convert p-coumarate to caffeate (24). However, these alternative routes to lignin biosynthesis do not fully compensate for a loss of CSE activity, because cse mutants are compromised in lignification and development. Likewise, the accumulation of caffeoyl shikimate that occurs in cse mutants suggests that HCT is relatively ineffective at metabolizing this substrate in vivo.

Lignin limits the processing of plant biomass to fermentable sugars (25, 26). Processing of cse mutant plants, which have reduced lignin content, might yield more sugars on saccharification. We compared cellulose-to-glucose conversion of senesced stems from both cse mutants and wild-type plants. Cell wall residues of senesced inflorescence stems of wild-type plants. Cell wall residues of senesced mutant plants, which have reduced lignin content, were treated with cellulase and converted to fermentable sugars (oligosaccharides) in vivo.

Fig. 4. Cellulose-to-glucose conversion during saccharification of the senesced inflorescence stems of cse mutants. h, hours. Error bars indicate SEM. *0.05 > P > 0.01, **0.01 > P > 0.001, ***0.001 > P > 0.001, unpaired two-sided t test.

The characterization of CSE in other species will reveal how widely the revision of the lignin biosynthetic pathway we propose here applies and whether CSE could be a generally useful target for reducing cell wall recalcitrance to digestion or industrial processing in biomass crops.

References and Notes


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Supplementary Materials
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Epigenetic Regulation of Mouse Sex Determination by the Histone Demethylase Jmjd1a

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Developmental gene expression is defined through cross-talk between the function of transcription factors and epigenetic status, including histone modification. Although several transcription factors play crucial roles in mammalian sex determination, how epigenetic regulation contributes to this process remains unknown. We observed male-to-female sex reversal in mice lacking the H3K9 demethylase Jmjd1a and found that Jmjd1a regulates expression of the mammalian Y chromosome sex-determining gene Sry. Jmjd1a directly and positively controls Sry expression by regulating H3K9me2 marks. These studies reveal a pivotal role of histone demethylation in mammalian sex determination.

The development of two sexes is essential for the survival and evolution of most animal species. Although several transcription factors, including the factor encoded by the Y chromosome gene Sry (1, 2), have been shown to play crucial roles in mammalian sex differen-
When analyzing Jmjd1a-deficient (Jmjd1aΔΔ) mice, which had been established from C57BL/6 (B6) x CBA F1 embryonic stem cells (11), we found that XY animals were frequently sex-reversed (table S1), either partially (12 of 58 animals) with a testis and an ovary (Fig. 1A) or completely (34 of 58 animals) with two ovaries (fig. S1). In contrast, all XY Jmjd1aΔΔ' and XY Jmjd1aΔΔ" mice had two testes (Fig. 1B and table S1). Notably, some of the completely sex-reversed animals were fertile (tables S1 and S2). The generation and comparison of XY Jmjd1a-deficient mice, carrying the Y chromosome from either CBA (YCBA) or B6 (YB6) on a B6 autosomal background (fig. S2), revealed that the sex-reversal phenotype was dependent on not only the loss of Jmjd1a but also the genetic origin of the Y chromosome combined with the B6 background. In total, 88% of XY CBA but only 14% of XY B6 Jmjd1a-deficient mice displayed abnormal sex differentiation (Fig. 1B). Spermiogenesis defects were observed in XY CBA as well as XY B6 Jmjd1a-deficient testes (fig. S1), as demonstrated previously (9, 12). XX Jmjd1a-deficient mice underwent normal sex differentiation and were fertile (table S1 and fig. S1).

To investigate the etiology of sex reversal, we examined expression of the testicular Sertoli cell marker Sox9 (13) and the ovarian somatic cell marker Foxl2 (14) in fetal gonads after sex determination at embryonic day 13.5 (E13.5) (Fig. 1C). XY Jmjd1a-deficient gonads contained both Sox9- and Foxl2-positive cells (Fig. 1D), indicative of ovotestes and therefore partial primary sex reversal, resulting from early failure of the testis-determining pathway. The number of Sox9-positive cells in XY B6 Jmjd1a-deficient gonads was higher than that in XY CBA. This phenotypic difference was sustained even after the ninth generation of backcrossing to B6 (fig. S3).

To address the molecular basis of this phenotype, we determined the expression levels of Sry and its downstream target gene, Sox9. A quantitative real-time fluorescence polymerase chain reaction (RT-qPCR) analysis revealed that the Sry expression levels were reduced to approximately 30% in XY Jmjd1a-deficient gonads at E11.5 [corresponding to 17 to 19 tail somite (ts) stages (Fig. 2A)]. Expression of Sox9 was significantly lower in XY CBA, as compared to XY B6, in control and mutant gonads. It is conceivable that the Sry expression levels in Jmjd1a-deficient gonads at E11.5 might be near the threshold level for inducing the male pathway, and therefore the genetic background-dependent difference of Sry expression may critically affect the subsequent sexual development. Sox9 expression was also reduced in XY Jmjd1a-deficient gonads (Fig. 2B).

A communifluorescence analysis demonstrated that the number of Sry- and Sox9-positive cells was reduced to ~25% in XY Jmjd1a-deficient gonads at E11.5 (Fig. 2C to F). The number of Sry-positive cells in XY CBA gonads was slightly, but significantly, lower than that of XY B6 gonads.
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that to Ki67 immunostaining analysis demonstrated nick end labeling (TUNEL) assay and antibody to Ki67 immunostaining analysis demonstrated that Jmjd1a deficiency led to neither an increase in apoptosis nor a decrease in proliferation (fig. S6). In addition, we established a transgenic mouse line (LN#9) in which the gonadal somatic cells (fig. S8), indicating that Jmjd1a contributed to a different mode of Sry regulation. Second, we attempted to rescue the mutant phenotype by experimentally restoring Sry function, by crossing the Hsp-Sry transgenic mouse line (16) into the Jmjd1a-deficient background. Forced expression of Hsp-Sry transgene rescued the defect of testis cord development in XY Jmjd1a-deficient gonads to the similar levels of those of XY control gonads (fig. S10). Furthermore, virtually no Foxl2-positive cells were observed in XY Jmjd1a-deficient gonads expressing the Hsp-Sry transgene (fig. S10), indicating that Sry acts epistatically to Jmjd1a in regulating male sex determination in mice.

We next investigated the expression profile of Jmjd1a protein during gonadal development. Jmjd1a was detected in gonadal somatic and germ cells but not in mesonephric cells at E11.5 (~4 × 10⁴ cells per gonad pair) (fig. S8), indicating that Jmjd1a deficiency did not affect gonadal somatic cell numbers. Thus, the critical role of Jmjd1a during mammalian sex determination is to ensure Sry expression above the threshold level.

To identify the critical step in the male sex-determining pathway that is controlled by Jmjd1a, we used two different approaches. First, we performed a microarray analysis to address whether Jmjd1a deficiency results in perturbed expression of known genes required for Sry expression. The analysis of a total of 41,181 probes revealed 131 genes, including Sry, with reduced (<0.5-fold) expression in XY Jmjd1aΔΔ, as compared to XY Jmjd1aΔ+ (table S3). However, Jmjd1a deficiency did not compromise expression of known Sry regulators (fig. S9), indicating that Jmjd1a contributes to a different mode of Sry regulation. Second, we attempted to rescue the mutant phenotype by experimentally restoring Sry function, by crossing the Hsp-Sry transgenic mouse line (16) into the Jmjd1a-deficient background. Forced expression of Hsp-Sry transgene rescued the defect of testis cord development in XY Jmjd1a-deficient gonads to the similar levels of those of XY control gonads (fig. S10). Furthermore, virtually no Foxl2-positive cells were observed in XY Jmjd1a-deficient gonads expressing the Hsp-Sry transgene (fig. S10), indicating that Sry acts epistatically to Jmjd1a in regulating male sex determination in mice.

To prove the direct link between Jmjd1a function and Sry expression, a chromatin immunoprecipitation (ChIP) analysis was performed, using purified gonadal somatic cells at E11.5. Jmjd1a was bound to regulatory regions within the Sry locus in wild-type cells (Fig. 4, A and B). Jmjd1a deficiency led to a significant increase in H3K9me2 levels within the Sry locus (Fig. 4C), without changing histone H3 occupancy (Fig. 4D). The H3K9me2 levels of the Sry locus were indistinguishable between XYB6 and XYCBA

**Fig. 2. Jmjd1a deficiency perturbs the expression of Sry.** (A and B) RT-qPCR analyses of Sry (A) and Sox9 (B) in XY gonads. Each of the samples included one pair of gonads/ mesonephros. Results were normalized to Gapdh, and the expression levels in XY Jmjd1aΔ+ were defined as 1. Numbers of examined embryos are shown above the bars. (C and D) Coimmunostaining profiles of Sry (C) and Sox9 (E) with the gonadal somatic cell marker, Gata4, in XYCBA gonads. (D and F) The ratios of the cells positive for Sry (D) and Sox9 (F) to the cells positive for Gata4. Scale bar, 50 μm. All data are presented as mean ± SE. *P < 0.05; **P < 0.01; ***P < 0.001 (Student’s t test).
gonads at E11.5 (fig. S14), demonstrating the conserved role of Jmjd1a between these genetic backgrounds. The unchanged levels of H3K9me3 at the Sry locus, with or without Jmjd1a, indicated H3K9me2-specific demethylation by Jmjd1a (Fig. 4E). Jmjd1a deficiency resulted in perturbed H3K4 methylation of the Sry locus (Fig. 4F). In contrast to Sry, the H3K9me2 levels of Sox9 were unchanged by Jmjd1a deficiency (fig. S15), indicating that Jmjd1a does not control Sox9 expression directly. Coordinated H3K9 demethylation/H3K4 methylation was commonly observed in other Jmjd1a target genes (fig. S15), suggesting that Jmjd1a-mediated H3K9 demethylation is required for subsequent H3K4 methylation for transcriptional activation. Since Sry is located on the heterochromatic Y chromosome, Jmjd1a-mediated H3K9 demethylation may induce deheterochromatinization of Sry to allow the access of the H3K4 methyltransferase and transcription factors (fig. S16).

This work shows a crucial role of a histone demethylase in Sry expression. Another chromatin regulator, Cbx2, reportedly plays a role in Sry expression in mice (19). However, in contrast to Jmjd1a, Cbx2 up-regulates the expression of several positive regulators of Sry, such as Dax1, Gata4, Wt1, and Nr5a1 (19), suggesting that they might be involved in different phases of mammalian sex determination but also demonstrates the importance of epigenetic regulation in spatiotemporal gene regulation during embryonic development.

Fig. 3. Jmjd1a is expressed in developing gonads and catalyzes H3K9 demethylation. (A) Coimmunostaining profiles of Gata4 and Jmjd1a on sections of XY<sup>BA</sup> gonads. Enlarged box indicates that Jmjd1a signals were observed in gonadal somatic cells as well as germ cells (asterisk). G, gonad; M, mesonephros. Scale bar, 50 μm. (B) Quantitative analysis of Jmjd1a transcripts in purified gonadal somatic cells. Expression is normalized to Gapdh. Numbers of examined embryos are shown above the bars. (C) Coimmunostaining profiles of Gata4 and H3K9me2 in XY<sup>BA</sup> gonads. G, gonad; M, mesonephros. Scale bar, 50 μm. (D) Quantitative comparison of the immunofluorescence intensities of H3K9me2 signals between gonadal and mesonephric cells. The intensities of H3K9me2 signals in Jmjd1a<sup>−/−</sup> mesonephric cells were defined as 1. MC, mesonephric cells; GC, gonadal cells. All data are presented as mean ± SE. *P < 0.05; **P < 0.01 (Student’s t test).

Fig. 4. Jmjd1a directly regulates H3K9 demethylation in the Sry locus. (A) Diagram of the Sry locus and the location of primer sets for ChIP-qPCR. (B) ChIP analysis with antibody to Jmjd1a, using purified XY<sup>BA</sup> gonadal somatic cells. GSC, gonadal somatic cells; MC, mesonephric cells. (C to F) ChIP analysis for H3K9me2 (C), pan-H3 (D), H3K9me3 (E), and H3K4me2 (F) at the Sry linear promoter region of purified XY<sup>BA</sup> gonadal somatic cells. All data are presented as mean ± SE. *P < 0.05; **P < 0.01 (Student’s t test).