

P021 Reverse genetic analysis of an archaeal MCM
**Tanja Kristensen, Reeja Cherian, Fiona Gray,
Kenneth Jensen and Stuart MacNeill**

*Department of Biology, University of Copenhagen,
Denmark.*

The MCM helicase plays a key role during chromosome replication in eukaryotic cells, most likely by unwinding DNA at the replication fork. In order to better understand the properties of this enzyme, we are using the genetically-tractable euryarchaeon *Haloferax volcanii* as a model system. Through a combination of comparative sequence analysis and X-ray crystallography, a number of interesting structural features have been identified in the N-terminal domain of MCM, specifically a zinc binding motif, a DNA binding β -hairpin loop and a β -hairpin loop with a role in inter-domain communication. Each of these has been subjected to extensive biochemical analysis *in vitro* but no *in vivo* analysis has been reported. Therefore, to investigate the importance of these structural features for MCM function *in vivo*, we have initiated a programme of reverse genetic analysis in order to test whether it is possible to perturb these elements without affecting cell viability and, if this is the case, what phenotype consequences result. The results of these studies demonstrate both essential and non-essential roles for conserved residues located within these structural motifs. In addition, we are undertaking reverse genetic analysis of the HTH domain located near the C-terminus of the MCM protein. The results of these studies will be presented.