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Article

# Histone Methylation Regulator PTIP Is Required for *PPARγ* and *C/EBPα* Expression and Adipogenesis

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## SUMMARY

**PPARγ and C/EBPα cooperate to control preadipocyte differentiation (adipogenesis). However, the factors that regulate *PPARγ* and *C/EBPα* expression during adipogenesis remain largely unclear. Here, we show PTIP, a protein that associates with histone H3K4 methyltransferases, regulates *PPARγ* and *C/EBPα* expression in mouse embryonic fibroblasts (MEFs) and during preadipocyte differentiation. PTIP deletion in MEFs leads to marked decreases of *PPARγ* expression and PPARγ-stimulated *C/EBPα* expression. Further, PTIP is essential for induction of *PPARγ* and *C/EBPα* expression during preadipocyte differentiation. Deletion of PTIP impairs the enrichment of H3K4 trimethylation and RNA polymerase II on *PPARγ* and *C/EBPα* promoters. Accordingly, *PTIP*$^{-/-}$ MEFs and preadipocytes all show striking defects in adipogenesis. Rescue of the adipogenesis defect in *PTIP*$^{-/-}$ MEFs requires coexpression of PPARγ and C/EBPα. Finally, deletion of PTIP in brown adipose tissue significantly reduces tissue weight. Thus, by regulating *PPARγ* and *C/EBPα* expression, PTIP plays a critical role in adipogenesis.**

## INTRODUCTION

Peroxisome proliferator-activated receptor γ (PPARγ) is considered the master regulator of adipogenesis. It is a member of the nuclear receptor superfamily of ligand-activated transcription factors and is both necessary and sufficient for adipogenesis (Farmer, 2006; Rosen et al., 2002). PPARγ has two isoforms, PPARγ1 and PPARγ2, generated by usage of two distinct promoters and alternative splicing (Zhu et al., 1995). PPARγ1 is ubiquitously expressed, while PPARγ2 expression is restricted to adipose tissues. However, both isoforms are strongly induced during preadipocyte differentiation in vitro, and both are highly expressed in adipose tissues in animals. PPARγ1 is induced earlier than PPARγ2 and is maintained at a level higher than PPARγ2 during preadipocyte differentiation (Morrison and Farmer, 1999). Data from PPARγ2 isoform-specific knockout (KO) mice indicate that PPARγ2 is not absolutely required for adipogenesis in vivo (Rosen and MacDougald, 2006), suggesting that PPARγ1 may be critical for adipogenesis. Although the functional difference between endogenous PPARγ1 and PPARγ2 remains unclear, ectopic expression of either PPARγ1 or PPARγ2 is sufficient to stimulate immortalized, nonadipogenic MEFs to differentiate into adipocytes (Mueller et al., 2002).

C/EBPα (CCAAT/enhancer binding protein α) is another principal adipogenic transcription factor and is strongly induced in the early phase of preadipocyte differentiation. Ectopic expression of C/EBPα stimulates nonadipogenic MEFs to undergo adipogenesis, while deletion of C/EBPα in mice results in almost complete absence of white adipose tissue (WAT) (Farmer, 2006). PPARγ and C/EBPα positively regulate each other's expression and cooperate to control preadipocyte differentiation (adipogenesis) (Farmer, 2006; Rosen and MacDougald, 2006). However, the factors and the underlying mechanisms that regulate the induction of *PPARγ* and *C/EBPα* expression during adipogenesis remain unclear.

PTIP (Pax transactivation domain-interacting protein) is a ubiquitously expressed nuclear protein that associates with active chromatin. Knockout of PTIP in mice leads to lethality by embryonic day 9.5 (Patel et al., 2007). PTIP carries six tandem BRCT domains that are predominantly found in proteins involved in DNA damage response. Indeed, ectopically expressed PTIP has been implicated in cellular response to DNA damage (Manke et al., 2003). However, the physiological function of endogenous PTIP has remained largely unclear.

Histone lysine methylation has been implicated in both gene activation and repression, depending on the specific lysine residue that gets methylated. For example, methylation on histone H3 lysine 4 (H3K4) associates with gene activation while methylation on histone H3 lysine 27 (H3K27) associates with gene repression (Li et al., 2007). We and others recently showed that endogenous PTIP is a component of a histone methyltransferase (HMT) complex that contains histone H3K4 methyltransferases MLL3 and MLL4 (also known as ALR) and the JmjC domain-containing protein UTX (Cho et al., 2007; Issaeva et al., 2007; Patel et al., 2007). Further, we and others have demonstrated that UTX is a histone H3K27-specific demethylase (Hong et al., 2007; Swigut and Wysocka, 2007). Thus, endogenous PTIP associates with two H3K4 methyltransferases and one H3K27 demethylase. Since both methylation of H3K4 and demethylation of H3K27 presumably associate with gene activation, these data strongly suggest a role of PTIP in gene activation.

In this report, we identify PTIP as a factor that regulates *PPARγ* and *C/EBPα* expression in MEFs as well as during preadipocyte differentiation. In MEFs, PTIP deletion leads to over 10-fold decrease of *PPARγ* expression and over 5-fold decrease

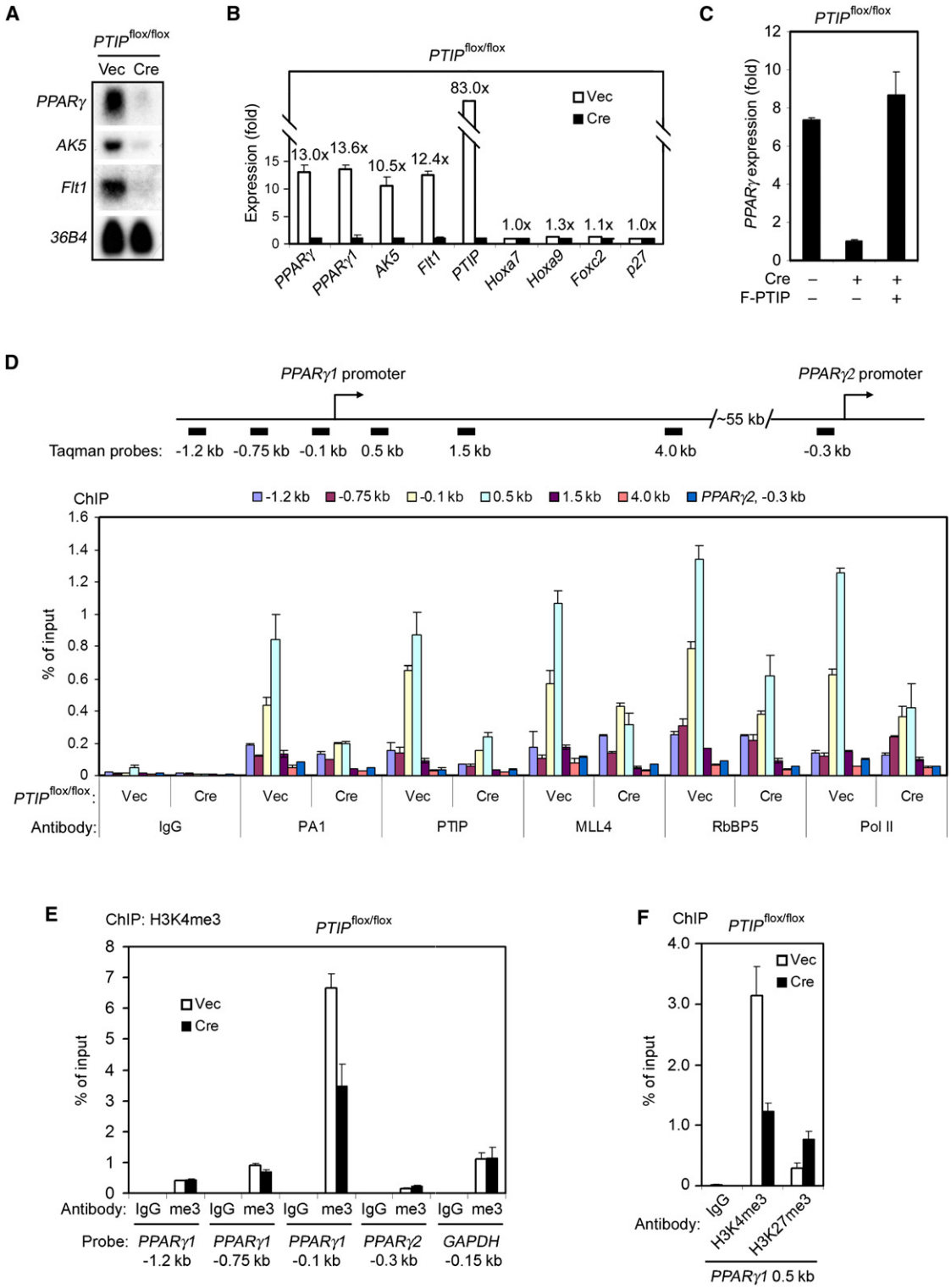


**Figure 1. PTIP Is Required for *PPARγ* Expression in MEFs**

(A and B) Immortalized *PTIP*$^{flox/flox}$ MEFs were infected with retrovirus MSCVpuro expressing Cre or vector (Vec) alone. Total RNA was extracted for confirmation of putative PTIP-regulated genes identified in microarray analysis (Table S1). Shown are northern blot (A) and qRT-PCR (B). *PPARγ2* mRNA level is below detection limit in qRT-PCR. *AK5* and *Flt1* stand for *adenylate kinase 5* and *FMS-like tyrosine kinase 1*, respectively. *36B4* is the loading control.

(C) Ectopic PTIP can rescue *PPARγ* expression in PTIP-deficient cells. *PTIP*$^{flox/flox}$ MEFs were sequentially infected with retroviruses MSCVhygro, expressing Cre, and MSCVpuro, expressing FLAG-tagged PTIP (F-PTIP). *PPARγ* expression was analyzed by qRT-PCR.