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The *Prdm13* histone methyltransferase encoding gene is a Ptf1a–Rbpj downstream target that suppresses glutamatergic and promotes GABAergic neuronal fate in the dorsal neural tube

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ABSTRACT

The basic helix–loop–helix (bHLH) transcriptional activator Ptf1a determines inhibitory GABAergic over excitatory glutamatergic neuronal cell fate in progenitors of the vertebrate dorsal spinal cord, cerebellum and retina. In an *in situ* hybridization expression survey of PR domain containing genes encoding putative chromatin-remodeling zinc finger transcription factors in *Xenopus* embryos, we identified *Prdm13* as a histone methyltransferase belonging to the *Ptf1a* synexpression group. Gain and loss of Ptf1a function analyses in both frog and mice indicates that *Prdm13* is positively regulated by Ptf1a and likely constitutes a direct transcriptional target. We also showed that this regulation requires the formation of the Ptf1a–Rbp-j complex. Prdm13 knockdown in *Xenopus* embryos and in *Ptf1a* overexpressing ectodermal explants lead to an upregulation of *Tlx3*/*Hox11L2*, which specifies a glutamatergic lineage and a reduction of the GABAergic neuronal marker *Pax2*. It also leads to an upregulation of *Prdm13* transcription, suggesting an autonegative regulation. Conversely, in animal caps, Prdm13 blocks the ability of the bHLH factor Neurog2 to activate *Tlx3*. Additional gain of function experiments in the chick neural tube confirm that *Prdm13* suppresses $Tlx3^+$/glutamatergic and induces $Pax2^+$/GABAergic neuronal fate. Thus, Prdm13 is a novel crucial component of the Ptf1a regulatory pathway that, by modulating the transcriptional activity of bHLH factors such as Neurog2, controls the balance between GABAergic and glutamatergic neuronal fate in the dorsal and caudal part of the vertebrate neural tube.

Introduction

The dorsal spinal cord is an integrative center that transmits and processes diverse somatosensory information. These neurons can be grouped into excitatory neurons and inhibitory neurons, which use glutamate and GABA/glycine as transmitters respectively. These inhibitory and excitatory neurons are interconnected and a correct balance between them is essential for correct processing of somatosensory inputs from the periphery. Six distinct classes of dorsal interneurons (early dI1-6 and late dIL^A and dIL^B) arise in the dorsal spinal cord from six progenitor domains (dP1-6). Among them, early-born dI4 and dI6 and late-born dIL^A neurons are GABA/glycinergic neurons (Helms and Johnson, 2003; Hori and Hoshino, 2012). These three classes of postmitotic interneurons express the homeodomain transcription factors *Lbx1*, *Pax2* and *Lhx1/5* that regulate GABA/glycinergic differentiation (Pillai et al., 2007; Batista and Lewis, 2008; Bröhl et al., 2008).

While postmitotic dorsal interneurons can be distinguished by the combinatorial expression of homeodomain transcription factors, bHLH factors have a crucial role in progenitors in the

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specification of excitatory *versus* inhibitory neurons. Ptf1a (pancreas transcription factor 1a), a bHLH transcriptional activator initially identified as a cell fate determinant of the pancreas (Krapp et al., 1998; Kawaguchi et al., 2002; Sellick et al., 2004; Afelik et al., 2006) that is restricted to dI4 and DIL^A interneuron progenitors in the dorsal spinal cord, plays an essential role in the specification of GABAergic and glycinergic inhibitory neurons while suppressing glutamatergic excitatory neurons. Mice lacking Ptf1a show a near complete loss of dI4 and dIL^A GABAergic interneurons and an increase of dI5 and dIL^B excitatory interneurons. Conversely, overexpression of *Ptf1a* in the chick neural tube induces GABAergic and glycinergic neurons at the expense of Tlx3-positive glutamatergic excitatory dI5 and dIL^B neurons (Glasgow et al., 2005; Hori et al., 2008; Huang et al., 2008). The requirement for Ptf1a in the generation of GABAergic and glycinergic inhibitory neurons has been also demonstrated in other regions of the developing CNS where it is expressed, including the cerebellum, cochlear nucleus and the retina (Hoshino et al., 2005a, 2005b; Fujitani et al., 2006; Nakhai et al., 2007; Pascual et al., 2007; Dullin et al., 2007; Fujiyama et al., 2009). Ptf1a has been shown to be required for *Pax2*, *Lhx1* and *Lhx5* expression and for the suppression of glutamatergic cell fate determinants *Tlx3* (also known as *Rnx* and *Hox11L2*) and *Lmx1b* (Cheng et al., 2004, 2005; Hori et al., 2008). The molecular mechanism by which Ptf1a activates one lineage specific gene expression and represses expression of genes of the other alternate lineage remains unclear.

Ptf1a is a component of a heterotrimeric transcription complex, called PTF1-J, that includes Ptf1a, a commonly expressed bHLH E-protein and a member of the mammalian Suppressor of Hairless family, Rbp-j (or its paralogue, Rbp-l, to form PTF1-L in the pancreas) that mediates Notch signaling (Beres et al., 2006; Masui et al., 2007). This PTF1-J transcription complex is required *in vivo* for the specification of GABAergic neurons in a Notch signaling independent manner (Hori et al., 2008). The PTF1-J complex binds a bipartite DNA sequence composed of an E-box for bHLH heterodimer binding and a TC-box for Rbp-j binding. Only a few direct targets of the PTF1-J complex in the dorsal spinal cord have been identified so far. These include *Nephrin* and *Neph3* genes encoding immunoglobulin type transmembrane proteins, whose function in the development of *Ptf1a* expressing GABAergic interneurons is unknown (Nishida et al., 2010) and *Ptf1a* itself (Meredith et al., 2009; Masui et al., 2008). The atonal related bHLH proneural gene *Neurog2*, which is broadly expressed in dorsal interneurons, is another direct downstream target of PTF1-J (Henke et al., 2009). Embryos null for both *Neurog2* and another member of the bHLH family, *Ascl1* (*Mash1*), coexpressed with *Neurog2* in dP3-dP5 progenitors, have an apparent loss of dI4 neurons. This phenotype is however not observed in the single mutant embryos (Helms et al., 2005) suggesting that Neurog2 has a redundant function with Ascl1 in dP4. As *Ascl1* is not affected in *Ptf1a* mutants, this suggests that additional targets of Ptf1a need to be identified to understand the dramatic loss of the dI4 cells observed in *Ptf1a* mutants (Henke et al., 2009).

In an *in situ* hybridization expression survey of the expression of PR domain containing genes in the *Xenopus* embryo, the expression profile of the *Prdm13* gene was found to overlap with that of *Ptf1a* in the developing spinal cord and retina. *Prdm13* encodes a protein containing a N-terminal PR domain related to the SET domain found in a large group of histone methyltransferases, followed by four zinc finger repeats (Huang et al., 1998; Albert and Helin, 2010). *Prdm13* belongs to the *Prdm* gene family consisting of 16 orthologs in rodents, birds and amphibians that encodes important regulators in differentiation and disease. Prdm family members recognize specific DNA-sequences or act as non-DNA binding cofactors. They either function as direct histone methyltransferases (HMTases) or recruit histone-modifying enzyme to target promoters (Fog et al., 2011; Hohenauer and Moore, 2012). Recent reports have shown that some vertebrate Prdm family members play important roles as cell fate regulators in the developing nervous system. For example, Prdm1/Blimp1 specifies photoreceptor over bipolar neurons in the mouse and zebrafish retina (Brzezinski et al., 2010; Katoh et al., 2010). It also controls cell fate decision at the neural plate border in zebrafish embryos (Roy and Ng, 2004; Hernandez-Lagunas et al., 2011). Prdm8 regulates target gene expression in postmitotic cortical neurons by forming a repressor complex with the transcription factor Bhlhb5 (Ross et al., 2012). Hamlet, the *Drosophila* Prdm3/Evi1 and Prdm16/Mel1 homolog controls olfactory receptor neuron diversification by modulating Notch signaling (Endo et al., 2011).

Here, we identify the HMTase Prdm13 as a likely direct target of the Ptf1a–Rbp-j complex. We show that Prdm13 negatively regulates its own expression and that it plays an important role downstream of Ptf1a in the suppression of glutamatergic and induction of GABAergic differentiation. We also provide evidence suggesting that Prdm13 represses glutamatergic neuronal fate by blocking Neurog2 ability to induce the glutamatergic-neuron specifier *Tlx3* gene.

Materials and methods

Expression constructs

The *mPrdm13* expression vector used in frog microinjection experiments was obtained by PCR amplifying from EST BY731300, clone E130110K18, using primers 5′- GAATCGCACGGAACTTCTCG-CACTTCG-3′ and 5′-AGAATGCTTTCAGAATGAGAGGCTATGCCA-GAGTCT-3′ a 2359 bp fragment containing an open reading frame starting from the second methionine (position 49 in supplementary material Fig. S1, corresponding to the first methionine in the other species) into the EcoRI and XhoI sites of the pCS2-Flag vector. The *mPrdm13* expression construct used in chick electroporation was generated by PCR amplifying from the same EST using primers 5′-CTCGAGCCCGAGCGTCTGCCTGGC-3′ and 5′-GAATTCCTGGTCCCTGCTCAGTCCCT-3′ a 2190 bp fragment containing an open reading frame starting from the second methionine into the XhoI and EcoRI sites of the pCIG vector.

MT-Ptf1apCS2+, MT-$Ptf1a^{W224A}$pCS2+, MT-$Ptf1a^{W242A}$pCS2+ and MT-$Ptf1a^{W224A/W242A}$pCS2+ were generated by PCR amplification using the following primers: forward 5′-CAGAATTCCATG-GAAACGGTCC-3′ and reverse 5′-CCCTCTAGATCACATATCAAGGCAC-3′. The PCR fragments were inserted into the EcoRI and XbaI sites of the MTpCS2+ vector.

$Ptf1a^{W224A}$–GRpCS2+, $Ptf1a^{W242A}$–GRpCS2+ and $Ptf1a^{W224A/W242A}$–GRpCS2+ were generated using the "QuickChange XL Site directed Mutagenesis" kit (Stratagene) to introduce mutations that lead to an exchange of tryptophan to alanine at position 224 and/or 242 using the following primers: C1_up GGACATTCTCTCT-CAGCGACTGATGAGAAGCAACTGAG, C1_down CTCAGTTGCTTCTCAT-CAGTCGCTGAGAGAGAATGTCC, C2_up GTTGTCAGAACGGCCAAAGTG-GCGACTCCTGAGGATCC, C2_down GGATCCTCAGGAGTCGCCAC-TTTGGCCGTTCTGACAAC. Rbp-J–HApCS2+ was generated by PCR amplification using the following primers: forward 5′-CTGGATC-CATGCAACCTGGC-3′ and reverse 5′-CAACTCGAGGGACACTACT-GCTGC-3′. The PCR fragment was inserted into the BamHI and XhoI sites of the HApCS2+ vector. Ptf1a/bHLH-Neurog2-GRpCS2 was generated by first amplifying the bHLH domain of Neurog2 (GR-Neurog2p3′, Perron et al., 1999) with the following primers, which contained the surrounding sequences of the Ptf1a bHLH domain (in italics): Ptf1a/bHLH-Neurog2_for *CTGAGGTCGGACGCGGAGATG-CAGCAG*CGGCGCGTTAAAGCTAACAAC and Ptf1a/bHLH-Neurog2_rev *GCGGCAGATCGGACTGTACCATCTCGC*TAAGAGCCCAGATGTAGTTGTAG. The generated megaprimer were used in a second PCR reaction