Meeting report

Archaea at St Andrews

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A report of the Biochemical Society meeting 'The Molecular Biology of Archaea', St Andrews, UK, 19-21 August 2008.

The closest that most biologists ever come to working with archaea is probably when they use a DNA polymerase such as Pfu, from the hyperthermophilic archaeon *Pyrococcus furiosus*, in PCR. Misconceptions about archaea still abound: everyone now appreciates that they are not bacteria, but they are often still thought of as curious organisms that live only in extreme environments, inaccessible to genetic study and of interest only to those at the fringes of biological research. The extent to which these views are outdated was abundantly illustrated at a recent meeting on the molecular biology of archaea at St Andrews in Scotland. In fact, archaea are widely distributed in all environments and are of considerable geochemical importance. For some model species an impressive genetic toolkit is available, which in combination with the 50 or so completely sequenced and annotated archaeal genomes, is leading to significant advances in the understanding of archaeal gene function. And because of their relatedness to eukaryotes, study of many core biological processes in archaea can illuminate, often at a structural level, similar processes in their more complex eukaryotic cousins.

DNA replication, recombination, and repair

One of the great attractions of working on archaea is that they provide stripped-down forms of processes that occur in eukaryotes. Dale Wigley (Clare Hall Laboratories, Cancer Research UK, South Mimms, UK) discussed one such example, the replication origin binding protein (ORC) of *Aeropyrus pernix*, which could provide insight into the conserved elements of eukaryotic origin recognition. At the replication origin of the *A. pernix* chromosome are four ORB elements, short conserved repeats arranged in pairs on either side of a AT-rich region named a duplex unwinding element. Wigley's group has shown that only one of the two ORC proteins (ORC1) in *A. pernix* binds at the origin, where it recruits MCM (mini-chromosome maintenance) helicase. ORC1 is made up of two domains: a Wing-helix domain that interacts with DNA and MCM helicase, and an AAA+-ATPase domain, which surprisingly also shows significant interaction with DNA. The interaction between the AAA+ domain and the DNA causes both a widening of the minor groove and DNA unwinding. MCM helicase (an ATP-dependent 3'→5' helicase) was also discussed in a talk by Zvi Kelman (University of Maryland Biotechnology Institute, Rockville, USA) who focused on the differing properties of MCM in *Methanothermobacter thermolithotrophicus* (*Mt*) and *Thermoplasma acidophilum* (*Ta*). His group has found that *Ta*MCM requires a fork-like structure for efficient helicase activity, whereas *Mt*MCM requires a 3'-overhang. MCM helicase and ATPase activity is stimulated by cdc6 (an alternative name for the ORC) in *T. acidophilum*, but helicase activity is inhibited by cdc6 in *M. thermolithotrophicus*. *Mt*cdc6 is also able to dissociate the *Mt*MCM complex in an ATP-dependent manner. Another critical protein for DNA replication and repair, PCNA (named for its eukaryotic homolog, proliferating cell nuclear antigen), was covered by Malcolm White (St Andrews University, UK). PCNA acts as a 'molecular toolbelt', bringing relevant proteins to the DNA at sites such as stalled replication forks. White has found that *Sulfolobus solfataricus* PCNA interacts with repair nucleases (Hjc and Xpf) that excise the lesions likely to cause stalled replication, and controls their activity within the cell.

Patrick Forterre (University of Paris-Sud, Paris, France) introduced a DNA repair complex called KEOPS, which has recently been described in yeast. The KEOPS complex is an example of a DNA-processing system that may have been present in the common ancestor of the Archaea and the Eukarya, but not the Eubacteria. Forterre described the

identification in archaea of four of the five genes encoding the proteins of this complex. One of these genes encodes the universal protein, Kae1, previously misannotated in many genomes as a peptidase. Forterre and colleagues have in fact found that it is an AP-lyase that cleaves depurinated DNA. In some archaea, such as *Methanocaldococcus jannaschii*, the genes for the KEOPS kinase protein and Kae1 are fused, and this fusion protein has been characterized at the structural level by Forterre and colleagues.

Christa Schleper (University of Vienna, Austria) described the transcriptional response of *S. solfataricus* to ultra violet radiation. Pilus formation was significantly upregulated, leading to cellular aggregation. She suggested that there may be exchange of DNA between the aggregated cells, facilitating the repair of damaged DNA by homologous recombination. The aggregation is species specific, with cells of *S. tokodaii* and *S. acidocaldarius* forming discrete clumps in mixed cultures. Dennis Grogan (University of Cincinnati, USA) described an *in vivo* assay of homologous recombination in *S. acidocaldarius*, using electroporated donor DNA with a multiply marked *pyrE* gene. Remarkably, he has found that recombination occurs between markers that are extremely close together, and overall it appears likely that multiple, discontinuous tracts of donor DNA of varying sizes are responsible for recombination in this organism.

Reverse gyrase is a DNA topoisomerase that introduces positive supercoiling, and is unique to thermophilic organisms, being present in all hyperthermophilic and some moderately thermophilic archaea, but absent from all mesophiles. Maria Ciaramella (Institute of Protein Biochemistry, Naples, Italy) described the reverse gyrase of *S. solfataricus*. Its biological function is not known, although thermal stabilization of DNA at the growth temperature of hyperthrmophiles could be one role. Her group has shown that the protein has two domains: an amino-terminal helicase domain and a carboxyl-terminal topoisomerase domain which could be expressed separately and reconstituted into a functional unit.

Archaeal transcription and the roles of RNA

The transcriptional apparatus of archaea has many similarities to that of eukaryotic cells, but is simpler: there is only one RNA polymerase, which resembles eukaryotic RNA polymerase II (Pol II), most of the subunits of which have clear homologs in the archaeal enzyme. Finn Werner (University College London, UK) described how his group has reassembled the 12-subunit archaeal RNA polymerase complex from individual purified components into a form that can actively transcribe *in vitro*, a feat not yet possible with the eukaryotic RNA Pol II. This, allied with the fact that a complete structure exists for this RNA polymerase, is enabling his group to perform a detailed dissection of subunit roles and structure-function relationships. Werner noted the example of the F and E subunits, which are found in archaea and in eukaryotic RNA polymerases (for example, as RPB7 and RPB4 in RNA Pol II), but are absent from the bacterial enzyme. These subunits form a complex that protrudes from the main catalytic core of the RNA polymerase, and appear to have multiple roles. They promote closure of the clamp formed by subunits A and B, which in turn can affect DNA melting and open complex formation, which in the presence of the transcription factor TFE can lead to transcription. Interestingly, Werner has found that they can also stimulate processivity of the RNA polymerase, an activity that correlates with their ability to bind RNA. The close evolutionary relationship between archaeal and eukaryotic RNA polymerases was further illustrated by Michael Thomm (University of Regensburg, Germany) who has demonstrated, using a similar *in vitro* assembly system, that some eukaryotic subunits can successfully replace their archaeal homologs. An archaeal RNA polymerase from *P. furiosus* lacking subunit P, for example, fails to form open complexes (where the DNA strands are partially unwound prior to mRNA synthesis), but this ability can be restored by the homologous subunit Rpb12. The archaeal genes cannot rescue gene function in yeast mutants, however, although some incorporation of subunits does occur.

Attention was also paid to other aspects of RNA function and metabolism. Karl-Peter Hopfner (University of Munich, Germany) discussed his group's recent structural and biochemical work on the exosome of *Archaeoglobus fulgidus*, which is a nine-subunit complex responsible for RNA degradation. This protein complex binds RNA and degrades it into trinucleotides, and is remarkably analogous to the proteasome: RNA has to be threaded through a narrow pore into a central region of the protein complex where cleavage takes place. The exosome consists of a trimeric cap which binds the RNA, sitting atop a hexameric complex which contains the active site for degradation. Accessory factors may also be involved in melting the RNA to enable it to pass into the narrow pore.

Jörg Soppa (University of Frankfurt, Germany) discussed how the comparison of free mRNA to polysome-bound mRNA using microarrays can be used to determine translational efficiency, and showed how such comparisons in two different haloarchaea indicate that many genes display significant departures from average translational efficiency; this is the first such study done in any prokaryote. He also described work on the occurrence of small RNAs in archaea, showing that *Haloferax volcanii* is likely to contain more than 150 small RNAs, some of which have been shown to give rise to phenotypes in stress resistance and carbon metabolism when mutated. Given the current excitement about novel roles of RNA in eukaryotes, this is surely an area where more developments are to be expected in archaea.

The theme of novel roles for RNA was also reflected in talks on virus and phage resistance. One of the more surprising findings of the past two years in prokaryote biology has been the discovery of a system for phage resistance in bacteria - surprising because, given the long history of phage and bacterial genetics, one might have expected that all the low-hanging fruit in this area would have been plucked long ago. This system, mediated by short repeat DNA elements called CRISPR sequences and a set of associated genes called *cas* (for CRISPR-associated) genes, was reviewed by John van der Oost (Wageningen University, the Netherlands), who has reconstituted the system in *Escherichia coli* to enable detailed study of its function. In this system, viral sequences become integrated between the CRISPR repeats, and processing of the long transcript of the CRISPR region by the Cas proteins produces small RNAs carrying short phage sequences that can interfere with the phage infectious cycle. This has been likened to a primitive form of immunity. Roger Garrett (Copenhagen University, Denmark) discussed the occurrence of CRISPR-like sequences in archaeal genomes, and showed not only how they often match sequences found in archaeal viruses and plasmids - which means they might have a regulatory role instead of, or in addition to, an immune one - but also how evidence exists in some archaeal viruses of defenses against the CRISPR system.

Despite the relatedness of archaea and eukarya, and the extent to which studies on fundamental processes at the genome level show clear similarities in mechanisms between the two, it might be assumed that work on archaea is unlikely to lead to new insights into processes that are normally considered unique to eukaryotic cells, such as vesicle formation by membrane invagination. However, it transpires that vesicle formation may be very much a part of some archaeal life-cycles: elegant microscopy described by Reinhard Rachel (University of Regensburg, Germany) shows that membrane invagination and vesicle formation may be involved in the cell division of the archaeon *Ignicoccus hospitalis*, and that vesicles are also sometimes seen in the vicinity of the contact sites between this microorganism and the parasitic archaeon *Nanoarchaeum equitans*, which uses *I. hospitalis* as a host. These observations served as a reminder that, even in a post-genomic world, there is still no substitute for actually looking at cells. The hunt is now on for the genetic determinants of this process.

Reverse genetics is of course a key part of functional genomics. Various tools for this are now well advanced in several archaea, notably halophiles and some methanogens. The meeting heard reports from Qunxin She (University of Copenhagen, Denmark) and Sonja Albers (University of Gronigen, the Netherlands) on further development of a number of reverse genetic tools, including expression and shuttle vectors and new methods for making knockouts, for different species of *Sulfolobus*. Importantly, these include S. *sulfotaricus*, widely used as a model organism but hitherto not very genetically tractable. It is becoming more routine to include genetic analysis alongside biochemical studies, and the ability to do complementation assays with site directed mutants means that the linkage between structural, biochemical and genetic studies is now in place for many archaeal species. All this promises well for the future and the ability of archaea to give structural and evolutionary insights into basic and universal cellular processes.