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Initiation of replication in Xenopus egg extracts at a spatially defined origin

Comment on: Sanuki Y, et al. RecQ4 promotes the conversion of the pre-initiation complex at a site-specific origin for DNA unwinding in Xenopus egg extracts. Cell Cycle 2015; 14(7):1010–23; PMID:25602506; http://dx.doi.org/10.1080/15384101.2015.1007003

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DNA replication is accomplished by the timely ordered assembly of various replication proteins at specific loci on the genome, called a 'replication origin'.[1] In higher eukaryotes some genomic loci have been identified as putative replication origins but there does not appear to be much sequence specificity at these origins. Considering that most intensive studies on replication have been carried out in yeast model systems, a novel *in vitro* system to interrogate a defined origin of replication can provide great insights into DNA replication in higher eukaryotes. Even though defined origins derived from some viruses have been used to study metazoan replication, extrapolating these results to native metazoan replication is difficult due to the dependency on viral factors for the origin licensing in these systems.

Eukaryotic origins of replication are first licensed by the helicase loading complex proteins ORC, CDC6, CDT1 to load the MCM2-7 helicase (the entire complex, including the MCM2-7 being considered part of the pre-replicative complex, or pre-RC).[1] Upon the activation of CDK2 and CDC7 kinases, the helicase is activated by the loading of CDC45 and GINS to form the active CMG (Cdc45-MCM2-7-GINS) helicase.[1] Yeast SLD2 and SLD3 proteins are intimately involved in the formation of CMG, in a reaction depended on phosphorylation by CDK kinase. GAL4 fused to components of ORC or to CDC6 were first shown to promote replication initiation from an array of GAL4-binding sites on episomes transfected into mammalian cells.[2] Subsequently, a similar strategy of artificially tethering GAL4-replication initiators (ORC or Cdt1), and chromatin acetyltransferases initiated replication from a chromosomal origin of replication at the c-Myc locus, though in this case a DNA Unwinding Element (DUE) was also necessary for replication initiation.[3] In this issue of Cell Cycle, Sanuki *et al.* introduce a novel *in vitro* replication system, which uses a similar strategy to create a defined site of replication initiation in *Xenopus* egg extracts.[4] They tether a GAL4-binding domain fused to CDC6 to a specific site on a plasmid. Representative proteins marking helicase loading and helicase activation, such as MCM2-7 subunits and CDC45, GINS are successfully assembled onto the GAL4-CDC6 bound locus in a site-specific manner. They validate that the assembled protein complexes on the artificial origin are functional by demonstrating that addition of geminin (an inhibitor of CDT1) or p21 (a CDK inhibitor) prevents the assembly of MCM subunits or loading of CDC45. Furthermore, Sanuki et al. demonstrate that DNA synthesis and unwinding predominantly occur at the GAL4 origin by examining site-specific BrdUTP incorporation and ssDNA cleavage of DNA bubble structures by P1 nuclease digestion. The specific initiation site is an advance, because traditional *in vitro* replication (of plasmids or sperm chromatin) in *Xenopus* egg extracts initiates at multiple (non-specific) sites on the DNA. While the effect of permanently tethered CDC6 at the artificial origin could complicate interpretations of the formation and activation of the pre-IC, the report by Sanuki et al. is the first that can monitor spatial and temporal changes in protein assembly and DNA structure at a specific site during replication initiation.

Many factors have been suggested to participate in DNA replication, however detailed mechanistic understanding of this process is lacking. RECQL4, a putative homolog of yeast SLD2, is essential for the initiation of DNA replication, and is conserved in higher eukaryotes.[5] Although yeast SLD2 is required for assembly of the active helicase CMG,[6] the exact role of RECQL4 in promoting replication initiation is debated in higher eukaryotes. Sanuki et al. show that *Xenopus* RECQL4 loading on an origin is independent of CDK activity and successful loading of RECQL4 is required for the recruitment of polymerase α and origin firing. Interestingly, *Xenopus* RECQL4 is dispensable for the loading of CDC45 at the origin as well as DNA binding of CMG. Overall, *Xenopus* RECQL4 seems to not be involved in the recruitment and assembly of the CMG complex, but is required for activating the CMG helicase to initiate unwinding and recruitment of RPA and DNA polymerase α. Although further work is needed to understand exactly how RECQL4 activates the CMG complex, the different function of Human[7] and *Xenopus* RECQL4 (compared to yeast SLD2) suggests that even conserved proteins may function differently across species. Future use of this powerful system will broaden our understanding of DNA replication initiation.

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