STRUCTURE-FUNCTION ANALYSIS OF CXXC FINGER PROTEIN 1

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Name: TATE Dissertation.pdf
Size: 11.12Mb
Format: PDF

View/Open

Permanent Link:	http://hdl.handle.net/1805/2044
Date:	2010-01-26
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Degree:	Ph.D.
Department:	Department of Biochemistry & Molecular Biology
Grantor:	Indiana University
Keywords:	protein domains ; cytosine methylation ; embryonic stem cells ; chromatin ; Set1 ; DNA methyltransferase ; Dnmt1 ; histone methyltransferase ; base excision repair ; DNA repair ; Ape1 ; differentiation ; CXXC finger protein 1 ; histone methylation
LC Subjects:	DNA-protein interactions ; Chromatin ; Embryonic stem cells ; DNA repair

Abstract:

This dissertation describes structure-function studies of CXXC finger protein 1 (Cfp1), encoded by the CXXC1 gene, in order to determine the functional significance of Cfp1 protein domains and properties. Cfp1 is an important regulator of chromatin structure and is essential for mammalian development. Murine embryonic stem (ES) cells lacking Cfp1 (CXXC1-/-) are viable but demonstrate a variety of defects, including hypersensitivity to DNA damaging agents, reduced plating efficiency and growth, decreased global and gene-specific cytosine methylation, failure to achieve in vitro differentiation, aberrant histone methylation, and subnuclear mis-localization of Setd1A, the catalytic component of a histone H3K4 methyltransferase complex, and tri-methylated histone H3K4 (H3K4me3) with regions of heterochromatin.

Expression of wild-type Cfp1 in CXXC1-/- ES cells rescues the observed defects, thereby providing a convenient method to assess structure-function relationships of Cfp1. Cfp1 cDNA expression constructs were stably transfected into CXXC1-/- ES cells to evaluate the ability of various Cfp1 fragments and mutations to rescue the CXXC1-/- ES cell phenotype. These experiments revealed that expression of either the amino half of Cfp1 (amino acids 1-367) or the carboxyl half of Cfp1 (amino acids 361-656) is sufficient to rescue the hypersensitivity to DNA damaging agents, plating efficiency, cytosine and histone methylation, and differentiation defects. These results reveal that Cfp1 contains redundant functional domains for appropriate regulation of cytosine methylation, histone methylation, and in vitro differentiation. Additional studies revealed that a point mutation (C169A) that abolishes DNA-binding activity of Cfp1 ablates the rescue activity of the 1-367 fragment, and a point mutation (C375A) that abolishes the interaction of Cfp1 with the Setd1A and Setd1B histone H3K4 methyltransferase complexes ablates the rescue activity of the 361-656 Cfp1 fragment. In addition, introduction of both point mutations (C169A and C375A) ablates the rescue activity of the full-length Cfp1 protein. These results indicate that retention of either DNA-binding or Setd1 association of Cfp1 is required to rescue hypersensitivity to DNA damaging agents, plating efficiency, cytosine and histone methylation, and in vitro differentiation. In contrast, confocal immunofluorescence analysis revealed that full-length Cfp1 is required to restrict Setd1A and histone H3K4me3 to euchromatic regions.

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