Cdc7p is an essential serine/threonine protein kinase whose activity is required throughout S phase for replication origin firing. Mutations in CDC7 cause cell cycle arrest after START but before the hydroxyurea arrest point. Cdc7p is regulated by varying levels of Dbf4p, the same way that CDKs are regulated by cyclins. Cdc7p is the catalytic subunit, activated by association with Dbf4p during late G1. The Cdc7p-Dbf4p complex interacts physically with replication origins: Cdc7p interacts with Orc2p, a component of the origin recognition complex, and Dbf4p interacts with origin DNA. Several lines of evidence suggest that members of the Mcm2-7 protein family are the most likely candidates for in vivo Cdc7p-Dbf4p substrates. Mcm2p and other Mcm2-7 proteins are in vitro substrates for Cdc7p-Dbf4p kinase activity, and the phosphorylation of Mcm2p in vivo depends upon Cdc7p-Dbf4p. Further, a mutation in MCM5/CDC46 can bypass the requirement for Cdc7p kinase activity in initiating DNA synthesis. Results of two-hybrid and GST-Mcm2p fusion affinity column chromatography showed that Cdc7p and Dbf4p interact physically with Mcm2p. Homologs of Cdc7p have been identified in S. pombe, Xenopus, mouse, and human, and a Dbf4p homolog exists in S. pombe; evidence suggests that the homologs may perform the same function as the S. cerevisiae proteins. The Xenopus Cdc7p homolog is required for DNA replication in vivo and in vitro, and mutations in the S. pombe homologcan block the initiation of DNA replication. The human and S. pombe Cdc7p homologs can phosphorylate Mcm2-7 proteins in vitro.