

Histone demethylase Jmjd3 is required for the development of subsets of retinal bipolar cells

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Di- and trimethylation of lysine 27 on histone H3 (H3K27me2/3) is an important gene repression mechanism. H3K27me2/3-specific demethylase, Jmjd3, was expressed in the inner nuclear layer during late retinal development. In contrast, H3K27 methyltransferase, Ezh2, was highly expressed in the embryonic retina but its expression decreased rapidly after birth. Jmjd3 loss of function in the developing retina resulted in failed differentiation of PKC-positive bipolar cell subsets (rod-ON-BP) and reduced transcription factor *Bhlhb4* expression, which is critical for the differentiation of rod-ON-BP cells. Overexpression of *Bhlhb4*, but not of other BP cell-related genes, such as transcription factors *Neurod* and *Chx10*, in Jmjd3-knockdown retina rescued loss of PKC-positive BP cells. Populations of other retinal cell subsets were not significantly affected. In addition, proliferation activity and apoptotic cell number during retinal development were not affected by the loss of Jmjd3. Levels of histone H3 trimethyl Lys27 (H3K27me3) in the *Bhlhb4* locus were lower in Islet-1-positive BP cells and amacrine cells than in the Islet-1-negative cell fraction. The Islet-1-negative cell fraction consisted mainly of photoreceptors, suggestive of lineage-specific demethylation of H3K27me3 in the *Bhlhb4* locus. We propose that lineage-specific H3K27me3 demethylation of critical gene loci by spatiotemporal-specific Jmjd3 expression is required for appropriate maturation of retinal cells.

histone methylation | progenitor | interneuron

Methylation of basic amino acid residues in histone is an important epigenetic mechanism to regulate gene expression. Recent studies have shown that histone lysine methylation regulates gene expression by influencing the accessibility of promoter or enhancer regions to transcriptional molecules (1). Di- and trimethylation of lysine 27 on histone H3 (H3K27me2/3) by the histone methyltransferase enhancer of the zeste homolog 2 (Ezh2/Kmt6) with polycomb repressive complex 2 (PRC2) is known as a mechanism of gene repression (2–4). Histone H3 trimethyl Lys27 (H3K27me3) represses gene expression by recruiting PRC1, which recognizes H3K27me3 and ubiquitinates lysine 119 on histone H2A (5). The role of H3K27me3 markers is well established in developmental processes (6). Dynamic switches in polycomb targets that restrict pluripotency and define the developmental potential of progenitor cells have been demonstrated by differentiation of ES cells into Pax6-positive radial-glia neuronal progenitor cells (7). The role of H3K27 in defining the timing of neurogenesis and gliogenesis was shown in cortical neurons by ablating Ezh2 at different developmental stages (8–10). Jmjd3 (Kdm6b) and Utx (Kdm6a) are H3K27 demethylases (11, 12). Jmjd3 is required to regulate the maintenance of the respiratory rhythm generator during late development and in the function of neuronal networks (13).

The vertebrate neural retina is organized into a laminar structure composed of six types of neurons and glial cells. In the mouse, these major retinal cell classes are generated from retinal progenitor cells between embryonic day (E) 11 and postnatal day (P) 10 in a conserved temporal order (14). The importance of histone

methylation during retinal development has been discussed in previous studies (details are provided in *SI Note 1*). Recently, it was reported that loss of function of *ezh2* in *Xenopus* causes defective proliferation of retinal progenitor cells and repression of proneural gene expression (15). *Xenopus ezh2* was expressed in the ganglia cell layer (GCL) and inner nuclear layer (INL) in the developing retina, and low-level expression of *jmjd3* in these layers has been reported (16). However, the functional roles of the H3K27 markers in mammalian retinal development have not been reported. In the present study, we examined the roles of H3K27 markers in retinal cell differentiation by ablating Jmjd3 during retinal development. We show that timed *Jmjd3* expression, which results in the demethylation of key genes and retinal maturation factors, plays a critical role in the differentiation of retinal subsets.

Results

Jmjd3 Expression in the INL of Developing Retina. We first examined the expression patterns of enzymes related to H3K27 modification. Only one enzyme (Ezh2) is known to act as a methyltransferase, whereas three enzymes (Jmjd3, Utx, and Uty) are demethylases at the H3K27 site (17). We examined the expression patterns of Jmjd3 and Utx in the developing mouse retina using in situ hybridization (Fig. 1*A* and *B* and Fig. *S1 A* and *B*). *Jmjd3* was expressed as early as E15 in the retina, and a weak signal was observed in the inner half of

Significance

The role of repressive gene regulation through histone modifications is known in various biological processes. Histone H3 trimethyl Lys27 (H3K27me3) represses gene expression, and our goal was to understand how this methylation regulates cell differentiation in the vertebrate retina. In this work, we focused on the role of demethylase (Jmjd3) of H3K27me3 in retinal development. Spatiotemporal expression patterns of Jmjd3 during retinal development were shown. Suppression of Jmjd3 expression during retinal development resulted in the failure of differentiation of retinal cell subsets. Lowered expression of genes essential for differentiation of the subsets by loss of expression of Jmjd3 was observed. Therefore, we propose that lineage-specific H3K27me3 demethylation of critical gene loci by spatiotemporal-specific Jmjd3 expression is required for appropriate maturation of retinal cells.

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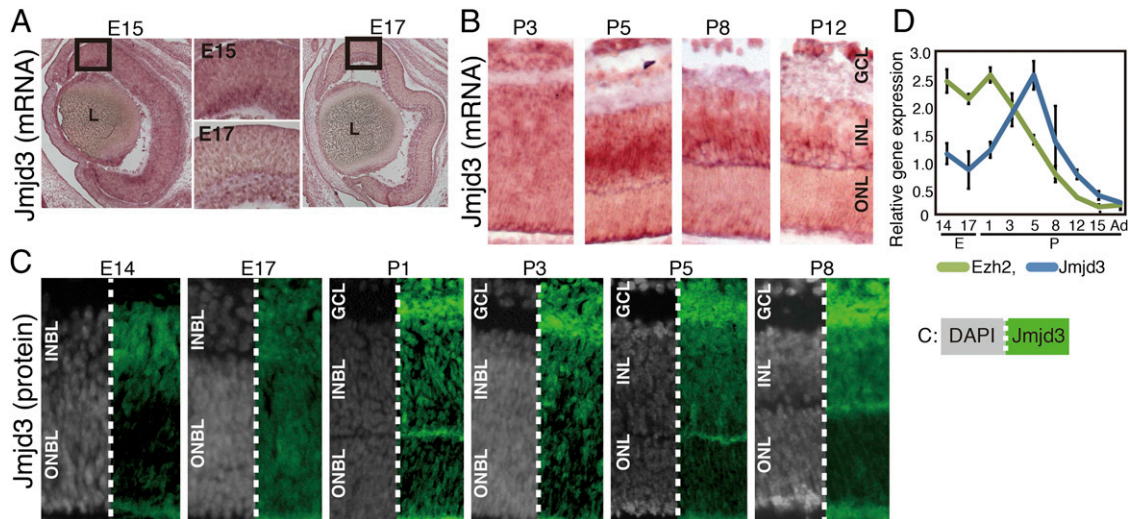


Fig. 1. Expression of *Jmjd3* and *Ezh2* in developing retina. In situ hybridization (A and B) and immunostaining (C) of *Jmjd3* in the developing mouse retina. Mouse retinas at indicated developmental stages were frozen-sectioned, and in situ hybridization and immunostaining were done. Signals in A and B are visualized using the 5-Bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro-blue tetrazolium chloride (NBT) system. In C, *Jmjd3* signals are shown in green and gray signals show nuclear staining by DAPI. (D) Expression of mRNA of *Jmjd3* and *Ezh2* was examined by RT-qPCR using total RNA extracted from the retina at different developmental stages. An average of three independent samples is shown with the SD. Ad, adult; L, lens; ONBL, outer neuroblastic layer.

the retina. At E17 and P3, the *Jmjd3* signal was weak and the region of expression was difficult to identify (Fig. 1 A and B). At P5, a signal was observed in the INL and GCL. At P8 and P12, signals in the INL decreased, although a faint signal was still detected (Fig. 1B). The *Utx* in situ hybridization signal was very weak, and at P8 and P12, faint signals were observed in the INL (Fig. S1A, arrows). Hybridization analysis with sense probes targeting *Jmjd3* gave relatively high background but did not generate specific signals, and probes for *Utx* gave no background or specific signals for the examined stages (Fig. S1B). We then performed immunostaining of *Jmjd3* with retinal markers. At E14 and E17, *Jmjd3* was expressed in GCL and at the inner side of the blastic layer (Fig. 1C). Costaining with the Ki67 proliferation antigen indicated that *Jmjd3* was expressed in postmitotic cells (Fig. S1C). At P1, *Jmjd3* was weakly expressed in GCL, and at P3, signals appeared at the inner neuroblastic layer (INBL) (Fig. 1C). At P5, the Müller glia marker *Ccnd3* (P5) and pan-bipolar (BP) marker *Chx10* (P5 and P8) colocalized with *Jmjd3*, but *PNR* (*Nr2e3*), which marks rod photoreceptors (18), did not in P5 and P8 (Fig. S1C). RT-quantitative PCR (qPCR) showed that *Jmjd3* expression peaked at the P5 retina, and was relatively low before and after this stage (Fig. 1D). *Ezh2* is known to be highly expressed in the retina in the INBL at E16 but is undetectable in adult retina (19). RT-qPCR showed that the level of *Ezh2* decreased after birth, and when retinal development was complete at about 2 wk after birth, *Ezh2* expression was negligible (Fig. 1D). Immunostaining showed that *Ezh2* was expressed in the whole retinal area at E14 and in all layers (GCL, INL, and outer neuroblastic layer) at E17 (Fig. S1D). At P5, *Ezh2* was expressed in the GCL and weakly in some INL cells. At P8, expression of *Ezh2* was observed in the outer half of the INL.

***Jmjd3* Knockdown in the Developing Retina Results in Loss of Rod-ON-BP Cells.** To examine the role of demethylation of H3K27 in retinal development, we performed *Jmjd3* loss-of-function analysis. At E17, the retina was transfected with U6 promoter-driven shRNA for *Jmjd3* (sh-*Jmjd3*) with an EGFP expression plasmid and cultured as explants. We first examined the effects of sh-*Jmjd3* on H3K27me3 in the retina. Immunostaining (Fig. S2A) showed that the patterns of H3K27me3 in the developing retina were comparable to those in previous reports (20). Next, the effects of sh-*Jmjd3* on the H3K27me3 expression pattern were examined. At

day 5, signals with stronger intensity for H3K27me3, especially in the INL, were observed in sh-*Jmjd3* transfected retina compared with sh-control transfected samples (Fig. S2B). At day 10, signal intensity was slightly stronger in the outer nuclear layer (ONL) (Fig. S2C). In the control, H3K27me3 signals were observed in the INL but very weakly in the ONL. However, in sh-*Jmjd3*-expressing retina, similar expression levels in the ONL and INL were observed (Fig. S2D). Quantification of H3K27me3-positive cells showed a higher number of positive cells in sh-*Jmjd3*-expressing samples than in sh-control samples at 5, 10, and 14 d of culture (Fig. S2E). We next examined retinal progenitor cell differentiation in the absence of *Jmjd3* after 14 d of culture. The gross morphology of the three-layer structure of the sh-*Jmjd3*-expressing retina was maintained, and we examined the differentiation patterns of the retinal cell subtypes based on immunostaining. Initially, we focused on the differentiation of cells localized to the INL, because *Jmjd3* was mainly expressed in the INL. *Chx10* is a pan-BP marker, and the number of *Chx10*-positive cells in the sh-*Jmjd3*-expressing retina was slightly lower than in the control (Fig. 2 A and E). PKC is a marker of rod-ON-BP cells, and PKC-positive cells were almost completely absent from retinas transfected with sh-*Jmjd3* (Fig. 2 B and E). *Bhlhb5* is a marker of cone-OFF-BP cells and GABAergic amacrine cells, and we detected an increase in the number of displaced amacrine cells in the GCL (Fig. 2C, green arrows and Fig. 2E). In the INL, amacrine and BP cells were not clearly distinguished, and the total number of *Bhlhb5*-positive cells in the INL was comparable (Fig. 2E). Cone-OFF-BP cells normally express Recoverin, as seen in the control retina at the border of the ONL and INL (Fig. 2D, yellow arrows). However, Recoverin-positive Cone-OFF-BP cells were not detected in sh-*Jmjd3*-transfected retina (Fig. 2E). Recoverin expression in the ONL was observed in rod photoreceptors as expected (Fig. 2D). *Isl1* is a marker of rod and cone-ON-BP and cholinergic amacrine cells (21, 22). The number of *Isl1*-positive ON-BP cells decreased by about 20% in sh-*Jmjd3*-transfected retina (Fig. S3A, yellow arrows and Fig. 2F). Because PKC-positive cells are a subset of *Isl1*-positive cells, the decrease in *Isl1*-positive cells likely corresponded to a decrease in PKC-positive cells. In contrast, the number of *Isl1*-positive amacrine cells in the INL and of displaced amacrine cells was comparable to that of the control (Fig. 2F). *HuC/D* is a marker of amacrine cells, and the number of displaced amacrine cells was increased in the sh-*Jmjd3*-transfected retina (Fig. S3B, yellow arrows), suggesting that

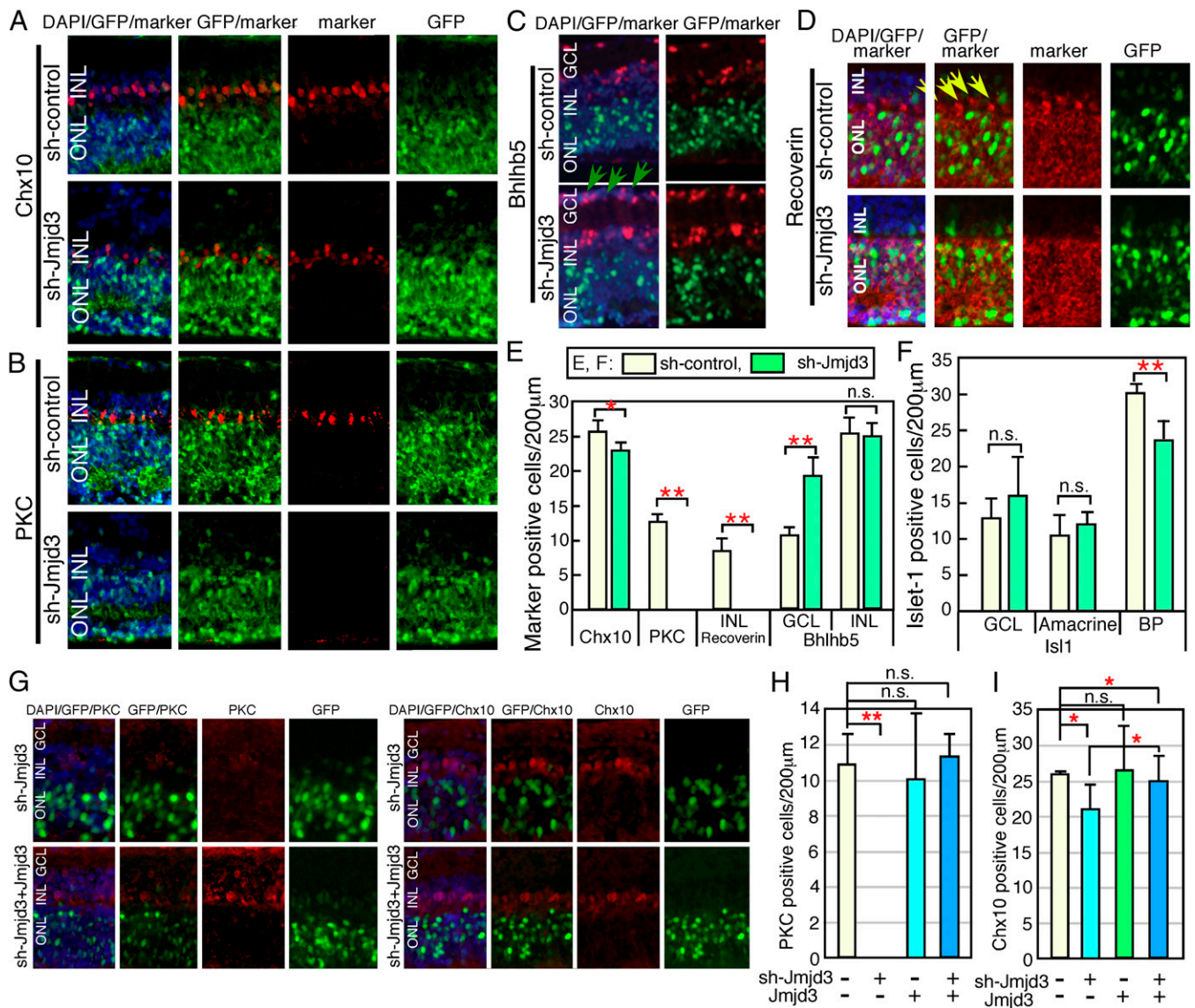


Fig. 2. Knockdown of *Jmjd3* expression during retinal development. Plasmids encoding CAG-EGFP/U6-shRNA-*Jmjd3* (sh-*Jmjd3*)/control U6 (sh-control) (A–D), or CAG-EGFP/sh-*Jmjd3*/*Jmjd3* expression plasmid (G–I) were introduced into the retina (E17) by electroporation. After 2 wk of explant culture, the retina was frozen-sectioned and immunostained using antibodies to antiretinal subset markers as indicated. In E, F, H, and I, the number of marker-positive cells in the electroporated region (200 µm) as judged by EGFP fluorescence was counted in sections. Green arrows in C indicate displaced amacrine cells, and yellow arrows in D indicate cone-OFF-BP cells. More than five sections from three independent samples were counted, and values with SDs are shown. ** $P < 0.01$, * $P < 0.05$, and $P > 0.05$ (n.s.) were calculated by the Student *t* test. n.s., not significant.

Isl1-positive and HuC/D-positive displaced amacrine cells are at least partially different populations. We obtained similar results using a second *Jmjd3* shRNA. The number of other cell types, such as Müller glia [glutamine synthetase (GS)], rod photoreceptors, and retinal ganglion cells (RGCs), were comparable to those of the control samples (Fig. S3 C–E and G). Neurite extension of RGCs was also comparable between control and sh-*Jmjd3*-expressing retina (Fig. S3F). We then examined the proliferation and apoptosis of retina expressing sh-*Jmjd3* by examining BrdU incorporation, as well as the expression of Ki67 and active caspase-3 (Fig. S4). We transfected sh-*Jmjd3* at two different retina developmental stages (E14 and E17), and we examined proliferation and apoptosis at six time points (Fig. S44). The number of active caspase-3-positive cells at E17~10 d showed a slightly but statistically significant difference, but no other samples showed significant differences between control and sh-*Jmjd3*-expressing retina (Fig. S4 B–E).

We then examined cotransfection of *Jmjd3* expression plasmids and sh-*Jmjd3*, and found that expression of *Jmjd3* in the sh-*Jmjd3*-transfected retina recovered expression of PKC (Fig. 2 G and H) and Chx10 (Fig. 2 G and I). Therefore, we identified specific loss of PKC-positive rod-ON-BP cells and Recoverin-positive cone-OFF-BP cells as a consequence of *Jmjd3* down-regulation.

Decreased Expression of *Bhlhb4* and *Vsx1* Due to Suppression of *Jmjd3* Expression. We explored whether expression of BP cell differentiation-related genes was affected by *Jmjd3* down-regulation. A plasmid encoding sh-*Jmjd3* was introduced into the retina at E15 and was cultured for 12 d. The expression levels of various genes were then examined by RT-qPCR (Fig. 3A). As expected, *Jmjd3* expression significantly decreased. *Bhlhb4* is essential for the maturation of rod-ON-BP cells, which were labeled for PKC (23), and *Vsx1* plays a pivotal role in the differentiation of cone-OFF-BP

cells, which were marked by Recoverin (24). We found that the expression levels of *Bhlhb4* and *Vsx1* were strongly suppressed in the presence of sh-Jmjd3 (Fig. 3A). Therefore, the loss of PKC-positive rod-ON-BP cells and Recoverin-positive cone-OFF-BP cells may be explained by suppressing the expression of *Bhlhb4* and *Vsx1*, respectively, by sh-Jmjd3. The expression levels of *Chx10*, *Og9x*, and *Isl1* were lower in the sh-Jmjd3-expressing cells (Fig. 3A), whereas difference of expression of the remaining genes between control and sh-Jmjd3-expressing samples was not statistically significant.

Using qPCR, we examined the transition of *Bhlhb4* and *Vsx1* expression during retinal development using whole retinas at various developmental stages. *Bhlhb4* and *Vsx1* expression was not detectable until P5, and their expression levels increased markedly with retinal development (Fig. 3B).

We next determined the levels of H3K27me3 at the *Bhlhb4*, *Vsx1*, and other BP-related gene loci in BP cells. To obtain retinal cells enriched for BP cells, we used *Isl1*, which is a marker of rod- and cone-ON-BP and cholinergic amacrine cells (21, 22). We stained permeabilized retinal cells at P9 with an anti-*Isl1* antibody, followed by staining with a secondary antibody conjugated with phycoerythrin. The *Isl1*-positive and *Isl1*-negative cells were then purified using a cell sorter (Fig. S5A and B), and ChIP analysis using an anti-H3K27me3 antibody was performed. The H3K27me3 level at the *Bhlhb4* and *Vsx1* loci was lower in *Isl1*-positive cells than in *Isl1*-negative cells (Fig. 3C). Because *Vsx1* is also expressed in type 7 cone-ON-BP cells (24, 25), it is

possible that differentiation of type 7 cone-ON-BP cells was defective in sh-Jmjd3-expressing retina. However, this was difficult to confirm, given the lack of a specific marker for this BP subset. Furthermore, although *Isl1* is strictly expressed in the ON-BP subset in the mature retina (21, 22, 26), *Isl1*-KO mice also have OFF-BP subset defects, suggesting that *Isl1* is transiently expressed in OFF-BP cell precursors (27) and that the precursors of Recoverin-positive cells are found in the *Isl1*-positive fractions. The level of H3K27me3 at the *Cdkn2a* (*Ink4a*) locus was not significantly different between the *Isl1*-positive and *Isl1*-negative populations, and that at the *Elavl3* locus was slightly higher in *Isl1*-positive cells. The H3K27me3 level at the *Mash1* locus was significantly lower in *Isl1*-positive cells (Fig. 3C). All other genes showed higher values in *Isl1*-positive cells than in *Isl1*-negative cells, but the difference was not statistically significant (Fig. S5C). We also examined levels of H3K27me3 at the *Chx10*, *Neurod*, and *GS* loci, but we could not detect reliable qPCR signals because the H3K27me3 levels at these gene loci were too low in both cell fractions.

We then explored whether the pattern of H3K27 levels in the *Bhlhb4* locus is specific for H3K27me3 by examining Histone H3 trimethyl Lys4 (H3K4me3) levels. ChIP analysis of H3K4me3 using *Isl1*-positive/negative retinal cells at P9 was performed. Interestingly, H3K4me3 marker in the *Bhlhb4* locus was much higher in the *Isl1*-positive fraction than in the *Isl1*-negative fraction (Fig. 3D), suggesting that H3K4me3 modification also contributes to specific up-regulation of *Vsx1* and *Bhlhb4* in cells

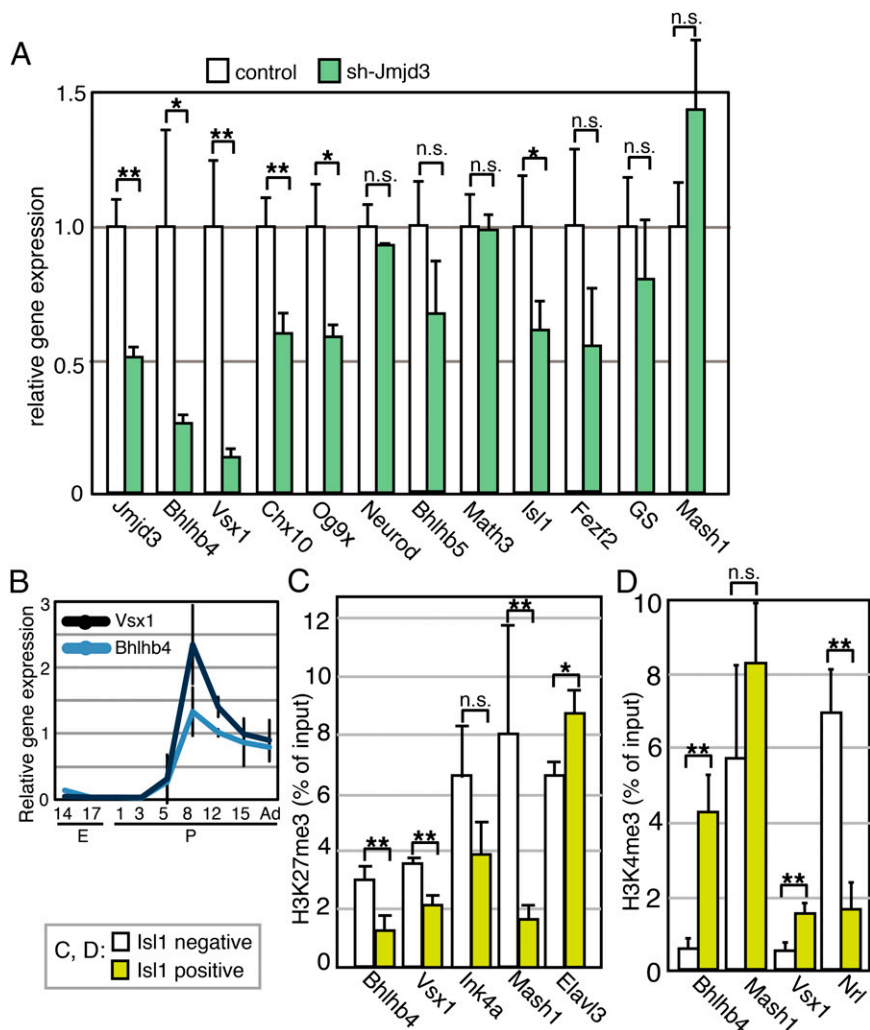


Fig. 3. Molecular signatures in sh-Jmjd3-transfected retina. (A) Plasmids encoding sh-Jmjd3 or sh-control with CAG-EGFP were introduced into the retina at E15; the retina was then cultured for 12 d as retinal explants, and RT-qPCR was performed. All values of qPCR were normalized by the normalization factor value calculated by housekeeping genes, *Gapdh* and *Sdha*. (B) Transition of expression of *Bhlhb4* and *Vsx1* was examined by RT-qPCR during retinal development. H3K27me3 (C) or H3K4me3 (D) modification of gene loci in purified *Isl1*-positive or *Isl1*-negative cells was examined by ChIP analysis. Experiments were done three times independently, and the average and SD are shown. Abcam antibody (ab6002) for H3K27me3 was used in C. ** $P < 0.01$, * $P < 0.05$, and $P > 0.05$ (n.s.) were calculated by the Student *t* test.

in the *Isl1*-positive cell fraction. The H3K4me3 level of the *Vsx1* locus was also higher in the *Isl1*-positive fraction, but that of *Nrl*, which is a photoreceptor-specific gene (28), was higher in *Isl1*-negative cells (Fig. 3*D*).

Bhlhb4 Expression Reverses the Loss of PKC-Positive Cells in sh-Jmjd3-Transfected Cells. We then focused on the PKC-positive BP cells for a more detailed molecular analysis. We investigated whether Bhlhb4 caused the loss of PKC-positive cells in sh-Jmjd3-expressing retina by forcing Bhlhb4 expression in sh-Jmjd3-expressing cells. We first examined the effects of Bhlhb4 expression alone in retinal progenitor cells (Fig. 4*A–D*). Bhlhb4-expressing and EGFP-expressing plasmids were transfected into the retina at E17, and the retina was cultured as an explant for 2 wk. Differentiation of the retina was then examined by immunostaining of frozen sections (Fig. 4*A* and *B*). Forced Bhlhb4 expression did not cause apparent morphological abnormalities of the retina, and PKC immunostaining showed comparable differentiation of rod-ON-BP cells (Fig. 4*A* and *C*). The Bhlhb4-expressing and sh-Jmjd3-expressing plasmids were then cotransfected into E17 retina. Bhlhb4 expression reversed the loss of PKC-positive cells by sh-Jmjd3 (Fig. 4*A* and *C*), suggesting that the loss of PKC-positive cells by sh-Jmjd3 was caused by the loss of Bhlhb4 expression. The slight decrease in the number of *Isl1*-positive BP

cells caused by sh-Jmjd3 was reversed by Bhlhb4 expression (Fig. 4*B* and *D*). We also performed rescue experiments using *Neurod* and *Chx10*, but we did not observe PKC-positive cells by expressing *Neurod* or *Chx10* in the presence of sh-Jmjd3 (Fig. 4*E* and *F*).

Discussion

In the present study, we examined the role of H3K27me3 modification in retinal development and analyzed the effects of loss of function of a demethylation enzyme. We observed a critical role of Jmjd3 for the maturation of PKC-positive BP cell subset development. Because H3K27me3 suppresses gene expression (29, 30), our results were indicative of the importance of timed demethylation to activate genes responsible for proper differentiation of BP cell subsets. Down-regulation of *Bhlhb4* and *Vsx1* expression, both of which are critical for BP subset differentiation, occurred in the absence of Jmjd3. Thus, we propose a model in which H3K27 methylation at loci critical for BP cell maturation occurs in retinal progenitor cells, which is retained during differentiation. Timed demethylation of genes in a specific subset of cells (i.e., BP precursor cells) releases the genes from the transcriptionally silent state (Fig. S6). This is strongly supported by our findings of a lower level of H3K27me3 at critical loci in the BP-enriched cell fraction and of Jmjd3 expression in the

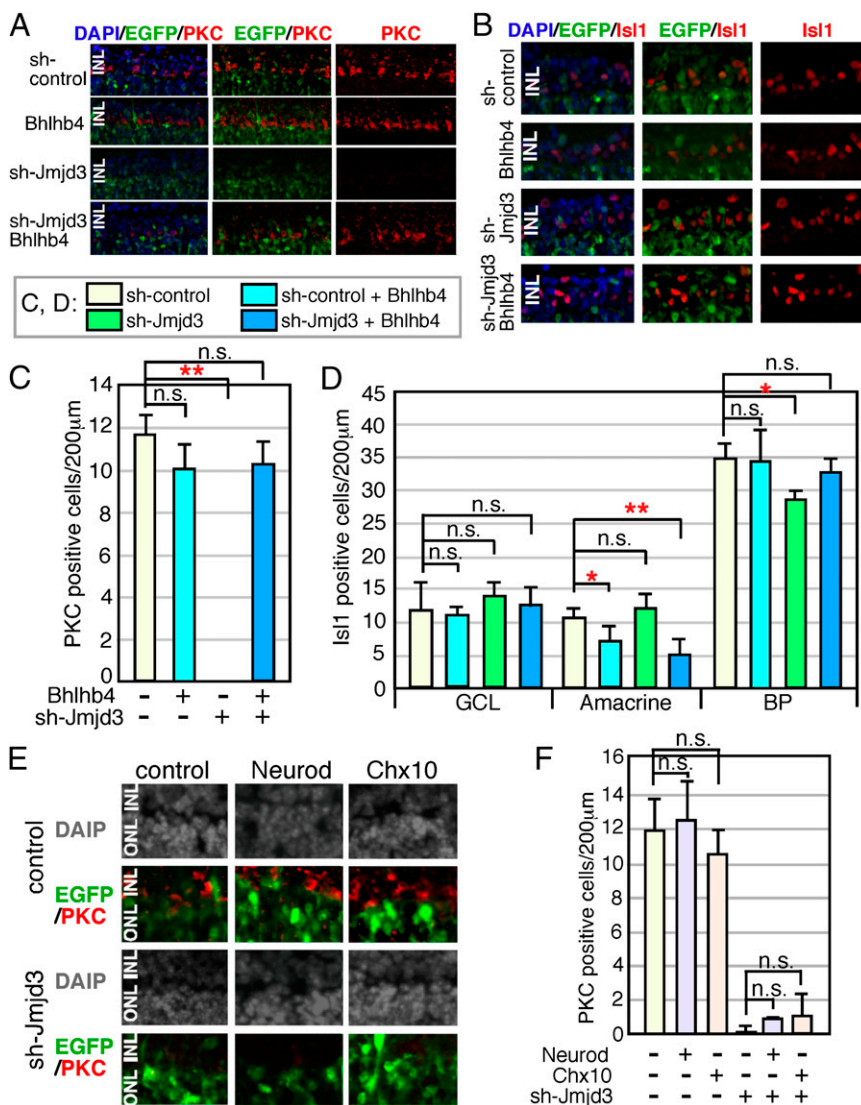


Fig. 4. Supplemental expression of Bhlhb4 into sh-Jmjd3-transfected retina. Mouse retina at E17 was isolated, and plasmids encoding CAG-EGFP and sh-Jmjd3 or sh-control with or without CAG-Bhlhb4 (*A–D*) and CAG-*Neurod* or CAG-*Chx10* (*E* and *F*) were introduced into the retina by electroporation. The total amount of transfected plasmids was adjusted as the same for all experiments by the addition of empty vector. (*A*, *B*, and *E*) After 2 wk of explant culture, the retina was frozen-sectioned and immunostained using antibodies to antiretinal subset markers as indicated. (*C*, *D*, and *F*) Number of marker-positive cells in the electroporated region (200 µm) was counted. More than five sections from three independent samples were counted, and values with SDs are shown. ** $P < 0.01$, * $P < 0.05$, and $P > 0.05$ (n.s.) were calculated by the Student *t* test.

INL of the postnatal retina. The *Mash1* locus also showed a lower level of H3K27me3 in the BP-enriched cell fraction. *Mash1* plays critical roles for BP cell differentiation (31, 32), therefore suggesting that this phenomenon is biologically relevant to achieve cell lineage-specific expression of *Mash1*. However, the mRNA level of *Mash1* in sh-Jmjd3-expressing retina was comparable to that of the control, suggesting some additional mechanisms for regulation of *Mash1* transcription.

In contrast to the expression pattern of *Jmjd3*, H3K27 methyltransferase, *Ezh2* was highly expressed in the embryonic retina. These results suggest that both methylation and demethylation are based on the spatiotemporal dynamics of related enzyme expression patterns. Regulation of the expression pattern dynamics of critical genes for neural fate determination by polycomb and timed H3K27 demethylation in the CNS were suggested previously (7, 10). Furthermore, critical roles for *Jmjd3* during early neural development in ES cell neurogenesis and chick spinal cord development have been demonstrated (33, 34). Our results suggest that during retinal differentiation, *Jmjd3* directs differentiation of subsets of neurons to regulate neuron/glia cell fate. During the late phase of neural development, *Jmjd3* is required in the embryonic respiratory neuronal network (13). In the immune system, *Jmjd3* is induced by bacterial products and inflammatory cytokines, and it is essential for M2 macrophage polarization in response to helminth infection and chitin, which is mediated by *Irf4* (35, 36). In addition, *Jmjd3* is essential for proper bone marrow macrophage differentiation (36), which suggests that the important role of timed *Jmjd3* expression for the maturation of specific cell subsets applies to the hematopoietic system.

We found an increased number of displaced amacrine cells (HuC/D- or *Bhlhb5*-positive cells) in sh-*Jmjd3*-treated retina. These cells are the result of failed maturation of PKC-positive

BP precursor cells, although the molecular basis for this phenomenon remains unknown. The presence of other *Jmjd3* targets in the retina is currently being explored. ChIP sequencing using a purified subfraction of retinal cells could be used to identify *Jmjd3* targets, although refinement of the ChIP technique is required to generate reliable results from such a low number of cells. In this study, we used retinal explants in combination with sh-RNA-mediated gene suppression. This system provides an excellent model system for molecular analysis but is limited, because we cannot analyze the effects of sh-RNA on early-born retinal subsets. Because *Jmjd3* was strongly expressed in the GCL, it may play a role in the development of RGCs and amacrine cells. This could be addressed by analyzing conditional KO of *Jmjd3* in the future.

Materials and Methods

All animal experiments were approved by the Animal Care Committee of the Institute of Medical Science, University of Tokyo, and conducted in accordance with the Association for Research in Vision and Ophthalmology statement for the use of animals in ophthalmic and vision research. The U6 promoter driving the shRNA vector was used for targeting *Jmjd3*, and the pCAG vector was used for overexpression. Plasmids were introduced into retinal explants by electroporation. Frozen sections in optimal cutting temperature compound were used for immunostaining. ChIP-qPCR and RT-qPCR results were normalized in accord with minimum information for publication of quantitative real-time PCR experiment guidelines. Further details are described in *SI Materials and Methods*.

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