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DOI: 10.1242/dev.181107

Document Version Publisher's PDF, also known as Version of record

Link to publication record in King's Research Portal

Citation for published version (APA):

Prajapati, R. S., Hintze, M., & Streit, A. (2019). PRDM1 controls the sequential activation of neural, neural crest and sensory progenitor determinants. *Development (Cambridge, England)*, *146*(24), Article dev181107. https://doi.org/10.1242/dev.181107

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RESEARCH ARTICLE

PRDM1 controls the sequential activation of neural, neural crest and sensory progenitor determinants

Ravindra S. Prajapati, Mark Hintze and Andrea Streit*

ABSTRACT

During early embryogenesis, the ectoderm is rapidly subdivided into neural, neural crest and sensory progenitors. How the onset of lineage determinants and the loss of pluripotency markers are temporally and spatially coordinated in vivo is still debated. Here, we identify a crucial role for the transcription factor PRDM1 in the orderly transition from epiblast to defined neural lineages in chick. PRDM1 is initially expressed broadly in the entire epiblast, but becomes gradually restricted as cell fates are specified. We find that PRDM1 is required for the loss of some pluripotency markers and the onset of neural, neural crest and sensory progenitor specifier genes. PRDM1 directly activates their expression by binding to their promoter regions and recruiting the histone demethylase Kdm4a to remove repressive histone marks. However, once neural lineage determinants become expressed, they in turn repress PRDM1, whereas prolonged PRDM1 expression inhibits neural, neural crest and sensory progenitor genes, suggesting that its downregulation is necessary for cells to maintain their identity. Therefore, PRDM1 plays multiple roles during ectodermal cell fate allocation.

KEY WORDS: Chick, Gastrulation, Ectoderm, Patterning, PRDM1

INTRODUCTION

In human and mouse embryonic stem cells, exit from pluripotency and entry into differentiation programmes is accompanied by dramatic changes in the chromatin landscape (Andrey and Mundlos, 2017; Habibi and Stunnenberg, 2017; Kalkan and Smith, 2014; Kim et al., 2008; Li and Izpisua Belmonte, 2018; Schlesinger and Meshorer, 2019; Surani et al., 2007; Theunissen and Jaenisch, 2017). As cells gradually lose the expression of pluripotency genes, developmental genes are primed for activation by changes in histone tail modifications. Subsequently, crossrepressive interactions between different transcription factors are thought to establish mutually exclusive fates. However, recent evidence suggests that pluripotency and differentiation networks overlap to varying degrees before final fate specification. A major challenge still remaining is how to translate these processes defined in vitro to the developing embryo, in which exit from pluripotency is not only controlled in time but is also synchronised with cell and tissue rearrangements that lay down the body plan (Habibi and Stunnenberg, 2017; Posfai et al., 2014; Rossant and Tam, 2017; Theunissen and Jaenisch, 2017; Wamaitha and Niakan, 2018).

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Received 2 June 2019; Accepted 27 November 2019

Amniote epiblast cells have the potential to form all embryonic lineages, and a small network of transcription factors including PouV (Oct4; also known as POU5F3), Nanog, Sox2 or Sox3 (Dunn et al., 2014; Kalkan and Smith, 2014; Kim et al., 2008; Rossant and Tam. 2017), and ERNI (also known as Ens-1) in birds (Fernandez-Tresguerres et al., 2010; Jean et al., 2015; Trevers et al., 2018) maintains them in a pluripotent undifferentiated state. As in humans, the chick epiblast is a flat disc, and this morphology is ideal to visualise rapid changes in gene expression in time and space as epiblast cells activate lineage-specific programmes. During gastrulation, the epiblast is transformed into three germ layers with non-ingressing epiblast cells forming the ectoderm, which generates precursors for the central and peripheral nervous system in quick succession, starting from the epiblast centre and progressing towards its periphery (Basch et al., 2006; Litsiou et al., 2005; Puelles et al., 2005; Streit et al., 1998; Streit and Stern, 1999; Stuhlmiller and García-Castro, 2012; Trevers et al., 2018; reviewed by Pla and Monsoro-Burg, 2018; Streit, 2018). The definitive neural plate, the primordium for the central nervous system, forms centrally surrounding the organiser (Fernandez-Garre et al., 2002; Rex et al., 1997; Sanchez-Arrones et al., 2012; Streit et al., 1997; Uchikawa et al., 2003), whereas neural crest and sensory progenitor fates emerge slightly later from cells at the neural plate border (Basch et al., 2006; Ezin et al., 2009; Khudyakov and Bronner-Fraser, 2009; Litsiou et al., 2005; reviewed by Pla and Monsoro-Burg, 2018; Simões-Costa and Bronner, 2013, 2015; Streit, 2018). Neural plate border cells contain progenitors for neural, neural crest and sensory placode lineages and uniquely retain much of the pluripotency network throughout gastrula and neural plate stages, endowing them with stem cell-like properties (Buitrago-Delgado et al., 2015, 2018; Hintze et al., 2017; Trevers et al., 2018). As different fates are allocated, cells lose the expression of pluripotency markers, while activating expression of fate specifiers such as the definitive neural marker Sox2, the neural crest marker Foxd3 and the sensory progenitor genes Six1 and Eva1/2 (Buitrago-Delgado et al., 2015, 2018; Hintze et al., 2017; Trevers et al., 2018). How is the sequential transition towards lineage determination controlled?

Although some of the signalling events have been identified, we know relatively little about the cell intrinsic mechanisms that coordinate the temporal and spatial order in which neural, neural crest and sensory progenitors are specified. In chick, the coiled-coil domain proteins ERNI and BERT control the timing of *Sox2* expression in the neural plate through its N2 enhancer (Papanayotou et al., 2008). At early gastrulation stages, the N2 enhancer is occupied by the chromatin remodelling enzyme Brm and the nuclear factors geminin and ERNI, which in turn recruit transcriptional repressors to prevent premature activation. Towards the end of gastrulation, *BERT* expression is initiated and replaces ERNI in this complex, allowing *Sox2* to be expressed. Identification of the gene networks that regulate neural crest and sensory progenitor specification reveals that both fates are initially under



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the control of neural plate border genes, which act in different combinations to confer neural crest or sensory progenitor identity. Foxd3 is a key neural crest determination factor (Kos et al., 2001; Lukoseviciute et al., 2018; Mundell and Labosky, 2011; Sasai et al., 2001; Simões-Costa et al., 2012; Teng et al., 2008) and its enhancers are regulated by a combination of Pax3/ 7, Msx1 and Zic1 as well as the pluripotency factors Sox2, Nanog and Oct3/4 (Fujita et al., 2016; Simões-Costa et al., 2012). In contrast, the sensory progenitor determinant Six1 is directly controlled by Dlx5/6, negatively regulated by Msx1, and probably indirectly by Gata3 and Tfap2a (Kwon et al., 2010; Pieper et al., 2012; Sato et al., 2010). However, how cells transit from an early epiblast state associated with pluripotency to activating cell fate-specific programmes in a temporal and spatial order remains unclear.

Here, we identify the transcription factor PRDM1 as a key component for the orderly transition from a pluripotency-like state to defined neural lineages. At its N terminus PRDM1 contains a methyltransferase-like PR/SET domain, which lacks enzymatic activity, whereas five C-terminal C2H2 zinc fingers mediate DNA binding and recruitment of chromatin-modifying enzymes (reviewed by Bikoff et al., 2009). Mostly acting as a transcriptional repressor (Ancelin et al., 2006; Győry et al., 2004; Kurimoto et al., 2015; Ren et al., 1999), it is known for its crucial role in B- and T-lymphocyte differentiation, germ cell fate determination, as well as in limb, heart and pharyngeal development (Kallies and Nutt, 2007; Magnúsdóttir et al., 2013; Nutt et al., 2007; Ohinata et al., 2005; Robertson et al., 2007; Saitou et al., 2005; Senft et al., 2019; Shaffer et al., 2002; Vincent et al., 2005). In zebrafish, PRDM1 also controls neural crest cell formation by directly regulating Foxd3 (Hernandez-Lagunas et al., 2005; Olesnicky et al., 2010; Powell et al., 2013). Here, we show that in the chick *PRDM1* expression is remarkably similar to that of the pluripotency associated gene ERNI (Streit et al., 2000): both are highly expressed in the pre-gastrula epiblast together with other pluripotency associated transcripts, but are gradually lost from ectodermal cells as neural, neural crest and sensory progenitor lineages are established. We find that *PRDM1* is required for the loss of some pluripotency markers and for the acquisition of neural, neural crest and sensory progenitor identity. PRDM1 acts as a transcriptional activator by recruiting Kdm4a to the promoter regions of neural and sensory progenitor genes, which in turn removes repressive histone marks and facilitates their expression in a stage-specific manner. Once expressed, specifiers of each neural fate - Sox2, Foxd3 and Six1 – downregulate *PRDM1*, whereas prolonged PRDM1 expression inhibits expression of the specifiers. Therefore, during specification of ectodermal lineages PRDM1 has three distinct activities: it is required for the loss of some pluripotency-associated genes, it directly activates neural determinants, and it must later be lost to allow progression towards definitive neural, neural crest and sensory progenitor identity.

RESULTS

Loss of *PRDM1* expression accompanies neural, neural crest and sensory progenitor specification

We have recently found that the transcription factor PRDM1 is highly enriched in the pre-streak epiblast together with other pluripotency markers such as ERNI, Sox3 and Nanog (Trevers et al., 2018). PRDM1 is an important regulator of cell fate decisions (Kallies and Nutt, 2007; Magnúsdóttir et al., 2013; Nutt et al., 2007; Ohinata et al., 2005; Robertson et al., 2007; Saitou et al., 2005; Senft et al., 2019; Shaffer et al., 2002; Vincent et al., 2005) and we therefore compared its expression during early chick development with those of pluripotency, neural, neural crest and sensory progenitor makers. PRDM1 expression begins before primitive streak formation, but after the onset of ERNI, Sox3 (Fig. 1M-P) and Nanog (Jean et al., 2015; Streit et al., 2000; Trevers et al., 2018) around Eyal-Giladi and Kochav (EG) stage XIII (Fig. 1A,B); all four transcripts are expressed almost identically in the entire epiblast. As the neural plate marker Sox2 is activated (Fig. 1E-H), *PRDM1* expression decreases in the *Sox2* domain and becomes gradually confined to the sensory progenitor domain at HH5 (Fig. 1C,D). For a short time, PRDM1 is co-expressed with the sensory progenitor markers Six1 and Eya2 (Fig. 1I-L), but is gradually lost as sensory placodes are specified (Fig. 1; Fig. S1A). These expression patterns agree with transcriptome profiling of prestreak chick epiblast, neural plate, sensory progenitors and non-neural ectoderm (Trevers et al., 2018; Fig. S1B). The dynamic changes of *PRDM1* expression are reminiscent of *ERN1* and other pluripotency markers (Jean et al., 2015; Lavial et al., 2007; Streit et al., 2000; Trevers et al., 2018), which, as PRDM1, are downregulated as cells are specified as neural and sensory progenitors.

PRDM1 is required for neural, neural crest and sensory progenitor fates and for the loss of some pluripotency markers at primitive streak stages

Acting in a protein complex, PRDM1 recruits histone-modifying enzymes to target genes and is generally associated with transcriptional repression (Ancelin et al., 2006; Győry et al., 2004; Kurimoto et al., 2015; Ren et al., 1999; reviewed by Bikoff et al., 2009; Mzoughi et al., 2016). Its expression, akin to that of pluripotency markers, suggests that it may maintain cells in a pluripotent state and prevent lineage specification. To test this hypothesis, we used a loss-of-function approach and assessed changes in gene expression, initially focusing on sensory progenitors. We knocked down PRDM1 using two antisense oligonucleotides (aONs) targeting intron-exon junctions (Fig. S2A). Two different aONs (alone or together) or control oligonucleotides (ONs) were electroporated broadly into the epiblast of early primitive streak stage chick embryos [Hamburger and Hamilton (HH) stage 3] and targeted sensory progenitors were harvested at HH6. As a first step to assess the effect of PRDM1 knockdown on many targets, we used multiplex NanoString nCounter to examine transcript levels of 382 genes, including markers for pre-streak epiblast and different ectodermal fates (Fig. S2B; Table S1). Surprisingly, we found that the sensory progenitor markers *Eya2*, *Six1* and *Irx1*, as well as their upstream regulators Gata3 and Dlx5/6 are significantly downregulated, as is the neural crest marker Snai2. In contrast, the pluripotency associated transcripts ERNI, Nanog and Eomes are upregulated, as are other genes expressed broadly in the pre-streak epiblast, such as MafA (Fig. S2B). These findings suggest that PRDM1 may be required for the loss of pluripotency markers and for the initiation of lineage specification.

To corroborate our NanoString results and to gain spatial information on changes in gene expression not only in sensory progenitors but also in neural and neural crest cells, we performed *in situ* hybridisation after PRDM1 knockdown. Embryos were again electroporated with control or experimental ONs at early streak stages (HH3) before the onset of definitive neural, neural crest and sensory progenitor markers. After 16-24 h, we assessed *Sox2* (neural), *Foxd3* (neural crest), and *Six1* and *Eya2* (sensory progenitor) transcripts. Whereas control ONs have no effect (Fig. 2A-D; *Sox2: n=0/8; Foxd3: n=0/5; Six1: n=0/5; Eya2: n=0/9*), *PRDM1* knockdown diminishes the expression of all four



Fig. 1. Expression of PRDM1, Sox2, Eya2 and Sox3 in the early chick embryo. (A-D) PRDM1 is broadly expressed in the epiblast at pre-primitive streak stages (A) and at primitive streak stages (B), but is downregulated as the neural plate (NP) is specified (C). At headfold stages (D) PRDM1 is confined to sensory progenitors (SP). (E-H) Sox2 is not expressed at pre-streak stages (E) and starts to be expressed in the ectoderm surrounding the organiser at primitive streak stages (F). As the neural plate forms (G) its expression increases and it is confined to the neural plate at headfold stages (H). (I-L) Eya2 is expressed in the hypoblast before primitive streak formation (I), and not expressed at primitive streak stages (J). At late primitive streak stages Eya2 is expressed in the mesoderm, but absent from the ectoderm (K). At headfold stages (L) it continues to be expressed in the head mesoderm and is expressed in sensory progenitors in the ectoderm. (M-P) Sox3 is broadly expressed in the prestreak (M) and primitive-streak-stage epiblast (N). At head process (O) and headfold (P) stages it is strongly expressed in the neural plate and weaker in sensory progenitors. Sections through the embryos at the level of the black lines (lowercase) are shown (bottom). Scale bars: 50 µm in A,B (all other sections are the same magnification as B).

transcripts in aON-targeted cells (Fig. 2F-I; *Sox2*: n=8/10; *Foxd3*: n=4/6; *Six1*: n=7/10; *Eya2*: n=8/10). PRDM1 is also required for the maintenance of *Dlx6* (control: n=0/3, aON: n=7/9) and *Gata3* (control: n=0/5; aON: n=6/6) expression, which are already expressed in the non-neural ectoderm at early primitive streak stages (Fig. S2B-F). In contrast, *PRDM1* knockdown causes expansion or upregulation of transcripts expressed in pluripotent pre-streak epiblast cells (Trevers et al., 2018) such as *Sox3* (Fig. 2E, J; control: n=0/5; aON: n=5/5), *MafA* (Fig. S2B,G,H; control: n=0/4, aON: n=4/5) and *ERNI* (Fig. S2B). Together, these results suggest an unexpected role for PRDM1 at early primitive streak stages. Although PRDM1 appears to inhibit stem cell-like properties, it is required for the activation of the neural, neural crest and sensory progenitor programmes.

Mutual repression between PRDM1 and Sox2, Foxd3 and Six1 maintains cell identity

As soon as neural cell fate specifiers are expressed, *PRDM1* expression is downregulated. To test whether PRDM1 loss is

required for cells to maintain lineage specification, we electroporated full-length *PRDM1* at primitive streak stages (HH4⁻; this leads to protein production \sim 3-5 h later at HH4/5) and found that this led to a reduction in Sox2 (5/5) and Eva2 (4/5)expression at HH6/7 (Fig. S3A-D) suggesting that PRDM1 inhibits definitive neural and sensory progenitor identity. How is *PRDM1* downregulation controlled? In zebrafish, PRDM1 is necessary for neural crest cell formation and it regulates Foxd3 directly. However, once it starts to be expressed, Foxd3 represses PRDM1 (Powell et al., 2013). We therefore tested whether a similar regulatory relationship exists in chick. HH3⁺ chick embryos were electroporated with Sox2, Foxd3 and Six1 constructs and the expression of PRDM1 was assessed at HH6/7 by in situ hybridisation. Mis-expression of Sox2 (9/9), Foxd3 (13/13) or Six1 (7/8), but not of GFP leads to loss of PRDM1 (Fig. 2K-N). Therefore, once ectodermal cells begin to acquire their unique identity at late primitive streak stages, direct or indirect repression of PRDM1 by Sox2, Foxd3 and Six1 (Fig. 20) allows lineage progression towards neural, neural crest and sensory progenitors.

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Fig. 2. PRDM1 is required for neural, neural crest and sensory progenitor fates, but later repressed by fate determinants. (A-J) Control (A-E) or PRDM1-targeting aONs (F-J) were electroporated into the epiblast of chick embryos at early primitive streak stages. At headfold stages the expression of *Sox2* (A, *n*=8; F, *n*=10), *Foxd3* (B, *n*=5; G, *n*=6), *Six1* (C, *n*=5; H, *n*=10), *Eya2* (D, *n*=9; I, *n*=10) and *Sox3* (E, *n*=5; J, *n*=5) was assessed by *in situ* hybridisation (blue). Fluorescein-labelled ONs are visualised by antibody staining in brown. Arrowheads in F-J indicate changes in gene expression after *PRDM1* knockdown. (K) Mis-expression of GFP (brown) does not affect the expression of *PRDM1* (blue). (L-N) Mis-expression of Sox2 (L, *n*=9), Foxd3 (M, *n*=13) and Six1 (N, *n*=8) leads to downregulation of *PRDM1* (blue; arrowheads) in targeted cells (brown). (O) PRDM1 is required for the expression of *Sox2*, *Foxd3* and *Six1* in the early epiblast, but is later downregulated by these factors. Panels on the left show each embryo before immunolabelling. Sections through the embryos at the level of the black lines (lowercase) are shown (bottom); h' and i' show higher magnification of the areas boxed in h and i; insets in k-n show a higher magnification of the boxed areas. Scale bars: 50 µm in A,B (all other sections are the same magnification as A); 10 µm in h',i'; 20 µm in inset in n (for k-n).

PRDM1 occupies Sox2 and Eya2 promoter regions at distinct stages

Our results show that PRMD1 is required early for the expression of neural progenitor genes. Does it act by direct interaction with regulatory or promoter regions? We find PRDM1 motifs within 2 kb upstream of the transcription start site (TSS) of Sox2, Foxd3, Six1 and Eya2, and of neural plate border genes such as Dlx5/6, Msx2and TFAP2a/e and transcripts expressed in a subset of sensory progenitors (e.g. Pax6, SSTR5, Dmbx1) (Fig. 3; Table S2). To assess whether PRDM1 occupies these sites in vivo, we focused on Sox2 and Eya2 as key factors required for neural plate and sensory progenitor specification and performed PRDM1 chromatin immunoprecipitation (ChIP) followed by qPCR (Fig. 3A-D). We dissected four distinct embryonic territories representing different cell states (Fig. 3B): epiblast cells before primitive streak formation (cEpi: *PRDM1*⁺, *Sox2*⁻, *Eya2*⁻), the neural plate border (NPB: PRDM1⁺, Sox2⁻, Eya2⁻) and early neural plate (eNP: PRDM1⁺, $Sox2^+$, $Eya2^-$) from HH4⁻, and the sensory progenitor domain (SP:

 $PRDM1^+$, $Sox2^-$, $Eya2^+$) from HH6. ChIP-qPCR reveals a significant enrichment of PRDM1 upstream of the Sox2 and Eya2 TSS when compared with IgG controls, but only in early neural plate and sensory progenitor cells, respectively (Fig. 3C,D). As a negative control we examined the TSS of the histone demethylase Kdm4a, which is ubiquitously expressed in epiblast cells at all the stages tested (Strobl-Mazzulla et al., 2010) and lacks a PRDM1 motif. We do not observe PRDM1 binding (Fig. 3I). Thus, when Sox2 and Eya2 begin to be expressed, PRDM1 is bound close to their TSS, suggesting that it may regulate their transcription directly.

Chromatin accessibility, DNA methylation and repressive histone mark H3K9me3 occupancy dynamically regulate Sox2 and Eya2 in vivo

Although *PRDM1* is expressed in all four cell populations tested, it binds close to the *Sox2* and *Eya2* TSS in a stage- and tissue-specific manner. We reasoned that changes in chromatin accessibility, DNA methylation and/or repressive histone marks may be important for



the regulation of neural and sensory progenitor gene expression and for PRDM1 binding. To assess these features as cells become successively specified we dissected pre-streak epiblast, neural plate and sensory progenitor cells. To investigate chromatin accessibility we performed assay for transposase-accessible chromatin (ATAC)qPCR, probing the genomic regions upstream of the *Sox2* and *Eya2* TSS that harbour PRDM1 motifs, and determined accessibility in neural plate and sensory progenitors relative to pre-streak epiblast cells. This analysis revealed that the PRDM1 site upstream of the

Fig. 3. PRDM1 recruits Kdm4a to the TSS of Sox2 and Eya2 to remove repressive marks in a time-specific manner. (A) PRDM1 binding motifs are detected upstream of the TSS of Sox2 and Eya2 (red bars). Inset shows PRDM1 motif. (B) Pre-primitive-streak-stage epiblast (cEpi, grey), early neural plate (eNP, blue), neural plate border (NpB, purple) and sensory progenitors (SP, purple) were dissected from chick embryos at different stages. (C-D) Chromatin isolated from cEpi, NpB and eNP was subjected to ChIP with IgG control (dark grey bars) and PRDM1 antibodies followed by qPCR using primers flanking the PRDM1 motifs upstream of the Sox2 TSS (C) or Eya2 TSS (D). PRDM1 binds to the Sox2 promoter in eNP cells and to the Eya2 promoter in SPs. Experiments were carried out in triplicate on three independent occasions. (E) ATAC-qPCR amplifying the region containing the PRDM1 motif upstream of the Sox2 and Eya2 TSS, respectively, from eNP and SPs. Quantification shows the fold change relative to ATAC-qPCR of the same region from pre-streak epiblast cells. The promoter region of Sox2 is accessible in eNP, but not SP cells, whereas the opposite is true for Eya2. (F) DNA methylation upstream of the Sox2 and Eya2 TSS was assessed by bisulfide conversion of non-methylated cytosine to uracil followed by PCR with primers that are specific for methylated (M) and non-methylated (U) DNA. At pre-streak stages (cEpi), the Eya2 TSS is largely methylated (red arrowhead), but nonmethylated in SPs (red arrowhead), whereas the Sox2 TSS is non-methylated in cEpi and eNP cells (red arrowhead). (G-H) ChIP-qPCR with control IgG (dark grey bars) and H3K9me3 antibodies (coloured bars) show the presence or reduction of repressive marks at the Sox2 TSS (G) and the Eya2 TSS (H). Repressive marks are present at the Sox2 TSS in cEpi (grey) and NpB cells. H3K9me3 is reduced in the eNP and to a lesser extend in SPs (purple), but increases in eNP when PRDM1 is knocked down (asterisk, red bar). Repressive marks are present at the Eya2 TSS in cEpi, NpB, eNP and HH6 NP (blue). H3K9me3 is reduced in the SP, but increases when PRDM1 is knocked down (asterisk, red bar). (I) PRDM1 ChIP-qPCR for the region upstream of the Kdm4a TSS in different tissues shows the absence of PRDM1 binding. Dark grey bars, IgG control; coloured bars, PRDM1 antibody. (J) H3K9me3 ChIPgPCR for the Kdm4a TSS in different tissues shows the absence of repressive marks and no changes over time. Dark grey bars: control IgG; coloured bars: H3K9me3 antibody. Knockdown of PRDM1 in eNP and SP (asterisks) has no effect on H3K9me3 deposition (red bars). (K) Western blot on protein lysates from mixed eNP/SP cells with Kdm4a antibodies; stripped blots were probed for y-tubulin. (L) Western blot on protein lysates from mixed eNP/SP cells with PRDM1 antibodies; stripped blots were probed for y-tubulin. (M) Western blots with PRDM1 (left) and Kdm4a (right) of immunoprecipitates with Kdm4a and Prdm1 antibodies, respectively, reveal interaction of both proteins. Input, cell lysates used as input for Co-IPs; IgG, control immunoprecipitation. (N) Kdm4a ChIP-qPCR for the Sox2 TSS from cEpi and eNP reveals binding of Kdm4a in eNP. Dark grey bars, IgG control; coloured bars, Kdm4a. Knockdown of PRDM1 in eNP (asterisk) abolishes Kdm4a binding (red bar). (O) Kdm4a ChIP-qPCR for the Eya2 TSS from cEpi and SP reveals binding of Kdm4a in SPs. Dark grey bars, IgG control; coloured bars, Kdm4a. Knockdown of PRDM1 in SPs (asterisk) abolishes Kdm4a binding (red bar). All experiments were repeated at least on three independent occasions. Data are mean±s.d. (unpaired two-tailed Student's t-test).

Sox2 TSS opens in neural plate cells at HH4⁻, whereas the PRDM1 motif upstream of the *Eya2* TSS becomes accessible only in sensory progenitors at HH5/6 (Fig. 3E). Thus, changes in chromatin accessibility coincide with the onset of *Sox2* and *Eya2* expression and with PRDM1 occupancy.

Methylation of CpG islands in proximity of the TSS is generally associated with transcriptional repression, and PRDM1 binding is known to be methylation sensitive (Doody et al., 2010). Indeed, CpG islands are predicted at position -2 kb to -0.4 kb from the *Sox2* and -2 kb to -1.7 kb from the *Eya2* TSS, close to PRDM1 binding sites. To investigate CpG methylation we performed bisulphite assays, in which cytosine is converted into uracil, whereas 5-methylcytosine remains intact, in pre-streak epiblast, neural plate and sensory progenitor cells. The above genomic regions were probed by PCR using primers that are specific for methylated and non-methylated DNA. We find that, at pre-streak stages, *Sox2* is not methylated, whereas CpG islands upstream of the

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Eya2 TSS are, but their methylation is lost in sensory progenitors (Fig. 3F). Thus, both genes are differentially prepared for transcription in agreement with their onset of expression at primitive streak (*Sox2*) and neural plate stages (*Eya2*).

Histone3 lysine9 trimethylation (H3K9me3) is a hallmark of transcriptional silencing and is enriched at repressed and bivalent promoter regions. To assess histone methylation we performed ChIP using H3K9me3 antibodies revealing dynamic occupancy close to the *Sox2* and *Eya2* TSSs. In pluripotent epiblast and HH4⁻ neural plate border cells H3K9me3 is enriched at the TSS of both genes. Although H3K9me3 occupancy is reduced upstream of the *Sox2* TSS in the HH4⁻ neural plate, the *Eya2* TSS loses H3K9me3 later in HH5/6 sensory progenitors (Fig. 3G,H). To ensure specificity, we used the ubiquitously expressed *Kdm4a* as control; we did not observe any changes in H3K9me3 (Fig. 3J). Thus, histone demethylation at the TSS of *Sox2* and *Eya2* clearly reflects the time- and tissue-specific transcriptional status of both genes.

Together, these results show dynamic epigenetic changes in proximity of the TSS of the neural plate specifier *Sox2* and the sensory progenitor specifier *Eya2* consistent with the onset of their transcription and PRDM1 binding. In pre-streak epiblast cells, the genomic regions close to the TSS of both genes are closed, decorated by repressive H3K9me3 marks and PRDM1 does not bind. Although CpG islands close to the *Eya2* TSS are methylated at pre-streak stages, those proximal to *Sox2* are not, foreshadowing its expression at primitive streak stages. H3K9me3 marks are lost concomitant with PRDM1 binding, suggesting that PRDM1 plays an active role in preparing the onset of *Sox2* and *Eya2* transcription.

PRDM1 recruits Kdm4a to remove repressive histone marks

PRDM1 is known to form multi-protein complexes that are generally involved in transcriptional repression. Our results suggest, however, that during neural and sensory progenitor specification, PRDM1 plays an activating role. To assess whether PRDM1 is required for histone demethylation upstream of the *Sox2* and *Eya2* TSSs, we electroporated PRDM1 aONs into neural and sensory progenitors at HH3 and collected early neural plate and sensory progenitors after 16-24 h. ChIP-qPCR reveals that H3K9me3 increases in the absence of PRDM1 when compared with controls (Fig. 3G,H). In contrast, there is no change in H3K9me3 at the *Kdm4a* TSS (Fig. 3J). We therefore propose that PRDM1 mediates activation of *Sox2* and *Eya2* transcription by promoting the removal of repressive histone marks.

PRDM1 itself does not have enzymatic activity but is known to interact with different chromatin modifiers (Ancelin et al., 2006; Győry et al., 2004; Magnúsdóttir et al., 2013; reviewed by Bikoff et al., 2009; Mzoughi et al., 2016). The histone demethylase Kdm4a specifically removes trimethylation from H3K9 and is broadly expressed in the chick epiblast (Strobl-Mazzulla et al., 2010). We therefore first assessed whether PRDM1 and Kdm4a interact. Western blot analysis from mixed neural plate border and sensory progenitors confirms the expression of both proteins (Fig. 3K,L), while co-immunoprecipitation (Co-IP) reveals that PRDM1 and Kdm4a bind to each other (Fig. 3M). We next examined whether Kdm4a is located close to the Sox2 and Eva2 TSS using ChIP qPCR. In pre-streak epiblast, Kdm4a does not occupy either region, but binds close to the Sox2 TSS in early neural plate cells and close to the Eva2 TSS in sensory progenitor cells (Fig. 3N,O). Thus, both PRDM1 and Kdm4a are found close to the TSS of Sox2 and Eya2 when their transcription becomes activated.

To test whether PRDM1 is required for Kdm4a binding, we knocked down PRDM1 in neural and sensory progenitors by

electroporation of aONs at HH3 and collected both tissues at HH4⁻ and HH6, respectively. Kdm4a ChIP-qPRC revealed that, in the absence of PRDM1, Kdm4a binding to both genomic regions is lost (Fig. 3N,O). These results show that PRDM1 is required to recruit Kdm4a to the TSS of *Sox2* and *Eya2*.

Together our results provide a model for sequential specification of neural and sensory progenitor fates in the embryonic ectoderm, with PRDM1 as a central player (Fig. 4). Before activation of cell type-specific transcripts their TSSs are inaccessible and decorated with repressive marks such as H3K9me3. As they become accessible, PRDM1 binds upstream of the TSS of key neural and sensory progenitor genes, recruits the histone demethylase Kdm4a, which in turn demethylates H3K9me3 to facilitate transcriptional activation.

DISCUSSION

During embryo development, exit from pluripotency and sequential activation of distinct differentiation programmes must be tightly controlled to coordinate cell fate decisions with morphogenetic processes. Our findings place the transcription factor PRDM1 into the centre of the network regulating these processes in the embryonic ectoderm. Dissection of PRDM1 function in time and space allows us to distinguish different PRDM1 activities. Initially, PRDM1 is required for cells to lose some pluripotency markers, and at the same time for activating the neural, neural crest and sensory progenitor programme. Once cells are specified, PRDM1 must be downregulated rapidly to maintain these fates: prolonged expression prevents the differentiation of neural lineages. Thus, PRDM1 function changes rapidly, presumably because of interaction with different co-factors.

PRDM1 balances loss of pluripotency markers and the activation of neural programmes

In amniote embryonic stem cells, different epigenetic mechanisms including transcriptional repressors, histone and DNA methylation



maintain pluripotency, while simultaneously preventing premature expression of differentiation markers (Andrey and Mundlos, 2017; Habibi and Stunnenberg, 2017; Kalkan and Smith, 2014; Kim et al., 2008; Li and Izpisua Belmonte, 2018; Schlesinger and Meshorer, 2019; Surani et al., 2007; Theunissen and Jaenisch, 2017). In the embryo, the loss of pluripotency is tightly coordinated with morphogenetic events. As the three germ layers form, cells in the ectoderm are rapidly specified as central and peripheral nervous system progenitors while pluripotency gene expression decreases. In chick, the pluripotency associated factors PouV, Nanog, Sox3 and ERNI are expressed in the blastoderm before primitive streak formation (Jean et al., 2015; Lavial et al., 2007; Streit et al., 2000), as is *PRDM1* (this study). As with *ERNI*, *PRDM1* expression is lost in epiblast cells as differentiation programmes are activated. Our results suggest that PRDM1 plays a dual role: while PRDM1 knockdown leads to upregulation of pluripotency markers, neural, neural crest and sensory progenitor specifiers fail to be expressed. It is therefore possible that PRDM1 inhibits pluripotency markers, while activating genes that are characteristic for neural fates. A similar scenario has been observed in primordial germ cells and during their conversion into pluripotent cells (Nagamatsu et al., 2015; Surani et al., 2007). PRDM1 deletion in primordial germ cells enhances their dedifferentiation into pluripotent embryonic germ cells, paralleling our findings. Conversely, its overexpression in embryonic stem cells suppresses parts of the pluripotency network and prevents the conversion of *in-vitro* induced primordial germ cells into pluripotent embryonic germ cells. Together, these findings highlight PRDM1 as an important node in the network that controls the balance between pluripotency and differentiation for several different lineages.

Molecular events controlling neural, neural crest and sensory progenitor fates

Around the time of gastrulation, ectodermal cells begin to activate neural, neural crest and sensory progenitor genes in a temporal sequence. Here, we demonstrate that, before this, the TSSs of the neural marker Sox2 and the sensory progenitor marker Eva2 are not accessible, are decorated with the repressive histone marks H3K9me3 and are not bound by PRDM1. H3K9me3 is linked to gene silencing and is known to bind the heterochromatin protein 1 family of transcriptional repressors (HP1) (Bannister et al., 2001; Lachner et al., 2001; Nielsen et al., 2001), and this is likely to prevent inappropriate transcription of both genes. As development proceeds, both promoter regions open in a time- and tissue-specific manner allowing PRDM1 to bind and recruit demethylases such as Kdm4a leading to removal of repressive H3K9me3. Although we show evidence for a PRDM1-Kdm4a interaction, other demethylases are expressed in the chick epiblast (Trevers et al., 2018) and may play similar roles. Previous studies have shown that, at pre-streak stages, the N2 enhancer of Sox2 is occupied by a complex including geminin and ERNI, which in turn recruits HP1 γ to repress Sox2 transcription (Papanayotou et al., 2008). During gastrulation, the coiled-coil protein BERT displaces ERNI from the complex together with HP1 γ . Here, we show that, at the same time, PRDM1 enables the removal of repressive marks at the promoter region, suggesting that both mechanisms work in concert to activate Sox2 transcription in early neural plate cells. It is tempting to speculate that a similar mechanism acts to promote the transcription of neural plate border, neural crest and sensory progenitor genes. Indeed, the neural plate border genes Dlx5/6, Gata3, TFAPa/e and Msx2 harbour PRDM1 motifs close to their TSS, as do Six1, Eva2, and the neural crest factor gene *Foxd3*, suggesting that PRDM1 may

control the onset of their expression directly. Whether geminin, ERNI and BERT at enhancer regions cooperate with PRDM1 remains to be elucidated, although the dynamic expression of ERNI is consistent with this hypothesis. Together, these observations place PRDM1 into the centre of the transcriptional network controlling the onset of neural, neural crest and sensory progenitor specification.

PRDM1: activator or repressor?

PRDM1 is generally considered to act as a transcriptional repressor. Here, we provide evidence that, in early epiblast cells, it also functions as a transcriptional activator, and we elucidate the underlying mechanism. PRDM1 contains a proline-serine rich domain and five C2H2 zinc fingers; the latter being responsible for DNA binding, whereas both are involved in the recruitment of additional cofactors (Ancelin et al., 2006; Győry et al., 2004; Kurimoto et al., 2015; Ren et al., 1999; reviewed by Bikoff et al., 2009; Mzoughi et al., 2016). In primordial germ cells, PRDM1 is required for the repression of somatic genes and forms a complex with the arginine-specific histone methyltransferase Prmt5, which in turn mediates methylation of histone H2A and H4 tails (Ancelin et al., 2006). In B cells, it represses genes associated with cell cycle progression and B cell maturation such as Myc, CIITA and Pax5 by recruiting histone deacetylases to their promoters (Bikoff et al., 2009; Győry et al., 2004; Lin et al., 1997; Yu et al., 2000). In addition, interacting with groucho proteins or the G9a methyltransferase, it represses the expression of interferon γ (Győry et al., 2004; Ren et al., 1999).

In contrast, our results reveal that PRDM1 acts as an activator in neural, neural crest and sensory progenitor cells. As in chick, zebrafish PRDM1 is required for neural crest cell formation by interacting with the enhancers of the neural crest factors Foxd3 and Tfap2a (Powell et al., 2013). We show that PRDM1 plays an important role in recruiting the histone demethylase Kdm4a to the TSS of the neural specifier Sox2 and the sensory progenitor specifier *Eva2*, leading to reduced H3K9me3 occupancy and gene activation. Both Kdm4a binding and loss of the repressive mark H3K9me3 are PRDM1 dependent, explaining why PRDM1 is necessary for the onset of the expression of both genes. Although we do not provide evidence for direct Foxd3 activation by PRDM1, a similar mechanism may operate in neural crest cells. In Xenopus, Kdm4a overexpression leads to upregulation of Foxd3 and the neural crest gene Slug1, accompanied by a loss of H3K9me3 at the Foxd3 promoter (Powell et al., 2013). In chick, Kdm4a is required for neural crest cell development and mediates H3K9me3 demethylation close to the TSS of the neural crest specifiers Sox10 and Snai2 (Strobl-Mazzulla et al., 2010). In this scenario, it is likely that PRDM1 and Kdm4a form part of activator complex that in turn activates transcription of Foxd3, Sox10 and Snai2 (Matsukawa et al., 2015). Interestingly, in sensory progenitor cells the promoters of neural crest cell specifiers are occupied by PRDM12, which represses their expression by promoting H3K9me3 deposition and thus prevents inappropriate expression of neural crest genes.

Although promoting transcriptional activation of neural determinants in early epiblast cells, shortly thereafter PRDM1 appears to play an inhibitory role. During normal development *PRDM1* is rapidly downregulated as lineage-specific genes become expressed, and we show that the fate specifiers themselves play a crucial role: mis-expression of Sox2, Foxd3 or Six1 leads to loss of *PRDM1*, although it remains unclear whether these factors are required for *PRDM1* downregulation. Likewise, in zebrafish, Foxd3

represses *PRDM1* (Powell et al., 2013). When *PRDM1* expression is maintained beyond its normal time neural, neural crest and sensory progenitor fates are inhibited. It is possible that PRDM1 activates a repressor of fate specifiers or recruits transcriptional repressors such as histone deacetylases or groucho family members to inhibit their expression directly. Thus, at early neural plate stages, PRDM1 and Sox2, Foxd3 and Six1 mutually repress each other directly or indirectly and the loss of PRDM1 after cell fate specification allows neural, neural crest and sensory progenitor cells to maintain their identity. Thus, tight regulation of PRDM family members and their interacting partners is required for fine tuning gene expression at the neural plate border and for mediating cell fate choices.

Conclusion

During embryo development, exit from pluripotency and sequential activation of distinct differentiation programmes must be tightly controlled in time and space to coordinate cell fate decisions with morphogenetic processes. PRDM1 emerges as a key node in the network regulating these processes in the embryonic ectoderm (Fig. 4). PRDM1 is required for the loss of some pluripotency markers, while at the same time activating the neural, neural crest and sensory progenitor programme. Once initiated PRDM1 is downregulated rapidly, allowing central and peripheral nervous system precursors to maintain their identity. In embryonic stem cells and primordial germ cells, PRDM1 downstream targets have been extensively characterised and are partially overlapping. It will be interesting to evaluate how these networks diverge in the neural lineage.

MATERIALS AND METHODS

Embryo collection and whole mount in situ hybridisation

Fertile hens' eggs were obtained from Henry Stewart farms (Norfolk, UK) and incubated at 38°C until they reached the stage required (Hamburger and Hamilton, 1951). Embryos were collected in nuclease-free phosphate buffered saline (PBS) and fixed in 4% paraformaldehyde at room temperature for 4-5 h. Whole-mount *in situ* hybridisation was carried out as previously described (Streit and Stern, 2001). To generate antisense digoxigenin-labelled probes the following plasmids were used: PRDM1 (Chen et al., 2017), Six1 (Sato et al., 2010), Eya2 (Mishima and Tomarev, 1998), Dlx5 (McLarren et al., 2003), Sox2 (Rex et al., 1997), Foxd3 (Kos et al., 2001), ERNI (Streit et al., 2000) and Gata3 (Sheng and Stern, 1999).

Electroporation of antisense oligonucleotides and expression vectors

Primitive-streak-stage embryos were electroporated in Tyrode's saline using five pulses of 5-7 mV for 50 ms with an interval of 750 ms and cultured in modified New culture (Stern and Ireland, 1981) until the 1- to 5-somite stages. Two splicing-blocking aONs were designed to knockdown *PRDM1* by pre-mRNA mis-splicing: aON1 (5'-ACTGTAATGCACTTACTGAGGTTC-3') targets the exon6-intron6 and aON2 (5'-TCTTAGTCTCCACC-ACCTAC-CTTCA-3') targets exon7-intron7 boundary. Control ONs were 5'-CCTCTTACCTCAGTTACAATTTATA-3' (GeneTools). For electroporation, each ON was used at a final concentration of 1 mM in distilled water containing 6% sucrose, 0.04% Fast Green and 0.5 mg/ml carrier plasmid (puc19). All ONs were labelled with fluorescein; to visualise targeted cells we performed immunocytochemistry using anti-fluorescein antibodies (Roche, 426346910).

For mis-expression, the coding regions of *PRDM1*, *Six1*, *Foxd3* and *Sox2* were cloned into pCAB-IRES-eGFP vectors, which drives ubiquitous expression of the gene of interest and eGFP. For electroporation, plasmids were used at a concentration of 2 mg/ml in distilled water containing 6% sucrose and 0.04% Fast Green. Anti-GFP (Life Technologies, a11122, 1:1000) and HRP-coupled secondary antibodies (Jackson ImmunoResearch, 111-035-003, 1:2500) were used to visualise targeted cells.

NanoString nCounter

HH3⁺/4⁻ embryos were electroporated with aONs targeting PRDM1 or control ONs, allowed to grow until HH6 and targeted sensory progenitor cells were dissected using a fluorescence microscope. Each sample contained 5-10 tissue pieces (5000-7000 cells), which were immediately lysed in lysis buffer and processed for NanoString nCounter as previously described (Hintze et al., 2017). Each experiment was repeated on three independent occasions. Counts were normalised to the positive controls contained within the hybridisation mix and negative control probe values were used to determine the background threshold level. Transcripts with expression values below the threshold were removed from further analysis. Counts were then normalised to the total amount of mRNA in each sample and differential expression between control and experimental conditions was determined using an unpaired two-tailed Student's *t*-test, comparing the average of three biological replicates (*P*<0.05, >1.2-fold change).

Chromatin immunoprecipitation

For ChIP, 15-20 explants of pre-streak epiblast, HH4⁻ neural plate border and early neural plate, and HH6 sensory progenitors were dissected in Tyrode's saline. Tissues were homogenised in nuclear extraction buffer [NEB: 0.5% NP-40, 0.25% Triton X-100, 10 mM Tris-HCl (pH 7.5), 3 mM CaCl₂, 0.25 M sucrose, 1 mM DDT, 0.2 mM PMSF, 1× protease inhibitor (PI)] using a Dounce homogeniser and fixed with 0.9% formaldehyde for 10 min at room temperature. The fixing reaction was quenched with 125 mM glycine, the tissues were washed three times in PSB-PI (1 mM DDT, 0.2 mM PMSF, 1× PI). Cells were re-suspended in NEB and nuclei were released by Dounce homogenising using a tight pestle. Nuclei were washed with PSB-PI and lysed in SDS lysis buffer [1% SDS, 10 mM EDTA in 50 mM Tris-HCl (pH 8)] for 1 h on ice before being diluted to 0.9 ml with ChIP Dilution Buffer [CDB: 0.01%SDS, 1.2 mM EDTA, 167 mM NaCl, 1 mM DDT, 0.2 mM PMSF and 1× PI in 16.7 mM Tris-HCl (pH 8)] and sonicated to obtain 500-1000 bp chromatin fragments. Triton X-100 was added to a final concentration of 1% before the chromatin was used for ChIP. Protein-A magnetic beads (100 µl) were blocked with 0.5% bovine serum albumin, coated with 5 mg antibody (PRDM1, ab13700; Kdm4a, ab24545; H3K9me3, ab8898; control IgG, ab171870; Abcam), added to the chromatin and allowed to bind overnight at 4°C. Beads were washed six times with RIPA buffer [500 mM LiCl, 1 mM EDTA, 1%NP-40, 0.7% Na-deoxycholate, 1× PI in 50 mM HEPES-KOH (pH 8)], followed by three washes in 10 mM Tris-HCl (pH 8) containing 1 mM EDTA and 50 mM NaCl. Chromatin was released from the beads in elution buffer [10 mM EDTA, 1% SDS in 50 mM Tris-HCl (pH 8)] at 65°C for 30 min. The eluted chromatin was reverse cross-linked by incubating at 65°C overnight before being incubated with RNaseA (0.2 mg/ml, Thermo Fisher Scientific, 12091021) and Proteinase K (0.2 mg/ml, Sigma-Aldrich, P4032). DNA was purified using phenol-chloroform and assayed with qPCR using primers for different genomic regions flanking PRDM1 motifs (Table S3). The genomic region -2 kb from the TSS of each gene was extracted from GalGal6 and screened for PRDM1 motifs using RSAT (rsat.sb-roscoff.fr). The PRDM1 matrix was obtained from JASPER (jaspar.genereg.net).

Western blot and Co-IP

For western blot, 50 HH4-6 neural plate border/sensory progenitor tissues were lysed in SDS-PAGE loading buffer by heating to 100°C for 10 min. The lysate was separated using 10% SDS-PAGE and proteins were transferred to immunoblot PVDF membrane. Blots were blocked with 5% milk powder in PBS for 1 h at room temperature, followed by incubation with primary antibodies to PRDM1 (Abcam, ab13700, 1:200) and Kdm4a (Abcam, ab24545, 1:400) at 4°C overnight. After washing in PBS, 0.2% Triton X-100, blots were incubated with HRP-conjugated secondary antibodies (donkey anti-goat-HRP: Abcam, ab6885, 1:1000; goat anti-rabbit-HRP: Jackson ImmunoResearch, 111-035-003, 1:1000) for 1 h at room temperature, washed again, developed using clarity western ECL (Bio-Rad, 170-5-60) and imaged with Bio-Rad ChemiDoc touch imaging system.

For Co-IP, tissues were lysed in Co-IP buffer [100 mM NaCl, 0.2% Triton X-100, 0.5% NP-40, 2 mM β -mercaptoethanol, 1 mM DTT, PI in 20 mM Tris-HCl (pH 7.5)], incubated with PRDM1 or Kdm4a antibody (5 mg)

overnight at 4°C. Bound proteins were precipitated using Protein-A coated Dynabeads (100 μ l, Thermo Fisher Scientific, 10001D). After three washes with Co-IP buffer, beads were suspended in SDS-PAGE loading buffer and proteins were processed for western blot.

ATAC qPCR

To assess the accessibility of the chromatin upstream of the *Sox2* and *Eya2* TSSs we performed ATAC qPCR from sensory progenitors, neural plate and pre-streak epiblast. Each experiment used 15 sensory progenitor and neural plate explants (HH6) and two central pre-streak epiblast pieces (~10,000 cells each). Tissues were dissociated and nuclei were isolated in cold lysis buffer [10 mM NaCl, 3 mM MgCl₂, 0.1% IPGEPAL CA-630 in 10 mM Tris-HCl (pH 7.4)]. Nuclei were washed with lysis buffer, recovered by centrifugation at 500 *g* and treated with transposase (Tn5 Transposase, Illumina, FC-121-1030) for 10 min as previously described (Buenrostro et al., 2013). DNA was purified using a mini-elute PCR purification kit (Qiagen) and qPCR was performed using primers for the region upstream of the *Sox2* and *Eya2* TSS.

DNA methylation assay

To examine the methylation status, CpG islands were predicted within 2 kb upstream of the *Sox2* and *Eya2* TSS using MethPrimer (Li and Dahiya, 2002). Central pre-streak epiblast, early neural plate (HH4⁻) and sensory progenitors (HH6) were dissected and genomic DNA was prepared using the DNeasy blood & tissue kit from Qiagen (69504). Genomic DNA was treated with bisulfide to convert unmethylated cytosine to uracil using the EpiJET Bisulfide Conversion Kit (Thermo Fisher Scientific, K1461) and used for PCR using two pairs of primers flanking the PRDM1 motif upstream of the *Sox2* and *Eya2* TSS and amplifying either methylated or unmethylated DNA. Primers were designed by MathPrimer (Li and Dahiya, 2002). PCR was carried out using Phusion U Hot Start DNA Polymerase (Thermo Fisher Scientific, F-555S/L) and analysed by gel electrophoresis.

Acknowledgements

The authors are grateful to Ewa Kolano for excellent technical assistance, to Claudio Stern and Karen Liu for critical reading of the manuscript, and the Streit group and Jeremy Green for many discussions.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: R.S.P., A.S.; Methodology: R.S.P.; Formal analysis: R.S.P., M.H.; Investigation: R.S.P., M.H.; Writing - original draft: A.S.; Writing - review & editing: A.S.; Supervision: A.S.; Project administration: A.S.; Funding acquisition: A.S.

Funding

This work was funded by project grants to A.S. from the National Institutes of Health (DE022065; DC011577) and the Biotechnology and Biological Sciences Research Council (BB/I021647/1). Deposited in PMC for release after 12 months.

Supplementary information

Supplementary information available online at http://dev.biologists.org/lookup/doi/10.1242/dev.181107.supplemental

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Supplementary Figures





Figure S1. *PRDM1* **expression. A.** In situ hybridisation showing that at HH10 *PRDM1* expression is absent in sensory placodes, except for epibranchial precursors. **B.** PRDM1, Eya2, Sox2 and Sox3 expression in pre-streak epiblast (cEpi), HH6-7 neural plate (NP), HH6-7 sensory progenitors (SP) and HH6-7 non-neural ectoderm (NNE) based on transcriptome data from Trevers et al., 2018. Grey line indicates FPKM value of 10.



Figure S2. PRDM1 knockdown.

A. Two antisense oligonucleotides were designed to knockdown PRDM1. aON1 targets the boundary of exon 6 and intron 6. RT PCR of control (1) and aON1 targeted tissue shows both wildtype and exon 6 deletion (*). aON2 targets exon 7- intron 6 boundary leading to exon 7 deletion. RT PCR shows both wildtype and exon 7 deleted products.

B. Bar diagram showing NanoString nCounter data for selected genes. PRDM1 was knocked down using a combination of aON1 and aON2 at early primitive streak stages; electroporated sensory progenitors were harvested at headfold stages and analysed by NanoString. Pluripotency genes are upregulated, while neural plate border, sensory progenitor and some neural crest genes are downregulated as compared to controls. **C.-F.** PRDM1 knockdown leads to loss of neural plate border markers *Gata3* and *Dlx6*. Control ONs (C, c, E, e) or PRDM1 aON1/2 (D, d, F, f) were electroporated at early primitive streak stages (brown). Embryos were fixed at headfold or early somite stages and gene expression was assessed by in situ hybridisation. While the expression of *Gata3* (n=5) and *Dlx6* (n=3) is normal in controls, expression is lost in PRDM1 knockdowns (D, d: n=6; F, f: n=9; arrowheads; insets in d and f show higher magnification of the sections in the region where gene expression is reduced). Panels on the left show embryos prior to visualisation of ON targeted cells by fluorescein immunolabelling (brown); c-f show sections through the same embryos shown in C-F at the level of the black line. Scale bar in c: 50μm; all sections are the same magnification.



Figure S3. Misexpression of PRDM1 at late primitive streak stages lead to repression of neural and sensory progenitor markers.

GFP controls (A, a, C, c) or PRDM1-IRES-RFP (B, b, D, d) were electroporated into primitive streak stage embryos. At headfold stages embryos were assess for *Sox2* and *Eya2* expression by in situ hybridisation. Gene expression is normal in controls, however, PRDM1 misexpression results in downregulation of *Sox2* and *Eya2* (arrowheads). a-d show sections through the same embryos as shown in A-D. Scale bar in a: 50µm; all sections are the same magnification.

Supplementary Tables

Table S1 NanoString n-Counter data comparing sensory progenitors electroporated with

 control and PRDM1 antisense oligonucleotides and target sequences for each gene on

 the probe set.

Click here to Download Table S1

Table S2 PRDM1 binding site analysis.Click here to Download Table S2

Table S3 PCR primers.

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