Towards the mechanism of replicative helicase activation in eukaryotes: A functional analysis.

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DNA Replication is amongst the most essential processes of all living organisms and, when misregulated, presents a major cause of genomic instability and cancer. A key step in eukaryotic DNA replication activation is the establishment of bidirectional replication forks. The minichromosome maintenance 2-7 complex (MCM2-7) is the core helicase that unwinds DNA ahead of the replication machinery. Initially assembled on double-stranded DNA as an inactive double hexamer, MCM2-7 is converted to an active Cdc45-MCM-GINS helicase encircling single-stranded DNA. However, this process and the role of DNA during activation is poorly understood. Here, we found that out of 6 possible Mcm interfaces the Mcm2/5 interface functions as the DNA exit gate for helicase activation using a chemical genetics approach *in vivo*. We show that single stranded DNA, produced by extrusion from the Mcm2/5 interface, is an essential trigger of DNA replication activation in budding yeast. Using high-resolution next generation sequencing approaches, we observed that DNA unwinding initiates from the N-terminal regions of the MCM2-7 double hexamer. Furthermore, using ChIP-qPCR and mass spectrometry approaches, we characterised two protein complexes, which represent an early and late stage of DNA replication initiation. Our results demonstrate for the first time how the inactive MCM2-7 double hexamer is converted to the active core of the replication fork *in vivo*, elucidating the mechanism of eukaryotic replicative helicase activation. Due to the manifold structural homologies of most proteins between species, we anticipate this mechanism to be conserved throughout the eukaryotic kingdom. This will be in particularly interesting for the future development of anti-cancer drugs and treatments.