

P028 Biochemical characterization of various components of the *Sulfolobus solfataricus* pre-replicative complex

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The initiation of DNA replication in eukaryotes is triggered at origins of replication after assembly of the pre-Replicative Complex, including Orc1-6, Cdc6 and Cdt1, factors which are all essential for the recruitment of the MCM2-7 helicase. Archaea initiate DNA replication using simple orthologs of the eukaryotic initiators. *Sulfolobus solfataricus* possesses one MCM, one Cdt1 homolog named WhiP and three Orc1 homologs. The atomic structure of a DNA-bound Orc1 hetero-dimer has been recently determined, describing a DNA-sequence dependent protein oligomerisation event at the origin of replication. Building on these results, we have characterised a number of structure-based mutants of the Orc1 proteins (kindly provided by our collaborators Erin Dueber and James Berger), to determine the role of distinct functional elements involved in the interaction with origin DNA. We identified mutants which affect the oligomeric state of Orc1 initiators, and we show that they mimic the behaviour of wild-type proteins in specific nucleotide states. These findings lead us to suggest the nature of a conformational change within a DNA-bound Orc1 protein, associated with nucleotide binding and hydrolysis. In addition, we determined the sequence specificity of the WhiP protein and investigated the interplay between WhiP and Orc1 proteins at various origins of replication. Altogether, these results lay the ground for describing the network of macromolecular interactions, which lead to the recruitment and loading of the MCM helicase.