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XSip1 neuralizing activity involves the co-repressor CtBP and occurs through BMP dependent and independent mechanisms

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Abstract

The DNA-binding transcription factor Smad-interacting protein-1 (Sip1) (also named Zfhx1b/ZEB2) plays essential roles in vertebrate embryogenesis. In *Xenopus*, XSip1 is essential at the gastrula stage for neural tissue formation, but the precise molecular mechanisms that underlie this process have not been fully identified yet. Here we show that XSip1 functions as a transcriptional repressor during neural induction. We observed that constitutive activation of BMP signaling prevents neural induction by XSip1 but not the inhibition of several epidermal genes. We provide evidence that XSip1 binds directly to the BMP4 proximal promoter and modulates its activity. Finally, by deletion and mutational analysis, we show that XSip1 possesses multiple repression domains and that CtBPs contribute to its repression activity. Consistent with this, interference with XCtBP function reduced XSip1 neuralizing activity. These results suggest that Sip1 acts in neural tissue formation through direct repression of BMP4 but that BMP-independent mechanisms are involved as well. Our data also provide the first demonstration of the importance of CtBP binding in Sip1 transcriptional activity *in vivo*.

Keywords: BMP4; CtBP; Neural induction; Sip1; Sox2; Zfhx1b; ZEB2

Introduction

The vertebrate neural plate arises from the embryonic ectoderm during gastrulation. Molecular studies have revealed that inhibition of BMP signaling in the ectoderm is required for neural fate acquisition. This BMP inhibition occurs through distinct mechanisms, including inhibition of BMP ligands by various secreted ligand-binding proteins, inhibition of transcription of the *BMP* gene itself, and negative modulation of intracellular signaling by Smad proteins (Munoz-Sanjuan et al., 2002; De Robertis and Kuroda, 2004; Linker and Stern, 2004; Stern, 2005). Downstream of neural induction, the intracellular components that establish neural cell fate are not well characterized. Several neural effectors have been identified such as Geminin (Kroll et al., 1998), Zic1 to 3 (Nakata et al., 1997; Kuo et al., 1998), Sox1 to 3 (Penzel et al., 1997; Kishi et al., 2000; Bylund et al., 2003; Graham et al., 2003), SoxD (Mizuseki et al., 1998a,b), and the Smad-binding protein Sip1

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(Eisaki et al., 2000; van Grunsven et al., 2000; Sheng et al., 2003).

Sip1 (Zfhx1b/Zeb2) belongs to the Zfhx1 family of multi-domain transcriptional repressors characterized by a homeo-domain-like domain and by two zinc finger clusters each of which binds with high affinity to CACCTG and CACANNTG binding sites and can form complexes with Smads (Remacle et al., 1999; Verschueren et al., 1999), the co-repressor CtBP (C-terminal binding protein) (Postigo and Dean, 2000; van Grunsven et al., 2003), and the co-activators p300 and pCAF (p300/CBP associated factor) (van Grunsven et al., 2006).

In embryos of *Xenopus*, chick and mouse, *Sip1* mRNA is detected at gastrula in the prospective neurectoderm (Eisaki et al., 2000; van Grunsven et al., 2000; Sheng et al., 2003; Van de Putte et al., 2003). Homozygotic deletion of *Sip1* in the mouse is embryonic lethal and the embryos show severe neural crest cell defects and fail to generate or maintain intact neural ectoderm (Van de Putte et al., 2003). In human, mutations in *ZFHX1B* exon coding sequences, most of which cause C-terminal truncation of the protein, lead to Mowat–Wilson Syndrome (Mowat et al., 2003).

Although Sip1 has been documented primarily as a transcriptional repressor (Verschueren et al., 1999; Comijn et al., 2001; van Grunsven et al., 2003; Vandewalle et al., 2005), it can also act as a transcriptional activator *in vivo* (Long et al., 2005; Yoshimoto et al., 2005). Overexpression of *Sip1* in certain epithelial cells induces epithelial to mesenchymal transition by directly repressing *E-cadherin* (Comijn et al., 2001) and other genes coding for crucial proteins of epithelial cell–cell junctions (Vandewalle et al., 2005). In animal cap explants of *Xenopus* early embryos, ectopic synthesis of XSip1 induces neural specific gene expression and represses in the embryo the expression of the panmesodermal marker gene *brachyury* (*Xbra*), of *BMP4* and of other genes in the presumptive epidermis (Eisaki et al., 2000; Lerchner et al.,

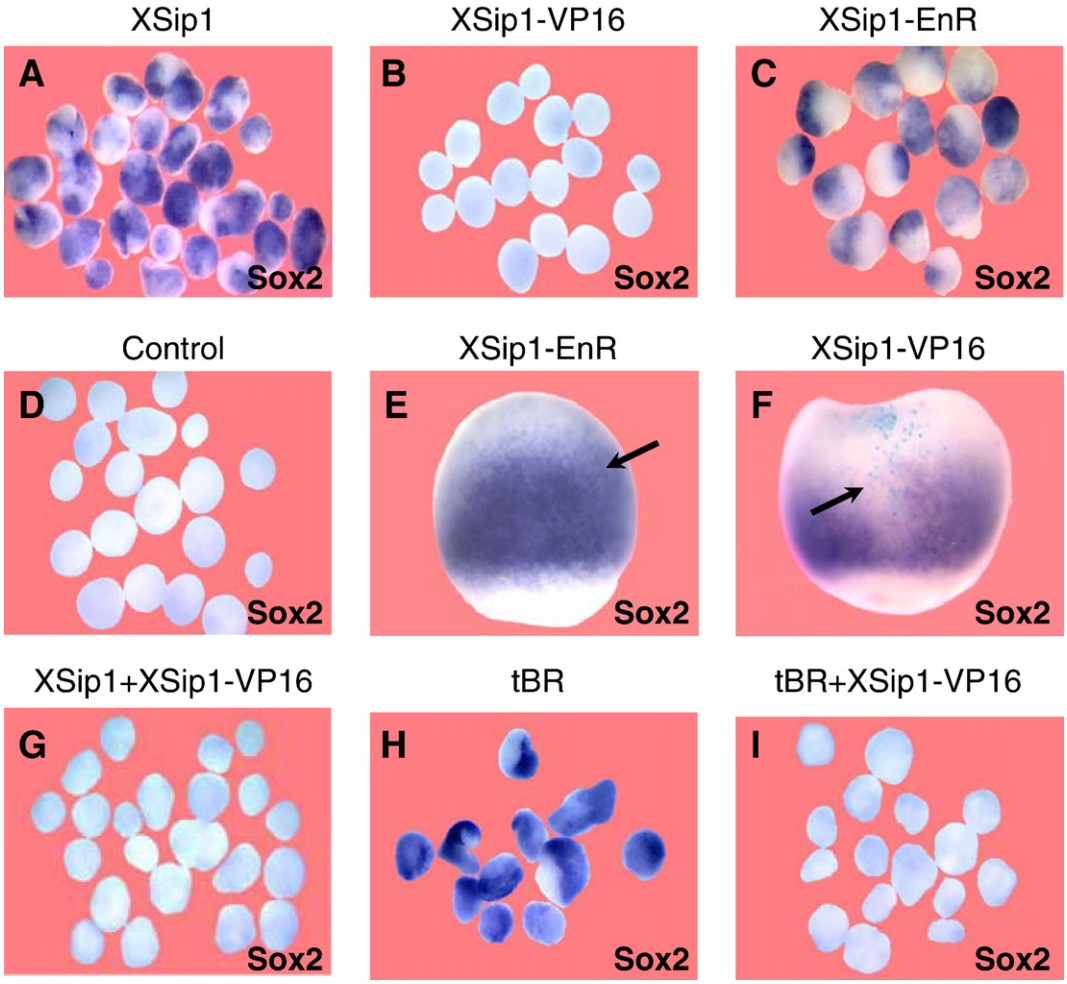

Fig. 1. XSip1 acts as a repressor during neural differentiation of the ectoderm. Whole-mount *in situ* analysis of *Sox2* expression in embryos (St. 11) or animal caps (St. 14). Embryos were injected at the four-cell stage with the indicated RNA. (A–D) Injection of 250 pg of *XSip1-VP16*, in contrast to wild-type *XSip1* and *XSip1-EnR*, does not induce *Sox2* in animal caps. (E, F) Embryos injected with 250 pg of *XSip1-VP16* or *XSip1-EnR* RNA. *LacZ* RNA was co-injected and X-gal staining was performed to reveal distribution of the injected RNA. Note the expansion of *Sox2* expression on the injected area in the *XSip1-EnR* injected embryo (arrow) and the reduction in the *XSip1-VP16* injected embryo. (G–I) Animal caps derived from embryos co-injected with 50 pg of *XSip1* RNA or 200 pg *tBR* RNA together with 250 pg of *XSip1-VP16* RNA. XSip1-VP16 prevents induction by XSip1 or tBR of *Sox2*. Respective inductions (A) 100%, $n=28$; (B) 0%, $n=29$; (C) 90%, $n=30$; (D) 0%, $n=44$; (E) 80% embryos with expanded *Sox2*, $n=18$; (F) 100% embryos with downregulation of *Sox2*, $n=40$; (G) 0%, $n=35$; (H) 100%, $n=26$; (I) 0%, $n=35$.