The NAD^+-dependent Sir2p Histone Deacetylase is a Negative Regulator of Chromosomal DNA Replication. Donald L. Pappas, Jr., Ryan Frisch and Michael Weinreich* Laboratory of Chromosome Replication, Van Andel Research Institute, Grand Rapids, MI 49503

The establishment of chromosome replication during S phase of the cell cycle requires a multi-step process and begins with the ordered assembly of a large protein complex at origins of replication during a narrow window from late mitosis to early G1 phase. The Origin Recognition Complex (ORC) determines the sites of DNA replication initiation by binding to origin DNA and recruiting additional initiation factors to the chromatin prior to DNA synthesis. The first step in this "chromosome replication cycle" is the formation of a large pre-replicative complex (pre-RC) at individual replication origins throughout the genome during exit from mitosis. Pre-RC formation occurs when Cdc6p(ATP) binds to ORC and facilitates the recruitment of the MCM DNA helicase to origins. It is not known how Cdc6p promotes MCM binding to origins or what role Cdc6p ATP binding or hydrolysis has during initiation. To better understand the genetic requirements for pre-RC formation, we selected chromosomal suppressors of a temperature sensitive *cdc6-4* mutant that altered a conserved residue in the Walker A ATP binding motif. We recovered loss-of-function mutations in the chromatin modifying genes *SIR2*, *SIR3*, and *SIR4* that suppressed the *cdc6-4* temperature sensitive lethality to varying degrees. This suppression was independent of the well-known transcriptional silencing roles for the SIR proteins at the *HM* loci, at telomeres, or at the rDNA locus. A deletion of *SIR2* gave the best suppression and uniquely rescued both the DNA synthesis defect of the *cdc6-4* mutant and its severe plasmid instability phenotype. A *SIR2* deletion suppressed additional initiation mutants affecting pre-RC assembly but not mutants that act subsequently. One model to explain these findings is that Sir2p promotes an inhibitory chromatin structure for pre-RC formation at replication origins. Thus, when Cdc6p function is compromised, a loss of Sir2p promotes a more "open" chromatin structure that allows loading of the MCM proteins. Since there are 7 *SIR2* orthologues in metazoans, a conservation of the ability to repress DNA replication from particular origins could afford one mechanism to establish patterns of DNA replication initiation within the developing organism or in response to cell autonomous signals.