Prdm Proto-Oncogene Transcription Factor Family Expression and Interaction with the Notch-Hes Pathway in Mouse Neurogenesis

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Abstract

Background: Establishment and maintenance of a functional central nervous system (CNS) requires a highly orchestrated process of neural progenitor cell proliferation, cell cycle exit, and differentiation. An evolutionary conserved program consisting of Notch signalling mediated by basic Helix-Loop-Helix (bHLH) transcription factor activity is necessary for both the maintenance of neural progenitor cell character and the progression of neurogenesis; however, additional players in mammalian CNS neural specification remain largely unknown. In Drosophila we recently characterized Hamlet, a transcription factor that mediates Notch signalling and neural cell fate.

Methodology/Principal Findings: Hamlet is a member of the Prdm (PRDI-BF1 and RIZ homology domain containing) proto-oncogene transcription factor family, and in this study we report that multiple genes in the Prdm family (Prdm6, 8, 12, 13 and 16) are expressed in the developing mouse CNS in a spatially and temporally restricted manner. In developing spinal cord Prdm8, 12 and 13 are expressed in precise neural progenitor zones suggesting that they may specify discrete neuronal subtypes. In developing telencephalon Prdm12 and 16 are expressed in the ventricular zone in a lateral to medial graded manner, and Prdm8 is expressed in a complementary domain in postmitotic neurons. In postnatal brain Prdm8 additionally shows restricted expression in cortical layers 2/3 and 4, the hippocampus, and the amygdala. To further elucidate roles of Prdm8 and 16 in the developing telencephalon we analyzed the relationship between these factors and the bHLH Hes (Hairy and enhancer of split homolog) effectors of Notch signalling. In Hes null telencephalon neural differentiation is enhanced, Prdm8 expression is upregulated, and Prdm16 expression is downregulated; conversely in utero electroporation of Hes1 into the developing telencephalon upregulates Prdm16 expression.

Conclusions/Significance: Our data demonstrate that Prdm genes are regulated by the Notch-Hes pathway and represent strong candidates to control neural class specification and the sequential progression of mammalian CNS neurogenesis.

Introduction

The nervous system of mammals contains a large number of neurons in a diverse array of neuron classes. Transcription factors play central roles in generating this complexity by controlling neural progenitor cell proliferation, patterning, and defining neuron fate [1,2]. For example, it is well established that an evolutionary conserved basic Helix-Loop-Helix (bHLH) transcription factor cascade downstream of Notch signalling is necessary for both the maintenance of neural progenitor cell character and the progression of neurogenesis. High Notch activity maintains neural progenitors through an effector pathway consisting of the bHLH Hairy and enhancer of split homologue transcription factors Hes1 and Hes5. Notch upregulates the Hes factors that then function as DNA-binding repressors and antagonize the expression of proneural bHLH genes [3]. Hence, low Notch activity reduces Hes activity and leads to upregulation of proneural bHLH factors such as Ngn2 (Neurogenin2) and Mash1 (Mammalian achaete-scute homolog1); these factors then repress neural progenitor cell maintenance and promote neuron differentiation [4].

Much of our understanding of the mechanisms of Notch and bHLH function in the mammalian central nervous system (CNS) is derived from seminal studies examining neurogenesis in the peripheral nervous system (PNS) of the fruit fly Drosophila melanogaster [5]. In the fly we recently identified Hamlet, a transcription factor as acting to instruct neuron and glial cell fate and mediating Notch signalling in a neural lineage-specific manner [6,7]. Hamlet is a member of the relatively uncharacterized transcription factor family known as the Prdm (PRDI-BF1 and RIZ homology domain containing) family [8]. Prdm family
members are characterized by an N-terminal PR domain, and in addition all but one (Prdm11) contain zinc fingers (Fig. 1). The PR domain is 20–30% identical to the SET (Su(var)3-9, Enhancer-of-zeste, and Trithorax) domain, a histone methyltransferase catalytic module [9].

We hypothesized that the Prdm gene family may also significantly participate in the development of the mammalian nervous system as this family meets the molecular criteria to be active in neurogenesis. Prdm family members are known to control cell proliferation both in cancer [10–15] and in normal development [16]. Furthermore, Prdm family members are also used to define cell fate. For example, a great deal of interest has been generated by the abilities of Prdm16 to control the switch between skeletal muscle and brown fat in mice [17,18], and Prdm1 to act as a switch between fast and slow twitch muscle in zebrafish (Danio rerio) [19]. In addition, Notch signalling is an essential control mechanism in neurogenesis, and Prdm family function in cell fate can occur through mediation of Notch signalling. For example, both Drosophila hamlet and its Caenorhabditis elegans homologue EGL-43 mediate Notch-controlled cell fate decisions [7,20,21].

Roles in nervous system development have already been demonstrated or suggested for a few members of the Prdm family. Both hamlet and EGL-43 are required for sensory neuron differentiation [6,22]. Furthermore, Prdm3 (Mds1/Evi1), the mouse homologue of hamlet, is also expressed in the PNS within the developing cranial and dorsal root ganglia [23], and knockout of Prdm3 in mice leads to nervous system hypoplasia [24]. prdm1 (blimp1) is expressed in sensory neuron precursors in both zebrafish [25] and Drosophila [26]. In zebrafish prdm1 expression at the edge of the neural plate specifies the precursor cells competent to form primary sensory neurons [25,27]. In these cells prdm1 functions within the context of a Notch-bHLH pathway since sensory neurogenesis also requires downregulation of Notch signalling and subsequent induction of ngn1 [28].

In this study we present evidence for the function of multiple relatively uncharacterized Prdm gene family members during mammalian neurogenesis. By employing mRNA in situ hybridization (ISH) analysis we show that several Prdm family members are expressed in spatially restricted and related domains of neuronal progenitors in the developing CNS consistent with a role in neural class specification. In addition, we find that a subset of Prdm family members remain expressed in the postnatal brain. Furthermore, by analyzing Hes loss- and gain-of-function embryos, we demonstrate that Prdm family gene expression in the developing telencephalon is controlled by the Notch-Hes pathway and regulated during the sequential progression of neurogenesis. We suggest that the genes of the Prdm family represent strong new candidates to function in neural progenitor cell proliferation and neural differentiation in the mammalian CNS.

**Results**

**Prdm5–16 expression in midgestation mouse embryos**

Fifteen mouse members of the Prdm family (Fig. 1, Table S1) were identified from the National Center for Biology Information (NCBI) http://www.ncbi.nlm.nih.gov/ and Mouse Genome Informatics (MGI) http://www.informatics.jax.org/ databases. The expression patterns of Prdm1–4 have been investigated in detail; of these, Prdm1, 3 and 4 are expressed in the nervous system [23,29,30] (data not shown). Prdm2 expression has not been reported in the CNS and our preliminary studies detected no CNS-specific expression (data not shown).

We designed two or three independent, gene-specific primer pairs for Prdm5–16. Using these primers we carried out reverse transcription polymerase chain reaction (RT-PCR) on total
mRNA isolated from stage embryonic (E) day 11.5 (whole embryo) and 13.5 (head only) tissue. In both samples we detected expression of all Prdm3–16 genes (data not shown). Furthermore, we confirmed that we had amplified cDNA from the correct predicted gene by sequencing the RT-PCR-generated amplicons from each primer pair.

**Prdm8, 12 and 13 show restricted nervous system expression from early embryogenesis**

To examine the expression of Prdm3–16 in detail, we carried out whole mount in situ hybridization (WISH) at E9.5 and E10.5. Three Prdm genes (8, 12 and 13) showed spatially restricted expression in nervous system tissue (Fig. 2). Prdm8 and 13 showed specific expression in spinal cord at E9.5 when neurogenesis starts (Fig. 2A, C), and maintained their expression in this tissue at E10.5 (Fig. 2D, F).

Prdm12 was also expressed in the spinal cord at E9.5. In addition, weak expression of Prdm12 was observed in the caudal forebrain and midbrain (Fig. 2B, blue and yellow arrowheads). At E10.5, these two expression domains of Prdm12 in forebrain and midbrain became stronger (Fig. 2E, red, blue and yellow arrowheads, respectively). For better visibility, we next dissected out the brain at E10.5 to analyze detailed expression of Prdm12 (Fig. 2G). Prdm12 was expressed in several regions of the diencephalon, p3 (Fig. 2G, red and green arrowheads), p1 (Fig. 2G, blue arrowhead), hypothalamus (Fig. 2G, pink arrowhead), and a small dorsal region in the midbrain (Fig. 2G, yellow arrowhead). To obtain precise spatial information about the Prdm12 expression domain in the diencephalon we performed two-color ISH to compare Prdm12 expression with Shh (sonic hedgehog). Shh marks the Zli (zona limitans intrathalamica), the definitive border of p2 and p3 (Fig. 2H–K) [31]. Our analysis clearly revealed that Prdm12 was expressed in postmitotic neurons adjacent to, but not overlapping, the Zli (Fig. 2I, J). In addition, Prdm12 was expressed in the p1 region in the diencephalic VZ but was excluded from the dorsal midline (Fig. 2I, K).

In addition to CNS expression, we also detected Prdm12 expression in the PNS. Prdm12 was expressed in a repeated pattern lateral to the spinal cord in the dorsal root ganglia (DRG) (Fig. 2E, black arrowheads) and in the head region in the cranial ganglia (Fig. 2B, E, white arrowheads).

**Prdm8, 12 and 13 family members are expressed in interrelated domains along the dorsal-ventral axis of the spinal cord**

To understand the detailed expression pattern of the Prdm gene family members in the spinal cord we performed further ISH on sections (Fig. 3). In the ventral neural tube distinct classes of motor- and interneurons are derived from distinct VZ progenitor cell populations. Each progenitor cell population is defined by the expression of subsets of homeodomain (HD) transcription factors [32]. We used two-colour ISH at the cervical level to map the expression domains of Prdm8, 12 and 13 in the VZ relative to previously described HD factors.

The dorsal limit of the Prdm8 (Fig. 3A–C) expression domain was identical to that of Dlx1 (Developing brain homeobox 1), which marks the neuronal progenitor domain p0 (Fig. 3B, J) [33]. The Prdm8 expression domain extended to a ventral limit significantly dorsal to Olig2 (Oligodendrocyte transcription factor 2) (data not shown) and was exclusive to the expression domain of Msx2/YX2 transcription factor related, locus 2) (Fig. 3C, J) [34,35]. Hence, the Prdm8 expression domain encompassed the VZ of progenitor regions p0, p1, p2 and pMN (Fig. 3J). Prdm12 expression (Fig. 3D–E) had a dorsal limit at the Dlx1 expression domain (Fig. 3D) and a ventral limit significantly dorsal to Olig2 (Fig. 3E); hence, Prdm12 was expressed only in the p1 (Fig. 3J). In addition, Prdm12 was expressed in the DRG (Fig. 3D, E, orange arrowheads). Prdm13 was localized in the VZ of the dorsal spinal cord with a ventral expression border at the Dlx1 dorsal limit (Fig. 3F, J).
In the spinal cord, expression of *Hes5* marks the proliferating neural precursors in the ventricular zone (VZ) and *class III β-tubulin* marks postmitotic neurons in the mantle zone. In the ventral spinal cord, *Prdm8* was expressed not in the VZ, but solely in a small subset of postmitotic neurons (Fig. 3G). However, both *Prdm12* and *Prdm16* had more complex expression patterns encompassing both *Hes5*-positive proliferating VZ cells and adjacent postmitotic cells in the mantle zone (Fig. 3K–M). Furthermore, the localization of *Prdm8* in the VZ was not consistent along the dorso-ventral axis. At the dorsal limit of *Prdm8* expression, where *Dbx1* was also expressed, *Prdm8* was present only weakly in the VZ but much more strongly in the mantle zone a where it expands beyond the extent of *Dbx1* expression (yellow arrowheads in B, C, L). In the dorsal spinal cord, *Hes5* expression (N) and *Prdm13* expression (O) overlap. *Prdm13* expression is weak in the proximal VZ (red arrowhead) and strong (purple arrowhead) at the interface between the VZ and the mantle zone (blue arrowhead).

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*Prdm8, 12 and 16* label interrelated domains in the developing forebrain

WISH on embryos isolated at E9.5, E10.5 and E11.5 demonstrated that *Prdm12* was expressed in the brain from E9.5.
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(Fig. 2) and that Prdm8 and 16 began to be expressed in the forebrain at E11.5 (data not shown). To analyze brain-specific expression of these factors in more detail we performed section ISH at E12.5 (Fig. 4A–C, F–H). We compared the expression pattern of the Prdm genes to those of Hes5, which marks proliferating VZ cells, and class III β-tubulin, which marks postmitotic neurons (Fig. 4D, E, I, J).

At E12.5 Prdm16 was expressed in the VZ throughout the rostral to caudal telencephalon (Fig. 4A, F). The expression was strongest at the pallium/subpallium boundary (psb) (Fig. 4A, F black arrowhead) and formed a lateral/strong to medial/weak gradient (Fig. 4A, F). Prdm16 was additionally expressed in the septum (Fig. 4A, white arrowhead), choroid plexus (Fig. 4F, red arrowhead), and pretectum in the diencephalon (Fig. 4F, purple arrowhead). Prdm16 was expressed in the dorsal telencephalic VZ with a steep lateral/strong to medial/weak gradient from the psb (black arrowhead). It also has expression in postmitotic neurons in the septum (white arrowhead) and pallium (blue arrowhead). (B) Prdm12 is expressed in lateral telencephalic VZ with a steep lateral/strong to medial/weak gradient from the psb (black arrowhead). It also has expression in postmitotic neurons in the septum (white arrowhead) and pallium (blue arrowhead). (G) Beside the expression of Prdm12 in the lateral VZ, a small expression domain in the prethalamus (pink arrowhead) is detected. (C, H) Prdm8 is expressed in the postmitotic neurons of the lateral and dorsal regions of the cortex (orange arrowheads). (D, E, I, J) The progenitor region and the postmitotic region is indicated by Hes5 (D, I) and class III β-tubulin (E, J) respectively. Scale bar in A, 250 μm (A–E); scale bar in F, 500 μm (F–J). Abbreviations: lv, lateral ventricle; psb, pallium-subpallium boundary; sep, septum; cge, caudal ganglionic eminence; th, thalamus; CPe, choroid plexus epithelium.

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Prdm8 and 12 are expressed in specific populations of neurons in the postnatal brain

To examine later stages of Prdm expression we extended our ISH analysis to E16.5 and early postnatal day (P) 6 brains. At E16.5 Prdm8 continued to be strongly expressed in postmitotic neurons in the developing forebrain (data not shown). Furthermore, at E16.5 Prdm16 was still weakly expressed in the telencephalic VZ; however, Prdm12 was no longer expressed in this structure (data not shown). At E16.5 both Prdm16 and Prdm12 continued to be expressed in the septum (data not shown).

At P6 Prdm8 expression was in a sharply defined lamina pattern in the neocortex (Fig. 5A). Comparison of the Prdm8 expression domain with Nissl staining and Tbr1 (T-box brain gene 1) [36] expression domains (Fig. 5B, white arrowheads, C) showed that Prdm8 was expressed in layers 2/3 and 4 (Fig. 5A). Prdm8-expressing cells were also scattered in dentate gyrus (DG) and in CA2 and CA3 regions of the pyramidal cell layer in the hippocampus (Fig. 5D). In addition, Prdm8 was expressed in the nucleus of the lateral olfactory tract (nLOT) (Fig. 5E, arrow) as confirmed by the identical expression of Tbr1 (Fig. 5F, arrow) and by Nissl staining (Fig. 5G, arrow). The nLOT is connected to the main olfactory bulb and the piriform cortex, and influences nonpheromonal olfactory-guided behaviours, especially feeding [37].

By P6 Prdm12 was no longer expressed. Prdm12, on the other hand, was expressed in specific populations of postmitotic neurons in the hypothalamus where it was restricted to the dorsomedial nucleus (Fig. 5H). Prdm12 was also expressed in the dorsal half of the zona incerta of the thalamus (Fig. 5I, arrows). Taken together, these results suggest additional roles of Prdm8 and 12 in differentiated neurons besides their roles in progenitors in the developing CNS.

Prdm16 is positively regulated and Prdm8 negatively regulated by Hes activity during telencephalic neurogenesis

Notch signalling is an essential control mechanism to regulate mammalian neurogenesis. We have previously shown that the Drosophila Prdm gene hamlet is a modifier of Notch signalling during Drosophila peripheral neurogenesis [7]. C. elegans EGL-43 was also demonstrated to be downstream of Notch signalling and to mediate the effect of Notch during vulva formation [20,21]. Finally, zebrafish prdm1 is required to enable Notch signalling pathway-mediated specification of sensory neurons [23,24].

In mammalian cortical neurogenesis high levels of Notch signalling maintain neural progenitors by upregulating the bHLH...
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Three Hes genes (Hes1, 3 and 5) can potentially substitute for each other in the ventricular zone [39]; hence we chose to analyze the expression of Prdm8 and 16 in Hes triple-null forebrain. To obtain Hes triple-null forebrains we crossed Hes1 (floxed/floxed); Hes3<sup>-/-</sup>; Hes5<sup>-/-</sup> mice with Emx1-Cre; Hes1<sup>-/-</sup>; Hes3<sup>-/-</sup>; Hes5<sup>-/-</sup> mice. We hereafter refer to the triple-null forebrains of the embryos derived from this cross as Hes cTKO forebrains. We examined E14.5 Hes cTKO forebrains for both Prdm8 and Prdm16 expression as well as the expression of the proneural factor Ngn2. It has previously been shown that in Hes cTKO mutant embryos cortical neurogenesis occurs prematurely [39]. Indeed, in Hes cTKO mutant telencephalon, compared to wildtype, there was a large increase in the number of cells expressing Prdm8 (Fig. 6A, A’, B, B’, n = 4) and Ngn2 (Fig. 6C, D, bracket, n = 4). These data show that, like Ngn2, Prdm8 expression is repressed by Hes activity, and they further imply that Prdm8 is activated during the temporal progression of neurogenesis in a fashion similar to Ngn2. Furthermore, in Hes cTKO telencephalon the progenitor cell population is reduced but not fully depleted [39]. Indeed, in Hes cTKO forebrains there was also a thinning of the Prdm16 expressing VZ region in the telencephalon (Fig. 6E, F, purple arrowheads) and a stronger reduction of Prdm16 in the VZ of the striatum (Fig. 6E, F, blue arrowheads, n = 4).

Hes1 promotes neural progenitor cell identity by repressing genes that promote cell cycle exit and neural differentiation [38]. Targets of Hes1 activity can be determined by electroporation into the telencephalon followed by examination of changes in putative target gene expression via ISH [38]. Our Hes1 loss of function (Hes cTKO) data implied that Prdm16 may be positively regulated by Hes1, and hence be part of a suite of genes expressed in neural progenitor cells. To examine if Prdm16 is positively
regulated by Hes we performed in utero electroporation of Hes1 cDNA along with EGFP cDNA (pEF-Hes1 and pEF-EGFP) into the telencephalon at E13.5 (Fig. 7A–D); we also electroporated EGFP cDNA alone as a control (Fig. 7E–H, bracket, n = 5, respectively). Eighteen hours later we sacrificed the embryos and carried out ISH to determine Prdm16 gene expression. Prdm16 was strongly upregulated by Hes1 overexpression (Fig. 7A, B, bracket and arrowheads, n = 5) implying that Prdm16 is positively regulated by Hes1 during neurogenesis and expressed in the neural progenitor cell population. As Hes1 protein is believed to act as a transcriptional repressor [3] positive regulation of Prdm16 by Hes1 may not be direct; it is possible that Hes1 acts by repressing a repressor of Prdm16 expression. At the same time we examined Ngn2 expression, and as previously reported Ngn2 was repressed by Hes1 overexpression (Fig. 7C, D, arrow, n = 4) [38]. Electroporation of EGFP alone did not cause any change in Prdm16 or Ngn2 gene expression (Fig. 7E–H).

**Discussion**

**Prdm family-mediated neural class specification of the developing spinal cord**

Our data suggest that several members of the Prdm family could play a role in neuronal specification. During spinal cord development distinct classes of neurons are generated from progenitor cells located at different dorso-ventral positions within the VZ. These domains are spatially defined by the restricted expression of members of the HD transcription factor family and the bHLH factor Olig2. The individual code of transcription factors expressed in each region of VZ defines the fate of the neurons that are generated in that specific region [32]. We can now add the Prdm transcription factors as another family in which multiple members delineate specific progenitor regions. The Prdm family is hence an interesting candidate for involvement in controlling neuron class identity in the spinal cord.

HD transcription factor proteins expressed in the spinal cord VZ are divided into two groups, classes I and II. A single class I and a single class II factor are paired in such a way that there is a sharp boundary between the domains that express each member of the pair. HD transcription factors are repressors, and the sharp boundaries of expression between each pair of class I and II factors are achieved by mutual cross-repression [32]. Interestingly, Prdm proteins associate with a wide range of chromatin-remodelling enzymes and also act predominantly as transcription repressors [16,40–44]. The expression domains of Prdm13 and 8 have a sharp mutual border, raising the interesting possibility that these factors may repress each other. In addition, Prdm13, 8 and 12 all have sharp borders with domains that express specific HD factors, raising the possibility of repression between Prdm and HD factors.

In addition to progenitor cell regions, Prdm8 and 12 are expressed in adjacent cells in the mantle zone. Furthermore, both Prdm8 and 13 are expressed at a high level by cells at the margin of the VZ. Therefore a second point in spinal cord neurogenesis that Prdm genes may be active is as the cells exit the proliferative zone and begin to differentiate as neurons. In this context, we note that in the Drosophila CNS hamlet specifies neuron class fate by acting only transiently at the point where intermediate precursor cells undergo a final division and the immature neuron is formed [6]. prdm1 also acts in a transient fashion to specify slow twitch muscle in zebrafish [19]. It is tempting to speculate that such transient Prdm protein activity could involve establishing a new stable chromatin state in the differentiating cells, either by direct PR domain-mediated remodelling or indirectly by recruiting remodelling enzymes [16,40–44].

**Prdm family-mediated patterning of the developing brain**

In the developing telencephalon patterning is controlled by transcription factors expressed not in discrete domains but rather in a graded fashion [45]. In the telencephalon both Prdm12 and 16 are expressed in lateral/strong to medial/weak gradients (Fig. 4). Furthermore, initial domains of Prdm12 expression are adjacent to the Zhi and isthmus, both of which act as signalling centres regionally patterning the developing brain. Hence, Prdm16 and especially Prdm12 are candidates that merit further examination for roles in brain patterning.

In the early postnatal brain Prdm8 is expressed in cortical layers 2/3 and 4, and Prdm12 in the hippocampus, part of the hypothalamus, and the thalamus (Fig. 5). Furthermore, outside the brain, Prdm12 is strongly expressed in both dental root and cranial ganglia. These expression domains imply that, in addition to a possible involvement in patterning, these factors may also play a role in the differentiation and function of specific neuron classes.

**Probable evolutionary conservation of individual Prdm family member functions during vertebrate neurogenesis**

A recent survey in zebrafish described homologues of the entire mouse Prdm gene family [46]. Zebrafish Prdm family members that have CNS specific expression during neurogenesis (prdm8a, 8b, 12, 13 and 16) are the homologues of those mouse Prdm family
members we describe in this study [46]. Furthermore, there is considerable conservation in the domains of expression for some of these homologues, suggesting probable evolutionary conservation of function. For example, zebrafish prdm9b and 13 are expressed in the spinal cord [46] similar to Prdm9 and 13 in mouse and it will be interesting to ascertain if they have analogous expression domains along the dorso-ventral axis. In the developing telencephalon, similar to mouse, zebrafish prdm16 is expressed during early neurogenesis (18 hours postfertilization) and downregulated later (by 24 hours postfertilization) [46]. These data suggest an early role for prdm16 in telencephalic neurogenesis and raise the question of whether prdm16 also marks neural precursors in zebrafish as it does in mouse. prdm3 is also expressed in early telencephalic development in zebrafish [46] but not mouse ([23] and data not shown); this overlapping telencephalic expression of prdm3 and 16 in zebrafish is interesting because they encode very similar proteins that are likely to share conserved mechanisms of action [40–42].

Putative conserved roles of Prdm family members in neuronal, smooth muscle, germ cell, and haemopoietic development, and in leukaemogenesis

Prdm16, originally named Mel1, was first identified as being expressed at highly elevated levels in leukaemia. Expression of high levels of a truncated form of Prdm16 that does not encode the PR domain (called sPrdm16 or Mel1s) is associated with acute myeloid leukaemia (AML) in humans [11,47] and causative of AML in mouse [48,49]. In this study we demonstrated that Prdm16 expression in the developing CNS is mediated by the Notch-Hes pathway. Interestingly, haemopoiesis also utilizes Notch signalling to maintain stem cell identity and to diversify cell types during lineage elaboration [50]. Moreover, certain mutations that constitutively activate Notch1 protein cause leukaemia; although this is usually T-cell acute lymphoblastic leukaemia rather than the AML associated with Prdm16 [51]. Hence, the relationship of Prdm16 to Notch signalling and a conserved role for Prdm16 in the maintenance of progenitor cell fate are very interesting prospects for further investigation in haemopoiesis and leukaemogenesis, as well as neurogenesis.

Intriguingly, Prdm3, originally named Mel1/Eis1, is the Prdm family member most closely related to Prdm16 [8]. Prdm3 also causes AML when a truncated form (called Eis1) that does not encode the PR domain is ectopically expressed [10,52]. Although Prdm3 is not expressed in developing mouse telencephalon [23] and data not shown), it is expressed in embryonic and adult haemopoietic progenitors in which it regulates proliferation [53]. The domain structure of the Prdm16 and Prdm3 proteins are very similar (Fig 1) and both regulate transcription through binding to the same co-factors, in particular the co-repressor C-terminal Binding Protein (CtBP) [40–42]. These results suggest that Prdm16 and Prdm3 proteins could function in neural progenitor cells, haemopoietic progenitors, and oncogenic haemopoietic progenitors via closely related mechanisms. Hence, previous studies of Prdm3 function in haemopoiesis and leukaemogenesis may be relevant to Prdm16 function in progenitor cells in the CNS.

Other Prdm family members also play roles in maintaining precursor cell proliferation and pluripotency. Prdm6 is expressed in a variety of smooth muscle-containing tissues where it acts to suppress differentiation and maintain the proliferative potential of vascular smooth muscle precursors [16]. Pluripotency is an essential feature of germ cells, and a recent report by Saitou and colleagues has described a two-step process in germ cell specification that involves the sequential activity of two Prdm family members [34]. First Prdm1 acts to repress the somatic gene expression program; second Prdm14 acts to promote the reacquisition of pluripotency and genome-wide epigenetic reprogramming. Notably, Prdm14 is also upregulated in human ES cells where it suppresses differentiation [55]. An interesting potential link between Prdm14 function and Prdm16 is that Prdm14 mediates the acquisition of germ cell pluripotency in part by upregulating Sox2 [54]. Sox2 also has a crucial role in neural precursor cell proliferation and maintenance [56]; hence in addition to the Hes factors, Sox2 is a very good candidate for interaction with Prdm16 during neurogenesis.

Conclusions

In this study we have shown that several members of the Prdm gene family [Prdm6, 8, 12, 13 and 16] have interrelated expression patterns during mouse CNS neurogenesis, which suggest roles in neuronal class specification and differentiation. Within the telencephalon we find that Prdm16 marks neuronal progenitor cells and Prdm8 postmitotic neurons. In this brain region Prdm16 expression is maintained by Notch-Hes signalling and transition to Prdm8 expression follows the down regulation of Notch. This relationship between Prdm genes and the Notch-Hes pathway will be interesting to investigate in wider developmental and oncogenic contexts. Interestingly, our study and a very recent study both show conservation of Prdm16 interaction with bHLH factors; Prdm16 interacts with Hes1 in this study and with Myf5 in the skeletal muscle to brown fat fate switch [18]. It is now important to ascertain if there are common mechanisms of Prdm16 (and Prdm3) interaction with the Notch pathway during brown fat determination, neurogenesis, haemopoiesis, and leukaemogenesis. Certainly, the data we present in this study show that the Prdm family interacts with the Notch-Hes pathway during neurogenesis, may control nervous system patterning, and may modulate neuronal progenitor cell proliferation and differentiation. Hence, the Prdm family is an excellent candidate for further investigation relating to the generation of nervous system complexity.

Materials and Methods

Sequence analysis

The nucleotide and peptide sequences used for primer design to generate ISH probes from Prdm gene family members were obtained from the NCBI and MGI databases. The accession numbers of the cDNAs or predicted gene sequences used for the design of the primers used in this study were as follows: Prdm3, NP_081823; Prdm6, NP_001028453; Prdm8, NP_084223; Prdm9, XP_619431; Prdm10, NP_00104296; Prdm11, CAM14371; Prdm12, XM_355325; Prdm13, NP_001074240; Prdm14, NP_001074678; Prdm15, XP_622716; Prdm16, NP_001870. All plasmids used to generate probes for ISH are freely available upon request from A.W.M. Protein domains for Figure 1 were identified by utilizing PFAM [57] and BLAST.

Molecular biology

Total RNA was prepared from ICR mouse embryos at E11.5 (whole embryos) and E13.5 (head) using an RNaseasy Mini Kit (QIAGEN). RT-PCR was performed using a OneStep RT-PCR kit (QIAGEN) as per manufacturer’s instructions. The individual PCR products were cloned into the plasmid vector pGEM-T Easy (Promega). Additional probes used were: Hes5, classIII β-tubulin, Nog2, Dox1, Olig2, Nsk2.2, Tbr1, and GFP. Both whole mount and section mRNA ISH were carried out using previously published one- or two-colour methods [36].

Mouse breeding

Hes mutant mice were generated as described previously [39]. In utero electroporation experiments were designed and executed as
described in recently published studies [38,39]. All animal research and husbandry was completed in accordance with the guidelines of the RIKEN Brain Science Institute and Kyoto University.

Supporting Information

Table S1  Sequence analysis that indicates the position of protein domains for Prdm1-16.

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