the native hexamers and the N-terminal subcomplex. In addition, conformational transitions coupled to ATP-binding were observed. This integrative approach revealed significantly different features between the archaeal and the eukaryotic proteasome regulatory systems.

P4

Protein phosphorylation in the archellum regulatory network of *S. acidocaldarius*

Lena Hoffmann[1], Katrin Anders[2], Andreas Schummer[1], M. Florencia Haurat[1], Julia Reimann[1], Chris van der Does[1], Bettina Warscheid[1], Lars-Oliver Essen[2], Sonja-Verena Albers[1]

[1]Albert-Ludwigs Universität, Freiburg, Germany, [2]Philipps Universität, Marburg, Germany

The archaellum regulatory network of *S. acidocaldarius* tightly controls the expression of the archaellum. In recent years several regulators of archaellum expression were identified, including repressors (ArnA, ArnB) and activators (ArnR, ArnR1). Furthermore, several eukaryotic like protein kinases and one serine/threonine-specific phosphatase play important roles in exerting control over expression of the archaellum operon.

Our main goal is to elucidate the role of phosphorylation as well as the interconnection of the regulatory proteins within this network with a specific focus on the repressors.

Applying X-ray crystallography and mass spectrometry we obtained structures of ArnB and the FHA domain of ArnA that gave insights into the localization of phosphopeptide-binding sites in ArnA and phosphorylated residues in the C-terminus of ArnB. Furthermore, we show that ArnA binds to the C-terminus of ArnB in a phosphorylation-dependent manner. Additionally, two kinases (ArnC and ArnD), specifically phosphorylated the repressor ArnB at S/T residues. While the deletion of arnC is resulting in a phenotype almost indistinguishable from the wild type, a ΔarnD strain displays hypermotility. Interestingly, the effect of the ArnD deletion are most pronounced during growth conditions under which the archaellum needs to be repressed leading to the conclusion that this kinase is mainly involved in repression of the archaellum presumably via phosphorylation of the main repressors ArnA and ArnB.

All in all, our results expand and underline the complexity of the phosphorylation-dependent regulatory network that enables and tightly controls expression of the *S. acidocaldarius* archaellum.

P5

Effect of protein phosphorylation on the archaellum regulatory network of *Sulfolobus acidocaldarius*

Lisa Franziska Bischof, Maria Florencia Haurat, Sonja-Verena Albers
Albert-Ludwigs University, Freiburg, Germany

Swimming in Archaea is mediated by the archaellum, a sophisticated motility structure which is assembled via a type IV pili mechanism. In *Sulfolobus acidocaldarius*, a network of proteins is responsible for the regulation of archaellum expression. Reversible protein phosphorylation is involved in both, the activation as well as the repression of archaellum protein expression. Two membrane-bound one-component systems, ArnR and ArnR1, regulate the expression of the archaellum filament forming protein (the archaellin) FlaB by binding with their N-terminal helix-turn-helix DNA-binding domain to a conserved region of the *flaB* promotor. Apart from the N-terminal Helix-turn-Helix domain, both proteins contain a putative sensor domain, a HAMP-domain and two transmembrane domains separated by a short loop located in the pseudoperiplasm. Remarkably, the DNA-binding domain of the two proteins is nearly identical on amino acid level, whereas the sensory domain as well as the HAMP domain vary. This indicates that different stimuli might be sensed by the two proteins. The phosphorylation status of the two proteins is thought to regulate their functional specificity and might be crucial for the interaction of ArnR and ArnR1 with each other or with other proteins of the archaellum regulatory protein network. The precise mechanism underlying ArnR and ArnR1 function is investigated, as this is a crucial step for understanding the regulatory cascade underlying archaellum expression and thus S. *acidocaldarius* motility.

P6

Identification of the *Sulfolobus acidocaldarius* archaellum stator complex formed by FlaG and FlaF

Patrick Tripp[1], Chi-Lin Tsai[2], Paushali Chaudhury[1], Ankan Banerjee[3], John Tainer[2], Sonja-Verena Albers[1]

[1]Albert-Luwigs-Universität Freiburg, Institut für Biologie II, Mikrobiologie, Freiburg, Baden-Württemberg, Germany, [2]The University of Texas MD Anderson Cancer Center, Molecular and Cellular Oncology, Houston, TX, USA, [3]Philipps-Universität Marburg, Fachbereich Chemie, AK Essen, Marburg, Hessen, Germany

Among Archaea motility is exclusively mediated by archaella, formerly known as archaeal flagella. Archaella are unique structures since they share similarities with type IV pili (T4P), while generating thrust like bacterial flagella.

Periplasmic archaellum components have yet to be identified. The monotopic membrane proteins FlaG and FlaF are conserved among all archaellated species and harbor an archaellin-domain, making them good candidates for periplasmic localization.

Heterologously produced soluble domains of FlaG (sFlaG) and FlaF (sFlaF) from *Sulfolobus acidocaldarius* were used for biochemical characterization. Sedimentation assays were performed to prove interaction between sFlaF and the major component of the *S. acidocaldarius* cell wall, the S-layer.

sFlaF crystallized as dimer and showed a β-sandwich fold. Site-directed mutagenesis within the FlaF dimer generated a monomeric FlaF species ($FlaF^{I86K}$) which is not able to restore motility in a $\Delta flaF$ strain.

Purified sFlaG was only stable in pH 3 environment. For Microscale Thermophoresis interaction studies, also sFlaF was shifted to pH 3 buffer and maintained stability. sFlaG and sFlaF interact with a K_D = 14 - 16 µM, while interaction between sFlaG and $sFlaF^{I86K}$ was not observed.
A heterotetrameric sFlaG/sFlaF-complex was reconstituted of which the crystal structure was obtained. The sFlaG/sFlaF-crystal structure was verified by site-directed mutagenesis. It was shown that the presence of the tetrameric complex is required but not sufficient for *S. acidocaldarius* motility.

Based on these results, we propose that FlaG and FlaF form the periplasmic archaellum stator complex that anchors the rotating machinery to the cell wall.

P7

Regulation of UV inducible pili system in *Sulfolobus acidocaldarius*

Thuong Le, Sonja-Verena Albers
Biology II, Freiburg, Egypt

In response to UV stress, species from the order of Sulfolobales including *Sulfolobus acidocaldarius* produce a special type IV pilus: the Ups pilus (UV-inducible pilus of Sulfolobales). These pili initiate species specific cellular aggregation. Within these aggregates cells can exchange DNA which can be used to repair UV-induced DNA damages via homologous recombination. All genes in the *ups*-operon were found to be highly induced upon UV irradiation. They encode: an ATPase (UpsE), a membrane protein (UpsF), two pilin subunits (UpsA, B) and a protein of unknown function (UpsX). Previous deep sequencing studies on *S. acidocaldarius* RNA suggest a primary TSS (transcription start site) in front of *upsX* and secondary TSSs in front of *upsE* and *upsA*. However, so far, it remained unclear how the *ups* operon is regulated.

With the use of LacS reporter assay, we found a conserved upstream motif (-44 to -38) of the *ups*-operon which plays an important role in the activity of the *upsX* promoter in response to UV stress. Nevertheless, substitution of these nucleotides does not have an effect on the transcription of other genes in the *ups*-operon which were believed to be transcribed together. Hence, the upstream-sequence is only essential for *upsX*-transcription while the *upsX* promoter is essential for transcription of the other *ups*-genes suggesting a more complex regulatory mechanism of the *ups* operon.

This study has given the first hints about regulation of the UV- response in *Sulfolobus* species, which could lead to further understanding of responsive mechanism to environmental stress in Archaea.

P8

Surface structures of *Haloarcula hispanica*

Anna Galeva[1], Dahe Zhao[2], Jingfang Liu[2], Alexey Syutkin[1], Mikhail Pyatibratov[1], Sergey Beznosov[1], Haibo Yang[2], Guiming Liu[2], Hua Xiang[2], Oleg Fedorov[1]

[1]*Institute of Protein Research, Russian Academy of Sciences, Pushchino, Moscow region, Russia,*

[2]*Institute of Microbiology, Chinese Academy of Sciences, Beijing, China*

During the evolution Archaea have developed various surface structures allowing them to survive in harsh environment. The most studied of them is the archaellum, a unique device of archaeal motility. The extracellular part of archaellum is a helical filament composed of multiple copies of the structural protein – archaellin. An interesting feature of Archaea is that different species can vary greatly in the number and organization of archaeallin genes. Studies of this phenomenon have shown that the presence of several archaellins can be critically necessary for motility in some species and unessential in others.

Currently, we investigate the motility apparatus of the haloarchaea *Haloarcula hispanica*. This organism has been previously an unexplored type of the archaellin gene organization with three archaellin genes, each of which is under its own promoter. Using each archaellin gene knockout, we showed that no one of archaellins is essential for motility. Thus, this organism has redundancy in archaellin genes, the biological role of which remains unknown.

In addition, we have found that the *H. hispanica* synthesizes a new type of surface filamentous structures (Tat-fibers). The uniqueness of these structures is that they are formed of a protein subunit, secreted using the Tat-pathway and does not contain an N-terminal alpha-helix, which is necessary for polymerization of archaellum and archaeal pili, formed by the Sec-pathway.

This study was supported by RFBR grant № 14-04-01604-a.

P9

Origin and evolution of the Wood-Ljungdahl pathway in the Archaea.

Panagiotis Adam, Guillaume Borrel, Simonetta Gribaldo
Institut Pasteur, Department of Microbiology, Unité de Biologie Moléculaire du Gène chez les Extrêmophiles, Paris, France

Among pathways of carbon fixation, the Wood-Ljungdahl (WL) pathway possesses one of the widest taxonomic distributions, being traditionally associated with methanogenic Archaea and acetogenic Bacteria alike. The two domains exhibit wide discrepancies in the enzymes and cofactors involved in the methyl branch. Nonetheless, the carbonyl branch is shared between Bacteria and Archaea, and is driven by the enzymatic complex of carbon monoxide dehydrogenase/Acetyl-CoA synthase (CODH) of which four subunits are shared. This lends support to the hypothesis that the pathway is ancient, possibly dating to the last common ancestor of Archaea as demonstrated by recent analyses, and possibly of both domains. In order to understand when the WL pathway emerged and how it evolved during archaeal diversification, we have initiated exhaustive phylogenomic analyses of the genes involved, using an updated sampling of archaeal genomes including the entire span of their so-called dark matter. Here we present preliminary results concerning the evolution of the CODH complex.

P10

Methanogenesis and the Wood-Ljungdahl pathway: an ancient, versatile, and fragile association

Guillaume Borrel[1,*], Panagiotis S. Adam[1,2], Simonetta Gribaldo[1,*]
[1]*Institut Pasteur, Department of Microbiology, Unité de Biologie Moléculaire du Gène chez les Extrêmophiles, Paris, France*
[2] *Université Paris Diderot, Sorbonne Paris Cité, Paris, France*

Methanogenesis coupled to the Wood-Ljungdahl (WL) pathway is one of the most ancient metabolisms for energy generation and carbon fixation in the Archaea. Recent results are sensibly changing our view on the diversity of methane-cycling capabilities in this Domain of Life. The availability of genomic sequences from uncharted branches of the archaeal tree has highlighted the existence of novel methanogenic lineages phylogenetically distant to previously known ones, such as the Methanomassiliicoccales. At the same time, phylogenomic analyses have suggested a methanogenic ancestor for all Archaea, implying multiple independent losses of this metabolism during archaeal diversification. This prediction has been strengthened by the report of genes involved in methane-cycling in members of the Bathyarchaeota (a lineage belonging to the TACK clade), representing the first indication of the presence of methanogenesis outside of the Euryarchaeota. In light of these new data, we discuss how the association between methanogenesis and the WL pathway appears to be much more flexible than previously thought, and might provide information on the processes that led to loss of this metabolism in many archaeal lineages. The combination of environmental microbiology, experimental characterization, and phylogenomics opens up exciting avenues of research to unravel the diversity and evolutionary history of fundamental metabolic pathways.

P11

Development of novel transaminases from extreme halophiles: A culture-based approach to biocatalyst discovery from the haloarchaea

Stephen Kelly, Julianne Megaw, Brendan Gilmore
Queen's University, Belfast, UK

Enzymes from haloarchaea have the ability to tolerate extreme industrial conditions, including the use of organic solvents and molar concentrations of salt. This makes them a fascinating area of study with respect to applications in biotechnology. We have cultivated a library of extreme halophiles from Kilroot salt mine, a thalassohaline environment formed around 220 million years ago. The microbiome of the mine, which has remained relatively undisturbed during this time, could allow for the discovery of ancient biocatalysts, with properties and substrate ranges unlike anything currently available.

Chiral amines have emerged as vital and versatile molecules in the synthesis of active pharmaceutical ingredients (APIs). Perhaps the most efficient approach to their production is through the use of transaminase enzymes. Halophilic transaminases have huge potential as robust and effective biocatalysts, with the ability to function under the extreme conditions which render mesophilic enzymes ineffective.

In this study, 5 haloarchaeal transaminase genes have been cloned from *Halobacterium noricense* and *Halorubrum saccharovorum*. Following expression in *Escherichia* coli, these proteins have been denatured and refolded to their active conformation. The activity of these recombinant proteins was then assessed against a variety of ketone substrates over a range of experimental parameters.

P12

Hot and Sweet – Elucidation of the N-glycosylation pathway in the thermoacidophilic crenarchaeaon S. acidocaldarius

Benjamin H. Meyer[1], and Sonja-Verena Albers[2]
[1]*Division of Molecular Microbiology, School of Life Sciences,University of Dundee, Dundee, UK*
[2]*Molecular Biology of Archaea, Albert-Ludwigs-Universität Freiburg Institut für Biologie II, Schänzlestr. 1, 79104 Freiburg, Germany*

The *N*-glycosylation process is conserved across all three domains of life. In contrast to Bacteria the N-glycosylation is distributed in almost all Archaea, which exhibit a high variety on dolichol pyrophosphate linked *N*-glycan, in terms of structure and composition. However, the *N*-glycosylation process in the ancient archaeal kingdom, the crenarchaeota is still uncovered. Here we will report the first results elucidating the *N*-glycosylation pathway in the thermoacidophilic archaeon *Sulfolobus acidocaldarius.* The S-layer protein SlaA*,* used as a reporter glycoprotein, possesses a remarkably high glycosylation density, averaging one site for each stretch of 30–40 residues. Each of the glycosylation sites observed was shown to be modified with a heterogeneous family of glycans, with the largest composed of a tribranched hexasaccharide (Glc-QuiS)-(Man)-(Man)-$GlcNAc_2$, which contain a chitobiose core reminiscent of the eukaryal *N*-glycans*.* A marker less deletion mutant of the *alg3* coding for UDP-Sulfoquinovose and agl16, encoding a glycosyltransferase revealed a reduced *N*-linked glycan on the S-layer. Phenotypical characterization of the deletion mutants revealed that a truncated N-glycan significant affects cell motility, cell-cell interaction and recognition, as well as cell growth at elevated salt concentration. Furthermore these studies give the first insight that the crenarchaeal N-glycosylation process in *S. acidocaldarius* is essential in contrast other archaeal species studies so far.

MICROBIOLOGY SOCIETY

Charles Darwin House, 12 Roger Street, London, WC1N 2JU, UK
www.microbiologysociety.org

The Microbiology Society is a membership organisation for scientists who work in all areas of microbiology. It is the largest learned microbiological society in Europe with a worldwide membership based in universities, industry, hospitals, research institutes and schools. The Society publishes key academic journals, organises international scientific conferences and provides an international forum for communication among microbiologists and supports their professional development. The Society promotes the understanding of microbiology to a diverse range of stakeholders, including policy-makers, students, teachers, journalists and the wider public, through a comprehensive framework of communication activities and resources.