*Journal of Reproduction and Development, Vol. 64, No 6, 2018*

**Opinions and Hypotheses**

# How does the promoter of an oocyte-specific gene function in male germ cells?

**Yuka MIKI[1)], Satoshi TSUKAMOTO[2)] and Naojiro MINAMI[1)]**

[1)] *Laboratory of Reproductive Biology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*
[2)] *Laboratory Animal and Genome Sciences Section, National Institute for Quantum and Radiological Science and Technology, Chiba 263-8555, Japan*

Abstract. Studying gene expression in germ cells is useful for elucidating mechanisms of transcriptional regulation, because different genes are activated in male and female germ cells. The promoter regions of an oocyte-specific gene, *Oog1*, have been characterized. Driving the expression of green fluorescent protein with these different promoter regions provided us with critical information on the regulation of gene expression. The 3.9 kb long promoter functions in both male and female germ cells in transgenic mice. What is the cause of this sexually dimorphic expression? There may be important factors within and perhaps also outside this 3.9 kb promoter region that are required to maintain proper sex-specific gene expression.
Key words: DNA methylation, Epigenetics, Gene expression, Oocyte, Promoter

(**J. Reprod. Dev. 64:** 463–468, 2018)

## Gene expression control

It has recently become clear that epigenetic modifications such as DNA methylation and histone methylation/acetylation are widely involved in the regulation of gene expression. In germ cells, epigenetic regulation of gene expression, in turn, regulates cellular differentiation; therefore, proper regulation of gene expression is essential for proper differentiation during development. A cascade of sex-specific factors regulates downstream genes leading to normal germ cell differentiation. Sexual fate is reversible without important sex-specific genes, which repress genes specific to the opposite sex [1–3]. For example, factor in germ line alpha (*Figla*) activates oocyte-specific genes and represses testis-specific genes to maintain genetic hierarchies in oocytes [4, 5]. The transcription of *Figla* peaks at around the time of birth and falls to a very low level at seven days of age. This transcription peak is accompanied by changes in the DNA methylation status of promoter regions; when transcription is repressed, the level of methylation within the CpG island of promoter regions is very high [6].

How is such sex-specific and/or time-dependent gene expression regulated in germ cells? *Oog1*, an oocyte-specific gene, is thought to function as a transcription factor during meiosis. Its expression also seems to be controlled by DNA methylation within its promoter. Here, we focus on the promoter of the oocyte-specific gene, *Oog1*, and discuss how epigenetic modification regulates this gene.

## *Oog1* expression

*Oog1* transcription begins at embryonic day 15.5 (E15.5), just after meiosis starts and continues during meiotic prophase arrest at diplotene. Its transcription seems to stop at meiotic resumption, because the amount of *Oog1* transcripts gradually decreases after fertilization. OOG1 contains a leucine zipper structure, which is a known DNA binding motif, suggesting that OOG1 is an oocyte-specific transcription factor [7].

## *Oog1* promoter

A 3.9-kb long promoter of *Oog1* was discovered by comparing the upstream sequences of all five copies of *Oog1*. *Oog1* is a multi-copy gene, with two copies on chromosome 4, and three copies on chromosome 12 in the mouse. All five copies contain a TATA-box in the proximal upstream region, which suggests that they are all transcribed [8]. The 20 kb upstream regions from the TATA boxes were compared using genomic sequence information obtained from the National Center for Biotechnology Information (NCBI) database. The 3.9 kb upstream sequence has been shown to share a high degree of conservation among the five copies. The 3.9 kb promoter region introduced into transgenic mice has three newborn ovary homeobox gene (NOBOX) binding elements (NBEs), one SP1 binding site, and eight E-boxes (or sequences similar to E-boxes). However, only two of the E-boxes (–118 bp and –3457 bp from the transcriptional start site) retain perfect homology among

Received: April 23, 2018
Accepted: August 17, 2018
Published online in J-STAGE: September 10, 2018
©2018 by the Society for Reproduction and Development
Correspondence: N Minami (e-mail: oog1nao@kais.kyoto-u.ac.jp)
This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: https://creativecommons.org/licenses/by-nc-nd/4.0/)

the five sequences (Fig. 1A). NOBOX is an oocyte-specific transcription factor [9]. SP1 is a zinc-finger transcription factor whose concentration gradually decreases as an oocyte grows [10]. E-box is a transcription factor binding site and *Figla* is known to activate zona pellucida glycoprotein gene-1, -2, and -3 (*Zp1, Zp2, and Zp3*) via E-boxes [5]. Among these regions of the five copies, some gaps have been observed. In fact, large gaps exist at –2.7 kb and –3.2 kb from the TATA box. These two regions, 2.7-kb and 3.9-kb long, were selected as viable candidates for the *Oog1* promoter (Oog1pro2.7 and Oog1pro3.9) [8].

## Generation of *Oog1* promoter-GFP transgenic mice

In order to verify the function of *Oog1* promoter regions, transgenic mice expressing the green fluorescent protein (*GFP*) reporter gene were generated. Oocyte-specific GFP expression was stronger in Oog1pro3.9 transgenic ovaries than that in Oog1pro2.7 ovaries (Fig. 2). GFP fluorescence was detected in oocytes from secondary to preovulatory follicles in Oog1pro2.7 transgenic ovaries; whereas, fluorescence was detected in oocytes from primordial to preovulatory follicles in Oog1pro3.9 transgenic ovaries. *GFP* transcripts in both transgenic lines were commonly detected from E15.5 to adulthood, showing that both promoters function in a similar way to those of endogenous *Oog1*.

## The 3.9 kb promoter is also functional in male germ cells

In addition, we found that the longer promoter is active in male germ cells as well (Fig. 3). Oog1pro3.9 has a high transcriptional activity in male germ cells and the GFP signal was detected in late pachytene spermatocyte, but not in mid-pachytene spermatocyte (Fig. 3), suggesting that the 3.9 kb *Oog1* promoter becomes active from late pachytene stage of meiosis. This implies that endogenous *Oog1* promoter also functions from the late pachytene stage of oogenesis. Indeed, endogenous *Oog1* in oocytes is activated at E15.5, which occurs near the pachytene stage of meiosis. In Oog1pro2.7 transgenic testis, however, *GFP* transcripts were slightly detected compared to those in Oog1pro3.9 transgenic testis [8]. Investigation of the differential regulation of the *Oog1* promoter in male versus female germ cells will help elucidate the regulation of sex-specific gene expression in germ cells.

## DNA methylation status in *Oog1* promoter

The DNA methylation status of the proximal region of the *Oog1* promoters may affect sex-dependent gene expression. Bisulfite-sequencing of the proximal promoter region performed by Ishida *et al*. [8] indicates that CpG at –597 bp is highly methylated, when expression of its downstream gene, either *Oog1* or GFP, is suppressed (Fig. 1 A, B). In male germ cells, expression of endogenous *Oog1* and *GFP* downstream of Oog1pro2.7 are suppressed, and their CpGs at –597 bp are highly methylated. On the other hand, the methylation of the same cytosine of the endogenous *Oog1* promoter, Oog1pro2.7 and Oog1pro3.9 in oocytes, and Oog1pro3.9 in male germ cells, is largely removed from the endogenous *Oog1* promoter and completely removed from artificial promoters. It seems that demethylated CpG at –597 bp enables the promoters to activate downstream genes. What is the cause of the difference in expression patterns? The methylation of CpGs at –597 bp may be associated with the expression of downstream genes.

## DNA methylation pattern change in germ cells

Germ cells show a sex-specific DNA methylation pattern, and the DNA of sperm and unfertilized eggs is hypermethylated [11]. After fertilization, DNA demethylation proceeds differently in paternal and maternal genomes; in the paternal genome, active DNA demethylation starts at pronuclear stage 2 (PN2) and almost all methylation is lost by the end of the pronuclear stage [12]. Subsequently, DNA methylation is gradually and passively removed from the two-cell stage to the blastocyst stage depending on DNA replication of the paternal and maternal alleles [13]. As part of the active DNA demethylation mechanism of the paternal genome, ten-eleven translocation methylcytosine dioxygenase 3 (TET3) changes 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) in order to induce DNA demethylation [14]. It has been shown that methyltransferase-like 23 (METTL23), which is an arginine methyltransferase that catalyzes the dimethylation of histone H3R17 (H3R17me2), recruits TET3 to chromatin containing the paternal allele; sperm protamines are replaced by maternal histone 3.3 with R17 dimethylated by METTL23 in the paternal pronucleus [15].

Why does demethylation not occur in the maternal genome? *Stella*, a PGC- and oocyte-specific gene, changes its chromatin conformation by binding to H3K9me2 and protects the maternal genome from TET3-mediated demethylation [16]. While most of the inherited DNA methylations are removed by the blastocyst stage, those of imprinted genes are protected from demethylation [13]. DNA methyltransferase 1 (*Dnmt1*) is a maintenance enzyme that methylates hemi-methylated CpG after DNA replication; in *Dnmt1* KO mice, imprinted genes such as *H19*, *Igf2*, and *Xist* are extensively demethylated [17].

Following implantation, the whole genome of the inner cell mass is subject to active *de novo* methylation mediated by DNA methyltransferases 3a and 3b (*Dnmt3a* and *Dnmt3b*); inactivation of *Dnmt3a* and *Dnmt3b* by gene targeting blocks this *de novo* methylation in early post-implantation mouse embryos [18]. After genomic methylation, primordial germ cells (PGCs) emerge in the epiblast at around E6.25 [19]. Germ cells are derived from PGCs, which initially have a DNA methylation status similar to somatic cells. Imprinted genes become demethylated for the first time in PGC at around E11.5 [20]. During this reprogramming of PGCs, TET1 is known to contribute to DNA demethylation; in *Tet1* null germ cells, DNA methylation is still maintained in differential methylated regions (DMRs) within imprinted genes and dysregulation of imprinted genes causes some progeny defects [21]. After DNA demethylation in PGCs, germ cells start to acquire genome-wide methylation, including sex-specific DNA methylation, from around E13.5 [22].

## Sex-specific pattern of DNA methylation

More than 1,600 CpG islands are differentially methylated in oocytes and sperms, in addition to known imprinting control regions (ICRs), which control imprinted genes expressed from either paternal or maternal allele [23]. In contrast to male germ cells, in which the whole genome shows hypermethylation

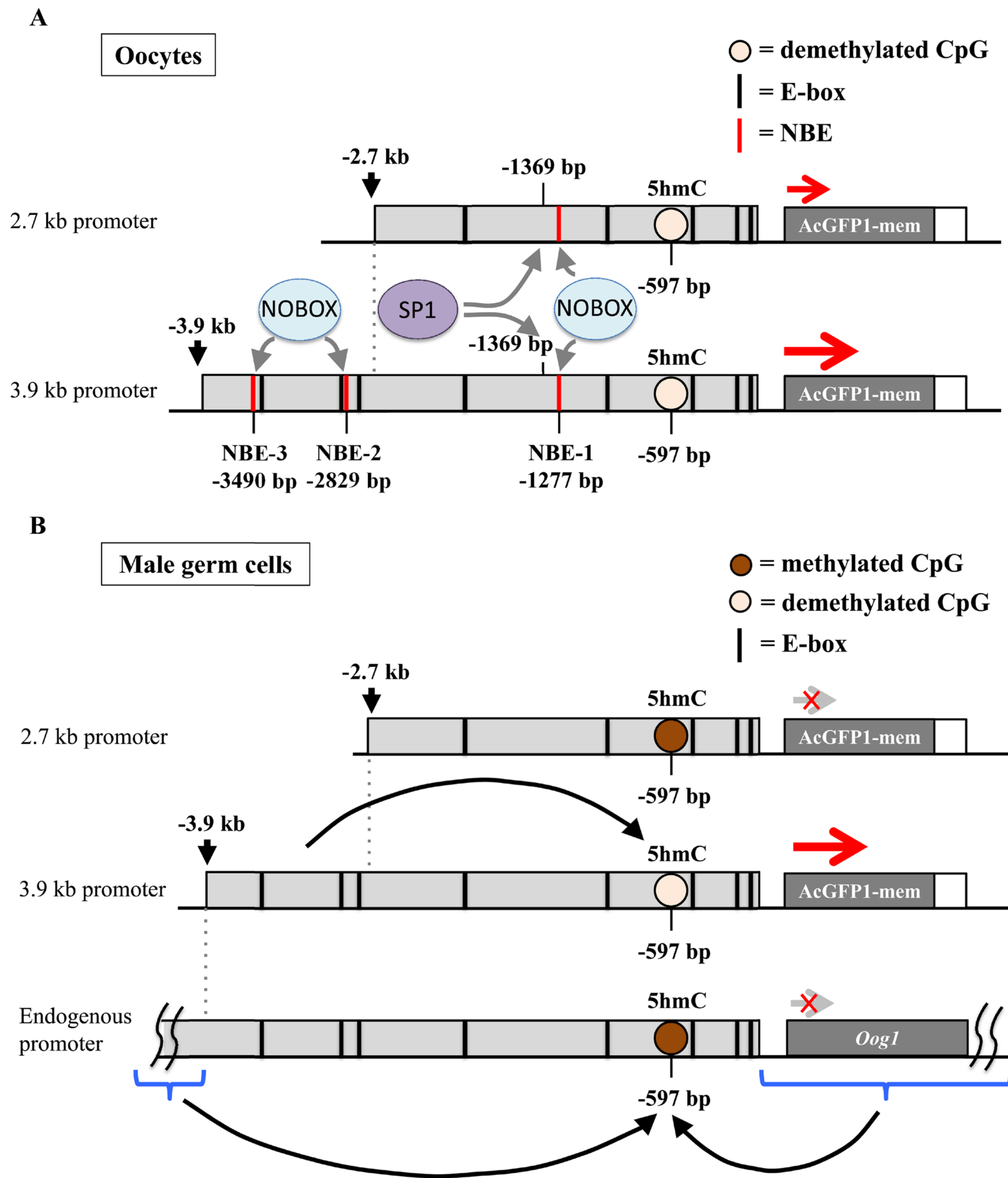


**Fig. 1.** Putative transcription factor binding sites and DNA methylation status at –597 bp related to the transcriptional activity. NBEs (–1277bp, –2829 bp and –3490 bp), SP1 binding element (–1369 bp), and E-boxes (–3457 bp, –2836 bp, –2711 bp, –2041 bp, –991 bp, –455 bp, –118 bp, and –44 bp) are shown in all promoter regions of *Oog1*. (A) In oocytes, the 3.9 kb promoter has higher transcriptional activity. The 3.9 kb promoter has two NBEs and three E-boxes in an additional 1.2 kb region upstream of the 2.7 kb promoter. (B) In male germ cells, only the 3.9 kb promoter has transcriptional activity and the CpG at –597 bp is demethylated specifically in the 3.9 kb promoter. Interestingly, there seems to be a relationship between DNA methylation status at –597 bp and the transcriptional activity. On the other hand, the CpG at –597 bp is methylated in endogenous *Oog1* promoter in male germ cells. Some regions outside the Oog1pro3.9 may contribute to the methylation of this CpG. Bisulfite-sequencing was performed using testes collected from 10-week-old male mice and oocytes collected from four to five-week-old female mice.

by the end of pachytene, except in the CpG-rich regions, DNA methylation in oocytes proceeds gradually as the oocytes grow [24, 25]. *Dnmt3l* (Dnmt3-like) has been proposed to cooperate with *Dnmt3a/Dnmt3b* to regulate oocyte-specific DNA methylation [26]. While DNA methylation in oocytes is gradually established, the *Oog1* promoter regions may remain demethylated and maintain *Oog1* expression during meiotic arrest using various strategies to avoid DNA methylation within the promoter region.

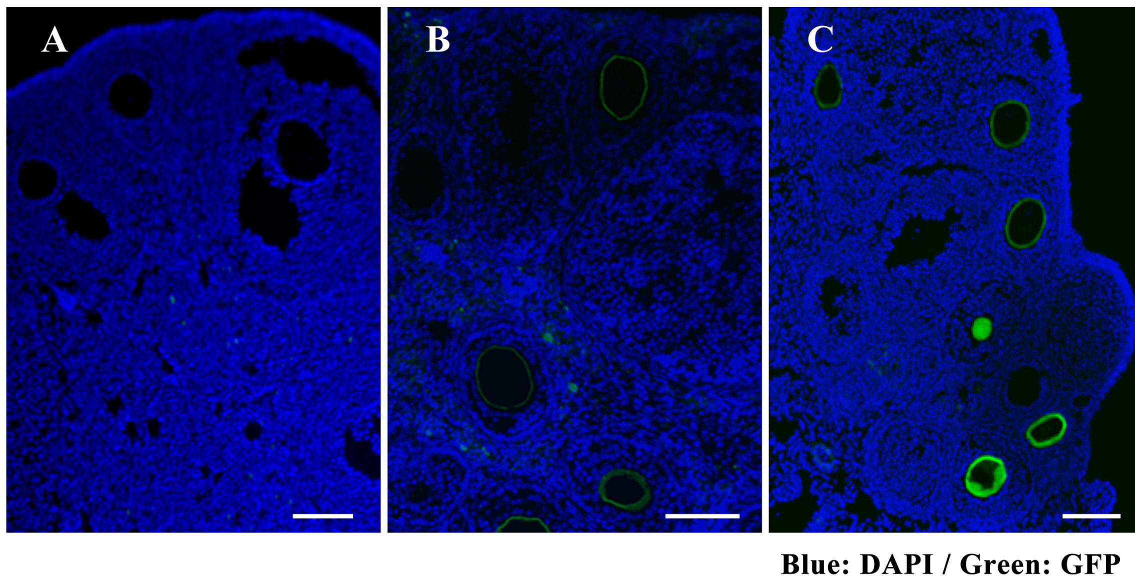


**Fig. 2.** Oog1 promoter activity in oocytes. Frozen sections of ovaries obtained from five-week-old WT mouse (A), Oog1pro2.7 transgenic mouse (B), and Oog1pro3.9 transgenic mouse (C). Strong GFP signal was detected in the oocytes of Oog1pro3.9 transgenic mouse compared to that in the oocytes of Oog1pro2.7 transgenic mouse. Scale bar: 100 µm.

## Protecting the demethylated status of DNA in oocytes

POU domain, class 2, transcription factor 1 (*Pou2f1*) binding site; POU domain, class 5, transcription factor 1 (*Pou5f1*) binding site; and the *Sox-2* binding site in ICR of maternally expressed *H19* gene have been shown to be essential for protecting maternal ICR from *de novo* DNA methylation after fertilization [27]. Although *Oog1* is an oocyte-specific gene and not an imprinted gene, similar mechanisms may maintain the DNA demethylation status of the *Oog1* promoter. NOBOX is an oocyte-specific transcription factor, and is known to activate a variety of oocyte-specific genes to maintain proper folliculogenesis; growth differentiation factor 9 (*Gdf9*) and *Pou5f1* have been shown to contain NBEs within their promoter regions, and the expression of these genes is downregulated in *Nobox* knockout ovaries, in which *Oog1* is also significantly downregulated [28–30]. Oog1pro2.7 also has one NBE and Oog1pro3.9 has two additional NBEs in the 1.2 kb region upstream of the 2.7 kb promoter (Fig. 1). The heavily methylated status of *Oog1* proximal promoter region in male germ cells might be due to the absence of NOBOX in male germ cells. It would be an important discovery if NOBOX proves to be involved in DNA methylation inhibition in promoter regions by binding to NBE. This putative function of NOBOX may also explain why Oog1pro3.9 shows a higher level of activity than Oog1pro2.7. NOBOX binding to the additional NBEs located in the upstream 1.2 kb region probably interfere significantly with DNA methylation in Oog1pro3.9.

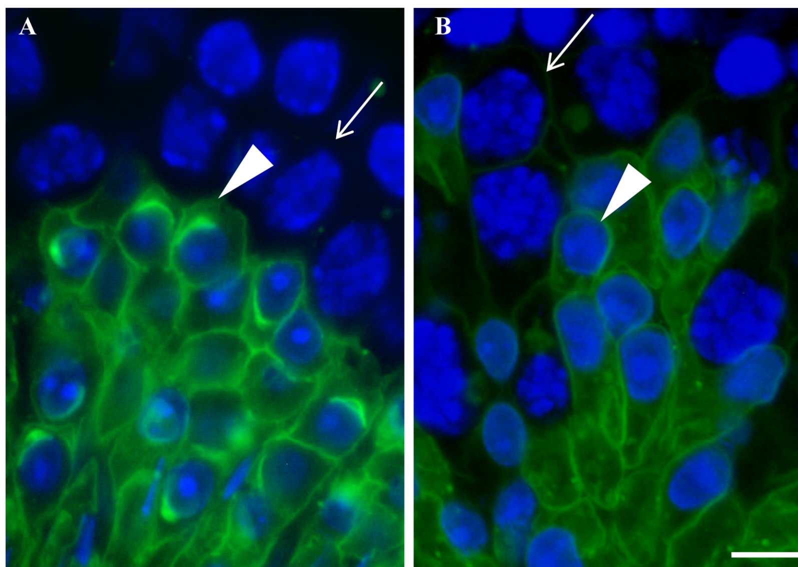


**Fig. 3.** Frozen sections of testes obtained from Oog1pro3.9 transgenic male mice. (A) Seminiferous tubule at stage VI. Arrow: mid-pachytene spermatocyte; Arrow head: step 6 spermatid. (B) Seminiferous tubule at stage IX. Arrow: late pachytene spermatocyte; Arrow head: step 9 spermatid. The 3.9 kb *Oog1* promoter may start to function from late pachytene stage in male germ cells as well as in oocytes.

## DNA methylation during spermatogenesis

The genome-wide DNA methylation during spermatogenesis almost finishes by the end of the pachytene spermatocyte stage and the DNA methylation pattern undergoes little change after this period [24]. In E16.5 prospermatogonia (PSG), genome-wide CpG methylation level is 30.1%, but in postnatal day 0.5 (P0.5) PSG, it rises to 76.1% and this methylation level is maintained until adult spermatozoa [24]. The transcription of *Stra8,* which is required to induce meiosis, peaks at P7.5 in differentiating spermatogonia and accordingly the methylation level of DMR upstream of *Stra8* decreases between P0.5 PSG and P7.5 spermatogonia [31]. *Oog1* expression may also be regulated by the methylation of CpG at –597 bp of the transcription start site (TSS) as DMR upstream of *Stra8* controls *Stra8* expression.

## The difference in functions of Oog1pro3.9 and the endogenous *Oog1* promoter in male germ cells

Why is endogenous *Oog1* not expressed in male germ cells despite the fact that *GFP* downstream of Oog1pro3.9 is expressed? The upstream region of the 3.9 kb endogenous *Oog1* promoter, *Oog1* gene body, and/or downstream of the gene body are expected to be involved. Regulatory elements located outside the gene body and its introns are known to regulate its gene expression in the form of its enhancers [32, 33]. Coactivators often mediate the physical interaction of enhancers with promoters and regulate transcription initiation. Some histone acetyltransferases also function as coactivators [34]. While CpG at –597 bp of the TSS is demethylated in the 3.9 kb promoter fragment, the corresponding CpG is methylated in endogenous *Oog1* promoter in male germ cells. Some regions within or outside the *Oog1* gene body may contribute to the methylation status of the CpG (Fig. 1B).

## A large region in the genome interferes with gene expression

As mentioned above, *Oog1* expression might be controlled by elements outside the 3.9 kb promoter region. Such a regulatory mechanism has been observed for *Igf2* expression. *Igf2*, an imprinted gene, is expressed only from the paternal allele and lies 70 kb upstream of the *H19* gene. *H19* is transcribed into noncoding RNA and is expressed only from the maternal allele. While the *H19* ICR fragment is kept hypomethylated in oocytes, it becomes methylated in male germ cells during spermatogenesis; the level of DNA methylation is maintained throughout fertilization leading to subsequent allele-specific expression [35, 36]. DMR 2 kb upstream of *H19* and enhancers downstream of *H19* regulate male germ cell-specific *Igf2* expression and oocyte-specific *H19* expression. On the maternal allele, this DMR is demethylated and the enhancers interact with the *H19* promoter. The paternal allele, in contrast, is methylated so the enhancers cannot interact with the promoter of *H19,* and instead activate *Igf2* upstream of the DMR [37]. It is, however, still unknown why these mechanisms are allele-specific. Imprinted genes maintain their allele-specific methylation status and therefore allele-specific expression despite the same environment in one cell. The methylation status of the CpG at –597 bp of the TSS has been shown to be associated with the oocyte-specific expression of *Oog1*. The maintenance of DNA methylation status, in general, is important for "specific expression". Sex-specific gene expression in germ cells may be controlled by similar mechanisms involved in imprinted genes establishing a sex-specific DNA methylation status.

## Expected achievement obtained from research on the *Oog1* promoter

Epigenetic modifications interact with each other and it is difficult to reveal such causal relationships. H3K9 and H3K14, when acetylated, enable transcription factors to bind to DNA by loosening the structure of nucleosomes [38]. Unmethylated DNA is assembled in nucleosomes that contain the acetylated histones H3 and H4. When *Pou5f1* expression is suppressed in embryonic stem cells, a complex of histone methyltransferase G9a and histone deacetylases is recruited. This complex also recruits DNMT3A and DNMT3B [39]. Therefore, the epigenetic modifications have interrelationships. We aim to examine histone modification and DNA methylation status within the *Oog1* gene segment and in the wider region around *Oog1* as well. While comparing the epigenetic modification status of endogenous promoters in oocytes and in male germ cells, determining the activities of Oog1pro2.7 and Oog1pro3.9 in transgenic mice will elucidate the effects of epigenetic modifications on the regulation of sex-specific gene expression.

Analysis of *Oog1* upstream sequences, including promoter regions, suggests that epigenetic modification, i.e., DNA methylation in CpG at –597 bp, is involved in the regulation of *Oog1* expression. If we can find common sequences around the five copies of *Oog1*, even if they are far away from the genes, they may coordinate with the *Oog1* promoter. Sex-specific gene expression in germ cells may depend on more complex mechanisms than those in somatic cells. Studies on sex-specific gene expression may elucidate a variety of gene expression mechanisms. If we can determine why *Oog1* is expressed specifically in oocytes and why Oog1pro3.9 promotes the expression of transgenes in male and in female germ cells, a breakthrough in epigenetics may be possible.

## References


1. **Kai T, Spradling A.** Differentiating germ cells can revert into functional stem cells in Drosophila melanogaster ovaries. *Nature* 2004; **428**: 564–569. [Medline] [CrossRef]
2. **Otori M, Karashima T, Yamamoto M.** The Caenorhabditis elegans homologue of deleted in azoospermia is involved in the sperm/oocyte switch. *Mol Biol Cell* 2006; **17**: 3147–3155. [Medline] [CrossRef]
3. **Uhlenhaut NH, Jakob S, Anlag K, Eisenberger T, Sekido R, Kress J, Treier AC, Klugmann C, Klasen C, Holter NI, Riethmacher D, Schütz G, Cooney AJ, Lovell-Badge R, Treier M.** Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. *Cell* 2009; **139**: 1130–1142. [Medline] [CrossRef]
4. **Hu W, Gauthier L, Baibakov B, Jimenez-Movilla M, Dean J.** FIGLA, a basic helix-loop-helix transcription factor, balances sexually dimorphic gene expression in postnatal oocytes. *Mol Cell Biol* 2010; **30**: 3661–3671. [Medline] [CrossRef]
5. **Liang L, Soyal SM, Dean J.** FIGalpha, a germ cell specific transcription factor involved in the coordinate expression of the zona pellucida genes. *Development* 1997; **124**: 4939–4947. [Medline]
6. **Pan B, Chao H, Chen B, Zhang L, Li L, Sun X, Shen W.** DNA methylation of germ-cell-specific basic helix-loop-helix (HLH) transcription factors, Sohlh2 and Figlα during gametogenesis. *Mol Hum Reprod* 2011; **17**: 550–561. [Medline] [CrossRef]
7. **Minami N, Aizawa A, Ihara R, Miyamoto M, Ohashi A, Imai H.** Oogenesin is a novel mouse protein expressed in oocytes and early cleavage-stage embryos. *Biol Reprod* 2003; **69**: 1736–1742. [Medline] [CrossRef]
8. **Ishida M, Okazaki E, Tsukamoto S, Kimura K, Aizawa A, Kito S, Imai H, Minami N.** The promoter of

the oocyte-specific gene, Oog1, functions in both male and female meiotic germ cells in transgenic mice. *PLoS One* 2013; **8**: e68686. [Medline] [CrossRef]

9. **Pangas SA, Rajkovic A.** Transcriptional regulation of early oogenesis: in search of masters. *Hum Reprod Update* 2006; **12**: 65–76. [Medline] [CrossRef]
10. **Worrad DM, Ram PT, Schultz RM.** Regulation of gene expression in the mouse oocyte and early preimplantation embryo: developmental changes in Sp1 and TATA box-binding protein, TBP. *Development* 1994; **120**: 2347–2357. [Medline]
11. **Smallwood SA, Tomizawa S, Krueger F, Ruf N, Carli N, Segonds-Pichon A, Sato S, Hata K, Andrews SR, Kelsey G.** Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. *Nat Genet* 2011; **43**: 811–814. [Medline] [CrossRef]
12. **Santos F, Hendrich B, Reik W, Dean W.** Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev Biol* 2002; **241**: 172–182. [Medline] [CrossRef]
13. **Lee HJ, Hore TA, Reik W.** Reprogramming the methylome: erasing memory and creating diversity. *Cell Stem Cell* 2014; **14**: 710–719. [Medline] [CrossRef]
14. **Gu TP, Guo F, Yang H, Wu HP, Xu GF, Liu W, Xie ZG, Shi L, He X, Jin SG, Iqbal K, Shi YG, Deng Z, Szabó PE, Pfeifer GP, Li J, Xu GL.** The role of Tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. *Nature* 2011; **477**: 606–610. [Medline] [CrossRef]
15. **Hatanaka Y, Tsusaka T, Shimizu N, Morita K, Suzuki T, Machida S, Satoh M, Honda A, Hirose M, Kamimura S, Ogonuki N, Nakamura T, Inoue K, Hosoi Y, Dohmae N, Nakano T, Kurumizaka H, Matsumoto K, Shinkai Y, Ogura A.** Histone H3 methylated at arginine 17 is essential for reprogramming the paternal genome in zygotes. *Cell Reports* 2017; **20**: 2756–2765. [Medline] [CrossRef]
16. **Nakamura T, Liu YJ, Nakashima H, Umehara H, Inoue K, Matoba S, Tachibana M, Ogura A, Shinkai Y, Nakano T.** PGC7 binds histone H3K9me2 to protect against conversion of 5mC to 5hmC in early embryos. *Nature* 2012; **486**: 415–419. [Medline] [CrossRef]
17. **Li E.** Chromatin modification and epigenetic reprogramming in mammalian development. *Nat Rev Genet* 2002; **3**: 662–673. [Medline] [CrossRef]
18. **Okano M, Bell DW, Haber DA, Li E.** DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 1999; **99**: 247–257. [Medline] [CrossRef]
19. **Saitou M, Barton SC, Surani MA.** A molecular programme for the specification of germ cell fate in mice. *Nature* 2002; **418**: 293–300. [Medline] [CrossRef]
20. **Lee J, Inoue K, Ono R, Ogonuki N, Kohda T, Kaneko-Ishino T, Ogura A, Ishino F.** Erasing genomic imprinting memory in mouse clone embryos produced from day 11.5 primordial germ cells. *Development* 2002; **129**: 1807–1817. [Medline]
21. **Yamaguchi S, Shen L, Liu Y, Sendler D, Zhang Y.** Role of Tet1 in erasure of genomic imprinting. *Nature* 2013; **504**: 460–464. [Medline] [CrossRef]
22. **Saitou M, Miyauchi H.** Gametogenesis from Pluripotent Stem Cells. *Cell Stem Cell* 2016; **18**: 721–735. [Medline] [CrossRef]
23. **Kobayashi H, Sakurai T, Imai M, Takahashi N, Fukuda A, Yayoi O, Sato S, Nakabayashi K, Hata K, Sotomaru Y, Suzuki Y, Kono T.** Contribution of intragenic DNA methylation in mouse gametic DNA methylomes to establish oocyte-specific heritable marks. *PLoS Genet* 2012; **8**: e1002440. [Medline] [CrossRef]
24. **Oakes CC, La Salle S, Smiraglia DJ, Robaire B, Trasler JM.** Developmental acquisition of genome-wide DNA methylation occurs prior to meiosis in male germ cells. *Dev Biol* 2007; **307**: 368–379. [Medline] [CrossRef]
25. **Sasaki H, Matsui Y.** Epigenetic events in mammalian germ-cell development: reprogramming and beyond. *Nat Rev Genet* 2008; **9**: 129–140. [Medline] [CrossRef]
26. **Hata K, Okano M, Lei H, Li E.** Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. *Development* 2002; **129**: 1983–1993. [Medline]
27. **Sakaguchi R, Okamura E, Matsuzaki H, Fukamizu A, Tanimoto K.** Sox-Oct motifs contribute to maintenance of the unmethylated H19 ICR in YAC transgenic mice. *Hum Mol Genet* 2013; **22**: 4627–4637. [Medline] [CrossRef]
28. **Rajkovic A, Pangas SA, Ballow D, Suzumori N, Matzuk MM.** NOBOX deficiency disrupts early folliculogenesis and oocyte-specific gene expression. *Science* 2004; **305**: 1157–1159. [Medline] [CrossRef]
29. **Lechowska A, Bilinski S, Choi Y, Shin Y, Kloc M, Rajkovic A.** Premature ovarian failure in nobox-deficient mice is caused by defects in somatic cell invasion and germ cell cyst breakdown. *J Assist Reprod Genet* 2011; **28**: 583–589. [Medline] [CrossRef]
30. **Choi Y, Rajkovic A.** Characterization of NOBOX DNA binding specificity and its regulation of Gdf9 and Pou5f1 promoters. *J Biol Chem* 2006; **281**: 35747–35756. [Medline] [CrossRef]
31. **Kubo N, Toh H, Shirane K, Shirakawa T, Kobayashi H, Sato T, Sone H, Sato Y, Tomizawa S, Tsurusaki Y, Shibata H, Saitsu H, Suzuki Y, Matsumoto N, Suyama M, Kono T, Ohbo K, Sasaki H.** DNA methylation and gene expression dynamics during spermatogonial stem cell differentiation in the early postnatal mouse testis. *BMC Genomics* 2015; **16**: 624. [Medline] [CrossRef]
32. **Lamb KA, Rizzino A.** Effects of differentiation on the transcriptional regulation of the FGF-4 gene: critical roles played by a distal enhancer. *Mol Reprod Dev* 1998; **51**: 218–224. [Medline] [CrossRef]
33. **Yasuda H, Oh CD, Chen D, de Crombrugghe B, Kim JH.** A Novel Regulatory Mechanism of Type II Collagen Expression via a SOX9-dependent Enhancer in Intron 6. *J Biol Chem* 2017; **292**: 528–538. [Medline] [CrossRef]
34. **Wang Z, Zang C, Cui K, Schones DE, Barski A, Peng W, Zhao K.** Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell* 2009; **138**: 1019–1031. [Medline] [CrossRef]
35. **Ferguson-Smith AC, Sasaki H, Cattanach BM, Surani MA.** Parental-origin-specific epigenetic modification of the mouse H19 gene. *Nature* 1993; **362**: 751–755. [Medline] [CrossRef]
36. **Matsuzaki H, Okamura E, Shimotsuma M, Fukamizu A, Tanimoto K.** A randomly integrated transgenic H19 imprinting control region acquires methylation imprinting independently of its establishment in germ cells. *Mol Cell Biol* 2009; **29**: 4595–4603. [Medline] [CrossRef]
37. **Hark AT, Schoenherr CJ, Katz DJ, Ingram RS, Levorse JM, Tilghman SM.** CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. *Nature* 2000; **405**: 486–489. [Medline] [CrossRef]
38. **Karmodiya K, Krebs AR, Oulad-Abdelghani M, Kimura H, Tora L.** H3K9 and H3K14 acetylation co-occur at many gene regulatory elements, while H3K14ac marks a subset of inactive inducible promoters in mouse embryonic stem cells. *BMC Genomics* 2012; **13**: 424. [Medline] [CrossRef]
39. **Feldman N, Gerson A, Fang J, Li E, Zhang Y, Shinkai Y, Cedar H, Bergman Y.** G9a-mediated irreversible epigenetic inactivation of Oct-3/4 during early embryogenesis. *Nat Cell Biol* 2006; **8**: 188–194. [Medline] [CrossRef]