

Activation of an Origin of Replication Visualised by Cryo-EM

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In eukaryotes, DNA loading and activation of the MCM replicative helicase are temporally separated to ensure that chromosomes are copied only once per cell cycle¹. Before replication initiation, MCM is loaded onto duplex DNA at replication origins forming an inactive head-to-head double hexamer². Upon S phase transition, helicase activation occurs in two steps, involving limited opening of the double helix first, and then topological separation of the two DNA strands^{3,4}. I will describe how imaging *in vitro* reconstituted reactions informs the mechanism of origin activation.

References:

- [1] Costa, A. & Diffley, J. F. X. The Initiation of Eukaryotic DNA Replication. *Annual review of biochemistry*, doi:10.1146/annurev-biochem-072321-110228 (2022).
- [2] Li, N. et al. Structure of the eukaryotic MCM complex at 3.8 Å. *Nature* 524, 186-191, doi:10.1038/nature14685 (2015).
- [3] Miller, T. C. R., Locke, J., Greiwe, J. F., Diffley, J. F. X. & Costa, A. Mechanism of head-to-head MCM double-hexameric formation revealed by cryo-EM. *Nature* 575, 704-710, doi:10.1038/s41586-019-1768-0 (2019).
- [4] Douglas, M. E., Ali, F. A., Costa, A. & Diffley, J. F. X. The mechanism of eukaryotic CMG helicase activation. *Nature* 555, 265-268, doi:10.1038/nature25787 (2018).
- [5] Lewis, J. S. et al. Mechanism of replication origin melting nucleated by CMG helicase assembly. *Nature* 606, 1007-1014, doi:10.1038/s41586-022-04829-4 (2022).