CXXC FINGER PROTEIN 1 UPREGULATES MAINTENANCE DNA METHYLATION

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Abstract

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DNA METHYLATION

This dissertation describes the role of CXXC finger protein 1 (CFP1) in maintaining global cytosine methylation. CFP1 is required for embryogenesis. Murine embryonic stem (ES) cells lacking the *CXXC1* gene (*CXXC1*$^{-/-}$) fail to differentiate and exhibit a ~70% reduction in global cytosine methylation. Maintenance DNA methyltransferase (Dnmt) activity is reduced by 60% in the absence of CFP1 and Dnmt1 protein expression is reduced by 50%. *De novo* Dnmt activity is not decreased in *CXXC1*$^{-/-}$ ES cells. Analysis of steady state Dnmt1 mRNA in ES cells lacking CFP1 revealed that the level of Dnmt1 transcript is elevated 50% compared to wild-type ES cells, in spite of reduced Dnmt1 protein levels. Pulse chase analysis demonstrated a 17% decrease in Dnmt1 protein stability in *CXXC1*$^{-/-}$ ES cells, suggesting that an additional mechanism is required to explain the 50% reduction in steady state protein levels. Global protein synthesis is decreased 15% and Dnmt1 protein synthesis is decreased 23% in *CXXC1*$^{-/-}$ ES cells. Further analysis of polysome profiles in *CXXC1*$^{-/-}$ ES cells revealed that the abundance of ribosomes is decreased compared to wild-type ES cells. The involvement of CFP1 in global protein synthesis provides a novel function for this epigenetic regulator.

Immunoprecipitation and immunofluorescence analyses revealed that CFP1 and Dnmt1 proteins interact and partially co-localize in the nucleus. The minimal regions of Dnmt1 that interact with CFP1 are found in the N- and C-terminus. The N-terminal region of Dnmt1 sufficient for interaction with CFP1 is required for targeting to

chromatin following DNA replication. The minimal regions of CFP1 sufficient for interaction with Dnmt1 also contain domains such as the CXXC domain or PHD domains, which are involved in chromatin association. These results suggest that CFP1 influences global cytosine methylation through several mechanisms involving Dnmt1. A combinatorial mechanism of decreased Dnmt1 protein stability and deficient Dnmt1 protein synthesis leads to decreased steady state Dnmt1 protein levels in *CXXC1*$^{-/-}$ ES cells. Additionally, the interaction between CFP1 and Dnmt1 may affect global cytosine methylation by directly affecting Dnmt1 activity or by influencing the chromatin targeting of Dnmt1.

David G. Skalnik, Ph.D. - Chair

Table of Contents