

INSIGHTS INTO THE ROLES OF HUMAN CDC6 AND REPLICATION PROTEIN A
IN INITIATION OF EUKARYOTIC DNA REPLICATION

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Since the discovery of the structure of DNA as an information-bearing molecule of the cell in 1953 by Watson and Crick, considerable effort was directed towards elucidating the mechanism by which DNA replication occurs.

Simian virus 40 (SV40) provides a powerful model system for study of eukaryotic DNA replication in which a viral protein, large T antigen (Tag), marshals the host's replication machinery to replicate the viral mini-chromosome. SV40 replication requires interaction of Tag with the host ssDNA binding protein, replication protein A (RPA). The C-terminal protein interaction domain of the RPA32 subunit (RPA32C) facilitates initiation of SV40 DNA replication, but whether it interacts with Tag is not known. Affinity chromatography and NMR were used to demonstrate physical interaction between RPA32C and the origin DNA binding domain of Tag. The structures of these domains were docked together using NMR data. Based on electrostatic complementarity in the complex, point mutations were designed to reverse charges in the binding sites, resulting in substantially reduced binding affinity. Corresponding mutations introduced into intact RPA impaired initiation of SV40 DNA replication and primosome activity,

implying a critical role for this interaction in assembly and progression of the SV40 replisome.

Cell division cycle 6 (Cdc6) protein plays an essential role in initiation of DNA replication by loading the minichromosome maintenance (MCM) complex of proteins onto chromatin. In order to accomplish its function, human Cdc6 (hCdc6) must also be phosphorylated by cyclin-dependent kinases. Phosphorylation of mammalian Cdc6 is also required for its export from the nucleus to the cytoplasm for replication to ensue. Analysis of several GFP-tagged phosphorylation deficient mutants of hCdc6 for sub-cellular localization in microinjected human cell lines suggests that phosphorylation alone is not sufficient for hCdc6 export from the nucleus. We propose that in human cells an additional mechanism, that could involve human prolyl isomerase, must be activated to initiate replication leading to the release of hCdc6 from the nucleus.

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