PROSPECTS
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Epigenetic Modulation by TFII-I During Embryonic Stem Cell Differentiation

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ABSTRACT

TFII-I transcription factors play an essential role during early vertebrate embryogenesis. Genome-wide mapping studies by ChIP-seq and ChIP-chip revealed that TFII-I primes multiple genomic loci in mouse embryonic stem cells and embryonic tissues. Moreover, many TFII-I-bound regions co-localize with H3K4me3/K27me3 bivalent chromatin within the promoters of lineage-specific genes. This minireview provides a summary of current knowledge regarding the function of TFII-I in epigenetic control of stem cell differentiation. J. Cell. Biochem. 113: 3056–3060, 2012.

KEY WORDS: EMBRYONIC STEM CELLS; *GTF2I*; *GTF2IRD1*; EPIGENETIC REGULATION; TFII-I; BIVALENT CHROMATIN

The TFII-I family transcription factors, encoded by *GTF2I* and *GTF2IRD1*, are implicated in various cellular and embryonic processes including cell differentiation and proliferation, angiogenesis, neural tube and skeletal development [Roy, 2012]. Both genes are deleted in Williams syndrome (WS), a neurodevelopmental disorder with a complex phenotype including mental deficiency, premature aging of skin, supravalvular aortic stenosis, dental and craniofacial malformations [Perez Jurado, 2003; Pober, 2010]. The WS phenotype is caused by the hemizygous deletion of 1.5–2.5 Mb segment in the 7q11.23 homologous region characterized by the presence of flanking low copy repeats that share high sequence similarity [Perez Jurado, 2003; Pober, 2010]. WS patients with smaller deletions that include the *GTF2I* and *GTF2IRD1* genomic region show facial dysmorphism, visual–spatial construction, and cognitive defects [Tassabehji et al., 2005; Dai et al., 2009; Antonell et al., 2010]. Collectively, these data point to TFII-I proteins as the prime candidates responsible for a specific spectrum of deficiencies observed in WS patients. In mice the loss of either *Gtf2i* or *Gtf2ird1* results in multiple abnormalities including embryonic lethality, growth retardation, brain hemorrhage, craniofacial, vasculogenic and neural tube closure defects [Enkhmandakh et al., 2009]. Several mouse models have been described to show that TFII-I genes contribute to facial development, motor function, hypersociability and behavioral abilities [Osborne, 2010; Howard et al., 2012].

DNA-BINDING MODE OF TFII-I FACTORS

The characteristic feature of TFII-I (a product of *GTF2I*) and BEN (a product of *GTF2IRD1*) is the presence of multiple atypical helix-loop-helix domains (I-repeats) that can serve as independent DNA-binding modules [Chimge et al., 2008; Roy, 2012]. The SELEX procedure performed with a set of isolated I-repeats delineated the RGATTR consensus [Vullhorst and Buonanno, 2005] which is in a perfect agreement with the *bona fide* BEN-binding sites located in the distal enhancers of *Hoxc8* [Bayarsaihan and Ruddle, 2000], *Troponin I slow* [O'Mahoney et al., 1998; Polly et al., 2003] and *Cfdp1*, *Sec23a*, and *Nsd1* genes [Makeyev and Bayarsaihan, 2011]. This consensus is also similar to the TFII-I and BEN binding motif from the distal element of the *Gsc* promoter [Ring et al., 2002; Ku et al., 2005]. Both proteins interact with the DICE element TRTYBTCTHYACMR located in the VH promoter of *IgH* genes [Tantin et al., 2004]. Furthermore, the SELEX experiment with the full-length BEN delineated the binding motif GGGRSCWGCGAYAGCCSSH that bears no sequence similarity to the DICE element or the RGATTR core consensus [Lazebnik et al., 2008].

Although TFII-I and BEN appear to recognize the same sequence, only TFII-I binds (together with USF1/USF2 heterodimer) to the upstream RBEIII (ACTGCTGA) motif necessary for transcription of human immunodeficiency virus type 1 [Chen et al., 2005]. In

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addition, TFII-I binds to the E-box (CANNTG) and pyrimidine-rich Initiator (YYANWYY) element [Roy et al., 1997]. It was proposed that TFII-I regulates a subset of estrogen-responsive genes through interaction with the Initiator sequence [Ogura et al., 2006]. TFII-I also modulates the expression of *c-Fos* by binding to the Initiator-like sequences TCAATCC and CCATATT that partially overlap with the serum induction and serum response elements, respectively [Grueneberg et al., 1997]. Regulation of *VEGFR-2* and β-globin promoters occurs by the recruitment of TFII-I to Initiator and E-boxes [Jackson et al., 2005; Crusselle-Davis et al., 2006].

With a rapid development of the high-resolution genome-wide mapping methods (ChIP-chip and ChIP-seq) it is possible to obtain a more comprehensive, unbiased assessment of DNA–protein interactions. Based on these massive sequencing projects it is becoming increasingly apparent that vertebrate transcription factors possess a more complex and diverse chromatin recognition properties than would be expected based on the classic promoter-bound model. For example, the total number of binding sites for E2F1, E2F4, and MyoD estimated to be between 17,000 and 34,000, respectively [Bieda et al., 2006; Cao et al., 2010; Lee et al., 2011]. It was noted that the great majority of chromatin occupancy is located in the intergenic regions indicating the possibility of long-range enhancer–promoter interactions. Our investigation revealed that in mouse ES cells the distribution of TFII-I binding regions is predominantly in intergenic regions and introns (Fig. 1A). TFII-I and BEN can either co-occupy the same sequence (e.g., *Gsc* promoter) or bind to two different sequences (e.g., *Cfl1* promoter) (Fig. 1B). *Gsc* and *Tnnt1* regulation is achieved by the recruitment of TFII-I and BEN to the R4 consensus (Fig. 1B). TFII-I alone binds to the single R4 consensus in the promoters of *Ezh2*, *T*, *Nsd1*, *Cited2*, and *Hoxa1*. In the *Tbx6* and *Acvr1b* promoters TFII-I interacts with the E-boxes. However, the far more common binding mode is achieved by the occupancy of

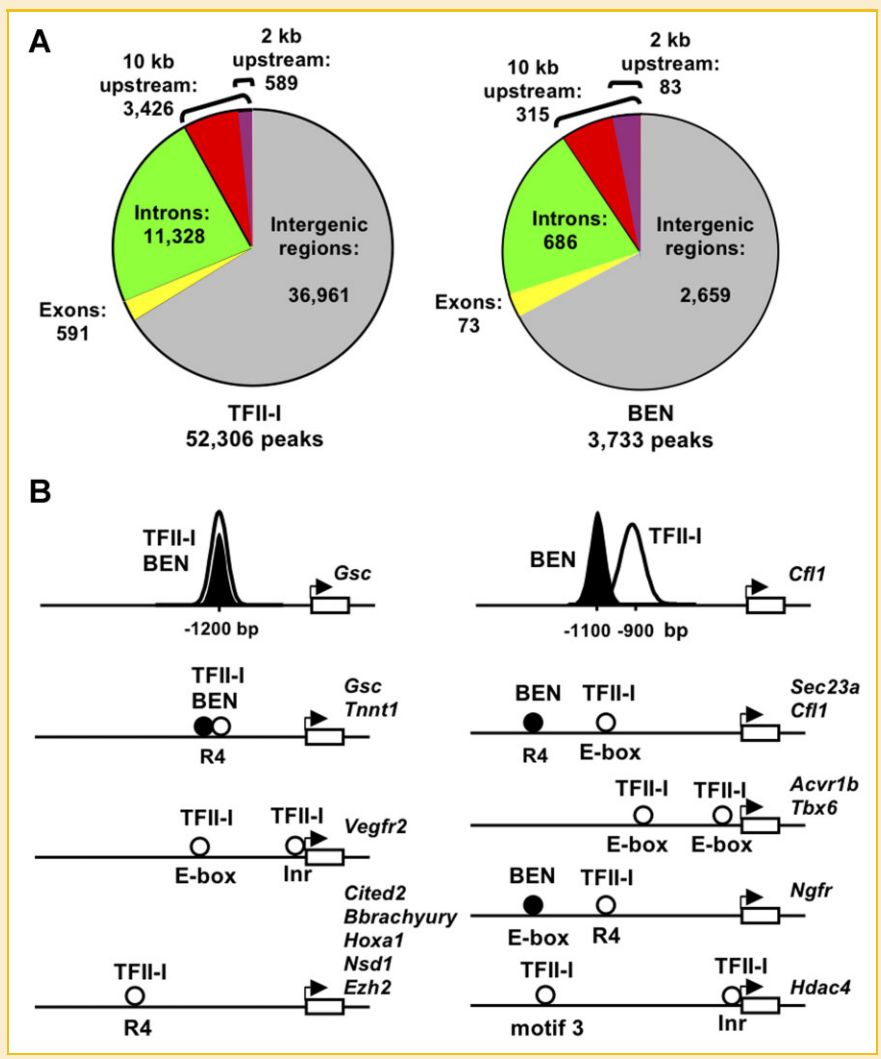

Fig. 1. Genome-wide mapping studies identified multiple genomic loci associated with TFII-I transcription factors across mammalian genomes. A: The pie chart depicting the ChIP-seq distribution of TFII-I and BEN in mouse ES cells. The majority of TFII-I and BEN binding occurs in the intergenic regions. B: The combinatorial binding of TFII-I and BEN over the promoter regions of target genes was deduced from the ChIP-chip experiments. R4—R4 core consensus RGATTR, E-box—CANNTG, motif 3—CCTGCCTCWGYC, and Int—Initiator YYANWYY.

different motifs within the same promoter. For example, TFII-I and BEN recognize E-boxes and R4 consensus in the promoters of *Cfl1* and *Sec23*. The similar binding was also observed in the promoters of *Ngfr*, *Vegfr2*, and *Hdac4* where TFII-I and BEN recognize different sequences including a novel DNA-binding motif (Fig. 1C).

TFII-I transcription factors are downstream of the TGFβ/activin signaling pathway [Ring et al., 2002; Ku et al., 2005]. Upon TGFβ/Activin stimulation, TFII-I forms a complex with Smad2 which targets the distal promoter element of *Gsc*. Recent ChIP-chip experiments identified TFII-I occupancy at the promoters of *Acvr1b*, *Smad5*, *Bmpr1*, *Chrd*, *Bmp2* critical in the TGFβ signaling (Fig. 2).

THE FUNCTION OF TFII-I IN EPIGENETIC REGULATION

Previous expression analysis of the *Gtf2i* null embryos defined a list of the potential TFII-I targets [Enkhmandakh et al., 2009]. Among them are histone-modifying enzymes including *Ezh2* and *Eed*, the core components of polycomb repressive complex 2 (PRC2), H3K4me3 demethylation gene *Aof2* and H3K36me3 methyltransferase gene *Nsd1*, the heterochromatin organizer *Suv39h1* gene and a subset of class I and class II histone deacetylase genes (Fig. 3A).

The correlation between promoter binding and expression of the corresponding genes is usually low, varying from 9.6% to 36.9% depending on the different datasets [Bussemaker et al., 2001; Conlon et al., 2003; Das et al., 2004, 2006]. Accordingly, we found no significant link between TFII-I binding and the level of gene activity in mouse ES cells. However, TFII-I co-localizes with bivalent chromatin H3K4/K27me3, a hallmark feature of genes critical in development and lineage differentiation [Bernstein et al., 2006; Mikkelsen et al., 2007]. The bivalent chromatin structure is established and maintained by the Polycomb (PcG) and Trithorax (Trx) group proteins [Fisher and Fisher, 2011]. We speculate that the selective recognition of bivalent domain by TFII-I marks active chromatin for proper execution of the differentiation program

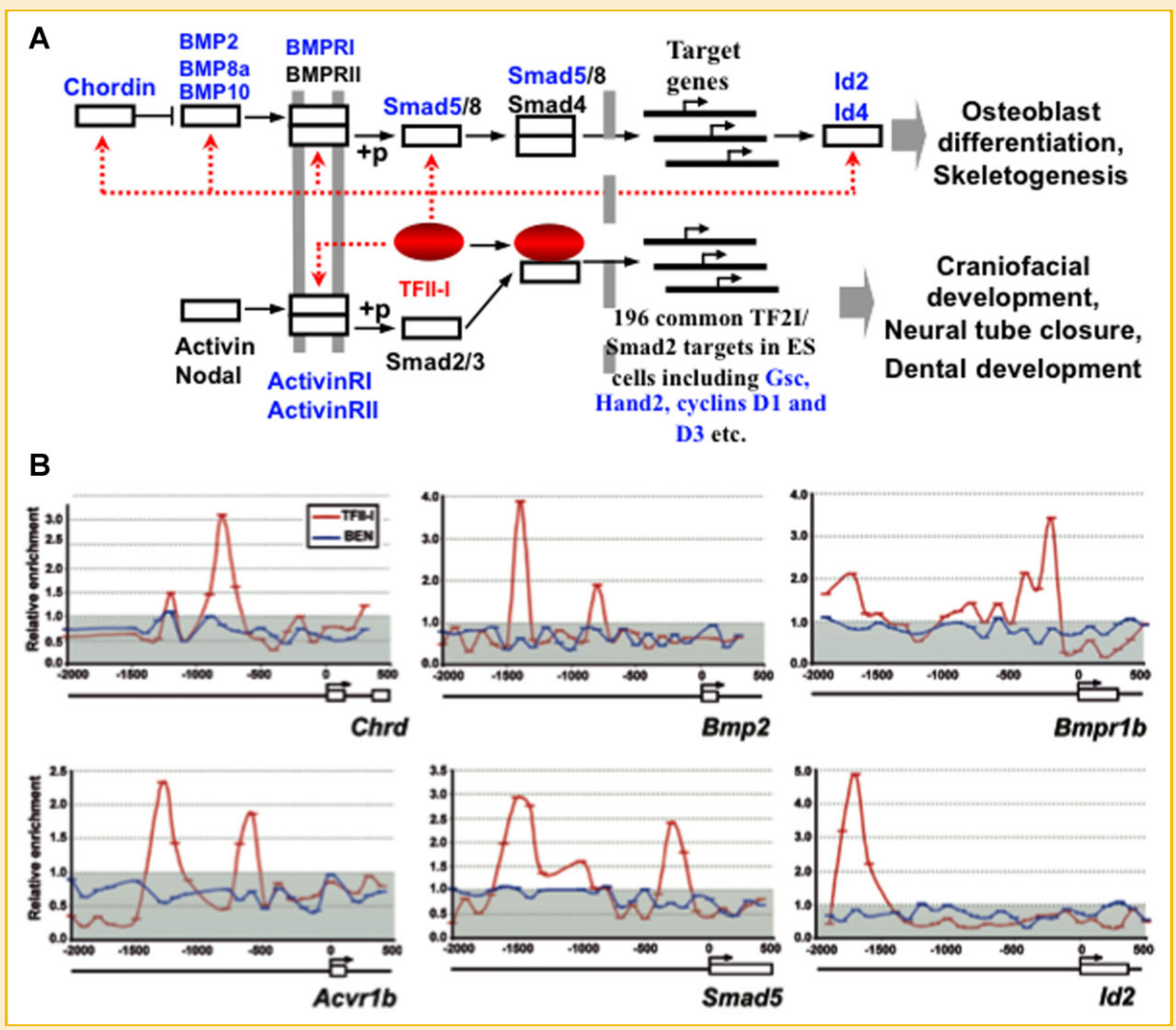

Fig. 2. The TFII-I function in the TGFβ/Activin signaling pathway. A: TFII-I binds (red arrows) to the regulatory regions of developmental genes associated with the TGFβ signaling pathway (marked by blue). B: ChIP-chip identified that TFII-I recognizes the promoters of *Acvr1b*, *Smad5*, *Bmpr1*, *Chrd*, *Bmp2*, and *Id2* important in the TGFβ signaling.

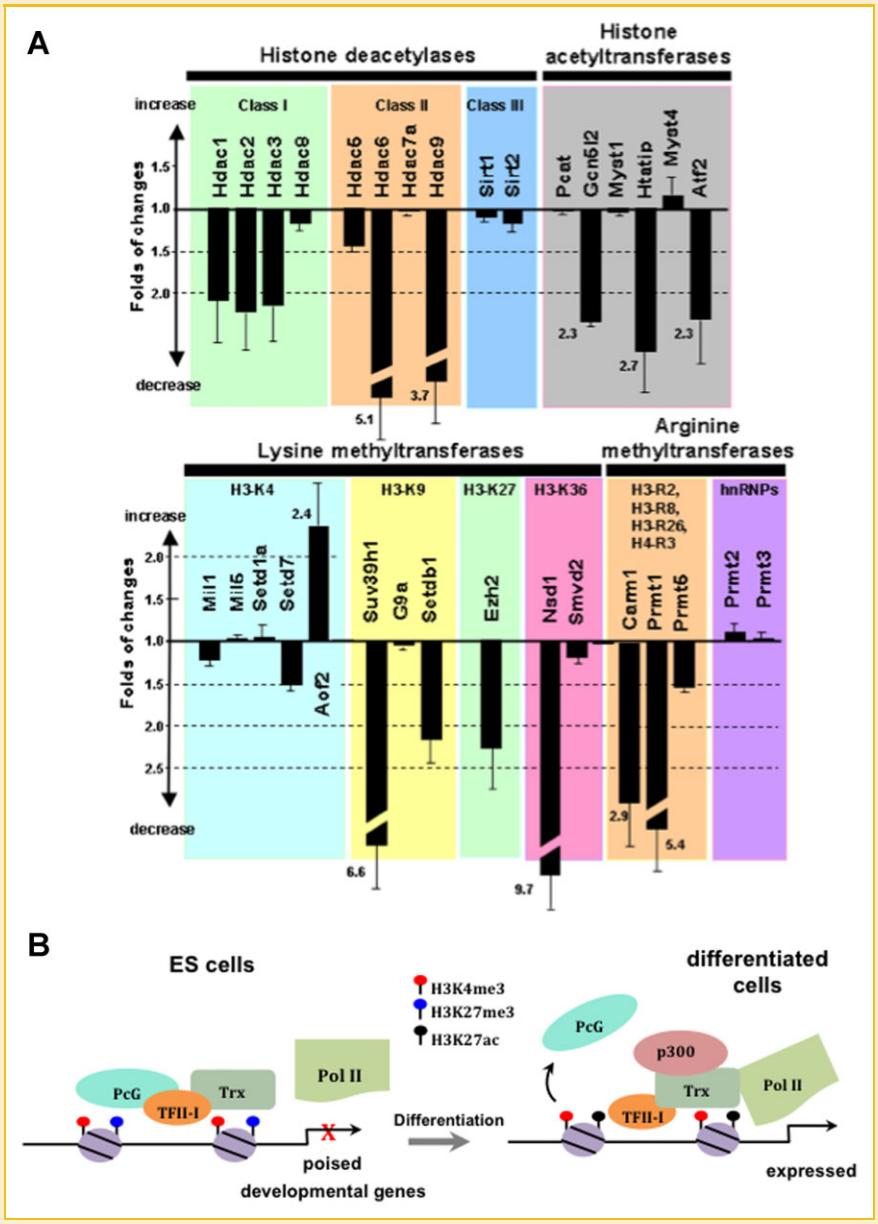

Fig. 3. TFII-I regulates expression of critical developmental genes through epigenetic control mechanisms. A: The inactivation of *Gtf2i* leads to down-regulation of histone deacetylases (class I and class II, but not class III), histone acetyltransferases and a distinct subset of histone methyltransferases. B: TFII-I may be recruited to the bivalent domain-containing genes for the fast and efficient activation in response to the differentiation-mediated Polycomb withdrawal. PcG, polycomb group proteins; Trx, Trithorax group proteins; Pol II, Polymerase II complex; p300, acetyltransferase complex. H3K4me3 mark is in red, H3K27me3 is in blue and H3K27ac, a landmark for active enhancers, is in black circle.

(Fig. 3B). For example, genes primed by TFII-I could be processed for a fast and efficient activation in response to the differentiation-mediated Polycomb withdrawal. We speculate that TFII-I may exert a distinct regulatory effect on the target genes by changing the status of bivalent chromatin (Fig. 3B).

PERSPECTIVE

The reduced expression of *GTF2I* and *GTF2IRD1* caused by the hemizygous deletion of the 7q11.23 region leads to dramatic changes in cellular differentiation program. The unbiased mapping of TFII-I and BEN genomic binding sites and identification of direct gene targets will advance our understanding of the molecular processes that underlie the pathogenesis of the WS phenotype. The contribution of TFII-I to the epigenetic mechanisms that occur during embryonic development requires further investigation. In this regard, a better understanding of the impact of histone modifications and DNA methylation in the differentiation of WS-specific neural crest progenitor cells could facilitate the identification of the critical signaling pathways and gene regulatory networks affected in the disease state.

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