Elucidating the DDK-dependent step in replication initiation

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By phosphorylating specific replication factors, cell cycle kinases ensure that eukaryotic DNA replication is initiated once and only once per mitotic cell division. New work in The EMBO Journal now reveals how DDK-mediated phosphorylation of Mcm2-7 helicase subunits is read out by Sld3, which provides further integration with CDK phosphorylation.

See also: TD Deegan et al (May 2016)
on replication origins from purified yeast proteins and demonstrated that DDK-dependent association of Sld3 and Cdc45 with pre-RCs can be recapitulated even in vitro. Since DDK efficiently phosphorylated origin-loaded Mcm2-7 complexes, such complexes could now be disassembled and tested for Sld3 interaction in binding assays. These analyses revealed that phosphorylated Mcm4 and Mcm6 strongly bound to Sld3. Mcm2, Mcm4 and Mcm6 bear N-terminal extension that has been shown to be extensively phosphorylated by DDK. Furthermore, Deegan et al. (2016) found that several synthetic Mcm4 and Mcm6 phosphopeptides with possible DDK-targeted sites also bind to Sld3 in vitro.

Deegan et al. (2016) mapped the Mcm2-7 binding site in Sld3 to its middle part, which also binds to Cdc45, and constructed specific mutations affecting either of these interactions. An unstructured loop in STD/CDB is known to be important for binding to Cdc45 (Itou et al., 2014), while basic amino acid residues after the STD/CDB were required for Mcm2-7 binding. Substitution of conserved basic residues with acidic residues in either region selectively reduced the binding to Cdc45 and Mcm2-7, respectively. This revealed that while Sld3 and Cdc45 are mutually dependent for loading onto phosphorylated Mcm2-7 in vivo (Kamimura et al., 2001), Sld3 can be specifically recruited to Mcm2-7 even in the absence of Cdc45 in vitro. Moreover, mutant Mcm4 and Mcm6 proteins with site-specific substitutions mimicking DDK phosphorylation were able to bypass the requirement for Cdc7, the catalytic subunit of DDK. In fact, such mutants support Sld3 association with Mcm2-7 in the absence of DDK phosphorylation in vitro. In an additional set of experiments, the authors could demonstrate that DDK-dependent phosphorylation is only required at the step of replication initiation, but not for subsequent elongation. Stalling plasmid replication after initiation by omitting DNA topoisomerase from the in vitro replication system (Yeeles et al., 2015), they found that Mcm2-7 dephosphorylation at this stage did not affect DNA replication efficiency once elongation was resumed by topoisomerase addition (Deegan et al., 2016). Finally, the Sld3 middle region is also phosphorylated by the replication checkpoint protein kinase Rad53, and the authors showed that this inhibits association with Mcm2-7 in addition to Dpb11 and Cdc45 binding.

The new results by Deegan et al. (2016) paper clearly demonstrate that Sld3 recognizes mainly DDK-phosphorylated peptides found on Mcm4 and Mcm6. The three-dimensional structure of the Sld3 phosphopeptide-binding region remains to be determined, but it is likely that part of the STD/CDB contributes to the interaction as well, and we can probably expect interesting new structural inferences given the lack of resemblance to already known phosphopeptide-binding domains. Moreover, because Sld3 binds to both Mcm4 and Mcm6, and two Sld3 molecules are connected by Sld7 in an anti-parallel orientation (Itou et al., 2015), recruitment of Cdc45 and GINS to double-hexameric Mcm2-7 complexes may occur in trans (Fig 1). Such a putative mechanism may secure simultaneous activation of both Mcm2-7 hexamers, to start DNA synthesis in both directions.

**References**


Deegan TD, Yeeles JTP, Diffley JF (2016) Phosphopeptide binding by Sld3 links Dbf4-dependent kinase to MCM replicative helicase activation. *EMBO J* 35: 961–973


