Extending the maternal–zygotic effect with genomic imprinting

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ABSTRACT: Maternal effect refers to the genetic phenomenon in which a phenotype in the progeny is caused by a genetic mutation in the maternal genome rather than a mutation of its own. Maternal effect genes are usually involved in the maternal-to-zygotic transition during embryonic development before zygotic genes are turned on. Although it is widely observed in invertebrate organisms, it is not common in vertebrate animals, especially in mammals. Genomic imprinting is an epigenetic phenomenon that is unique to eutherian mammals, marsupials and plants. One characteristic of genomic imprinting is parental origin-specific expression of imprinted genes. The molecular mechanisms underlying genomic imprinting are poorly understood. Mouse Zfp57 is the first example of a mammalian maternal–zygotic effect gene and it exhibits maternal–zygotic embryonic lethality around midgestation when both maternal and zygotic functions of Zfp57 are absent. Loss of Zfp57 also results in loss of differential DNA methylation at multiple imprinted regions. Interestingly, the midgestational embryonic lethality due to loss of both maternal and zygotic functions of Zfp57 occurs much later than the typically observed maternal–zygotic embryonic lethality during the maternal-to-zygotic transition period in early preimplantation embryos. I hypothesize that gradual loss of heritable genomic DNA methylation imprints over many cell divisions could account for this spatial and temporal discrepancy between the causative molecular defect and the observed phenotype in the Zfp57 mutant.

Key words: DNA methylation / embryo development / epigenetics / imprinting / maternal–zygotic effect

Introduction

Maternal effect, zygotic effect and maternal–zygotic effect

Parental influence on the development of progeny has been observed in both invertebrate and vertebrate animal kingdoms. There are more examples of maternal influence on the development of the progeny than those of paternal influence. In invertebrates and occasionally in vertebrates, maternal effects are conferred by the deposition of cytoplasmic transcripts or proteins in oocytes (Fig. 1). These then exert their effects on fertilized zygotes as well as on patterning of the early embryos (Priess et al., 1987; Nusslein-Volhard, 1991; Newman-Smith and Rothman, 1998; De Robertis et al., 2000; Bourc'his et al., 2001; Howell et al., 2001; Schier, 2001, 2007; Gosden, 2002; Stitzel and Seydoux, 2007). A zygotic mutant is generated when a heterozygous female is used in the cross to give rise to a homozygous progeny (see Cross I of Fig. 1). In contrast, a maternal–zygotic mutant is obtained when a homozygous female is used in the cross with a heterozygous male to produce a homozygote (see Cross II of Fig. 1). In the meantime, a heterozygote (−/+), lacking just the maternal function is also obtained in Cross II.

Maternal effects are usually manifested before transcription of the zygotic genes (Schier, 2007; Stitzel and Seydoux, 2007). In the mouse, zygotic transcription does not start until the 2-cell stage (Schultz, 2002). It has been reported that about one-third of the total maternal transcript is lost between the stages of 8-cell mouse embryos and blastocysts (Bachvarova and Moc, 1985). Some of the known maternal effect genes are required between 2-cell stage and 8-cell stage embryos (Christians et al., 2000; Tong et al., 2000; Wu et al., 2003). Hence, maternal gene products are required for the maternal-to-zygotic transition during embryonic development. However, some phenotypes caused by loss of maternal gene products are not manifest until well beyond the maternal-to-zygotic transition period, even into adulthood (Leader et al., 2002; Payer et al., 2003; Wagner et al., 2004). Indeed, in a recent large-scale screen for maternal effect genes in zebrafish, a significant number of the isolated mutants were found to cause developmental arrest at the midblastula transition, much later than the maternal–zygotic transition period (Wagner et al., 2004).

The apparent maternal–zygotic embryonic lethality conferred by the mutation in the Zfp57 gene is, to our knowledge, the first instance to be reported in mammals (Li et al., 2008). Among all genetic crosses...
tested, midgestation embryonic lethality was only observed in the maternal–zygotic mutants, i.e. homozygous mutant embryos derived from homozygous mutant females (unfilled circles in Crosses 3 and 5 of Fig. 2). This embryonic lethality was not present in the zygotic mutants derived from heterozygous females (the circle with green dots in Cross 4 of Fig. 2) or in the heterozygous progeny lacking maternal Zfp57 (the circles with red dots in Crosses 2 and 5 of Fig. 2). In contrast, there are many examples of maternal–zygotic effect genes in invertebrates such as *Drosophila melanogaster* and *Caenorhabditis elegans* (Robbins, 1980; Perrimon et al., 1989; Moskowitz and Rothman, 1996; Perrimon et al., 1996; Melendez and Greenwald, 2000; Bellotto et al., 2002; Roberts et al., 2003). Many maternal effect mutants have been isolated in a recent genetic screen carried out in zebrafish (Wagner et al., 2004), however, none displays this maternal–zygotic lethal phenotype. As suggested by Wagner et al. (2004), it is possible that such screenings are not saturated and that the essential zygotic function for some maternal–zygotic effect genes may preclude the isolation of this class of mutants. Indeed, two studies suggest that this class of maternal effect genes do exist in zebrafish (Ciruna et al., 2002; Lunde et al., 2004).

**Genomic imprinting**

Cytoplasmic factor-mediated parental control in mammals is not as common as it is in invertebrates. The volume of the cytoplasm of mammalian oocytes is much smaller compared with that of their invertebrate counterparts. Therefore, there is less capacity to package maternal gene products in the cytoplasm of the vertebrate oocytes. Genomic imprinting, which is mediated by epigenetic modification of the genome in the germline, plays a more dominant role in the parental control of the development and survival of mammalian embryos (Tilghman, 1999; McGrath and Solter, 1984; Surani et al., 1984; Reik et al., 2001).

The term ‘imprinting’ was originally used by Crouse in 1960 to describe the inheritance of maternal and paternal chromosomes in *Sciara* (Cattanach, 1986). Failure of parthenogenetic embryos as well as failure of the embryos that inherited two complete sets of either maternal (gynogenetic embryos) or paternal (androgenetic embryos) chromosomes to develop properly suggested that parental-specific expression of genes does exist in mammals (Graham, 1974; Surani and Barton, 1983). Nuclear transfer experiments further demonstrated that maternal and paternal haploid genomes are not functionally equivalent in mammalian embryonic development (McGrath and Solter, 1984; Surani et al., 1984). It was then hypothesized that specific imprinting occurs during gametogenesis so that the presence of both sets of chromosomes is required for the proper development of fertilized oocytes. Genomic imprinting is observed in eutherian and marsupial mammals as well as in plants (Feil and Berger, 2007; Jullien and Berger, 2009; Renfree et al., 2009; Law and Jacobsen, 2010). Currently, about 100 imprinted genes have been identified in the mouse (listed in the Harwell imprinting website http://www.mgu.har.mrc.ac.uk/research/imprinting/). It was estimated that about 1% of genes are imprinted and some unknown imprinted genes have yet to be identified (Koerner et al., 2009). Genomic imprinting could be either maternal or paternal, with the phenotypic consequences manifested relatively late in development or even into adulthood (Reik et al., 1993; Bourc'his et al., 2001; Bartolomei, 2009). Interestingly, some imprinted genes show tissue-specific imprinting patterns and many unidentified imprinted genes may belong to this class of imprinted genes (Bartolomei, 2009).

Many of the imprinted genes are clustered and co-regulated by a cis-acting imprinting control region termed ICR (Ben-Porath and Cedar, 2000; Verona et al., 2003; Lewis and Reik, 2006). These include the widely studied *Igf2-H19*, *Dlk1-Dio3*, *IgfZr*, *Snprn* and *Kcnql* clustered imprinting regions (Verona et al., 2003; Lewis and Reik, 2006). One of the hallmarks of the ICRs is that they contain a germline-derived differentially methylated region (DMR) at the CpG sites of an ICR (Edwards and Ferguson-Smith, 2007). It has been shown that ICRs can exert long-range cis-acting regulatory control over regions as large as several million base pairs (Edwards and Ferguson-Smith, 2007; Bartolomei, 2009). Differential DNA methylation at the CpG sites in the ICR regions is established in the female or male germline and is protected from the genome-wide demethylation which occurs in early embryos (Tilghman, 1999). Whereas DNA methylation in non-imprinted regions is erased during this process, differential DNA methylation at the imprinted ICR regions is stably maintained during embryogenesis (Howell et al., 2001). Two distinct proteins that both display maternal effect in mouse have been found to be involved in the stable maintenance of DNA methylation imprints in mouse embryos. Maternal *Stella/PGC7* plays a partially protective role in maintaining DNA methylation imprints at multiple imprinted regions (Payer et al., 2003; Nakamura et al., 2007), whereas loss of both the maternal and zygotic functions...
of Zfp57 results in complete loss of methylation imprints at a large subset of imprinted domains (Li et al., 2008). The protective roles of Stella/PGC7 and ZFP57 in DNA methylation imprints are partially overlapping and not mutually exclusive, suggesting that at least two different complexes or pathways are involved in the maintenance of DNA methylation imprints in mouse embryos.

DNA methylation usually occurs at the CpG sites in mammals (Suzuki and Bird, 2008). Cytosine methylation is carried out by DNA methyltransferases (Chen and Li, 2004; Klose and Bird, 2006). Loss of function studies in mouse as well as in cell culture have demonstrated that DNMT3A and DNMT3B, together with their stimulating DNA methyltransferases, whereas DNMT1 is the major maintenance DNA methyltransferase (Li et al., 1992, 1993; Okano et al., 1999; Bourc’his et al., 2001; Kaneda et al., 2004). In contrast, it is less clear which proteins may be involved in DNA demethylation in mammals. Recently, it has been shown that some components of the DNA repair pathway, in particular GADD45, may contribute to this process (Barreto et al., 2007; Rai et al., 2008; Gehring et al., 2009; Ma et al., 2009). Interestingly, it was just reported that elongator complex protein 3 (ELP3) functions in the active demethylation of male pronuclei in early embryos (Okada et al., 2010). Furthermore, activation-induced deaminase (AID) which was originally discovered to play a role in generating antibody diversity in the immune system is found to be involved in demethylation in the primordial germ cells as well as in reprogramming of induced pluripotent cells (Bhutani et al., 2010; Popp et al., 2010).

There is ample experimental evidence suggesting that there is some cross-talk between DNA methylation and histone modifications. Indeed, DNMT3L binds to the N-terminal tail of histone H3 without methylation at H3K4 (Ooi et al., 2007). It has also been shown that the histone demethylase KDM1 (LSD1) is required for the maintenance of global DNA methylation through regulating the stability of the DNMT1 protein via lysine demethylation by KDM1 (Nicholson and Chen, 2009; Wang et al., 2009). Interestingly, a related protein named KDM1B that is specifically expressed in the germine plays an important role in the establishment and maintenance of DNA methylation genomic imprints specifically in the female germine (Ciccone et al., 2009). Furthermore, depletion of histone H1 in...
embryonic stem cells affects the expression of multiple imprinted genes (Fan et al., 2005). Future work aim at identifying the key histone modifications essential for DNA methylation in the imprinted regions will shed light on mechanistic link between these two epigenetic processes in genomic imprinting.

There are many imprinting related diseases. Hypomethylation at the IGF2-H19 imprinted region can lead to a growth retardation disease called Silver–Russell syndrome (SRS) whereas hypermethylation at the same imprinting control region can give rise to the Beckwith–Wiedemann syndrome (BWS) with an overgrowth phenotype (Das et al., 2009). Molecular defects in the SNRPN imprinted region are associated with Prader–Willi syndrome (PWS) and Angelman syndromes (AS) (Das et al., 2009). Both syndromes are characterized by neurologic, developmental and behavioral problems with early childhood obesity and short stature as prominent features in PWS patients and severe mental retardation, seizures and ataxia as prominent features in AS patients. Loss of imprinting has also been associated with predisposition to cancer (Robertson, 2005).

**New developments**

In a screen for genes down-regulated during ES cell differentiation, we identified the Zfp57 gene (Li and Leder, 2007). Zfp57 encodes a zinc finger protein that has a Kruppel associated box (KRAB) domain in the amino-terminus followed by C2H2-type zinc fingers in the carboxyl end (Li et al., 2008). KRAB zinc finger proteins are unique to vertebrates and possibly only present in tetrapod animals (Bellefroid et al., 1991; Looman et al., 2002; Shannon et al., 2003). This family of proteins underwent lineage-dependent expansion during evolution. Xenopus has only a few KRAB box-containing proteins whereas there are over 300 members in the human genome. KRAB zinc finger proteins can recruit a co-repressor called KAP1/TIF1β/Trim28 which in turn can recruit HP1, MBD3, HDAC and other histone modifying and DNA methylation modifying enzymes (Friedman et al., 1996; Ryan et al., 1999; Abnik et al., 2001; Matsuda et al., 2001; Schultz et al., 2001; Schultz, 2002). These proteins can mediate the transition from euchromatin to heterochromatin at the target genes and the established repressive state is mitotically stable based on an in vitro cell culture study with an artificial KRAB zinc finger protein (Ayyananathan et al., 2003). Interestingly, an artificial KRAB zinc finger protein and the co-repressor KAP1/TIF1β/Trim28 could indeed initiate de novo DNA methylation during mouse embryogenesis (Wiznerowicz et al., 2007). KAP1/TIF1β/Trim28 and the KRAB zinc finger protein ZFP809 have also been shown to be involved in silencing of retroviruses in embryonic stem cells (Wolf and Goff, 2007, 2009).

Zfp57 is a maternal–zygotic effect gene in mouse (Li et al., 2008). Loss of just the zygotic function of Zfp57 causes partial neonatal lethality. Eliminating both the maternal as well as the zygotic functions of Zfp57 results in highly penetrant maternal–zygotic embryonic lethality (unfilled circles in Crosses 3 and 5 of Fig. 2). Interestingly, Zfp57 is required for the maintenance of DNA methylation imprints at multiple imprinted regions (Li et al., 2008). Loss of just the zygotic function of Zfp57 causes partial loss of differential DNA methylation at both the IG-DMR and the Snrpn DMR in zygotic mutant midgestation embryos (Fig. 3B), whereas eliminating both the maternal and the zygotic functions of Zfp57 results in complete loss of differential DNA methylation in these two imprinted regions (Fig. 3C). As indicated by Zygote (1) in Fig. 4, differential DNA methylation is present at both the IG-DMR and the Snrpn DMR of the zygote formed after fertilization of the sperm and the oocyte derived from a wild-type male mouse and a wild-type female mouse, respectively. Similarly, differential DNA methylation is also present in the zygote [see Zygote (2) in Fig. 4] formed from the cross between a heterozygous female mouse and a homozygous male mouse. Consistent with the partial neonatal lethality observed in the zygotic mutant (Li et al., 2008), loss of just the zygotic function of Zfp57 causes partial loss of differential DNA methylation at both the IG-DMR and the Snrpn DMR of the zygotic mutant midgestation embryo [Fig. 3B and the −/− midgestation embryo derived from Zygote (2) in Fig. 4]. In contrast, highly penetrant embryonic lethality is observed in maternal–zygotic mutant midgestation embryos (Li et al., 2008). Consistent with this, eliminating both the maternal and the zygotic functions of Zfp57 leads to complete loss of germline-derived DNA methylation imprints at both the IG-DMR and the Snrpn DMR even though differential methylation is present at the IG-DMR in the zygote formed after fertilization from the cross between a homozygous female mouse and a homozygous male mouse [Fig. 3C and the −/− midgestation embryo derived from Zygote (3) in Fig. 4]. These data suggest that imprinting control regions are direct downstream target regions of ZFP57. Indeed, ZFP57 can bind to the Snrpn imprinted region in a chromatin immuno-precipitation (ChIP) assay (Li et al., 2008). Another interesting finding is that differential methylation at the Snrpn DMR can be re-acquired in the presence of zygotic ZFP57 in heterozygous midgestation embryos despite the fact that the Snrpn DMR is not methylated in the oocyte or in the zygote derived from a null female mouse [the −/+ midgestation embryos derived from Zygote (4) in Fig. 4]. Furthermore, this re-acquisition occurs in an allele-specific manner so that differential DNA methylation is only re-acquired at the Snrpn DMR of the maternal chromosome. This result suggests that either there is additional differential DNA methylation outside the defined Snrpn DMR that serves as an imprinting mark or there is some kind of DNA methylation-independent imprinting memory that can initiate de novo methylation at the Snrpn DMR. We favor the second hypothesis which is also supported by a recent finding on differential DNA methylation at the Snrpn DMR (Li et al., 2008; Kaufman et al., 2009). Consistent with these results, the imprinted regions still retained a memory of their parental origins even after their DNA methylation imprints had been erased (Davis et al., 2000; Lucifero et al., 2004). It has also been found previously that the imprinting status could be maintained at some placental genes by repressive histone marks in the absence of DNA methylation imprinting marks (Lewis et al., 2004; Reik et al., 2004; Umlauf et al., 2004). Interestingly, de novo DNA methylation appears to be dispensable for the establishment of maternal imprinting present on the X chromosome in the mouse that is essential for preferential inactivation of the paternal X chromosome during preimplantation development and trophoblast differentiation (Chiba et al., 2008).

It was reported that mutations in the human homolog of Zfp57 are also associated with hypomethylation at multiple imprinting control regions (Mackay et al., 2008). It was also shown that mutations in human ZFP57 cause transient neonatal diabetes (TND), cardiovascular defects and other developmental abnormalities (Hirasawa and Feil, 2008; Mackay et al., 2008). We found that loss of Zfp57 results in
loss of differential DNA methylation at multiple imprinted regions including the Dlk1-Dio3 imprinted domain (Li et al., 2008). Human patients with deletions and epimutations affecting the Dlk1-Dio3 imprinted domain exhibited phenotypes reminiscent of human patients carrying upd(14), i.e. uniparental disomy of chromosome 14 (Kagami et al., 2008). Intriguingly, the Dlk1-Dio3 (also called Dlk1-MEG3) imprinted domain was just found to be associated with susceptibility to type 1 diabetes (Wallace et al., 2010). It will be interesting to find out in the future if loss of Zfp57 will confer susceptibility to type 1 diabetes as well.

The clinical symptoms of TND include growth retardation and diabetes at birth followed by remission and reoccurrence later in life (Das et al., 2009). Mutations in the human Zfp57 gene are associated with TND. Besides Zfp57, the imprinted PLAGL1 region is another major candidate disease locus for TND (Mackay et al., 2005, 2008). Thus, dysregulation of genomic imprinting could be the leading cause of TND. Future research focused on differential DNA methylation at other imprinting regions, especially the target regions of Zfp57 such as the Dlk1-Dio3 imprinted domain, may lead to identification of additional genetic lesions that can cause TND. This can serve as the basis for prenatal testing of human patients that are predisposed to TND.

**Figure 3** Heritable genomic imprinting defect present in the Zfp57 mutants can delay the onset of phenotypes due to loss of maternal Zfp57. As shown in the diagrams, maternal Zfp57 gene product (green dots) is deposited in the zygote and persists in preimplantation embryos. However, it is presumably degraded in post-implantation embryos (small green dots) and completely missing in midgestational embryos. Zygotic Zfp57 gene product (red dots) is expressed in preimplantation embryos and continues to be produced in post-implantation and midgestational embryos. Paternal chromosomes are indicated by blue lines. Black circles, methylated CpGs; White circles, unmethylated CpGs; Shaded circles, partially methylated CpGs. (A) Differential DNA methylation is present in the maternally derived Snrpn DMR (SN) and paternally derived IG-DMR (IG) of the Dlk1-Dio3 imprinted region upon fertilization and genomic DNA methylation imprints are stably maintained at both DMRs throughout embryogenesis in wild-type (+/+) embryos. (B) Maternal Zfp57 gene product is present in the oocyte derived from a heterozygous (+/−) female mouse even though it contains a deleted allele at the Zfp57 locus. Owing to partially overlapping patterns of maternal and zygotic Zfp57 gene product, it is hypothesized that both Snrpn DMR (SN) and IG-DMR (IG) start to lose differential DNA methylation in post-implantation zygotic mutant embryos and DNA methylation imprints are partially lost in the midgestational zygotic mutant embryos at both DMRs. (C) DNA methylation imprint is not established at the Snrpn DMR in the oocyte derived from a homozygous (−/−) female mouse. Differential methylation at the Snrpn DMR remains absent in maternal–zygotic mutant embryos throughout embryogenesis. In contrast, differential methylation at the IG-DMR is present in the zygote and persists in preimplantation embryos since the DNA methylation imprint at the IG-DMR is normally established in the sperm of homozygous male mice. However, this DNA methylation imprint at the IG-DMR starts to be lost in post-implantation maternal–zygotic mutant embryos and is completely missing in midgestation maternal–zygotic mutant embryos due to the absence of both maternal and zygotic Zfp57 gene product.

**Implications**

Maternal–zygotic embryonic lethality in invertebrate animals usually occurs very early (Newman-Smith and Rothman, 1998). However, a majority of embryonic lethality due to loss of both maternal and zygotic functions of Zfp57 does not occur until midgestation (Li et al., 2008). Although we found maternal ZFP57 is present in preimplantation embryos (Li et al., 2008), it is unlikely that maternal ZFP57 will persist long enough to be present in midgestational embryos (Fig. 3A and B). There appear to be spatial and temporal differences between the presumed time window of the maternal Zfp57 activity (Fig. 3A and B) and the observed phenotype (embryonic lethality around midgestation) caused by loss of both the maternal and the zygotic functions of Zfp57 (Fig. 3C). I hypothesize that gradual loss of heritable DNA methylation imprints (Fig. 3C) that are usually stably maintained during embryogenesis in wild-type individuals (Fig. 3A) can account for the spatial and temporal discrepancy between the molecular defects and the observed phenotypes in response to loss of both the maternal and the zygotic functions of Zfp57 (Fig. 3C). Because of the heritability of differential DNA methylation imprint at the imprinted regions, the target genes in the imprinted regions can be...
Figure 4  Methylation cycle at the imprinted IG-DMR and Snrpn DMR. Paternal chromosomes are indicated by blue lines. IG, IG-DMR; SN, Snrpn DMR; Black circles, methylated CpGs; White circles, unmethylated CpGs; Shaded circles, partially methylated CpGs; −/−, a homozygous Zfp57 mutant embryo; +/−, a heterozygous Zfp57 embryo derived from a null female. Differential DNA methylation at the CpG sites of ICRs is reset during gametogenesis. In sperm, the CpG sites at the IG-DMR are methylated whereas those at the Snrpn DMR are unmethylated. In contrast, methylation occurs at the Snrpn DMR but not at the IG-DMR in the oocytes derived from wild-type females. Upon fertilization, differential methylation is present at the ICRs of the wild-type zygote indicated as Zygote (1). These germline-derived differential DNA methylation imprints are resistant to genome-wide pronuclear demethylation in the zygote and stably maintained in wild-type embryos. Similar to what is observed in the oocytes derived from wild-type females, methylation imprint is normally established at the Snrpn DMR in the oocytes derived from heterozygous female mice in the presence of the maternal ZFP57. Without the paternal ZFP57, methylation imprint is still established at the IG-DMR of the sperm derived from homozygous male mice. Therefore, DNA methylation imprint is presumed to be present at both the IG-DMR and the Snrpn DMR of the mutant zygote indicated as Zygote (2) formed upon fertilization in the cross between a heterozygous female and a homozygous male. DNA methylation imprint is not established at the CpG sites of the Snrpn DMR in the oocytes derived from Zfp57 homozygous mutant female mice lacking the maternal ZFP57 and accordingly it is also absent in the heterozygous (+/−) zygote indicated as Zygote (3) formed upon fertilization in the cross between a wild-type male and a homozygous mutant female. Intriguingly, differential DNA methylation is re-acquired allele-specifically at the maternal chromosome of the Snrpn DMR in the presence of the zygotic ZFP57 (−/+), in about 50% of the heterozygous (+/−) midgestational embryos derived from null female mice (Li et al., 2008).

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expressed at late stages of embryogenesis or even in adulthood. Accordingly, the phenotypes due to abnormal expression of the target genes can manifest later in development in spite of the presence of molecular defects in early embryos. For example, the Ube3a imprinted gene at the Snrpn imprinted region that has been implicated in AS with clinical symptoms such as seizure and mental retardation is only expressed in the brain even though the imprinting status has already been established in preimplantation embryos (Rougeulle et al., 1997; Butler, 2009; Weaver et al., 2009; Fig. 3).

Spatiotemporal separation of the molecular defect (directly caused by loss of a given gene) and the observed phenotype is a particularly interesting finding in our genetic analysis of the Zfp57 mutant with respect to development and disease. Usually there is close correlation between gene expression patterns and observed phenotypes due to loss of a given gene. In the case of Zfp57, it does not need to be active in a particular tissue or organ, e.g., the heart, as long as the target genes of Zfp57 such as the imprinted genes located at the Dlk1-Dio3 imprinted region are expressed in the cardiovascular system and are responsible for the observed cardiovascular phenotypes. Indeed, maternal Zfp57 is present in both oocytes and preimplantation embryos (Li et al., 2008; Fig. 3). Consistent with its expression, maternal Zfp57 appears to influence the maintenance of DNA methylation imprints at multiple imprinted regions in early embryos (Li et al., 2008; compare Fig. 3B with Fig. 3C). In addition, it is required for the establishment of DNA methylation imprints at the Snrpn DMR in the female germline [Li et al., 2008; Fig. 3C and Zygote (3) in Fig. 4]. Therefore, the molecular defects in epigenetic memory caused by loss of maternal Zfp57 are already present in early embryos. As shown in Fig. 3C, loss of differential DNA methylation at the Snrpn DMR is present in the oocyte as well as in the preimplantation embryo and remains absent throughout embryogenesis in the maternal–zygotic mutant embryo. However, the phenotypes (PWS and AS syndromes) due to loss of imprinting at the Snrpn DMR occur after birth (Butler, 2009).

Furthermore, our previous finding demonstrated that the maternal function of Zfp57 present in the zygotic mutant prevented the midgestational embryonic lethality which was observed when both the maternal and the zygotic functions of Zfp57 were absent in the maternal–zygotic mutant (Li et al., 2008; comparing the zygotic mutant in Cross 4 of Fig. 2 with the maternal–zygotic mutant in Crosses 3 and 5 of Fig. 2). Our preliminary results also suggest that it can rescue some of the cardiovascular phenotypes present in the maternal–zygotic mutant embryos (Cullen and Li, unpublished data). Consistent with these results, the maternal function of Zfp57 present in the zygotic mutant can partially rescue the DNA methylation defect present in the imprinting control regions (comparing Fig. 3B with Fig. 3C). Therefore, it appears that the maternal function of Zfp57 can influence late-stage embryogenesis by modulating the establishment and/or maintenance of DNA methylation imprints in preimplantation embryos.

I believe more genes with this kind of distinctive developmental role will be uncovered provided that they are involved in the establishment and/or maintenance of heritable epigenetic memory such as DNA methylation and various histone modifications. It is the loss of function of their target genes rather than genetic mutations themselves that are directly responsible for the observed developmental phenotypes. Since it is estimated that there are over 300 members in the mouse or human genome, other KRAB zinc finger proteins are plausible candidates that may exhibit developmental and epigenetic regulation potentials similar to ZFP57. More studies should be aimed at identifying the molecular functions as well as developmental roles of these KRAB zinc finger proteins in mammals. It is possible that these proteins are partly responsible for the diversity and complexity present in mammalian species (Looman et al., 2002; Reik, 2007).

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**References**


Ma DK, Guo JU, Ming GL, Song H. DNA excision repair proteins and Gadd45 as molecular players for active DNA demethylation. *Cell Cycle* 2009;8:1526–1531.


Matsuda E., Agata Y., Sugai M., Katakai T., Gonda H., Shimizu A.. Targeting of genomic imprints. *KDM1B is a histone H3K4 demethylase required to establish maternal imprinting*.


Moskowitz IP, Rothman JH. Lin-12 and glp-1 are required zygotically for early embryonic cellular interactions and are regulated by maternal GLP-1 signaling in *Caenorhabditis elegans*. *Development* 1996;122:4105–4117.


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.


