Cooperation of E2F-p130 and Sp1-pRb Complexes in Repression of the Chinese Hamster dhfr Gene

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**Background** In mammalian cells reiterated binding sites for Sp1 and two overlapping and inverted E2F sites at the transcription start site regulate the dhfr promoter during the cell growth cycle.

**Method** Here we have examined the contributions of the dhfr Sp1 and E2F sites in the repression of dhfr gene expression. In serum-starved cells or during serum stimulation, the Chinese hamster dhfr gene was not derepressed by trichostatin A (TSA), an inhibitor of histone deacetylase (HDAC).

**Results** Immunoprecipitation experiments showed that HDAC1 and hypo-phosphorylated retinoblastoma protein (pRb) are associated with Sp1 in serum-starved CHOC400 cells. In transfection experiments, reporter plasmids containing the reiterated dhfr Sp1 sites were stimulated 10-fold by TSA, while a promoter containing four dhfr E2F sites and a TATA box was responsive to E2F but was completely unaffected by TSA. HDAC1 did not coprecipitate with p130-E2F DNA binding complexes, the predominant E2F binding activity in cell extracts after serum starvation, suggesting that p130 imposes a TSA insensitive state on the dhfr promoter. In support of this notion, recruitment of GAL4-p130 to a dihydrofolate reductase-GAL4 reporter rendered the promoter insensitive to TSA, while repression by GAL4-pRb was sensitive to TSA. Upon phosphorylation of pRb and p130 after serum stimulation, the Sp1-pRb and p130-E2F interactions were lost while the Sp1-HDAC1 interaction persisted into S phase. Together these studies suggest a dynamic model for the cooperation of pRb and p130 in repression of dhfr gene expression during withdrawal from the cell cycle.

**Conclusion** We propose that, during initial phases of cell cycle withdrawal, the binding of dephosphorylated pRb to Sp1-HDAC1 complexes and complexes of E2F-1 -to -3 with DP results in transient, HDAC dependent suppression of dhfr transcription. Upon withdrawal of cells into G0, recruitment of p130 to E2F4/DP-1 complexes at the transcription start site results in a TSA-insensitive complex that cooperates with Sp1-HDAC-pRb complexes to stably repress dhfr promoter activity in quiescent cells.