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Déborah Bourc'His, Charlotte Proudhon

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Sexual dimorphism in parental imprint ontogeny and contribution to embryonic development

Déborah Bourc'his* and Charlotte Proudhon.

INSERM U741
Paris 7 University
2 Place Jussieu
75251 Paris Cedex 05, France

* Corresponding author

Tel : +33 1 44 27 47 31

Fax : +33 1 44 27 82 10

E-mail address : bourchis@ijm.jussieu.fr

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Abstract

Genomic imprinting refers to the functional non-equivalence of parental genomes in mammals that results from the parent-of-origin allelic expression of a subset of genes. Parent-specific expression is dependent on the germ line acquisition of DNA methylation marks at imprinting control regions (ICRs), coordinated by the DNA-methyltransferase homolog DNMT3L. We discuss here how the gender-specific stages of DNMT3L expression may have influenced the various sexually dimorphic aspects of genomic imprinting : 1) the differential developmental timing of methylation establishment at paternally and maternally imprinted genes in each parental germ line, 2) the differential dependence on DNMT3L of parental methylation imprint establishment, 3) the unequal duration of paternal versus maternal methylation imprints during germ cell development, 4) the biased distribution of methylation-dependent ICRs towards the maternal genome, 5) the different genomic organization of paternal versus maternal ICRs, and finally 6) the overwhelming contribution of maternal germ line imprints to development compared to their paternal counterparts.

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1. Introduction

Genomic imprinting imposes in mammals a requirement for biparental reproduction that results from the monoallelic and parent-specific expression of imprinted genes. Imprinted genes are a typical illustration of epigenetic regulation where two identical sequences in a common nuclear environment are differentially expressed. Some 90 imprinted genes are now identified in mouse and human genomes. Estimates of their total number range from 100 to 200 (Luedi et al., 2005; Morison et al., 2005). Imprinted gene regulation can suffer from both genetic and epigenetic alterations and the functional haploid state of these genes makes them particularly vulnerable to environmental effects, *in vitro* culture conditions and manipulations inherent to assisted reproduction and somatic nuclear transfer technologies (Trasler, 2006). Disrupted imprinted gene expression is associated with a variety of developmental and neurological congenital disorders as well as with tumorigenesis (Jiang et al., 2004). The penetrance and severity of a growing number of psychiatric and behavioral pathologies have moreover been linked to parent-specific effects of transmission. Reasons for the evolution of imprinting in mammals have been widely debated since the initial description of imprinting effects in mouse in the early 80's (Barton et al., 1984; McGrath and Solter, 1984). The parental conflict theory applies to species in which there is unequal provision for parental resources after conception, as it is the case in mammals, where nutrients are provided exclusively by the mother initially through exchanges via the placenta and then through lactation (Moore and Haig, 1991). The unbalanced parental investment in pre-natal and post-natal care would have led to the evolution of parent-specific expression during mammalian specification, with paternally expressed genes having a growth-enhancement effect and maternally expressed genes tending to reduce *in utero* and post-natal growth potentialities. An increasing number of findings fail to fit into this theory, and an alternate model offers that genomic imprinting served as a co-adaptation between the mother and the offspring and between males and females, without the notion of conflicting parental interests (Swaney et al., 2007).

Some common themes tend to be found at imprinted genes. They have by definition a *cis*-acting imprinting control region (ICR) that bears differential DNA methylation marks on parental alleles and is converted into parent-specific expression in combination with other epigenetic modifications (Trasler, 2006). Primary parent-specific methylation marks are established during gametogenesis when parental genomes are physically separated, and we showed by genetic means that this process is under the influence of the methylation regulator DNMT3L (Bourc'his et al., 2001; Bourc'his and Bestor, 2004). Some genomic features tend to characterize imprinted genes: association with CpG island promoters, presence of non-coding RNAs in their vicinity, organization in clusters around shared long-range effect ICRs, association with tandem repeat arrays or underrepresentation of

retrotransposons of the SINES class (Short INterpersed Elements) (Greally, 2002; Wood and Oakey, 2006). But these are neither exclusive nor obligatory criteria for the identification of imprinted genes. Although a systematic study has not been performed, stage- and tissue-specific expression seems to be a common trait for imprinted genes (Wood et al., 2006). Parent-specific expression is usually limited to perinatal life with a predilection for placental tissues. Imprinted genes are most of the time silenced or biallelically expressed in adult tissues, with the noticeable exception of the brain where functional genomic imprinting is commonly preserved (Davies et al., 2005). Finally, there are roughly as many genes exclusively expressed from the maternal genome than from the paternal one. However this equal force in parental expression masks many aspects of sexual dimorphism in the establishment, longevity, distribution, genomic organization, and contribution to development of paternal versus maternal-specific germ line imprints (Bourc'his and Bestor, 2006). We suggest that the differential timing of expression of the imprinting regulator *Dnmt3L* in the male and female germ lines may have shaped the biased distribution of methylation imprints towards the maternal genome. As a consequence, maternal germ line imprints have a dominant contribution to development compared to paternal ones, notably in the establishment of the fetomaternal interface through the control of placenta differentiation and physiology.

2. DNA methylation as a support for the determination and maintenance of imprinted expression

Mammalian methylation patterns are established and maintained by a family of proteins called DNA (cytosine-5)-methyltransferases (DNMTs) that add a methyl group to cytosines present in the context of CpG dinucleotides (Goll and Bestor, 2002). The subgroup of DNMT3 proteins comprises two active enzymes establishing methylation on unmethylated substrates (*de novo* methylation), DNMT3A and DNMT3B, and one regulatory factor, DNMT3L (DNMT3-Like). DNMT3L lacks the catalytic motifs essential to the methylation reaction, but acts as a stimulator of *de novo* methylating activities (Kareta et al., 2006). Clonal propagation of methylation is dependent on the maintenance activity of DNMT1. This protein recognizes hemimethylated substrates generated by the semi-conservative mode of DNA replication and reproduces methylation patterns on the newly synthesized DNA strand at each cell division.

Germ line-derived and allele-specific patterns of DNA methylation characterize imprinting control regions and are inversely correlated with the state of allelic expression. Biochemical and functional data support DNA methylation as the essential epigenetic mark for the control of parent-specific expression. The heritability and reversibility of DNA

methylation patterns allow the successive phases of acquisition, maintenance and erasure of imprints, which will be outlined in more detail below. Engineered mutations in *Dnmt1* abolish global maintenance of methylation patterns including at ICRs (Li et al., 1992). Targeted inactivation of *Dnmt3A* or *Dnmt3L* genes affects gametic methylation establishment and embryos derived from the fertilization of *Dnmt3A* or *Dnmt3L* deficient oocytes have a disrupted expression of maternally imprinted genes, consisting in a switch to biallelic expression or complete repression (Bourc'his et al., 2001; Kaneda et al., 2004). Methylation imprint-deficient embryos die at mid-gestation, illustrating the essential and dose-dependent role of imprinted genes in mammalian development. Allele-specific induction of posttranslational modifications of histones and recruitment of their chromatin-binding partners is also associated with imprinted expression (Fournier et al., 2002). But inactivation of genes encoding different histone acetyltransferases or methyltransferases has failed to show evidence of a failure to maintain imprinted expression in mutant embryos (Goll et al., 2002). Moreover, during spermatogenesis, histones are globally replaced by protamines to allow a tighter nuclear condensation from the spermatid stage onwards, and are then reloaded onto the paternal pronucleus in the zygote shortly after fertilization (Rousseaux et al., 2005). A small and species-specific fraction of the genome is not subject to histone-to-protamine exchange but imprinted regions are apparently not part of this histone-persistent compartment of the spermatozoal chromatin (Miller and Iles, 2007). Histone modifications seem therefore unlikely to serve as a support for the transmission of paternal imprints from spermatozoa to the zygote.

3. Differential chronology of methylation imprint establishment in parental germ lines

The life cycle of imprinting has been studied through the methylation analysis of individual paternally and maternally imprinted genes in the developing germ line and somatic tissues (Fig. 1) (Davis et al., 1999; Li et al., 2004; Lucifero et al., 2002). Primordial germ cells (PGCs) emerge from a subpopulation of cells from the epiblast at 6 days of gestation (dpc). Like other embryonic cells, they show a biparental pattern of DNA methylation at imprinted control regions (Hajkova et al., 2002). Paternal alleles from paternal ICR are methylated while maternal alleles are unmethylated, and vice-versa for maternal ICR. As PGCs colonize the genital ridges around 10.5dpc, somatic methylation patterns are erased (Kato et al., 1999; Lee et al., 2002) and establishment of methylation patterns subsequently occurs in the differentiating germ line according to the sex of the individual. At the time of fertilization, paternal ICR are specifically methylated in spermatozoa and unmethylated in the oocyte, while maternal ICR are methylated in the ovulated oocyte but not in spermatozoa. Other genomic sequences, and in particular retrotransposable elements, follow the same kinetics of germ line methylation changes as imprinted genes (Hajkova et al., 2002; Lees-Murdock et

al., 2003). But contrary to the methylation of the rest of the genome that undergoes dramatic remodeling from fertilization to the embryo implantation (Oswald et al., 2000; Rougier et al., 1998), imprinted methylation marks of both paternal and maternal origin are unaltered during this period (Tremblay et al., 1997). They keep on being faithfully maintained in the developing individual, except in the germ line where a new cycle of erasure/establishment will occur.

There are profound differences in hormonal, temporal, structural and functional control of germ cell proliferation and differentiation between the sexes. Acquisition of gametic methylation imprints also encounters gender-specific differences in the developmental stages where it occurs (Fig. 1). Gametic methylation is acquired very early during spermatogenesis. As methylation patterns are just erased, male germ cells enter in mitotic quiescence (G0/G1 arrest) around 14.5 dpc in a cell type called prospermatogonia. *De novo* methylation is initiated in this fetal period and will be completed within a few days after birth, as these cells become spermatogonial stem cells (Li et al., 2004). Spermatogonial stem cells produce upon their asymmetrical divisions a daughter cell that maintains a self-renewing activity and a spermatogonia that will enter into spermatogenetic differentiation to undergo meiosis and produce mature spermatozoa with paternal methylation imprints (De Rooij, 2001). In the female germ line, gametic *de novo* methylation is comparatively a late event occurring in post-natal growing oocytes (dictyate stage) (Kono et al., 1996; Lucifero et al., 2004). The competence to initiate maternal imprinted methylation is dependent on the oocyte size and therefore on follicular development. As dictyate oocytes are arrested at the diplotene stage of prophase of first meiosis, it seems that gametic *de novo* methylation requires a quiescent nuclear environment in both male and female germ lines. This is however the only common theme in parental methylation acquisition. Male germ line methylation occurs in perinatal life in a 2n context of cells that have just undergone massive demethylation and are far upstream the entry into meiosis. In the female germ line, methylation patterns are established after birth a long time after somatic methylation pattern erasure, in cells that have already undergone meiotic recombination and have a 4n DNA content. This differential chronology of methylation establishment in the male versus female germ lines is likely to underlie the sex-specific acquisition of parental imprinted marks.

4. Sex-specific expression and effects of Dnmt3L, a germ line regulator of methylation activities

Expression, evolutionary and functional arguments point towards a central role of DNMT3L in regulating methylation acquisition in the mammalian germ line, and in shaping many aspects of sexual dimorphism in imprinted gene expression. DNMT3L has no

methyating activity *per se* but interacts with and stimulates the *de novo* DNA-methyltransferases DNMT3A (Kareta et al., 2006). *Dnmt3A*, *Dnmt3B* and *Dnmt3L* are all expressed at the time genomic imprints are established in both parental germ lines (La Salle et al., 2004; La Salle et al., 2006; Sakai et al., 2004). However, *Dnmt3A* and *Dnmt3B* have a broader window of expression during gametogenesis than the stages where imprints are acquired. They are also expressed during embryonic development and in some adult somatic tissues (Okano et al., 1999). *Dnmt3L* expression is restricted to prospermatogonia and growing oocytes, in exact coincidence with gametic methylation acquisition at imprinted genes and other genomic sequences (Fig. 1) (Bourc'his et al., 2001; La Salle et al., 2006). As maternal methylation acquisition was shown to be oocyte-size dependent, a sharp increase in *Dnmt3L* transcription is also observed in oocytes having reached a critical diameter of 60-80 μ m (Lucifero et al., 2007). Comparison of the DNA methylation system of diverse vertebrate species revealed the emergence of the *Dnmt3L* gene in the mammalian clade, potentially by duplication from *Dnmt3A* (Yokomine et al., 2006). This phylogenetic restriction argues for the coincident evolution of *Dnmt3L* with the acquisition of genomic imprinting in mammals. Finally, genetic data confirm the biological role of DNMT3L in gametic *de novo* methylation and its importance for mammalian fertility (Bourc'his et al., 2004; Bourc'his et al., 2001). The identification of *Dnmt3L* as the first imprintor gene provided the genetic proof of the existence of genomic imprinting and considerably advanced our understanding of the mechanism of imprinting establishment.

The nature of the sequences affected by the invalidation of *Dnmt3L* shows unexpected gender differences, providing an additional level of sexual dimorphism in the ontogeny and effects of parental imprint acquisition. We showed that DNMT3L is required for retrotransposon methylation and repression in male germ cells but largely dispensable for paternally imprinted methylation (Bourc'his et al., 2004), while it is necessary for maternally imprinted methylation but has only a very limited influence on retrotransposon methylation in female germ cells (Bourc'his et al., 2001; Lucifero et al., 2007). As a consequence, *Dnmt3L* deficiency induces a markedly different phenotype in males and females. *Dnmt3L*^{-/-} males present with a complete azoospermia, associated with an interruption of spermatogenesis at the pachytene stage triggered by major asynapsis of homologous chromosomes. *Dnmt3L*^{-/-} females produce mature oocytes but their heterozygous *Dnmt3L*^{-/+} progenies systematically die at mid-gestation, mostly from placental dysfunction (Fig. 2). *Dnmt3L* deficiency behaves therefore as one of the few maternal-effect mutations in mammals. The embryonic lethality of *Dnmt3L*^{-/+} embryos results from a disrupted expression of maternally imprinted genes that failed to acquire proper methylation imprints in the maternal *Dnmt3L*^{-/-} germ line. The

unequal dependence of paternal versus maternal germ line methylation marks towards DNMT3L is likely to reflect the sexually dimorphic expression pattern of DNMT3L.

The mechanism by which DNMT3L targets specific genomic sequences in each parental germ line is unknown. Differential pre-existing histone modifications could be involved as suggested by the exclusion of DNMT3L from H3 histone tails methylated on lysine 4 (Ooi et al., 2007). Changes in global levels of histone acetylation and incorporation of specific histone variants are known to be associated with meiosis (Akiyama et al., 2004; Mahadevaiah et al., 2001). As a consequence, the two cell types in which paternal and maternal *de novo* methylation occurs (mitotic versus meiotic) have very different patterns of histone modifications. Prospermatogonia and growing oocytes have also a very distinct composition in terms of stage-specific transcription or chromatin-associated factors that could explain the differential binding to paternal and maternal ICR and subsequent visibility for DNMT3L recognition and *de novo* methylation in male and female germ lines. The molecular mechanism behind the sex-specific timing of *Dnmt3L* expression is still debated. The open reading frames of DNMT3L proteins are identical in female and male germ lines, but an alternative promoter located in an intron of the upstream *AIRE* gene is specifically recruited for dictyate oocyte *Dnmt3L* transcription (Shovlin et al., 2007). Although no obvious consensus sequences for oestrogen, androgen or gonadotrophin receptor binding were identified at the sex-specific promoters (Proudhon and Bourc'his, unpublished data), hormone-activated *cis* sequences could be involved in the differential initiation of *Dnmt3L* expression in prospermatogonia and growing oocytes.

5. Differential genomic organization of paternal and maternal imprinting control regions

The DNMT3L-induced differential kinetics of methylation acquisition in male and female germ lines may not only be responsible for the sex-specific acquisition of methylation imprints in each parental germ line. It may also have contributed to the evolution of specific genomic features distinguishing paternal and maternal ICRs. While the total number of paternally and maternally imprinted genes is approximately even, most of the primary methylation marks acquired in the germ line are of maternal origin. More than 15 imprinted clusters are dependent on ICRs harboring maternal germ line methylation, while only 3 imprinted loci have been reported to be controlled by paternal germinal marks: *H19/Igf2*, *Gtl2/Dlk1* and *A19/Rasgrf1* (Reik and Walter, 2001). The rest of paternally imprinted genes are controlled through the production *in cis* of a non-coding RNA (ncRNA), silenced itself on the maternal allele by DNA methylation inherited from the oocyte. The repression induced by these regulatory paternally ncRNAs can be 1) post-transcriptional, when the ncRNA is

antisense to an adjacent transcript, promoting the local formation of a double-strand RNA and triggering an RNAi response of degradation of this transcript, or 2) transcriptional, when the firing of the ncRNA promoter prevents the activation of other promoters in the vicinity, or when the ncRNA transcript elongation promotes the local formation of repressed chromatin. This latter mode of control has been documented at an imprinted locus on chromosome 17 in the mouse: the *Air* ncRNA transcribed on the paternal chromosome interferes bidirectionally with the expression of neighbor genes, including *Igf2R* (Sleutels et al., 2002), by inducing the organization of an heterochromatin-like domain extending over 400 kilobases. The promoter of *Igf2R* acquires some zygotic methylation marks on the paternal allele, secondary to its repressed status. Maternal methylation of the ICR prevents the expression of the *Air* ncRNA on the maternal chromosome and subsequently allows expression of *Igf2R* and other genes of the cluster.

Paternal germ line methylation marks are not only rare and underrepresented compared to maternal germ line methylation marks; the genomic constitution of the few paternal ICRs is also very distinct from their maternal counterparts (Bourc'his et al., 2006). Maternal ICRs tend to be located at promoter regions and are enriched in CpG dinucleotides, fulfilling the criteria of CpG island annotation. On the other side, the three paternal ICRs reported so far map to intergenic regions and are CpG-poor, these genomic characteristics being conserved in the murine and human species. The evolutionary and biological significance of the parental difference in ICR genomic location is unclear. The difference in CpG composition provides however a simple mechanistic model for the biased distribution of germ line methylation imprints towards the maternal genome and the subsequent evolution of a DNA-methylation independent mode of regulation of paternally repressed genes. Cytosines present in the context of CpG sites are targeted by DNA methylation in mammalian genomes. Methylated cytosines behave as endogenous mutagens, being spontaneously desaminated into thymidines, which fail to be recognized by the DNA repair machinery during DNA replication and are therefore amplified by cell divisions (Gonzalzo and Jones, 1997). As a consequence, methylated CG motifs tend to be lost during the course of evolution, in favor of TG or CA motifs depending on which DNA strand the transition from methylated C to T happened.

We emphasized that the duration of methylation marks is markedly different in the male versus the female germ lines (Fig. 1). Methylation is acquired very early during spermatogenesis, in precursors of the self-renewing spermatogonial stem cells. Paternally methylated CpGs have to endure many replication cycles in spermatogonial stem cells and proliferating spermatogonia, and to persist from a few weeks to several years before the production of mature spermatozoa. In humans, an average of 23 mitotic divisions was estimated to occur per year before the formation of mature spermatozoa, until the end of a

man reproductive life (Eichenlaub-Ritter et al., 2006). On the contrary, maternal methylation patterns are established just a few days before ovulation, in growing oocytes that have reached a critical diameter and have stopped dividing since the last pre-meiotic replication occurring during fetal life. The longer duration of paternal methylation patterns and their higher exposure to replication cycles during gametogenesis may have favor the erosion of paternal ICRs by C →T transition during evolution, while maternal ICRs have been better conserved owing to the shorter longevity of methylation patterns in the female germ line. This in turn would explain why paternal and maternal genomes rely on different molecular strategies to control parent-specific expression. There is no explanation as to why *H19*, *Gtl2* and *Rasgrf1* imprinted expression is (still?) controlled by paternal germ line methylation. An initial higher CpG content of their ICRs may have allowed them to persist until now. Genome-wide screens for new imprinted genes will help clarifying whether the underrepresentation of paternal germ line ICRs is evolutionary relevant or reflects a sampling bias in former methods of imprinted gene identification.

6. Asymmetric contribution of paternal and maternal germ line imprints to early development

The paucity of paternal germ line imprints raises the question of their relative contribution to development. A collection of embryonic models of individual and combined parental imprint deficiencies is available and their comparative analysis allows the evaluation of the net and combined effects of paternal and maternal germ line imprints (Fig. 3). The evidence that both parental genomes are strictly required for mammalian development was historically evidenced in mouse through the construction by nuclear transfer of uniparental conceptuses (Barton et al., 1984; McGrath et al., 1984). Parthenogenetic (two maternal genomes) and androgenetic (two paternal genomes) conceptuses failed to develop past mid-gestation and showed moreover an opposite imbalance in embryo:placenta representation depending on the parental origin of the nuclei. These phenotypes in mirror have led to the conclusion that the maternal genome is required for the development of the embryo, while the paternal genome promotes the development of extraembryonic structures (placenta). These models do not address however the specific influence of maternal and paternal germ line imprints on development. Uniparental conceptuses are indeed a mixture of inadequate imprinting: they not only show a lack of imprinting of the absent parental genome origin, but they also have a double dose of imprinting of the parental genome present in two copies. For example, androgenotes (two paternal genomes) have a lack of maternal imprinting that results in a double dose of genes negatively regulated by maternal imprints but also a double contribution of paternal imprinting that results in no expression of paternally imprinted

repressed genes. These conceptuses show an exclusive development of extraembryonic structures with no embryonic contribution. Whether this phenotype is due to a lack of maternal imprinting and/or an excess of paternal imprinting can not be delineated.

Because of the involvement of DNMT3L in the establishment of maternal methylation imprints during oogenesis, *Dnmt3L*^{-/+} embryos derived from fertilized *Dnmt3L*^{-/-} oocytes have a complete lack of maternal methylation imprints transmitted by their mother, but a normal dose of paternal imprinting inherited from the wild-type sperm (Bourc'his et al., 2001). These maternal imprint-free embryos give the opportunity to assess the specific biological contribution of maternal imprinting to development. The presence of a normal dose of paternal germ line imprints in *Dnmt3L*^{-/+} models allows gastrulation and development of the embryo proper until 9.5dpc. The embryonic lethality at this point is related to a failure of specialized extraembryonic structures to establish functional connections with the maternal blood system and results therefore from nutritional deprivation. This event normally occurs around 8.5dpc through the formation of the chorioallantoic structure that will later develop into the umbilical cord. Several extraembryonic defects are observed in *Dnmt3L*^{-/+} embryos, such as an hyperproliferation of the terminally differentiated trophoblast giant cells, an overgrowth of the chorionic plate and abnormal vascularization of the visceral yolk sac (Fig. 2). *Dnmt3L*^{-/+} embryos have therefore in common with androgenetic conceptuses an hyperproliferation and hyperdifferentiation of extraembryonic tissues, which suggests a key role of maternal germ line imprints on the control of adequate trophoblast/placenta growth. Embryos lacking specifically maternal imprints can also be derived by fertilizing neonate non-growing oocytes (ng/sp) (Kono et al., 1996), which are developmentally upstream from DNMT3L-induced establishment of maternal methylation (Fig. 1). These ng/sp embryos die also before 9dpc but their phenotype was not documented.

Maternal germ line methylation imprints seem essential for the early post-implantation development, and notably for the establishment of the feto-maternal interface through the regulation of extraembryonic differentiation and functionality. Construction of paternal imprint-free embryos emphasized that on the other side, paternal germ line imprints may be dispensable at least for early development. Parthenogenetic embryos containing one set of chromosomes from maternally imprinted fully grown oocytes and one set of chromosomes arising from epigenetically naive non-growing oocytes develop normally until 13.5dpc (Kono et al., 1996). These specific paternal imprint-free fg/ng embryos exhibit an enhanced development compared to regular fg/fg parthenogenotes with no paternal imprinting and two doses of maternal imprinting (Fig. 3). Development can be further improved to term (but with a low efficiency) by deleting one copy of the *H19* locus on the ng nucleus, resulting in the restoration of a normal monoallelic expression of this gene and therefore artificially reproducing a paternal imprint of this locus (Kono et al., 2004). Future combined genetic

manipulation of *H19*, *Gtl2* and *Rasgrf1* expression in these paternal imprint-free embryos should definitely clarify whether the methylation-dependent influence of the paternal germ line is restricted to the control of the expression of these three loci in the embryo or extends to additional uncharacterized loci. In this regard, it should be pointed here that embryos lacking both paternal and maternal germ line imprints were derived by nuclear transfer from early colonizing PGCs, in which methylation at imprinted genes and other genomic loci has not yet been established (Kato et al., 1999; Lee et al., 2002; Yamazaki et al., 2003). These complete imprint-free embryos have a developmental competence similar to the one displayed by *Dnmt3L*^{-/+} maternal imprint-free embryos, with an embryonic lethality at 9.5dpc resulting from increased extraembryonic growth. One can not exclude that the epigenetic defect is affecting other sequences than imprinted genes in these PGC-derived embryos, and the derivation of purely imprint-free embryos through the duplication of the maternal imprint-free genome of *Dnmt3L*^{-/-} oocytes should provide a definite answer regarding the combined and net effects of paternal and germ line imprints in early development. Nonetheless, the phenotypic coincidence suggests that the lack of paternal imprints does not further impair early development once maternal imprints are absent.

7. Conclusions

The DNA-methyltransferase homolog DNMT3L appears to be a key factor for the acquisition of imprinting not only during germ cell development but also in the course of evolution. The gender-specific developmental stages of expression of DNMT3L during gametogenesis is likely to underlie the various sexually dimorphic aspects of parental imprint ontogeny and contribution to development as summarized in Table 1. Sex-specific *cis* sequences for *Dnmt3L* transcription initiation probably determines the sex-specific timing of *Dnmt3L* expression and in turn the differential kinetics of acquisition of paternal and maternal imprints regarding the occurrence of meiotic recombination. The different nuclear chromatin and protein composition of prospermatogonia and growing oocytes provides a plausible mechanism for the differential methylation of paternal and maternal ICRs in each parental germ line. Differential duration of paternal and maternal methylation germ line imprints may have also influenced the loss of paternal ICRs and favored the conservation of maternal ICRs during evolution. The overrepresentation of maternal ICRs could finally explain the major contribution of maternal germ line imprints to early development.

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Table 1. Various aspects of sexual dimorphism in parental imprint ontogeny and contribution to embryonic development

	Paternal germ line imprints	Maternal germ line imprints
Timing of methylation acquisition in the germ line	Pre-meiotic	Meiotic
Dependence on Dnmt3L	Limited	Major
Duration of germ line methylation marks in mouse	35 days / 3 years	Few days
Number of imprint control regions	3	> 15
Genomic organization of imprinting control regions	CpG poor	CpG rich
	Intergenic regions	Promoter regions
Influence on early development	Limited	Major

Legends

Fig. 1. Developmental kinetics of methylation imprints and *Dnmt3L* expression in male and female germ lines. Primordial germ cells (PGCs) have a biparental pattern of imprinted methylation (blue/red) at the time of their specification occurring around 6dpc and during their migration phase. Methylation patterns are erased as they colonize the genital ridges around 10.5dpc. Methylation is established at paternally imprinted genes in perinatal male germ cells named prospermatogonia (Prospg). Prospg are arrested at the G0/G1 phase of mitosis, reside in male gonads from 14.5dpc until a few days after birth and express *Dnmt3L* (grey shade). At this time, they differentiate into self-renewing spermatogonial stem cells (SSC). Paternal germ line methylation is maintained from a few weeks to a several years as spermatogenesis proceeds through the phases of proliferating spermatogonia (Spg), spermatocytes undergoing meiotic recombination, spermatids and finally spermatozoa (Sperm). Methylation is established at maternally imprinted genes in resting post-natal growing oocytes that start expressing *Dnmt3L* as they reach a critical diameter (>60µm), and therefore only a few days before ovulation. These oocytes have already undergone meiotic recombination. Ng : non-growing oocyte, Fg : fully grown oocyte, MII : ovulated oocyte in metaphase II of meiosis.

Fig. 2. Anomalies of extraembryonic structures in 8.5 dpc *Dnmt3L*^{-/+} embryos. Top panel: The trophoblast giant cells (TGC) recognized by their characteristic large nucleus size are organized into a single layer of cells in the ectoplacental cone of wild-type (WT) embryos. In aged-matched *Dnmt3L*^{-/+} embryos, the overproduction of TGC is indicative of an hyperdifferentiation of trophoblast cells. Their angiogenic activity promotes the local accumulation of maternal blood that is not anymore constrained into discrete sinuses. Low panel: Abnormal vessel development and fewer fetal erythrocytes of the visceral yolk sac (VYS) of *Dnmt3L*^{-/+} embryos.

Fig 3. Review of the different embryonic models of imprinting deficiencies. Four categories of embryos are delineated: biparental (normal), paternal imprint-free, maternal imprint-free and complete imprint-free. Paternal germ line imprints are depicted in blue, maternal germ line imprints in red. Embryos are organized from left to right from normal development to the poorest development. The stage of embryonic lethality and the relative contribution of placental (pink) and embryonic structures (yellow) is indicated for each conceptus. The embryo size of *Dnmt3L*^{-/+}, PGC-derived and *Dnmt1*^{-/-} embryos is slightly smaller than a normal embryo due to the altered functionality of the extraembryonic structures. The fg^{ngH19Δ13} embryo was obtained by transfer and parthenogenetic activation of a fg oocyte nucleus and a ng oocyte nucleus carrying a deletion of the ICR of the *H19* locus. *Dnmt3L*^{-/+} embryos are derived from fertilization of *Dnmt3L*^{-/-} oocytes and show a specific lack of maternal germ line imprints with normal paternal germ line imprints. *Dnmt1*^{-/-} embryos are deficient in global methylation maintenance and present with an hypomethylation at paternally and maternally imprinted genes but also at other genomic sequences. The 8 cell-stage arrest of the ng/ng embryo is not compatible with the other complete imprint-free models and may reflect a technical problem. fg: fully grown oocyte, ng: non-growing oocyte, sp: spermatozoon, Partheno: original parthenogenote, Andro: original androgenote, PGC: primordial germ cells.

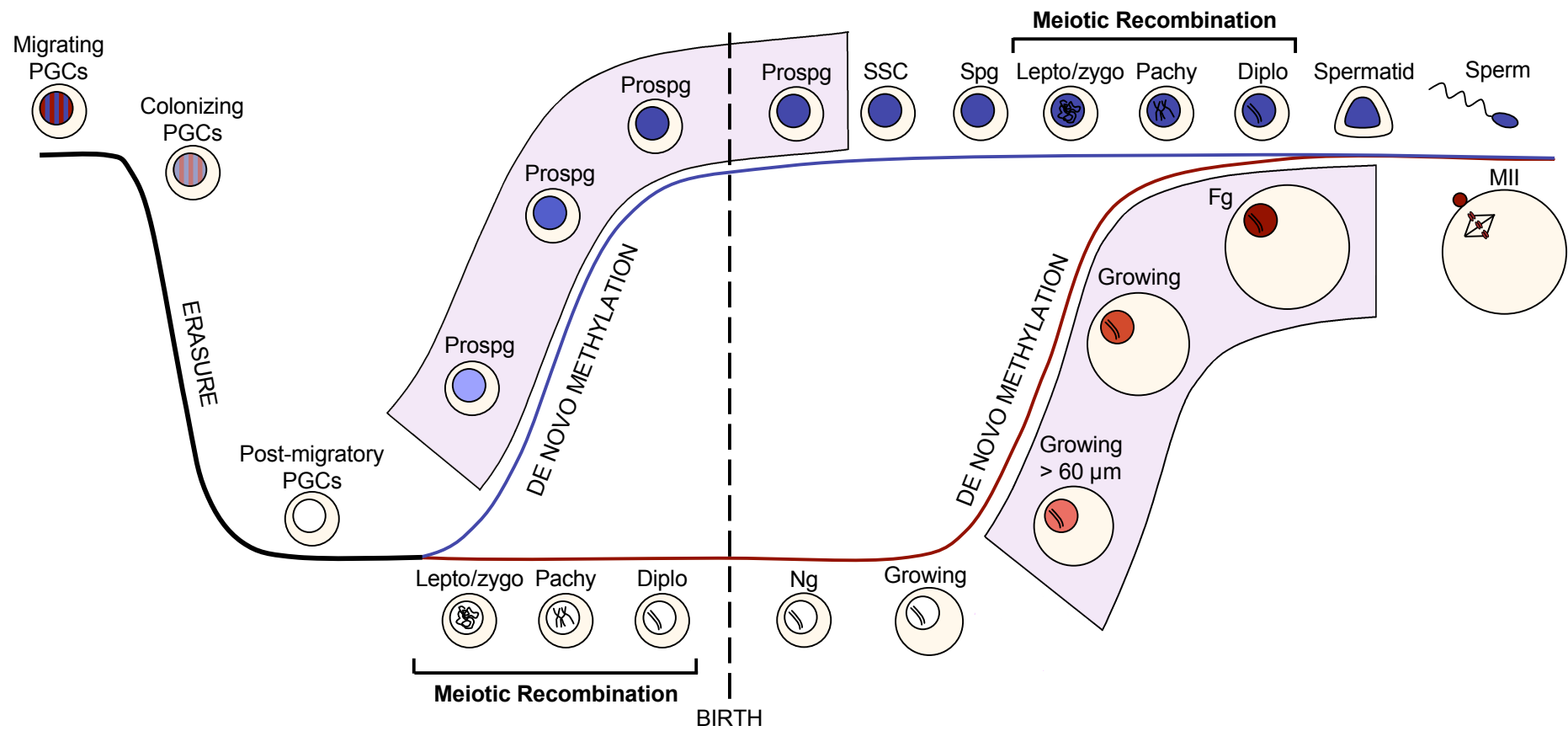


Figure 1
Bourc'his and Proudhon

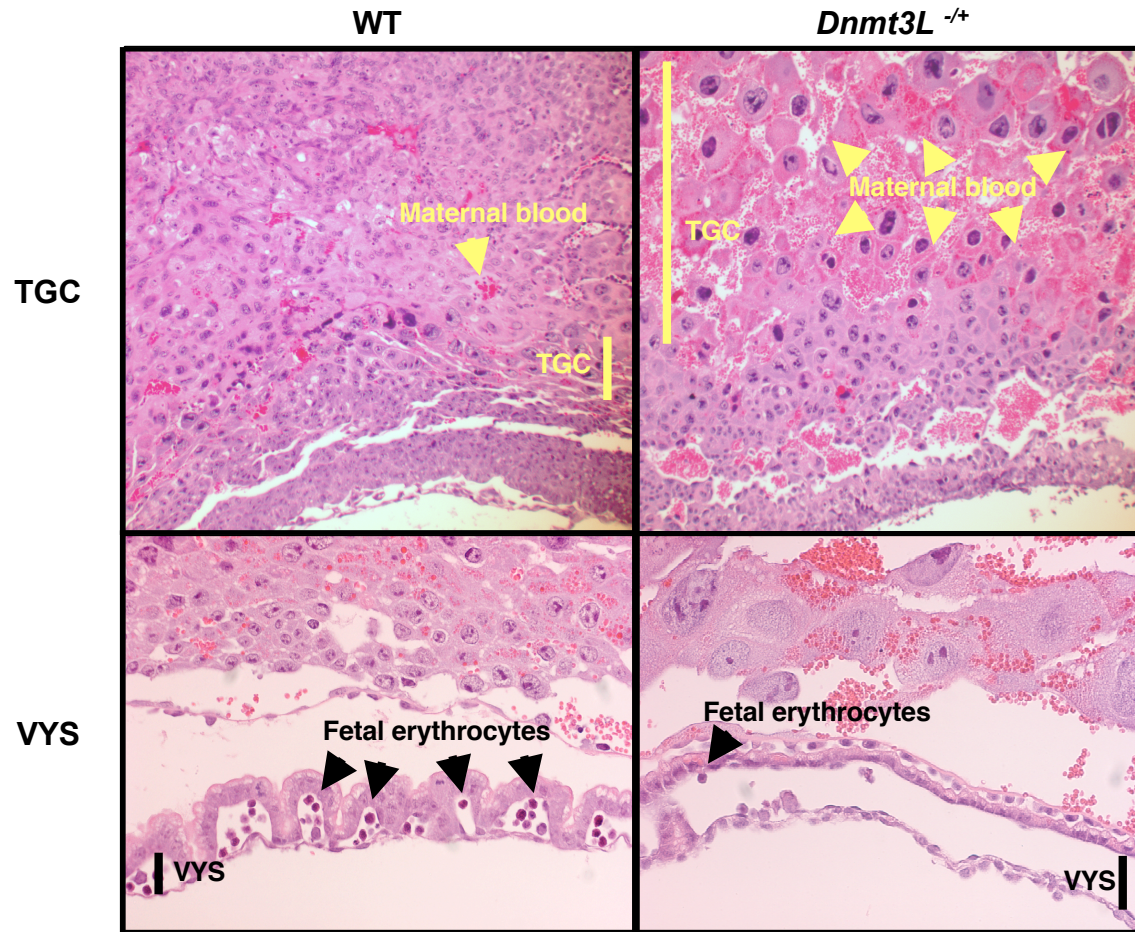


Figure 2
Bourc'his and Proudhon

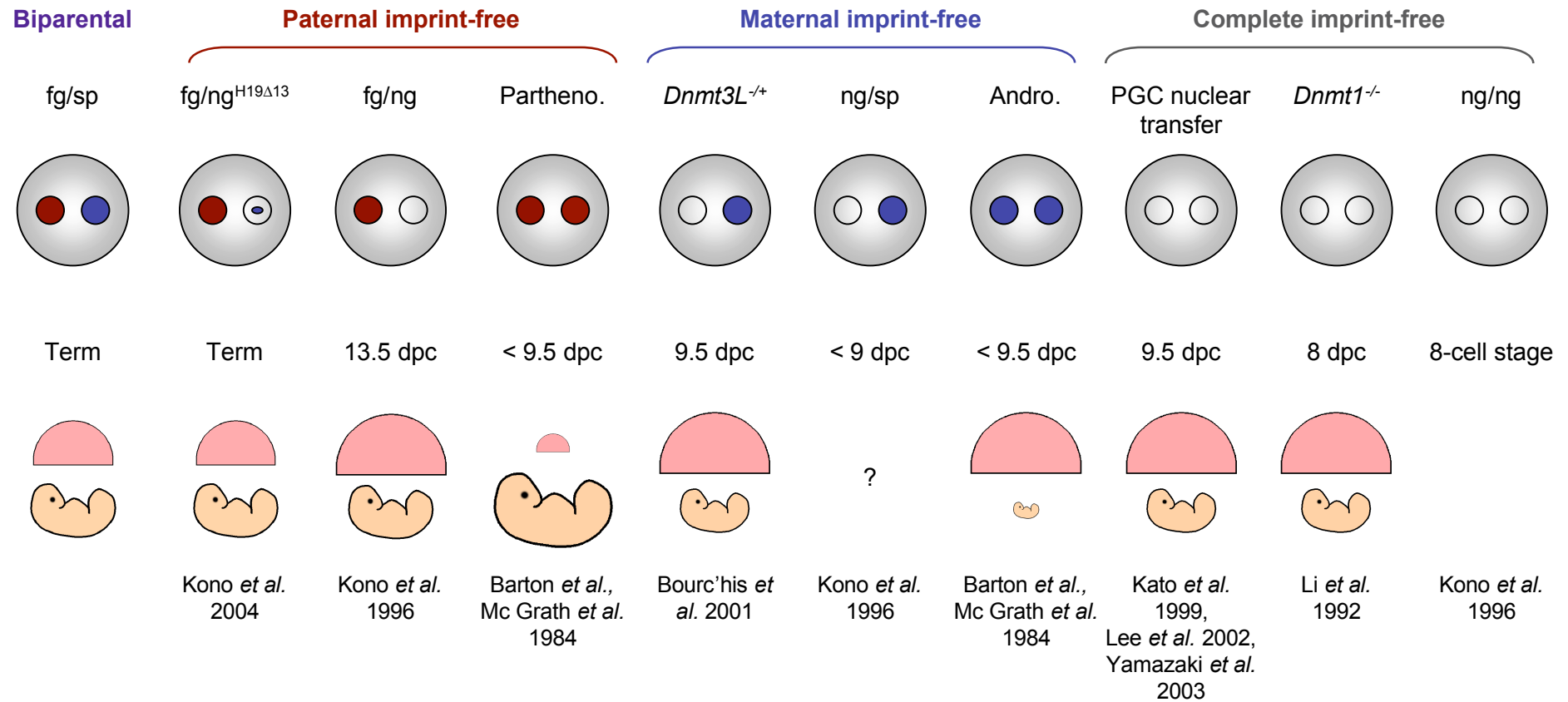


Figure 3
Bourc'his and Proud'hon