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Differential Oocyte-Specific Expression of Cre Recombinase Activity in *GDF-9-iCre, Zp3cre,* and *Msx2Cre* Transgenic Mice[1]

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ABSTRACT

Oocyte-specific deletion of ovarian genes using Cre/loxP technology provides an excellent tool to understand their physiological roles during folliculogenesis, oogenesis, and preimplantation embryonic development. We have generated a transgenic mouse line expressing improved Cre recombinase (iCre) driven by the mouse *growth differentiation factor-9* (*GDF-9*) promoter. The resulting transgenic mouse line was named *GDF-9-iCre* mice. Using the floxed *ROSA* reporter mice, we found that Cre recombinase was expressed in postnatal ovaries, but not in heart, liver, spleen, kidney, and brain. Within the ovary, the Cre recombinase was exclusively expressed in the oocytes of primordial follicles and follicles at later developmental stages. The expression of iCre of *GDF-9-iCre* mice was shown to be earlier than the Cre expression of *Zp3Cre* and *Msx2Cre* mice, in which the *Cre* gene is driven by *zona pellucida protein 3* (*Zp3*) promoter and a homeobox gene *Msx2* promoter, respectively, in the postnatal ovary. Breeding wild-type males with heterozygous floxed *germ cell nuclear factor (GCNF)* females carrying the *GDF-9-iCre* transgene did not produce any progeny having the floxed *GCNF* allele, indicating that complete deletion of the floxed *GCNF* allele can be achieved in the female germline by *GDF-9-iCre* mice. These results suggest that *GDF-9-iCre* mouse line provides an excellent genetic tool for understanding functions of oocyte-expressing genes involved in folliculogenesis, oogenesis, and early embryonic development. Comparison of the ontogeny of the Cre activities of *GDF-9-iCre, Zp3Cre,* and *Msx2Cre* transgenic mice shows there is sequential Cre activity of the three transgenes that will allow inactivation of a target gene at different points in folliculogenesis.

conditional knockout, Cre, folliculogenesis, gamete biology, gametogenesis, oocyte development, ovary, ovum, oogenesis

INTRODUCTION

To understand the physiological roles of genes in mammals in vivo, embryonic stem cell (ES) technology has been commonly used for generating mice functionally deficient in specific gene products by insertion of neomycin or hprt gene cassettes into the locus of a specific gene to terminate the expression of an endogenous gene (i.e., knockout mice) [1–3]. However, deletion of a large number of genes using a conventional ES cell-targeting strategy causes early embryonic lethality, which prevents elucidation of the roles of these gene products at later developmental stages and in adults [4, 5]. Cre/loxP technology [6, 7] provides an excellent strategy to determine physiological functions of these genes in vivo. By generating a line of floxed mice, which has normal expression of the gene of interest and bypasses embryonic lethality, and a line of Cre transgenic mice, in which the Cre recombinase is specifically driven by a tissue-specific gene promoter, investigators can generate tissue-specific knockout mice and then characterize the function of genes of interest. One of the critical factors to determine whether cell-specific knockouts can be generated successfully using the Cre/loxP strategy is the level of Cre recombinase within the cells in the Cre transgenic mice. Recently, a codon-improved Cre recombinase (iCre) [8] has been used to replace the prokaryotic Cre [7] to enhance the expression level of Cre recombinase, thus improving the recombination efficiency of loxP sites.

Folliculogenesis is the development of the follicle, an essential functional unit of the ovary containing an oocyte and granulosa and thecal cells, from the primordial stage through the primary, preantral, antral, and preovulatory follicle stages [9]. Along with follicular development, oocytes also undergo growth in the postnatal ovary, giving rise to mature eggs [10, 11]. Oocyte-expressing genes are involved not only in folliculogenesis and oogenesis but also in preimplantation embryonic development [12–15]. There are many genes expressed in the oocytes that are not oocyte specific [4, 16, 17]. Deletion of these genes using the conventional ES-targeting strategy may cause embryonic lethality, which prevents analysis of their functions in oogenesis and folliculogenesis [5]. Even if germline deletion of oocyte-expressing genes could bypass embryonic lethality, their female reproductive phenotypes (if there are any) may not be interpreted as a result of the loss of their gene products in the oocytes, but rather could be due to the loss of their expression in other tissues, such as hypothalamus and pituitary (if expressed). Also, if genes of interest are expressed in oocytes at all stages of follicular development, their roles in later follicular stages, such as secondary and preovulatory follicles, will be unlikely to be characterized using the conventional knockout mouse models. In addition, it is impossible to determine the role of maternal gene products during preimplantation embryonic development using the conventional ES cell knockout strategy. In an effort to understand the physiological roles of genes, which are expressed in oocytes, during folliculogenesis, oogenesis, and preimplantation embryonic development, we have sought to adapt the Cre/loxP system to delete floxed genes specifically in oocytes, at different stages of follicular development. Several Cre transgenic mouse lines, such as *Zp3Cre* and *Msx2Cre*, in which the *Cre* gene is driven by *zona pellucida protein 3* (*Zp3*) promoter and a homeobox

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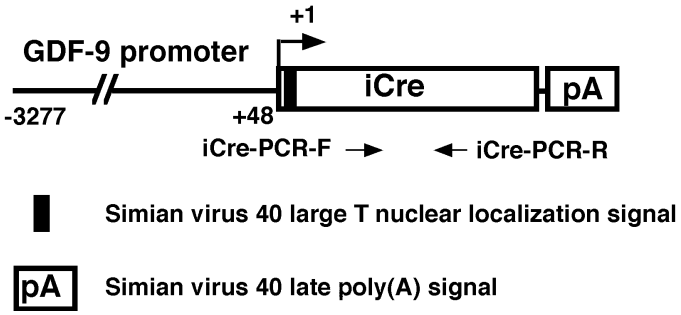

FIG. 1. Schematic representation of the *GDF-9-iCre* transgene. Mouse *GDF-9* promoter fragment (-3277-+48) was inserted in the upstream of an improved Cre (*iCre*) expression cassette, which contained a consensus Kozak sequence, simian virus 40 (SV40) large T nuclear localization signal, the iCre coding sequences, and a SV40 poly(A) tail. PCR primers, iCre-PCR-F and iCre-PCR-R, were used to determine the presence of the iCre transgene in mice.

gene *Msx2* promoter, respectively, have been generated [18, 19]. Zp3Cre was shown to express the Cre recombinase in the oocytes at primary and/or later follicular stages [12, 18]. Recently, these transgenic mice have been used successfully to delete an oocyte-expressing gene, *germ cell nuclear factor* (*GCNF*), in the female germline [12, 20]. Both *Zp3Cre* and *Msx2Cre* transgenic mouse lines are valuable for generating conditional knockouts in the oocytes at primary and/or later follicular stages. However, both transgenic mouse lines are unlikely to be useful for achieving complete deletion of oocyte genes at early follicular stages, such as at primordial and even early primary stages. To date, there is no *Cre* transgenic mouse line expressing Cre recombinase in the oocytes in the primordial follicles during postnatal ovarian development. In this report, we describe the generation and characterization of an iCre-expressing transgenic mouse line under the promoter of an oocyte-expressing gene, *growth differentiation factor-9* (*GDF-9*) [21, 22] and its comparison with Zp3Cre and Msx2Cre.

MATERIALS AND METHODS

Animals

The floxed ROSA, *R26R* [23], mice were generously provided by Dr. Phillip Soriano (Fred Hutchinson Research Cancer Center, Seattle, WA). The floxed *GCNF* mice were generated as described previously [12, 20]. Transgenic mice, *Zp3Cre* [18] and *Msx2Cre* [19], were generously provided by Drs. Barbara Knowles (The Jackson Laboratory) and Gail Martin (University of California at San Francisco), respectively. Healthy adult C57B6/SJL mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All mice were maintained on a 14L:10D cycle, with free access to food and water, in the vivarium of the Baylor College of Medicine. All experiments in this study were approved by the Animal Welfare Committee of Baylor College of Medicine.

Generation of Transgenic GDF-9-iCre *Mice*

A 1.1-kilobase (kb) improved Cre (*iCre*) fragment, which contains a consensus Kozak sequence [24], the nuclear localization signal peptide of the a simian virus 40 (SV40) large T antigen, and coding sequences of the *iCre* gene [8], was generated by PCR using primers iCre-F (5′-gatatcaagcttgtccaccatggtg-3′) and iCre-R (5′-caactctagactcagtttcagtccccatc-3′), and pBlue.iCre plasmid (generously provided by Dr. R. Sprengel) [8] as a template. This 1.1-kb *iCre* fragment was then digested with *Hin*dIII and *Xba*I, and the digested iCre fragment was subcloned into the *Hin*dIII/*Xba*I sites of the pGL3-Basic-Luc vector (Promega, Madison, WI) to replace the original luciferase coding sequences to generate a promoterless mini-iCre expression cassette, pGL3B-iCre. GDF-9 promoter fragment (-3277-+48) was amplified from the pGDF-9-LUC plasmid (generously provided by Dr. Martin Matzuk) [12] and cloned into the *Kpn*I/*Hin*dIII sites of the pGL3B-iCre plasmid to generate *GDF-9-iCre* transgene expression vector, pGL3B-GDF-9-iCre. The nucleotide sequences of this transgene vector, pGL3B-GDF-9-iCre, were confirmed by sequence analysis.

A 4.68-kb *Kpn*I/*Sal*I DNA fragment from the iCre expression vector, pGL3B-GDF-9-iCre, which contains 3.3-kb *GDF-9* promoter, 1.1-kb iCre coding sequence, and a SV40 late poly(A) signal (Fig. 1), was purified from an agarose gel and then microinjected into the pronuclei of fertilized eggs of C57-B6/SJL mice. The microinjected eggs were then transferred to pseudopregnant mothers to generate *GDF-9-iCre* founder mice. The resulting transgenic mouse lines were named *GDF-9-iCre.*

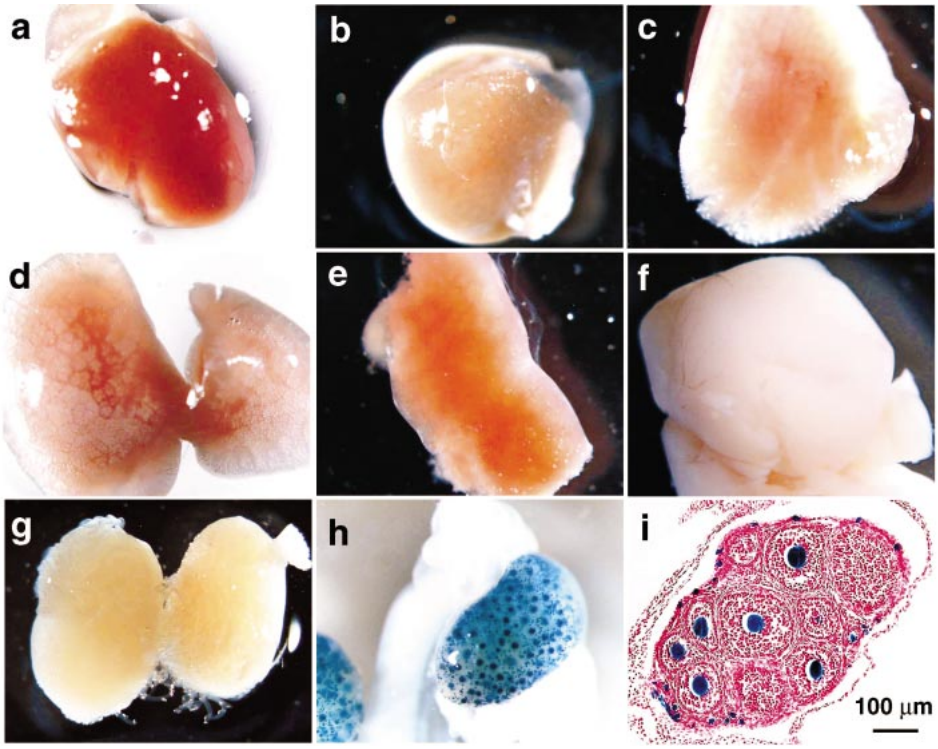

FIG. 2. The β-galactosidase staining showing the ovary-specific expression of iCre recombinase in *GDF-9-iCre* transgenic mice. Mouse heart (**a**), kidney (**b**), liver (**c**), lung (**d**), spleen (**e**), brain (**f**), and ovary and its surrounding oviduct, fat, and uterus (**h**) from 24-day-old *GDF-9-iCre; R26R* female, and mouse testis (**g**) from 24-day-old *GDF-9-iCre;R26R* male were stained for β-galactosidase activity. Note the positive blue staining signal in the ovary. After staining, the ovary was post-fixed, embedded, and sectioned. Tissue sections were then counterstained with nuclear fast red. A histological section of the stained ovary (**h**) is included in (**i**).

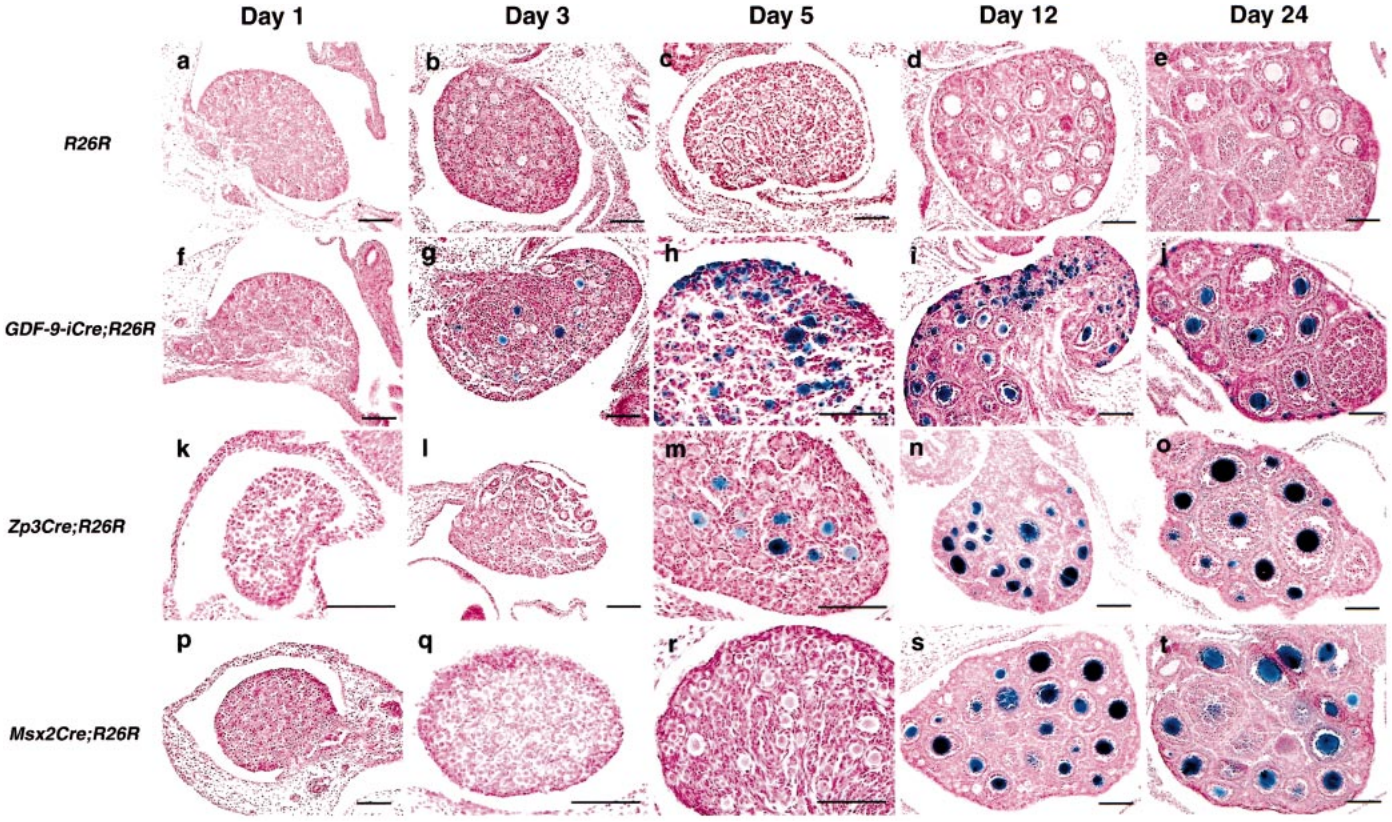

FIG. 3. Ontogeny of expression of Cre recombinase in ovaries of *GDF-9-iCre, Zp3Cre,* and *Msx2Cre* transgenic mice at various developmental stages. Mouse ovaries from *R26R* (**a**–**e**), *GDF-9-iCre;R26R* (**f**–**j**), *Zp3Cre;R26R* (**k**–**o**), and *Msx2Cre;R26R* (**p**–**t**) mice at Day 1 (**a**, **f**, **k**, **p**), Day 3 (**b**, **g**, **l**, **q**), Day 5 (**c**, **h**, **m**, **r**), Day 12 (**d**, **i**, **n**, **s**), and Day 24 (**e**, **j**, **o**, **t**) were subjected to β-galactosidase staining. After staining, ovaries were postfixed, embedded, and then sectioned. Tissue sections were then counterstained with nuclear fast red. Scale bars = 100 μm.

Mouse Genotyping

Mouse tail genomic DNA was extracted [25] and genotyped by PCR. *GDF-9-iCre* mice were genotyped by PCR analysis using primers iCre-PCR-F (5′-tctgatgaagtcaggaagaacc-3′) and iCre-PCR-R (5′-gagatgtccttcactctgattc-3′). PCR reactions for the *iCre* transgene were carried out for 30 cycles (94°C, 1 min; 58°C, 2 min; 72°C, 2 min) in a buffer containing 2.5 mM $MgCl_2$, while the PCR conditions for genotyping the Cre transgene in *Zp3Cre* and *Msx2Cre* mice were described previously [12]. PCR conditions for genotyping the $GCNF^{+}$, $GCNF^{fl}$, $GCNF^{lox}$, and *R26R* allele were reported previously [12, 25].

β-Galactosidase Staining

GDF-9-iCre, *Zp3Cre*, and *Msx2Cre* male mice were crossed with *R26R* females to generate *GDF-9-iCre;R26R*, *Zp3Cre;R26R*, and *Msx2Cre;R26R* mice, respectively. Ovaries at Days 1, 3, 5, 12, and 24 after birth were collected and then subjected to β-galactosidase staining according to the manufacturer's protocol (Specialty Media, NJ). After staining, tissues were postfixed, dehydrated, embedded, and then sectioned. Tissue sections were counterstained with 0.1% nuclear fast red. These tissues and their sections were examined under a Zeiss Axioskop 2 microscopy (Carl Zeiss, Inc., Thornwood, NY).

RESULTS

Generation of GDF-9-iCre *Transgenic Mouse Lines*

Microinjection of the *GDF-9-iCre* transgene (Fig. 1), which contains 3.3-kb *GDF-9* promoter (-3277-+48) [12], 1.1-kb iCre coding sequence [8], and a SV40 late poly(A) signal, into the pronuclei of fertilized eggs of C57-B6/SJL mice followed by transfer of the microinjected eggs into pseudopregnant mothers produced eight founder pups. Genomic PCR analysis showed that four of these eight pups carried the *GDF-9-iCre* transgene (data not shown). Among these four founders, three were females (5090, 5091, and 5092) and the other one was male (5085). All founders except the male founder successfully transmitted the transgene to their offspring after breeding with wild-type mice.

Cre Recombinase Activity in the Ovary of GDF-9-iCre *Mice*

R26R reporter mice [23] were used to determine the tissue and cell specificity of the Cre recombinase in three germline-transmitted *GDF-9-iCre* mouse lines. Breeding of the founder mice carrying the *GDF-9-iCre* transgene with *R26R* mice produced *R26R* and *GDF-9-iCre;R26R* mice. A variety of tissues, including heart, kidney, liver, lung, spleen, brain, testis, and ovary, of 24-day-old *R26R* and *GDF-9-iCre;R26R* mice were collected postmortem and subjected to β-galactosidase staining. Positive blue staining in tissues indicates that Cre recombinase is active in turning on the *LacZ* gene expression in *R26R* mice. As expected, no β-galactosidase staining was observed in all tested tissues of *R26R* mice (data not shown). All tested tissues of *GDF-9-iCre;R26R* mice generated from the *GDF-9-iCre* mouse line 5091 had a various degree of blue staining (data not shown), indicating ectopic expression of Cre recombinase in this transgenic mouse line. In contrast with the ectopic Cre expression in the *GDF-9-iCre* transgenic mouse line, 5091, the other *GDF-9-iCre* transgenic mouse lines, 5090 and 5092, displayed tissue-specific Cre expression (Fig. 2). β-Galactosidase staining of tissues derived from the *GDF-9-iCre* transgenic mouse line, 5092, is shown in Figure 2. No β-galactosidase activity was observed in the heart, kidney, liver, lung, spleen, brain, testis, uterus, or

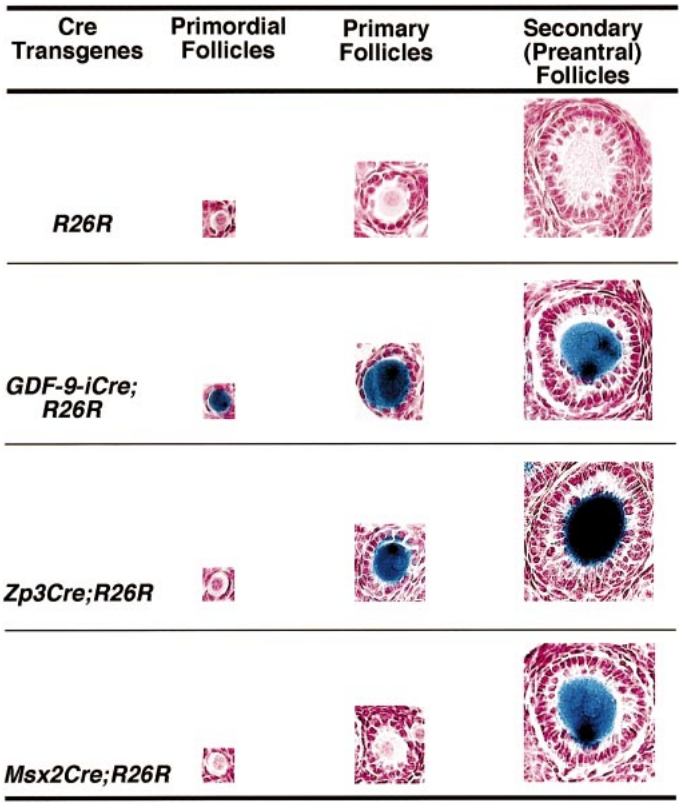

FIG. 4. The β-galactosidase staining showing the Cre recombinase activity in the primordial, primary, and secondary follicles in *GDF-9-iCre, Zp3Cre,* and *Msx2Cre* transgenic mouse lines. Ovaries from 3- to 24-day-old *R26R, GDF-9-iCre;R26R, Zp3Cre;R26R,* and *Msx2Cre;R26R* mice were subjected to β-galactosidase staining. After staining, ovaries were postfixed, embedded, and then sectioned. Tissue sections were then counterstained with nuclear fast red. Note the presence of positive blue staining in the primordial follicle of *GDF-9-iCre;R26R* but not *Zp3Cre;R26R* or *Msx2Cre;R26R* mice. All images were taken at the same magnification (×400).

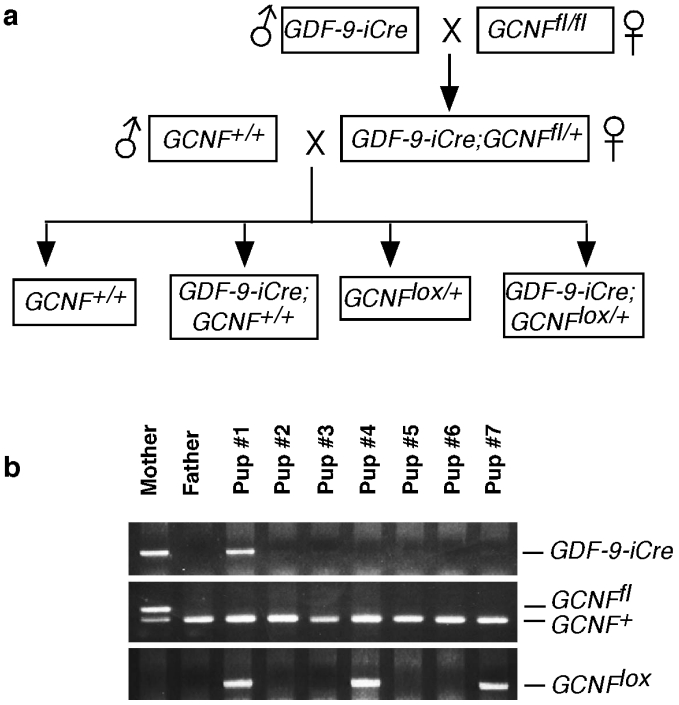

FIG. 5. Deletion of the floxed *GCNF* allele by *GDF-9-iCre* in the female germline. A breeding scheme (**a**) and a representative PCR genotyping result (**b**) of a litter of pups from breeding of a wild-type male with a *GDF-9-iCre;GCNF*$^{fl/+}$ female. Note the lack of a *GCNF*^{fl} allele in all pups and the presence of a *GCNF*^{lox} allele in some pups (1, 4, and 7), indicating complete deletion of the floxed *GCNF* DBD fragment in the female germline, regardless of the presence of *iCre* transgene in the progeny.

oviduct of 24-day-old *GDF-9-iCre;R26R* mice (Fig. 2, a–g). However, positive β-galactosidase staining was observed in the ovary of 24-day-old *GDF-9-iCre;R26R* mice (Fig. 2h). Ovarian sections showed that positive blue staining was observed only in the oocytes, not the somatic cells (Fig. 2i). These results suggested that Cre recombinase from this transgenic mouse line is active only in the oocytes turning on the *LacZ* expression in *GDF-9-iCre;R26R* mice. Similar results were obtained using the *GDF-9-iCre* transgenic mouse line, 5090 (data not shown). Therefore, we have successfully generated a *GDF-9-iCre* transgenic mouse line that will be useful for achieving oocyte-specific deletion of the floxed genes of interest in the postnatal ovary.

Stage-Specific Expression of Cre Recombinase in GDF-9-iCre, Zp3Cre, *and* Msx2Cre *Transgenic Mice*

To determine the ontogeny of the expression of Cre recombinase in the ovary during postnatal development, ovaries of *GDF-9-iCre;R26R* derived from the 5092 *GDF-9-iCre* transgenic line were collected at Days 1, 3, 5, 12, and 24 after birth and then subjected to β-galactosidase staining. Similarly, the Cre recombinase activities of *Msx2Cre* transgenic mice [19] and *Zp3Cre* transgenic mice [18] in the ovaries at Days 1, 3, 5, 12, and 24 after birth were also analyzed. Ovaries from *R26R* littermates were used as negative controls for β-galactosidase staining. As expected, there was no blue staining in the ovarian sections from *R26R* mice at the age of 1–24 days after birth (Fig. 3, a–e). In *GDF-9-iCre;R26R* mice, positive blue staining was observed in the oocytes of 3- to 24-day-old ovaries, but not in 1-day-old ovary (Fig. 3, f–j). At Day 3, positive blue staining was observed in the oocytes of some primordial follicles in *GDF-9-iCre;R26R* mice (Fig. 3g). At Days 5, 12, and 24, positive blue staining was detected in the oocytes of a number of primordial follicles in *GDF-9-iCre; R26R* ovaries (Figs. 3, h–j, and 4). Positive blue staining was also observed in the oocytes, not the somatic cells of all follicles at the primary, secondary, and later follicular stages (Figs. 3 and 4). In the *Zp3Cre;R26R* females, positive blue staining was observed in the ovaries at the ages of 5 days or older (Fig. 3, k–o). There was no positive blue staining in the primordial follicles in any *Zp3Cre;R26R* females tested. Similar to previous reports [12, 18], positive blue staining was observed only in the oocytes at the primary, secondary, and later follicular stages, but not in the adjacent somatic cells in *Zp3Cre;R26R* ovaries (Figs. 3 and 4). In *Msx2Cre;R26R* mice, positive blue staining was observed in the ovaries of 12-day- and 24-day-old animals, but not 1-, 3-, and 5-day-old animals (Fig. 3, p–t). Positive blue staining was observed in the oocytes of follicles at the early secondary and later follicular stages, but not in the oocytes of primordial follicles nor in the primary follicles of *Msx2Cre;R26R* ovaries (Figs. 3 and 4). Therefore, the Cre recombinase in the *GDF-9-iCre* mice was active earlier than that of *Zp3Cre* and *Msx2Cre* in the ovary not only at the postnatal age but also at the follicular stage, indicating that *GDF-9-iCre*, *Zp3Cre*, and *Msx2Cre* can be used to

delete oocyte-specific genes of interest at specific stages of follicular development in the postnatal ovary.

Deletion of the Floxed GCNF *Allele in Female Germline by* GDF-9-iCre *Mice*

The floxed *GCNF* mice [20] were used to test the efficiency of the Cre recombinase in *GDF-9-iCre* transgenic mice in female germline. Breeding of *GDF-9-iCre* males with the floxed *GCNF* females produced *GDF-9-iCre-GCNF*$^{fl/+}$ mice (Fig. 5a). *GDF-9-iCreGCNF*$^{fl/+}$ females were then crossed with wild-type male mice, and the progeny of this breeding was genotyped to determine whether the floxed *GCNF* allele was lost in the progeny and a recombined *GCNF*^{lox} allele was detected in the progeny (Fig. 5a). If Cre recombinase is active in the oocyte, the floxed *GCNF* allele (*GCNF*^{fl}) will be converted to the recombined *GCNF*^{lox} allele regardless of the presence or absence of *GDF-9-iCre* transgene in the progeny. We found that no floxed *GCNF* allele was detected in any of the 47 pups generated from three *GDF-9-iCreGCNF*$^{fl/+}$ females, and a recombined *GCNF*^{lox} allele was observed in 22 of these 47 progeny. A representative genotyping result is shown in Figure 5b. The recombined *GCNF*^{lox} allele but not the floxed *GCNF* allele was detected in pups 1, 4, and 7, even though there was no *GDF-9-iCre* transgene in pups 4 and 7 (Fig. 5b). These results show that the Cre recombinase expressed in adult *GDF-9-iCre* females is sufficient to delete the floxed *GCNF* allele in the female germline.

DISCUSSION

Recently, several research groups, including our laboratory, have successfully used the Cre/loxP technology to delete genes of interest in mouse gonads, which greatly advances our understanding of their physiological functions in reproduction [12, 26–31]. The limiting factor in the generation of Cre/loxP knockout mouse models is the recombination efficiency of the Cre recombinase in the *Cre* transgenic mouse line. To enhance the expression level of Cre recombinase using the *iCre* gene with a mammalianized codon usage [8], instead of the prokaryotic *Cre* [7], driven by a strong gene promoter, will likely improve Cre recombination efficiency. In our goal of understanding the roles of oocyte genes during folliculogenesis and oogenesis in the postnatal ovary and during preimplantation embryonic development, we wanted to generate a transgenic mouse line that expresses Cre recombinase at a high level in the oocytes at the earliest possible follicular stage. Here, we report the generation and characterization of transgenic mice that express the *iCre* gene under the control of *GDF-9* promoter. Using *R26R* reporter mice, we were able to show that *GDF-9-iCre* mice (line 5092) can delete the floxed *pGK-neo* cassette upstream of the *LacZ* gene, turning on the *LacZ* gene expression in the postnatal ovary, specifically in oocytes as early as at the primordial follicular stage (Figs. 2–4). The oocyte-specific expression of the Cre recombinase in this *GDF-9-iCre* mouse line is in agreement with the reported expression of the endogenous *GDF-9* gene expression in the ovary [17, 21, 32]. The absence of detectable transgene activity in the testis in this *GDF-9-iCre* transgenic line, which is inconsistent with endogenous testicular *GDF-9* expression reported previously [17], is probably due to either the site of integration of the transgene into the genome or the length of the promoter fragment used. Comparing the Cre recombinase activity in the postnatal ovary of *GDF-9-iCre* mouse line with those of two other Cre transgenic mouse lines, *Zp3Cre* [18] and *Msx2Cre* [20], we observed that *GDF-9-iCre* mouse line expresses the Cre recombinase activity in the postnatal ovary not only at an earlier time after birth but also at the earlier stage of follicular development than *Zp3Cre* and *Msx2Cre* mouse lines (Figs. 3 and 4). To our knowledge, this is the first Cre transgenic mouse line that can express the Cre recombinase in the oocytes of primordial follicles in the postnatal mouse ovary.

Having determined that Cre recombinase in the *GDF-9-iCre* transgenic line can efficiently induce excision of the artificial DNA fragment in the *ROSA* locus, we asked the question whether the Cre recombinase in this *GDF-9-iCre* transgenic line could delete a floxed endogenous gene fragment. Using the floxed *GCNF* mice recently generated [20], we found that the floxed *GCNF* allele was completely deleted in the female germline by GDF-9-iCre (Fig. 5), similar to Zp3Cre and Msx2Cre reported previously [12, 20]. These results indicate that *GDF-9-iCre* transgenic mice can be used for conditional deletion of oocyte genes in the postnatal ovary, thus allowing dissection of the physiological functions of genes expressed in the oocytes during folliculogenesis, oogenesis, and preimplantation embryonic development.

Using the conventional ES cell-targeting strategies, it is impossible for us to determine the roles of oocyte genes at different stages of follicular development if these genes are expressed continuously in the oocytes during follicular development. The Cre/loxP system provides an excellent strategy to understand gene function during folliculogenesis and oogenesis. The sequential oocyte-specific Cre expression in primordial, primary, or secondary follicles of *GDF-9-iCre*, *Zp3Cre*, and *Msx2Cre* mouse lines characterized in this report (Figs. 3 and 4) would allow conditional ablation of oocyte genes at a specific follicular stage, thus facilitating understanding of their roles at each follicular developmental stage. In addition, these three transgenic mouse lines can be used for conditional expression of transgenes specifically in various follicular developmental stages in the ovary.

In conclusion, we generated and characterized a *GDF-9-iCre* transgenic mouse line that can express Cre recombinase in oocytes as early as at the primordial follicular stage. We also reported the sequential oocyte-specific Cre expression in primordial, primary, or secondary follicles of *GDF-9-iCre*, *Zp3Cre*, and *Msx2Cre* mouse lines. We believe that *GDF-9-iCre* transgenic mice along with *Zp3Cre* and *Msx2Cre* transgenic mice will allow us to dissect the physiological functions of genes expressed in the oocytes during folliculogenesis, oogenesis, and preimplantation embryonic development.

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