

Maternal effect genes: Findings and effects on mouse embryo development

Kyeoung-Hwa Kim, Kyung-Ah Lee

Department of Biomedical Science, College of Life Science, CHA University, Seoul, Korea

Stored maternal factors in oocytes regulate oocyte differentiation into embryos during early embryonic development. Before zygotic gene activation (ZGA), these early embryos are mainly dependent on maternal factors for survival, such as macromolecules and subcellular organelles in oocytes. The genes encoding these essential maternal products are referred to as maternal effect genes (MEGs). MEGs accumulate maternal factors during oogenesis and enable ZGA, progression of early embryo development, and the initial establishment of embryonic cell lineages. Disruption of MEGs results in defective embryogenesis. Despite their important functions, only a few mammalian MEGs have been identified. In this review we summarize the roles of known MEGs in mouse fertility, with a particular emphasis on oocytes and early embryonic development. An increased knowledge of the working mechanism of MEGs could ultimately provide a means to regulate oocyte maturation and subsequent early embryonic development.

Keywords: Embryonic development; Maternal factor; Mice; Oocytes; Zygotic gene activation

Introduction

Abundant transcripts and proteins are produced and stored in oocytes during their growth and development to function during completion of meiosis, fertilization, embryonic cell divisions, zygotic gene activation (ZGA), and early embryogenesis until implantation has occurred. Maternal effect genes (MEGs) refer to the genes whose products have little effect on oocyte development, maturation, ovulation, and fertilization, but which activate the embryonic genomes and initial organization of embryonic cell lineages. Accordingly, the absence of MEGs hinders early embryonic development [1]. Currently, increas-

ing numbers of MEGs have been identified using various gene knock-out approaches in the mouse (Table 1), including the tissue-specific Cre-loxP system, the gene knockdown system, transgenic ribonucleic acid interference (RNAi), and gene-trap mutagenesis (Table 2).

In this mini-review, we summarize the core information regarding each gene by categorizing MEGs according to the biotechnical tools used to uncover their functions (Tables 1, 2). We conclude by discussing skin-embryo-brain-oocyte homeobox (*Sebox*) and growth arrest-specific protein 6 (*Gas6*) as new candidates for investigation according to our findings using RNAi [2,3].

Traditional gene knockout

There are 14 MEGs that have been revealed by traditional knockout animals. These are: maternal antigen that embryos require (*Mater*), heat shock factor 1 (*Hsf1*), oocyte-specific deoxy nucleic acid (DNA) methyltransferase-1 (*Dnmt1o*), DNA-cytosine-5-methyltransferase 3-like (*Dnmt3l*), formin2 (*Fmn2*), postmeiotic segregation increased 2 (*Pms2*), T-cell leukemia/lymphoma 1a (*Tcl1a*), nucleophosmin/nucleoplasmin 2 (*Npm2*), germ and embryonic stem cell enriched protein (*Stella*), zygote arrest 1 (*Zar1*), ubiquitin-conjugating enzyme

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Corresponding author: **Kyung-Ah Lee**

Department of Biomedical Science, College of Life Science, CHA University, 6-9 105 Road, Nonhyeon-ro, Gangnam-gu, Seoul 135-907, Korea
Tel: +82-2-557-3937 Fax: +82-2-563-2028 E-mail: leeka@ovary.co.kr

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Table 1. MEGs identified by using the traditional knockout approach and their resultant reproductive performance with the stage of their embryonic arrest

Gene	References	Fertility		Stage of developmental arrest
		Male	Female	
<i>Mater</i>	Tong et al., 2000 [4]	F	S	2C
<i>Hsf1</i>	Christians et al., 2000 [5]	F	S	Zygote
<i>Dnmt1o</i>	Howell et al., 2001 [6]	F	S	Post-I
<i>Dnmt3l</i>	Bourc'his et al., 2001 [7]	S	S	Post-I
<i>Fmn2</i>	Leader et al., 2002 [8]	F	SubF	MI
<i>Pms2</i>	Gurtu et al., 2002 [9]	S	F	Pre-I
<i>Tcl1a</i>	Narducci et al., 2002 [10]	F	SubF	4C to 8C
<i>Npm2</i>	Burns et al., 2003 [11]	F	SubF or S	Zygote
<i>Stella</i>	Payer et al., 2003 [12]	F	SubF	Pre-I
<i>Zar1</i>	Wu et al., 2003 [13]	F	S	Zygote
<i>Ube2a</i>	Roest et al., 2004 [14]	F	S	2C
<i>Zfp36l2</i>	Ramos et al., 2004 [15]	F	S	2C
<i>Uchl1</i>	Sekiguchi et al., 2006 [16]	NM	S	Zygote
<i>Filia</i>	Zheng and Dean, 2009 [17]	F	SubF	zygote to MO

MEGs, maternal effect genes; F, fertile; S, sterile; 2C, two cell stage; Post-I, postimplantation; SubF, subfertile; MI, metaphase I; NM, not mentioned; MO, morula.

E2A (*Ube2a*), zinc finger protein 36, C3H type-like 2 (*Zfp36l2*), ubiquitin carboxyl-terminal hydrolase L1 (*Uchl1*), *Filia*.

1. Mater

MATER was first identified as an oocyte antigen in the mouse model of autoimmune premature ovarian failure and determined as a MEG by analyzing null mutations in mice. *Mater* transcripts are expressed in the ovary and, in particular, the oocyte but not in other tissues. The MATER protein is encoded by a single-copy gene that is detected in the cytoplasm of growing oocytes and which is expressed until the late blastocyst stages [39,40].

Mater^{-/-} mice deliver pups at an approximately equal sex ratio without phenotypic abnormalities. *Mater*^{-/-} male mice are fertile, and *Mater*^{-/-} female mice also exhibit normal folliculogenesis, oogenesis, ovulation, and fertilization. In addition, the first cleavage event is normal during preimplantation embryonic development. However, *Mater*^{-/-} female mice are sterile because embryonic development is blocked at the two-cell (2C) stage despite normal embryonic morphology. In addition, the number of ovulated oocytes and embryos produced in *Mater*^{-/-} female mice are similar to that of normal females. These results demonstrate that *Mater* is essential for embryonic development

Table 2. MEGs found by using various approaches and their resultant reproductive performance with the stage of their embryonic arrest

Gene	Approach method	References	Lethality	Fertility		Stage
				Male	Female	
<i>Ezh2</i>	TKO	O'Carroll et al., 2001 [18]	Early embryonic lethal	NA	NA	NA
	CKO (Zp3 promoter)	Erhardt et al., 2003 [19]	NR	NM	F	NR
<i>E-cadherin</i>	TKO	Larue et al., 1994 [20]	Died around the time of implantation	NA	NA	NA
	CKO (Zp3 promoter)	De Vries et al., 2004 [21]	NR	NM	F	NR
<i>Dnmt3a</i>	TKO	Okano et al., 1999 [22]	Died at about 4 weeks of age	NA	NA	NA
	CKO (TNAP promoter)	Kaneda et al., 2004 [23]	NR	S	S	Post-I
<i>Brg1</i>	TKO	Bultman et al., 2000 [24]	Died during peri-implantation stage	NA	NA	NA
	CKO (Zp3 promoter)	Bultman et al., 2006 [25]	NR	NM	SubF	2C
<i>Dicer1</i>	CKO (Zp3 promoter)	Murchison et al., 2007 [26]	NR	NM	S	MI
<i>Padi6</i>	CKO (CMV promoter)	Esposito et al., 2007 [27]	NR	F	S	2C
<i>Atg5</i>	TKO	Kuma et al., 2004 [28]	Died within 1 day of delivery	NA	NA	NA
	CKO (Zp3 promoter)	Tsukamoto et al., 2008 [29]	NR	NM	S	4C to 8C
<i>Ago2</i>	TKO	Morita et al., 2007 [30]	Died after implantation at E	NA	NA	NA
	CKO (Zp3 promoter)	Kaneda et al., 2009 [31]	NR	NM	S	Zygote
	Gene KD system	Lykke-Andersen et al., 2008 [32]	NA	NA	NA	2C
<i>Tif1a</i>	Gene KD system	Torres-Padilla and Zernicka-Goetz, 2006 [33]	NA	NA	NA	2C to 4C
<i>Oct4</i>	TKO	Nichols et al., 1998 [34]	Died before egg cylinder formation	NA	NA	NA
	Gene KD system	Foygel et al., 2008 [35]	NA	NA	NA	Zygote to MO
<i>Sebox</i>	Gene KD system	Kim et al., 2008 [2]	NA	NA	NA	2C
<i>Gas6</i>	Gene KD system	Kim et al., 2011 [3]	NA	NA	NA	Zygote
<i>Basonuclin</i>	TG RNAi (Zp3 promoter)	Ma et al., 2006 [36]	NM	NM	SubF	2C
<i>CTCF</i>	TG RNAi (Zp3 promoter)	Wan et al., 2008 [37]	NM	NM	SubF	MO
<i>Floped</i>	TG MG	Li et al., 2008 [38]	NM	NM	S	2C

MEGs, maternal effect genes; TKO, traditional knockout; NA, not available; CKO, conditional knockout; NR, not related; NM, not mentioned; F, fertile; S, sterile; Post-I, postimplantation; SubF, subfertile; 2C, two cell stage; Gene KD system, gene knockdown system; MI, metaphase I; MO, morula; TG MG, gene-trap mutagenesis; TG RNAi, transgenic RNAi.

beyond the 2C stage in mice [4].

The subcortical maternal complex (SCMC) that forms during oogenesis has also been shown to be necessary for embryonic development beyond the 2C stage [38]. Located in the subcortex of oocytes and embryos, the SCMC is composed of MATER, FLOPED, transducin-like enhancer of split 6 (TLE6), and FILIA [17]. MATER, FLOPED, and TLE6 interact directly with each other, while FILIA interacts only with MATER. Therefore, MATER and its oocyte-specific binding partners are necessary for the first embryonic cleavage event.

2. *Hsf1*

HSF1 is a major transactivator of stress-inducible genes activated by heat and other stress stimuli [41]. *Hsf1*^{-/-} female mice have normal folliculogenesis and oogenesis [5]. In addition, oocytes from *Hsf1*^{-/-} female mice are arrested at metaphase II until fertilization, and the zygotes have two pronuclei and a second polar body, indicating that ovulation and fertilization in *Hsf1*^{-/-} female mice occurs normally. *Hsf1*^{-/-} females mated with *Hsf1*^{+/+} or *Hsf1*^{+/-} males produce embryos that are unable to develop properly beyond the zygotic stage. Additionally, the ultrastructure of the nuclei of 2C embryos from *Hsf1*^{-/-} female are abnormal. These findings led Christians et al. [5] to conclude that a 'maternal effect mutation' (i.e., the deficiency of *Hsf1* from the mother) controls early post-fertilization development and results in infertile *Hsf1*^{-/-} females.

3. *Dnmt1o*

DNMT1 maintains genomic methylation patterns in mammalian somatic cells. Though oocytes and preimplantation embryos lack DNMT1 expression, they do have its variant DNMT1O, which is found in the nuclei of early growing oocytes and in the cytoplasm of mature oocytes. DNMT1O is expressed in the cytoplasm at the 2C and 4C stages, in only the nucleus at the 8C stage, and again only in the cytoplasm at the blastocyst stage. This suggests a spatially diverse expression pattern for DNMT1O throughout the early stages of embryonic development [42]. In a study by Howell et al. [6], *Dnmt1o* was deleted by elimination of the oocyte-specific promoter and first exon using traditional knockout technology. This deletion mutant is called *Dnmt*^{Δ1o}. Knockout males are fertile, whereas *Dnmt*^{Δ1o} females exhibit a phenotype during gestation. *Dnmt*^{Δ1o} embryos are not affected during early embryonic development; they implant and develop after implantation. However, because of deficiencies in the uterine environment, nearly all *Dnmt*^{Δ1o} embryos die between embryonic days 14 and 21, and rare *Dnmt*^{Δ1o} survivors die within 24 hours after birth [6].

4. *Dnmt3l*

With sequence similarity to DNMT3A and DNMT3B, DNMT3L has been shown to catalyze de novo methylation of CpG islands. DNA

methyltransferases and regulatory factors are involved in the establishment of maternal imprints in growing diplotene oocytes and paternal imprints in perinatal prospermatogonia [43,44].

In a study by Bourc'his et al. [7], *Dnmt3l*^f mice were generated by replacing four exons of the *Dnmt3l* gene with a β-galactosidase-neomycin phosphotransferase fusion gene under the control of the *Dnmt3l* promoter. Both sexes of *Dnmt3l*^{f/g} mice are viable but sterile. In *Dnmt3l*^{f/g} males, neonatal testes contain a normal component of germ cells, while adult testes contain only Sertoli cells and have severe hypogonadism. Oogenesis in *Dnmt3l*^{f/g} females is normal, but there is a lethal maternal-effect in that *Dnmt3l*^{f/+} embryos from *Dnmt3l*^{f/g} females fail to develop at mid-gestation due to pericardial edema with exencephaly and other neural tube defects. Furthermore, the paternally imprinted *H19* gene is normally methylated in *Dnmt3l*^{f/+} progeny from *Dnmt3l*^{f/g} females, whereas the maternally imprinted genes, small nuclear ribonucleoprotein polypeptide N and paternally expressed gene 1, are unmethylated. These results suggest that *Dnmt3l* is required for the establishment of maternal imprints during oogenesis, but is not required for the maintenance of paternal imprints. Global genome methylation levels are also not affected by removal of *Dnmt3l* [7].

5. *Fmn2*

Fmn2 was identified as a gene that is expressed in the developing and mature central nervous system, including the spinal cord and brain [45]. FMN2 is highly homologous to the *Drosophila* cappuccino protein, which is a *Drosophila* MEG required for polarity of the egg and embryo [46]. FMN2 is essential for establishment of cell polarity, cytoskeleton organization, meiotic maturation, and regulation of asymmetrical spindle positioning in mouse oocytes [47,48].

FMN2 was determined to function like a MEG by analyzing *Fmn2*^{-/-} mice [8]. *Fmn2*^{+/-} mice are phenotypically normal, and *Fmn2*^{-/-} mice have normal development of the central nervous system and a normal sex distribution. *Fmn2*^{-/-} male mice are fertile, but *Fmn2*^{-/-} female mice are subfertile in that they only give birth to 1-3 offspring of which only a few survive.

In *Fmn2*^{-/-} mice, emission of the first polar body occurs in only 3% of oocytes, of which the rest are arrested at metaphase of meiosis I. These oocytes have a loss of normal barrel-shape spindles and centrally located chromosomes which cause a parthenogenic cell division. In spite of inaccurate positioning of the metaphase spindle during meiosis I, *Fmn2*^{-/-} oocytes form the first polar body. These findings suggest that FMN2 is necessary for microtubule-independent chromatin positioning. When *Fmn2*^{-/-} oocytes were microinjected with *Fmn2* messenger RNA (mRNA), these oocytes were rescued from meiotic incompetence and showed first polar body formation [8]. Furthermore, because of meiotic incompetence in *Fmn2*^{-/-} oocytes, embryos from

Fmn2^{-/-} oocytes appear polyploidy, such as triploid and pentaploid. *Fmn2*^{-/-} females have a small number of implantation sites in the uterus and contain embryos with morphological defects, such as developmental delay and placental resorption caused by gross morphological defects. These results led to the conclusion that the deletion of *Fmn2* arrests oocytes at metaphase of meiosis I and results in subfertility in *Fmn2*^{-/-} female mice [8].

6. *Pms2*

A DNA mismatch repair gene homolog, PMS2 is required for methyl-directed post-replication mismatch repair and was identified as a MEG using null mutation mice [49]. Homologous *Pms2* mutations do not affect embryonic or neonatal lethality. *Pms2*^{-/-} males fail to produce offspring by having a reduced number of normal spermatozoa (<25% that of wild-type males) in the epididymis. Progression of spermatogenesis is first disrupted at the primary spermatocyte stage, and *Pms2*^{-/-} males have abnormalities in chromosome synapsis at meiotic prophase I. Also, microsatellite instability (MSI) has a frequency of approximately 10% in the sperm from *Pms2*^{-/-} males, suggesting a role for PMS2 in the differentially methylated region (DMR) [49].

To determine the consequences of maternal *Pms2* deficiency on genetic stability, DNA isolated from the tail was analyzed for polymorphisms between maternal and paternal alleles using microsatellite markers [9]. In heterozygous offspring derived from *Pms2*^{-/-} oocytes, MSIs in the maternal alleles were detected with an average frequency of 9.1%, which is a frequency similar to that observed in *Pms2*^{-/-} males. However, MSI was not observed in control offspring. Although they have a functionally defective DMR, *Pms2*^{-/-} females are fertile with a normal pregnancy rate and litter size [49].

7. *Tcl1a*

TCL1A interacts with AKT to increase AKT kinase activity and plays an important role in the transduction of anti-apoptotic and proliferative signals in T-cells [50]. As a developmental regulator, TCL1A is expressed in the ovary, testis, early developmental stage embryo, fetal thymus, and bone marrow [51]. *Tcl1a* mRNA and protein are present from germinal vesicle (GV) oocytes up to stage 2C embryos, but their presence is dramatically decreased in later embryonic developmental stages [51].

In a study by Narducci et al. [10], *Tcl1a*^{-/-} mice were produced by removing exons 2, 3, and 4 of the coding region. *Tcl1a*^{-/-} males and females are viable and show no discernible histological abnormalities. *Tcl1a*^{-/-} males are fertile, and the ovaries of *Tcl1a*^{-/-} females are histological normal. Additionally, *Tcl1a*^{-/-} females appear to have normal folliculogenesis, oogenesis, oocyte maturation, ovulation, and fertilization. These results indicate that TCL1a does not affect follicle development and concurrent oogenesis. However, *Tcl1a*^{-/-} embryos fail

to develop beyond the 4C–8C stage, and development is slower than that for wild-type embryos, with *Tcl1a*^{-/-} embryos exhibiting a delayed or arrested blastomere proliferation [10]. *Tcl1a*^{-/-} females produce fewer offspring than *Tcl1a*^{+/-} and *Tcl1a*^{+/+} females. Moreover, the number of pups from *Tcl1a*^{-/-} females is smaller. These findings indicate that *Tcl1a*^{-/-} females are subfertile.

8. *Npm2*

NPM2, an oocyte-specific nuclear protein, was identified using a polymerase chain reaction suppression-subtraction ovary library [11]. Transcripts of *Npm2* are limited in growing oocytes. NPM2 protein is expressed in the nucleus of oocytes before GV breakdown (GVBD) and in the cytoplasm after GVBD. NPM2 is detected in both pronuclei after fertilization, remains in nuclei until the 8C stage, and is barely expressed in the blastocyst stage.

Npm2^{-/-} male mice are normal and fertile, whereas *Npm2*^{-/-} female mice are subfertile or infertile. The ovaries from *Npm2*^{-/-} females have normal oogenesis, and *Npm2*^{-/-} oocytes have normal maturation and fertilization. However, *Npm2*^{-/-} embryos are not cleaved beyond the 2C stage. Taken together, *Npm2*^{-/-} female mice have defects in early embryogenesis, such as an absence of coalesced nucleolar structures, loss of heterochromatin formation, and deacetylation of histone H3. The authors of these findings concluded that *Npm2* is a MEG that is vital for nuclear and nucleolar organization in oocytes and in embryo development for the specific stages from zygote to 2C transition [11].

9. *Stella*

STELLA was identified during early embryonic gonadal development in the mouse [52]. *Stella* is a novel gene expressed in primordial germ cells (PGCs), oocytes, and early developmental stage preimplantation embryos [53]. STELLA protein is expressed in the cytoplasm of oocytes and embryos, and especially in pronuclei of zygotes. *Stella*^{-/-} mice have a similar number of PGCs to that of normal mice, suggesting no relationship between *Stella* and development of early gonadal PGCs. Furthermore, testes and ovaries of *Stella*^{-/-} adult mice have no histological abnormalities, and *Stella*^{-/-} male mice have normal fertility. Indeed, *Stella*^{-/-} female mice have follicles at all developmental stages with normal ovulation and no significant differences in the number of oocytes compared to that of wild-type mice. However, it is obvious that *Stella*^{-/-} female mice have reduced fertility as *Stella*^{-/-} embryos exhibit developmental failure during early embryogenesis. *Stella*^{-/-} embryos are arrested at the zygote (49%), 2C (10%), and morula (27%) stages, and only 15% of *Stella*^{-/-} embryos develop to the blastocyst stage. When *Stella*^{-/-} females are mated with wild-type males, *Stella*^{-/-} females produce only a few offspring in a small litter size [12].

10. *Zar1*

A novel oocyte-specific gene, *Zar1* was identified by using subtractive hybridization and screening of a mouse ovarian cDNA library [13]. *Zar1* transcripts are detected at high levels from growing oocytes up to zygote embryos, but are markedly reduced in 2C embryos and are not present until the blastocyst stages. The rapid disappearance of *Zar1* expression at the 2C stage suggests an essential role in the oocyte-to-embryo transition [13].

Zar1^{-/-} males are fertile, whereas *Zar1*^{-/-} females are infertile. In *Zar1*^{-/-} females, all stages of follicle development and corpus lutea formation are detected. In addition, *Zar1*^{-/-} oocytes resume meiosis and progress to metaphase II and form two pronuclei after fertilization. Hence, the functions of folliculogenesis, oogenesis, fertilization, and the first cleavage event in *Zar1*^{-/-} females are indistinguishable from that of wild-type mice. However, most *Zar1*^{-/-} embryos are arrested mainly at the zygote stage, while some are arrested at the 2C stage, and no embryos develop to the 4C stage, which results in sterility of the *Zar1*^{-/-} female [13].

11. *Ube2a*

Ube2a, a RAD6-homologous gene, is required for DNA repair and functions as a ubiquitin-conjugating enzyme [54]. Ubiquitin-conjugation signaling requires the activity of ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-ligating (E3) enzymes and results in proteolytic degradation of protein. The body weight of *Ube2a*^{-/-} mice is lighter than their wild-type littermates by 12% [14]. *Ube2a*^{-/-} male mice are fertile, but *Ube2a*^{-/-} female mice are not able to produce litters. Ovaries from *Ube2a*^{-/-} female mice appear to have normal numbers of follicles and corpus lutea, indicating normal follicular development and ovulation. After fertilization, *Ube2a*^{-/-} embryos contain two pronuclei, which suggests normal fertilization. However, wild-type embryos develop into the hatched blastocyst stage, whereas *Ube2a*^{-/-} embryos remain at the 2C stage and some embryos are degraded with severe cytoplasmic fragmentation. These results suggest that maternal *Ube2a* is essential for preimplantation embryonic development [14].

12. *Zfp36l2*

ZFP36L2 can bind to mRNAs containing class II AU-rich elements, followed by degradation of the target mRNA [55]. *Zfp36l2*^{-/-} females are completely sterile, whereas *Zfp36l2*^{-/-} males are fertile. *Zfp36l2*^{-/-} females exhibit typical estrous cycles, normal sexual behavior, and normal anatomy of their reproductive tracts. Ovaries from *Zfp36l2*^{-/-} mutant females also appear anatomically and histologically normal with the presence of follicles at all developmental stages. Possible uterine and corpus luteum defects were ruled out by investigating successful pregnancies after transplantation of wild-type embryos

into *Zfp36l2*^{-/-} uteri. By performing classical wild-type ovary transplantation to rescue *Zfp36l2*^{-/-} female sterility, a potential ovarian etiology for *Zfp36l2*^{-/-} female infertility was also ruled out. *Zfp36l2*^{-/-} females were additionally shown to ovulate morphologically normal oocytes, but with an overall reduction in the number of oocytes compared with that from wild-type females. Although the oocytes from *Zfp36l2*^{-/-} females undergo meiosis and are fertilizable, the embryos do not progress beyond developmental stage 2C. These results indicate that embryonic arrest at stage 2C accounts for the infertility in the *Zfp36l2*^{-/-} female [15].

13. *Uchl1*

One of many deubiquitinating enzymes, UCHL1 is expressed in the ovary, testis, placenta, and neuron [56-58]. UCHL1 associates with monoubiquitin, prolongs the half-life of ubiquitin in neurons, and resists apoptotic stress in testicular germ cells [58,59]. UCHL1 is expressed in the plasma membranes of oocytes and early embryos, including the outer layer cells of the trophectoderm in blastocyst stage embryos, but not in the cytoplasm of oocytes and embryos [16].

The gracile axonal dystrophy (*gad*) mouse is an autosomal recessive mutant that shows sensory ataxia at an early stage, followed by motor ataxia at a later stage. The *gad* mutation is transmitted by the *Uchl1* gene on chromosome 5 [60,61]. Because of a deletion of the nucleotide sequences encoded by exons 7 and 8 in *Uchl1*^{gad/gad} knockout mice, the *Uchl1*^{gad} allele predicts a truncated UCHL1 protein [62]. *Uchl1*^{gad/gad} female mice have morphologically normal oocytes, follicles of all developmental stages, and corpus lutea in the ovaries. Additionally, the number of ovulated oocytes is not significantly different between *Uchl1*^{gad/gad} mice and wild-type mice. These observations suggest that the deletion of *Uchl1* does not affect the estrous cycle, oogenesis, folliculogenesis, or ovulation. However, when *Uchl1*^{gad/gad} female mice were mated with *Uchl1*^{gad/gad} males, Sekiguchi et al. [16] found that embryos had three or more pronuclei, indicating polyspermic fertilization. *Uchl1*^{gad/gad} female mice therefore had a significantly decreased litter size. The monoubiquitin level was also lower in oocytes from *Uchl1*^{gad/gad} females, suggesting that UCHL1 is a fusion protein on the plasma membrane and plays a role in blocking polyspermy.

14. *Filia*

Filia transcripts are expressed in growing oocytes and is degraded during oocyte maturation, whereas FILIA protein persists during early embryogenesis. FILIA binds to MATER and participates in SCMC formation that is crucial for preimplantation embryo development [38]. In a study by Zheng and Dean [17], *Filia* was deleted in mouse ESCs, such that the transcriptional and translational start sites as well as the first 2 exons of the coding region were removed. *Filia*^{-/-} male mice have normal fertility, whereas *Filia*^{-/-} females have markedly re-

duced (about 50%) fecundity. There are significantly reduced percentages of *Filia*^{-/-} embryos that reach the morula and blastocyst stages when compared with wild-type embryos, with *Filia*^{-/-} embryos being mostly delayed at the 3C to 4C stages.

FILIA is required for placement of MAD2, a crucial component of the spindle assembly checkpoint, at kinetochores. However, AURKA, PLK1, and γ -Tubulin are completely absent in affected embryos from *Filia*^{-/-} females, and significantly higher rates of hyperploidy with abnormal spindle formation and chromosome misalignment are detected in *Filia*^{-/-} embryos [17].

Conditional gene knockout

When using traditional knockout technology, F₁ heterozygous parents may not produce F₂ null mutant offspring, if the F₂ null homozygotes die during embryogenesis or are dead at birth. Therefore, another method is required to delete the genes that cause lethality when targeted by traditional knockout methods. This new method is the conditional knockout system. With this system, genes can be deleted using the tissue-specific Cre-loxP system under the control of tissue-specific promoters, such as the *Zp3* promoter in the case of oocyte-specific expression. Cre-loxP recombination is frequently used to generate a conditional knockout animal in which a target gene is only deleted in a specific tissue or at a specific time. When cells with loxP sites in their genome express Cre recombinase, the Cre recombinase excises the DNA fragment located between the two loxP sites [63]. As such, the conditional gene knockout technique reduces the risks associated with traditional knockout techniques, such as embryonic lethality and complex phenotypes.

1. Ezh2 (Enhancer of zeste 2)

A SET domain-containing protein, EZH2 and its interacting partner, embryonic ectoderm development (EED), are polycomb group proteins that form an EZH2-EED complex which is crucial for early development control, such as epigenetic regulation of gene expression [64]. EZH2 is mainly expressed in early development and interacts with DNMT1 and DNMT3A, which are DNA methyltransferases that are known MEGs [65]. The EZH2-EED complex is essential for histone H3-K27 methylation during imprinted X inactivation [66]. EZH2 proteins are also expressed in growing oocytes but not in surrounding somatic cells [19].

Ezh2 homozygous null mutations are embryonic lethal mutations [18]. Deletion of maternal *Ezh2* by conditional knockout (*Ezh*^{F/F}-*Zp3*-Cre) was obtained using the combination of Cre-recombinase driven by *Zp3* regulatory elements (*Zp3*-Cre) and the *Ezh2* gene (exons 8–11) flanked by loxP sites. When *Ezh2*-deficient oocytes were fertilized with wild-type sperm, *Ezh2*^{DEL/F} offspring without maternal *Ezh2* dis-

played severe growth retardation.

The EZH2-EED complex is associated by histone H3-me₂-K27 in X inactivation. However, EZH2 depletion disrupts histone H3 methylation, whereby H3-K9 and H3-K27 are almost undetectable either during oocyte development or in the zygote. Therefore, EZH2 interacts with EED and plays an essential role in early development and in pluripotent embryonic stem cells through mechanisms such as methylation of histone H3-K27 and histone H3-K9; however, this essential role does not appear to carry over to differentiated cells [19].

2. E-cadherin

A specific calcium ion-dependent cell adhesion molecule, E-cadherin plays an essential role in determination of cell-cell adhesion and cell shape. The cytoplasmic domain of E-cadherin binds to β -catenin, and these proteins form the E-cadherin-catenin adhesion complex with the actin filament network. The transcription of mouse *E-cadherin* is under the regulation of the transcription factor snail [67]. E-cadherin also contributes to the process of compaction at the 8C stage [21].

Homozygous null mutant mice for E-cadherin show an early embryonic lethality through failure to form an intact trophectoderm cell layer [20]. Using the combination of the oocyte-specific *Zp3*-Cre transgene and floxed alleles, live-born litters were obtained from maternal *E-cadherin*-deficient embryos [21,68]. In these studies, low levels of *E-cadherin* expression from the paternal allele in *E-cadherin*-deficient embryos were detected at the morula stage, and adhesion did not occur until the morula stage. *E-cadherin*-deficient embryos had delayed cell division, but progressed compaction of individual blastomeres. β -catenin expression in *E-cadherin*-deficient embryos is detected only in the pronuclei in zygotes and nuclei at the 2C stage, whereas it is detected at the surface of the 2C and 8C stages of normal embryos. E-cadherin and β -catenin are required for adhesion of individual blastomeres during compaction and trophectoderm formation [21].

3. Dnmt3a (DNA methyltransferase 3a)

DNMT3A plays crucial roles in genomic imprinting, X chromosome inactivation, and mammalian development [69]. Using Northern blot and semi-quantitative reverse transcription polymerase chain reaction analysis, *Dnmt3a* expression was detected as ubiquitous in the fetal testis, fetal ovary, and all adult tissues [70,71]. *Dnmt3a*^{-/-} mice develop to term and appear to be normal at birth. However, most *Dnmt3a*^{-/-} mice are runts after birth and die at about 4 weeks of age [22]. Therefore, the function of *Dnmt3a* was analyzed using the Cre-loxP system. In the early postnatal stage, *Dnmt3a*^{2lox/1lox}, *TNAP*-Cre conditional mutant mice appear to have a reduction of about 20% in body weight when compared with wild-type littermates, but survive to adulthood and reach a normal body weight. *Dnmt3a*^{2lox/1lox}, *TNAP*-

Cre conditional mutant female mice are infertile. Embryos are smaller than wild-type embryos at embryonic day (E) 9.5 and exhibit developmental defects, such as a smaller size, open neural tube, a lack of branchial arches, and pale skin at E10.5. In addition, the pattern of maternal methylation at DMRs and other sequences are affected during oogenesis upon disruption of *Dnmt3a*.

Dnmt3a^{2lox/1lox}, TNAP-Cre conditional mutant male mice are also sterile. The testis weight of mutant male mice is decreased compared with that of wild-type mice due to a reduced number of spermatogonia at postnatal day 11. Mutant male mice that are 11 weeks old have testes that contain only a few spermatocysts and no spermatids or spermatozoa. These observations indicate that *Dnmt3a* is essential for spermatogenesis and functions as a MEG [23].

4. Brg1 (BRM/SWI2-related gene 1; Smarca4)

BRG1, a catalytic subunit of mammalian SWI/SNF-related chromatin remodeling complexes, regulates transcription and remodeling of nucleosomes in an ATP-dependent manner [72]. *Brg1*^{-/-} mice die during the peri-implantation stage [24]. Neither the inner cell mass (ICM) nor trophoblast survive during blastocyst outgrowth studies. Experiments with other cell types, however, have demonstrated that BRG1 is not a general cell survival factor. Therefore, the function of *Brg1* as a MEG was demonstrated using the *Brg1*^{Zp3/Cre} conditional mutant.

In the study by Bultman et al. [25], *Brg1*^{Zp3/Cre} females produce normal 2C embryos, suggesting that BRG1 does not affect oocyte growth, maturation, meiosis I, ovulation, fertilization, or the first cleavage event. However, the majority of embryos (78%) from *Brg1*^{Zp3/Cre} females are arrested at the 2C and 4C stages and only 12% of embryos advance to the blastocyst stage. Furthermore, maternal *Brg1* transcripts are degraded and replaced by ZGA in wild-type mice, but not in maternally depleted embryos. It was concluded that BRG1 is essential for ZGA and is linked to histone modification, particularly to histone H3-K4 methylation. Furthermore, transcriptional activity of these embryos is reduced by about 35%. Using Expression Analysis Systematic Explorer analysis, Bultman et al. [25] found that BRG1 is involved in the regulation of transcription, RNA processing, and the cell cycle.

5. Dicer1

A conserved ribonuclease, Dicer1 is required for formation of the RNA-induced silencing complex (RISC), which is composed of Dicer1, EIF2C2/AGO2, and TARBP2. Dicer is required to process precursor microRNAs (miRNAs) to mature miRNAs and then load them onto EIF2C2/AGO2 [73]. In addition, Dicer processes long double-stranded RNA (dsRNA) into many small interfering RNAs (siRNAs) [74]. Through the RNAi pathway, miRNAs and siRNAs regulate turnover of many maternal transcripts in oocytes [26,75].

In a study by Murchison et al. [26], Dicer1 conditional knockout mice

were produced by combining a conditional *Dicer1*-floxed allele with a Cre recombinase transgene expressed from the *Zp3* promoter. The normal numbers of GV oocytes are recovered from *Dicer1* conditional knockout (*Dicer1*^{lox/lox}; *Zp3*-Cre) females. In addition, *Dicer1*^{-/-} oocytes show a normal structure with a normal shape of the GV. These results suggest that *Dicer1* expression in oocytes is not involved in oocyte growth, development, and response to gonadotropins. Most *Dicer1*^{-/-} oocytes undergo GVBD, but fewer *Dicer1*^{-/-} oocytes extrude a polar body, revealing an arrest at meiosis I in mature *Dicer1*^{-/-} oocytes. *Dicer1*^{-/-} oocytes also have multiple spindles with misaligned chromosomes. These results indicate that *Dicer1* is required for completion of oocyte mitotic maturation, spindle organization, and chromosome configuration. The authors of the study concluded that the deletion of *Dicer1* in oocytes affects the oocyte transcriptome, which results in meiosis I arrest and infertility in the *Dicer1*^{-/-} female mouse [26].

6. Padi6 (Peptidyl arginine deiminase 6)

A member of the peptidyl arginine deiminase family, PADI6 is involved in the posttranslational modification of arginine into citrulline through a process called citrullination [76]. PADI6 protein is detected in mammalian sperm, in oocytes at all stages from primordial to ovulatory follicles, and persists in embryos up to the blastocyst stage. PADI6 localizes to the cytoplasmic lattice of oocytes, which is a fibrillar matrix of so-called cytoskeletal sheets [77], and the PADI6 protein is important in ribosome storage in the cytoplasmic lattices of oocytes and the ZGA [78].

In *Padi6* conditional knockout mice, the *Padi6* gene is disrupted by using loxP and a *CMV*-Cre transgenic line [27]. Homologous *Padi6* mutant mice are viable and born at the expected Mendelian ratio. No phenotypical or histopathological abnormalities are observed in any tissue. Homologous *Padi6* mutant male mice are fertile, whereas female mice are infertile. PADI6 appears not to play a role in oocyte growth, maturation, and ovulation. Furthermore, PADI6 is not required for fertilization and pronuclear formation. However, homologous *Padi6* mutant zygotes do not progress beyond the 2C stage of development. Therefore, maternally-expressed PADI6 plays an essential role in the organization of the cytoskeletal sheets through citrullination of oocyte-specific keratins. The fertility defect with deficiency of citrullination results in dispersion of the cytoskeletal sheets, and these results indicate that *Padi6* is a mammalian MEG [27].

7. Atg5 (Autophagy-related 5)

ATG5 is a crucial component for autophagosome formation and is an acceptor molecule for ATG12 [79]. By the ubiquitin-proteasome system, maternal protein degradation accelerates after fertilization and is apparent at the 2C stage in mammals [80,81]. Autophagy, another important maternal protein degradation system, is a process

by which cytoplasmic constituents in the lysosome are degraded. Autophagy plays an essential role during early embryogenesis [29,82]. Although many studies have suggested a possible role for autophagy in development, traditional *Atg5*^{-/-} knockout mice are born at the expected Mendelian ratio, are normal at birth, but die within 1 day of birth as a consequence of low nutrient status [28].

Atg5 conditional knockout mice (*Atg5*^{fllox/-}-*Zp3*-Cre) were produced by crossing the mouse lines bearing an *Atg5*^{fllox} allele to a *Zp3*-Cre transgenic line [29]. Despite exhibiting completely defective autophagy, normal ranges of superovulated oocytes are recovered from *Atg5*^{fllox/-}-*Zp3*-Cre female mice and they are fertilized normally. These results suggest that autophagy is not important for oogenesis, oocyte maturation, and fertilization. *Atg5*^{fllox/-}-*Zp3*-Cre female mice also produce smaller pups when mated with wild-type males because the deficiency in autophagy is rescued by zygote-derived ATG5. In contrast, fertilization of *Atg5*-null oocytes with *Atg5*-null sperm results in the arrest of early embryonic development. *Atg5*-null embryos do not develop and remain at the 4C or 8C stage. These results suggest that autophagy deficiency causes arrest of early embryonic development at the 4C or 8C stage [29].

8. Ago2 (Argonaute 2)

AGO2 is a key factor of the RISC complex, which is essential for miRNA- or siRNAs-mediated repression of target genes. The RISC complex targets miRNAs or siRNAs to specific mRNAs that are cleaved or translationally inhibited [83]. Through reducing the polyA tail of mRNA and inhibiting translation, all four members of the AGO family (AGO1 to AGO4) can repress target gene expression [84]. Only AGO2, however, has slicer activity that cleaves the target mRNA [83]. It has been found that *Ago2* mRNA is expressed in oocytes and throughout all early embryonic developmental stages [32], and that AGO2 protein is localized to the cytoplasmic P-bodies in oocytes that serve as sites for mRNA destruction and decay [85].

By traditional gene targeting, Morita et al. [30] demonstrated that *Ago2*^{-/-} mice exhibit embryonic lethality at around E5.5. In another study of *Ago2* conditional knockout mice, *Ago2* function was disrupted by crossing mice with an *Ago2* floxed conditional allele and *Zp3*-Cre transgenic mice [84]. Ovaries from homologous *Ago2* conditional knockout (*Ago2*^{F/F}-*Zp3*-Cre) females appear to be normal, and MII oocytes are morphologically indistinguishable from wild-type oocytes in their appearance, maturity, size, and numbers. However, spindle formation and chromosome configuration are abnormal. Moreover, miRNA is decreased by about 80% in *Ago2* knockout oocytes as compared with wild-type oocytes. These results indicated that *Ago2* is essential for the biogenesis or stability of mRNAs during oogenesis. In addition, mutant embryos fail to undergo the first cleavage event and are fragmented. Therefore, *Ago2*^{F/F}-*Zp3*-Cre female mice are in-

fertile [31].

Following the microinjection of *Ago2* dsRNA into the cytoplasm of pronuclear embryos, embryonic development arrests at the 2C stage and development does not progress to the blastocyst stage. Specifically, *Ago2* dsRNA-injected embryos exhibit a 90% knockdown of *Ago2* mRNA [32]. These results led to the conclusion that *Ago2* is a mammalian MEG that is essential for degradation of a proportion of maternal transcripts that is mediated by the RNAi pathway.

Gene knockdown system

To assess the function of target genes during oocyte maturation and early embryogenesis, dsRNA, siRNA, antisense morpholino oligonucleotides, or specific antibodies are microinjected into oocytes or zygotes resulting in a loss-of-function for the target gene. This system is a well-established and useful tool for gene function analysis.

1. Tif1α (Transcriptional intermediary factor 1 alpha; Trim24)

A transcriptional regulator of nuclear receptors, TIF1α has been shown to interact with proteins related to chromatin structure [86]. *Tif1a* transcripts are expressed from GV oocytes to blastocysts. Additionally, *Tif1a* mRNA is present in all blastomeres, but is restricted to the ICM when blastocysts are formed. TIF1α protein is also expressed in the cytoplasm of oocytes, but it moves to the pronuclei at the zygote stage after fertilization. During embryogenesis, TIF1α protein remains in nucleolar-like bodies and is associated with transcription machinery factors and chromatin remodelers such as Snf2h and Brg1. Therefore, disruption of *Tif1a* leads to mislocalization of RNA polymerase II, Snf2h, and Brg1. Lastly, TIF1α modulates gene expression during ZGA via Snf2h [33].

Tif1a dsRNA was microinjected into the cytoplasm of zygotes in a study by Torres-Padilla and Zernicka-Goetz [33]. In this study, the majority of embryos injected with *Tif1a* dsRNA arrest between the 2C and 4C stage upon efficient knockdown of TIF1α protein. In addition, microinjection of antibodies into the cytoplasm of zygotes blocks TIF1α protein, and injected embryos arrest at the 2C to 4C stage of development. These results suggest that *Tif1a* is essential for early embryogenesis [33].

2. Oct4 (Octamer-binding transcription factor 4; Pou5f1)

The *Oct4* gene is expressed in a tissue-specific pattern during early mammalian development [87]. *Oct4* expression appears in oocytes and pluripotent early embryos. In mouse blastocysts, *Oct4* transcripts and proteins are only present in the ICM but not in the trophectoderm [88]. As one of the Yamanaka factors, *Oct4* induces epigenetic reprogramming of a somatic genome to an embryonic pluripotent state [89]. Using a traditional knockout approach, male and female

mice that are heterozygous for *Oct4* deletion are fertile but have 50% less pups than wild-type mice. *Oct4*-deficient embryos can develop up to the blastocyst stages, but ICM cells are not pluripotent. Therefore, *Oct4* disruption results in peri-implantation lethality before egg cylinder formation and offspring are not produced [34].

Oct4-morpholino was microinjected into the cytoplasm of zygotes by Foygel et al. [35]. The results of this study demonstrate that embryos lacking OCT4 are mainly blocked at the zygote and/or morula stages and do not develop to the blastocyst stage. The embryos are rescued and develop to the blastocyst stage when the embryos are co-injected with *Oct4* mRNA and morpholino. These results suggest that *Oct4* is important for early embryogenesis [35].

3. *Sebox* (Skin-embryo-brain-oocyte homeobox)

We recently applied RNAi as a tool for functional analysis of genes expressed in oocytes. To determine the role played by target genes in oocyte maturation and preimplantation embryo development, RNAi was used to knockdown target gene expression in the mouse. While we carried out RNAi for several genes, we could categorize transcripts in oocytes into three groups, with loss-of-function stopping meiosis at GV [90], MI [91-95], and MII [2,3]. Therefore, transcripts in oocytes could be categorized as genes required for embryonic development and for oogenesis, as suggested by Dean [96].

The *Sebox* gene encodes a protein with a homeodomain motif that binds DNA and regulates gene expression [97]. *Sebox* transcripts are highly expressed in GV oocytes to stage 2C embryos, but expression is markedly decreased in later embryonic developmental stages [2]. Following the microinjection of *Sebox* dsRNA into the cytoplasm of GV oocytes, we found that the oocytes develop to the MII stage with normal configurations of spindles and chromosomes without changes in the activity of important kinases, such as maturation-promoting factor (MPF) and mitogen-activated protein kinase. However, when *Sebox* dsRNA was microinjected into the pronuclear embryos, we found that embryo development is inhibited at the 2C stage. Therefore, we conclude that *Sebox* is crucial for normal early embryogenesis and propose *Sebox* as a new mammalian MEG candidate [2].

4. *Gas6* (Growth arrest-specific gene 6)

A ligand for AXL, TRY3, and MERTK receptor tyrosine kinases, GAS6 is a member of the vitamin K-dependent protein family and is implicated in growth arrest, cell proliferation, differentiation, and cell survival [98,99]. GAS6 plays a pivotal role in thrombosis, hematosi, and spermatogenesis [99,100]. Similar to the expression pattern of other MEGs, expression of *Gas6* is constitutive throughout oocyte maturation, gradually decreases from the pronuclear to the 4C-stage embryo, and slightly increases again from 8C to blastocyst-stage embryos [3].

To investigate the role of GAS6 in oocyte maturation and fertilization using RNAi, we microinjected *Gas6* dsRNA into the cytoplasm of GV oocytes. Interestingly, despite the marked reduction in *Gas6* expression after *Gas6* RNAi, nuclear maturation is not affected, including spindle structures and chromosome segregation. However, *Gas6* RNAi increases the phosphorylation of Tyr15 in p34^{cdc2} and MPF reactivation fails after the first polar body emission [3]. To confirm that cytoplasmic maturation was deficient, we evaluated the change in Ca²⁺ oscillation and cortical granule exocytosis and the rates of sperm penetration and pronuclear formation. Due to insufficient cytoplasmic maturation, *Gas6*-silenced MII oocytes exhibit markedly decreased cytoplasmic Ca²⁺ excitability, a lack of cortical granule exocytosis, and fertilization failure, especially pronuclear formation. Therefore, we propose *Gas6* as another new candidate MEG vital for oocyte cytoplasmic maturation and normal fertilization [3].

Transgenic RNAi

Basonuclin and CCCTC-binding factor (*Ctcf*) have been identified as MEGs using an oocyte-specific gene-targeting method. These transcripts have been depleted from growing oocytes using transgenic RNAi under the control of the *Zp3* promoter [36,37].

1. Basonuclin

Basonuclin, a zinc-finger protein, regulates rRNA transcription. Basonuclin co-localizes with RNA polymerase I (Pol I) activity in the nucleolus of mouse oocytes, and basonuclin mutation interferes with Pol I transcription [101]. *Basonuclin* mRNA has been detected in oocytes and zygotes, but its levels rapidly decrease at the 8C stage and disappear at the blastocyst stage. In a study by Ma et al., transgenic basonuclin-RNAi mice have apparently normal morphology, but female mice are subfertile with normal oocyte maturation and ovulation [36]. The ovaries from *basonuclin*-RNAi transgenic female mice exhibit normal size, weight, and number of follicles. However, some follicles have abnormal morphologies. For instance, *basonuclin*-deficient oocytes are biochemically abnormal and contain cytoplasmic dark granules, which are not detected in wild-type oocytes. Both RNA Pol I- and Pol II-mediated transcription is perturbed in *basonuclin*-deficient oocytes. Additionally, *basonuclin*-deficient embryos fail to develop beyond the 2C stage, which accounts for the observed subfertility. Thus, the authors suggest that basonuclin is a MEG due to the fact that *basonuclin* deficiency results in phenotypes that are similar to those for previously reported mouse MEGs [36].

2. *Ctcf*

CTCF is a multifunctional transcription factor with 11 zinc-finger DNA-binding domains. CTCF is proposed to be a candidate trans-act-

ing factor for X chromosome selection in the mouse because CTCF plays an essential role in controlling the epigenetic switch for X inactivation [102]. It is also involved in the regulation of epigenetics and early embryonic development [37]. Using the transgenic RNAi-based approach, *Ctcf*-deficient oocytes express reduced levels of CTCF with increased methylation of histone H19 and decreased competency of embryonic development [103].

The function of *Ctcf* as a MEG was identified using transgenic mice expressing *Ctcf* dsRNA under the control of the *Zp3* promoter [103]. *Ctcf*-deficient oocytes are able to undergo delayed GVBD, and are observed in anaphase or telophase of the first meiosis, which reduces the emission of the first polar body. *Ctcf*-deficient embryos reach the 2C stage, but are developmentally delayed at the 2C to 4C transition. These data suggest that disruption of *Ctcf* affects the ZGA at the 2C stage, thus arresting embryos at one cell division. Only 7% of cultured *Ctcf*-deficient embryos develop to the blastocyst stage and almost all of the *Ctcf*-deficient embryos arrest at the morula stage with high levels of apoptosis.

Gene-trap mutagenesis

Gene-trap mutagenesis is a technique that randomly generates loss-of-function mutations [104]. Gene-trap is introduced using an insertional gene trap cassette that consists of a reporter gene, a selectable genetic marker, and a poly A site or stop codon. When inserted into an intron of an actively expressed gene, the gene trap vector is transcribed from the endogenous promoter of that gene. A fusion transcript is formed such that the expressed inserted reporter and selectable marker are terminated prematurely at the inserted stop codon. This processed fusion transcript encodes a truncated and non-functional cellular protein [104].

1. Floped (oocyte expressed protein, Ooep)

FLOPED is a key component for SCMC that assembles during oogenesis. The SCMC consists of FLOPED, TLE6, Folia, and Mater and is essential for the first embryonic cell division. The expression of *Floped* transcripts is first detected at E15.5 and peaks 1 week after birth. *Floped* transcripts do not appear in other tissues including testis, but rather only in growing oocytes in mouse ovaries. During oocyte maturation and ovulation, *Floped* transcripts are degraded and are no longer detected by the 2C stage of early embryogenesis. FLOPED protein, however, persists during early embryogenesis up to the blastocyst stage [38].

The *Floped* gene contains three exons, of which a gene trap vector was inserted between exon1 and exon2. *Floped* mutant animals express a mutant FLOPED-β-galactosidase fusion protein. *Floped^{tm/tm}* mice are viable, survive to adulthood, and appear to be normal. Ovaries in

Floped^{tm/tm} female mice show normal histology with follicles at all developmental stages within the corpora lutea. These results suggest that *Floped^{tm/tm}* female mice have normal processes of folliculogenesis and ovulation. Oocytes from *Floped^{tm/tm}* female mice are also morphologically indistinguishable from their wild-type counterparts, and a comparable number of normal-appearing GV oocytes are recovered from these animals. In *Floped^{tm/tm}* female mice, progression from zygotes to 2C embryos is delayed by 6–8 hours, and blastomeres are cleaved into unequal sizes with demonstrable fragmentation. Additionally, *Floped^{tm/tm}* female mice produce no offspring and are sterile [38].

Conclusion

The molecular basis for the processes of oocyte maturation, fertilization, and successful early embryo development are mainly dependent on components that are stored in oocytes and encoded by MEGs. Mouse transgenesis plays an essential role in identifying the genes that are important for normal development in mammals. Using this approach, oocytes or embryos derived from mothers that are homozygous null for a given MEG have disrupted genetic expression of that MEG and provide for analysis of the loss-of-function phenotypes. However, the function of some MEGs cannot be addressed by mouse mutagenesis. The functions of these MEGs are studied using gene knockdown systems. Through current studies that use new molecular biological techniques, the working mechanisms by which MEGs regulate early embryo development have been revealed one at a

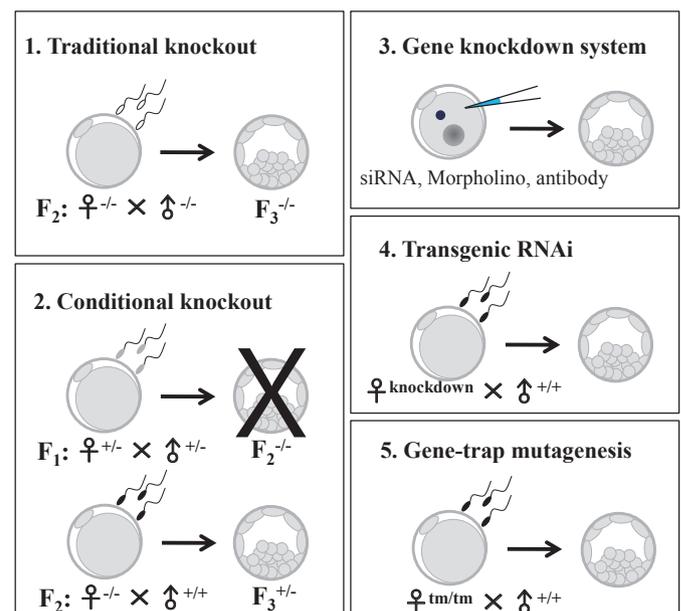


Figure 1. Summary of the various technical approaches for identifying maternal effect genes.

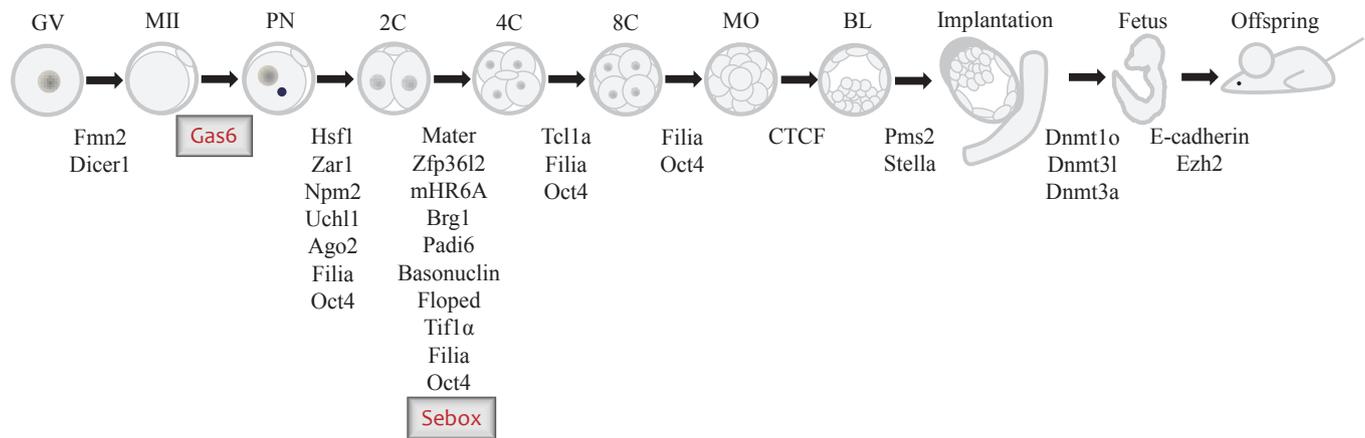


Figure 2. Schematic diagram showing arrested embryonic stages after interference of maternal effect gene expression by various gene knock-out approaches, including the tissue-specific Cre-loxP system, gene knockdown system, transgenic ribonucleic acid interference, and gene-trap mutagenesis in the mouse. GV, germinal vesicle; MII, metaphase II; PN, pronucleus stage; 2C, 2-cell stage; 4C, 4-cell stage; 8C, 8-cell stage; MO, morula stage; BL, blastocyst stage.

time. In this review, we have summarized the molecular techniques used for the identification of MEGs (Figure 1), findings of the loss-of-function of those genes, and the resulting phenotypes related to male and female fertility and reproduction.

Although only a small number of mammalian MEGs have been found to date, we believe that there will be an exponential increase in the discovery of other MEGs. Our recent identification of SEBOX and GAS6 as new candidate MEGs through the knockdown of these genes *in vitro* has added to the short list of known MEGs (Figure 2). Increasing the amount of evidence regarding the molecular mechanisms of MEGs in the mouse provides a better understanding of the regulation of oocyte maturation, fertilization, and normal early embryonic development in human reproduction.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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