Summary

The work presented in this thesis determined the effects on targeted down regulation of a vital gene product involved in the intiation of DNA synthesis in nontransformed cells. MCM4 protein, a member of a heterohexameric MCM complex with a proposed DNA helicase activity was targeted by antisense oligonucleotides in primary human cell line. It was found that targeted downregulation of MCM4 resulted in degradation of the remaining MCM complex proteins with a rapid and specific kinetic. This is a novel finding. Downregulation of MCM4 protein, solubilizes chromatin bound MCM protein complexes resulting in a block of DNA synthesis. Additionally, cells lacking MCM complexes exhibit drastically extended population doubling times resulting in a virtually complete proliferative block. Cells enriched in G1 phase by withdrawal of mitogenic stimulation fail to enter S-phase upon restimulation in the absence of MCM complex. In addition to contributing substantative data for the function of MCM proteins, these results should provide a reference for further studies in primary human cells of the inititation of DNA replication.