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REVIEW

Genomic imprinting in development, growth, behavior and stem cells

Robert N. Plasschaert and Marisa S. Bartolomei*,‡

ABSTRACT

Genes that are subject to genomic imprinting in mammals are preferentially expressed from a single parental allele. This imprinted expression of a small number of genes is crucial for normal development, as these genes often directly regulate fetal growth. Recent work has also demonstrated intricate roles for imprinted genes in the brain, with important consequences on behavior and neuronal function. Finally, new studies have revealed the importance of proper expression of specific imprinted genes in induced pluripotent stem cells and in adult stem cells. As we review here, these findings highlight the complex nature and developmental importance of imprinted genes.

KEY WORDS: Imprinted genes, Fetal growth, DNA methylation, Induced pluripotency, Behavior, Neuronal development

Introduction

Mammals inherit two sets of chromosomes, one from each parent, and therefore possess two copies of each gene. For the majority of these genes, both alleles are expressed or repressed, depending upon the cell type. However, a small number of genes, designated imprinted genes, are monoallelically expressed in a parent-of-origin-specific manner. The murine genome contains ∼150 such imprinted genes, although this number is likely to increase as more tissue-specific imprinting is described. Importantly, imprinting is well-conserved across mammals, with many, but not all, imprinted genes and imprinting mechanisms being conserved between mouse and human (Lee and Bartolomei, 2013). This conservation has greatly facilitated the study of imprinting, as researchers have used both experimental mouse models and human genetic disorders to expand our knowledge of imprinting. A significant consequence of imprinting is that mammalian development requires genetic contributions from both a mother and a father. Moreover, a number of rare congenital disorders (Table 1) are caused by parental-allele-specific mutation or misregulation of one or more imprinted genes (Butler, 2009).

Imprinted genes are typically located in clusters of 3-12 genes that are spread over 20 kb-3.7 Mb of DNA, although examples of single imprinted genes do exist (Edwards and Ferguson-Smith, 2007). The clusters harbor maternally and paternally expressed imprinted genes that encode both protein-coding and non-coding (nc) RNAs. Each well-studied cluster has a discrete imprinting control region (ICR) that governs imprinted expression and exhibits parent-of-origin-specific epigenetic marks, such as DNA methylation and post-translational histone modifications. Much work over the past 25 years has centered on the identification of imprinted genes and understanding the mechanisms that underlie imprinted expression. For example, how imprints are acquired and reset in the germline and subsequently maintained and read after fertilization has been the subject of intense study. Although less effort has been focused on elucidating the function of imprinted genes, recent experiments have revealed broad roles for imprinted gene expression in mammals. These studies have shown that the small number of genes that exhibit imprinted expression have a large influence on mammalian development. As more imprinted genes have been discovered, it has become clear that they exert their effects on numerous and varied processes, including fetal growth, pluripotency, differentiation and behavior. Here, we review the mechanisms of imprinted gene regulation and the diverse roles of imprinted genes during development, highlighting recent work that expounds on their functional importance.

Establishing, maintaining and erasing imprints

The identification of the first imprinted genes (*Igf2r*, *Igf2* and *H19*) in 1991 (Barlow et al., 1991; Bartolomei et al., 1991; DeChiara et al., 1991) sparked initial efforts towards elucidating the mechanisms of imprint establishment, maintenance and erasure, which together control the timing and placement of genomic imprinting (Fig. 1). Allele-specific DNA methylation of ICRs has been pursued as the best candidate mechanism for conferring parental-specific imprints following fertilization. Moreover, the hypothesis that parental-specific imprints are imposed when the parental genomes can be distinguished prompted investigators to assay methylation acquisition during gametogenesis, when the maternal and paternal genomes are in separate compartments and can be independently modified. It was initially shown that paternal-specific methylation of the *H19*/*Igf2* ICR is acquired prenatally in prospermatogonia prior to the onset of meiosis in the male germline (Davis et al., 2000). By contrast, maternal-specific ICR methylation occurs postnatally in growing oocytes, with different ICRs being methylated at a slightly different time during oocyte growth (Lucifero et al., 2004). In both germlines, DNA methylation is established through the action of the *de novo* DNA methyltransferase 3a (DNMT3A) and the accessory protein DNMT3L (Bourc'his et al., 2001; Hata et al., 2002; Kaneda et al., 2004; Okano et al., 1999). Although it remains poorly understood how specific sequences are chosen for allele-specific DNA methylation in the germline, recent work has indicated that transcription through ICR sequences provides a key instructive step for the DNA methyltransferase proteins (Chotalia et al., 2009; Henckel et al., 2012).

Following fertilization, the parental-specific imprints must be maintained despite the extensive genome reprogramming and DNA demethylation that occurs at this time (Bartolomei and Ferguson-Smith, 2011). In addition to the action of the maintenance DNA methytransferase DNMT1, which methylates the newly synthesized strand of DNA, it is likely that the recognition of unique *cis*-acting

Department of Cell & Developmental Biology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA.
*Present address: 9-123 SCTR, 3400 Civic Center Blvd, Philadelphia, PA 19104, USA.

‡Author for correspondence (bartolom@mail.med.upenn.edu)

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Table 1. Human genetic disorders associated with imprinted genes

Disorder	Associated imprinted domains	Clinical phenotypes
Beckwith–Wiedemann Syndrome (BWS)	*H19-IGF2*, *CDKN1C*	Overgrowth, hemihyperplasia, macroglossia, abdominal wall defects, increased risk for embryonic tumors
Silver–Russell Syndrome (SRS)	*H19-IGF2*, *GRB10*, *MEST* (*PEG1*), *PEG3*	Undergrowth and asymmetry
Prader–Willi Syndrome (PWS)	*SNRPN-UBE3A*	Neonatal feeding difficulty, hypotonia, hypothalamic dysfunction, intellectual delay, hyperphagia, obesity
Angelman Syndrome (AS)	*SNRPN-UBE3A*	Developmental delay, speech impairment, poor motor control, seizures
Transient neonatal diabetes (TND)	*PLAGL1*	Transient diabetes mellitus present at birth
Uniparental disomy Chr14 (UPD14)	*DLK1*	Scoliosis, early puberty, developmental delay, hypotonia
Pseudopseudohypoparathyroidism (PPHP)	*GNAS*	Parathyroid hormone resistance, low energy, muscle cramps, bone thinning

Shown are some of the major congenital disorders associated within misexpression of imprinted genes, the clinical phenotype of these disorders and the associated imprinting domains (named for specific genes in that imprinted gene cluster).

sequences by *trans*-acting factors provides protection from post-fertilization reprogramming. For example, the maternal factor PGC7 (also known as STELLA or DPPA3) plays a general role in maintaining DNA methylation in the early mouse embryo, acting via interactions with dimethylated histone 3, lysine 9 residues (Nakamura et al., 2012). In addition, zinc finger protein homolog 57 (ZFP57) appears to play a more specific role in regulating imprinted genes. *ZFP57* mutations have been identified in transient neonatal diabetes patients and are associated with defective DNA methylation at several imprinted loci (Mackay et al., 2008). In line with this, *Zfp57* null mice show loss of imprinting at many, but not all, loci (Li et al., 2008). It is possible that other yet-to-be-identified proteins also maintain DNA methylation at ICRs in the early embryo.

Finally, to complete the imprinting cycle, the somatic pattern of biparental imprints is erased in primordial germ cells (PGCs), which are recruited from somatic cells in the early embryo. Although this process of erasure is poorly understood, it appears that imprints are lost through a series of active and passive events, including those involving the action of the ten-eleven translocation (Tet) family of methylcytosine dioxygenases, which catalyze the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine (Dawlaty et al., 2013; Hackett et al., 2013; Yamaguchi et al., 2013), as well as the action of DNA repair machinery (Pastor et al., 2013). Furthermore, the methylation of newly replicated DNA by DNMT1 is repressed in PGCs, probably by repression of *Uhrf1*, a factor essential for recruiting DNMT1 to the replication fork (Kagiwada et al., 2012).

Mechanisms of imprinted gene regulation

Two well-defined mechanisms of imprinted gene regulation have been described: the insulator model and the ncRNA model (Lee and Bartolomei, 2013). The insulator model (Fig. 2A) is best illustrated at the *H19*/*Igf2* locus. In this example, the ICR on the maternal allele is unmethylated and is bound by the insulator protein CCCTC binding factor (CTCF). This binding prevents the downstream enhancers that are shared by both *H19* and *Igf2* from engaging the *Igf2* promoter, but allows the enhancers to access *H19* and activate its expression. On the paternal allele, the ICR is hypermethylated, which prevents CTCF from binding and the insulator from forming. Consequently, the *Igf2* gene is activated by the shared downstream enhancers. Thus, in this model, the epigenetic state of the ICR determines the expression pattern of the locus.

The other major imprinting model is the ncRNA model (Fig. 2B), which is employed at a number of loci, including *Igf2r*/*Airn* and *Kcnq1*/*Kcnq1ot1*. In this case, the promoter of a long ncRNA is located within the ICR. On the paternal allele, the ICR is unmethylated thus allowing expression of the ncRNA, which in turn silences the rest of the genes in the domain in *cis*. This occurs by either attracting machinery that lay down repressive chromatin marks (Nagano and Fraser, 2009) or by preventing RNA polymerase II recruitment at promoters (Latos et al., 2012), although these mechanisms are not fully understood. Methylation of the ICR on the opposing maternal allele results in silencing of the ncRNA, thereby allowing activation of proximal genes. Thus, in this example, the function of long ncRNAs is to facilitate silencing of adjacent genes.

Imprinted genes and embryonic development and growth

The conclusion that imprinted genes are essential for proper development was made after studies demonstrating the developmental arrest of uniparental mouse embryos. In mice, uniparental embryos can be experimentally produced through nuclear transfer of zygotic pronuclei. Androgenetic embryos (derived from two paternal pronuclei) and gynogenetic embryos (derived from two maternal pronuclei) lacked embryonic and extraembryonic tissues, respectively (Barton et al., 1984; McGrath and Solter, 1984), suggesting a central role for imprinted genes in early lineage commitment and growth. Consistently, the first identified imprinted genes were shown to be essential for normal fetal growth but, as we discuss below, roles for imprinted genes in placental growth and behavior have since been discovered (summarized in Fig. 3).

Fetal growth and development

The most well-studied example of an imprinted gene that regulates growth is the paternally expressed *Igf2* gene, which is a positive regulator of fetal growth. Inappropriate biallelic expression of *Igf2* results in broad embryonic overgrowth, whereas its reduction leads to growth restriction (DeChiara et al., 1991; Ferguson-Smith et al., 1991; Leighton et al., 1995). Interestingly, the effect of IGF2 on fetal growth is neutralized by a maternally expressed imprinted gene, *Igf2r*. *Igf2r* mutations are associated with overgrowth and embryonic death, but both phenotypes are rescued in an *Igf2* null background (Ludwig et al., 1996). Accordingly, IGF2R acts as an antagonistic receptor that binds IGF2 and targets it for lysosomal degradation (Foulstone et al., 2005). The *H19* gene, which

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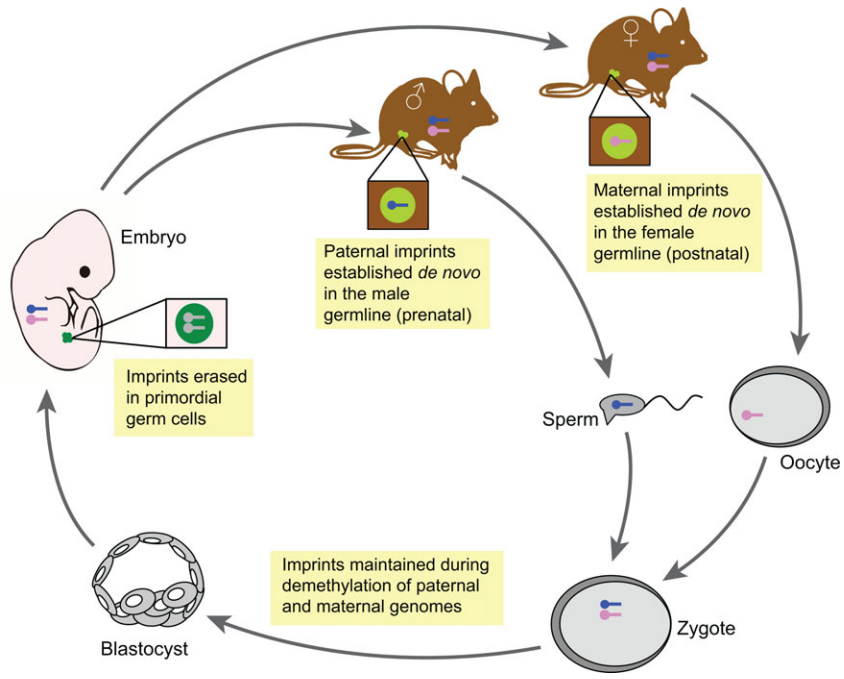

Fig. 1. Establishment, maintenance and erasure of genomic imprints during mouse development. Imprints are acquired in a sex-specific manner in the mature germline (light green circles) during development, with paternal imprints (blue chromosomes) being established prenatally and maternal imprints (pink chromosomes) established postnatally. These imprints are retained despite the global changes in DNA methylation that occur after fertilization, which include active demethylation of the paternal genome and passive demethylation of the maternal genome. These imprints are maintained in somatic tissues throughout adulthood. In primordial germ cells (PGCs, dark green circles), imprints are erased (gray chromosomes) and reset for the next generation.

expresses both a 2.3 kb ncRNA and a microRNA (miR-675) in a manner linked to the expression of *Igf2* (Fig. 2A), has been proposed to be a growth repressor (Hao et al., 1993). However, it is unclear whether the longer ncRNA, the miRNA, or both exhibit growth repressive properties.

Another imprinted gene with a broad developmental effect on embryonic growth is *Grb10*. Maternally expressed in most murine tissues, *Grb10* acts as a crucial growth restrictor. Maternal *Grb10* knockout embryos exhibit overgrowth, and deletion of the *Grb10* ICR results in biallelic expression and significant undergrowth (Charalambous et al., 2003; Shiura et al., 2009). Like IGF2, GRB10 probably exerts its effect on growth through the insulin pathway, binding the insulin receptor and both insulin-like receptors IGF1R and IGF2R (Holt and Siddle, 2005). Recent work has also shown that GRB10 is a substrate for mammalian target of rapamycin (mTOR), with mTOR-mediated phosphorylation and stabilization of GRB10 leading to reduced insulin signaling (Yu et al., 2011).

Although *Igf2*, *Igf2r*, *H19* and *Grb10* are important for modulating growth control pathways, many other imprinted genes affect embryonic growth through other mechanisms. It has been proposed that a number of these imprinted genes, including *Peg1* (*Mest*), *Gtl2* (*Meg3*), *Cdkn1c*, *Plagl1* and *Dlk1*, are coordinately regulated in multiple tissues along with *Igf2* and *Grb10* to regulate growth in a proposed 'imprinted gene network' (Arima et al., 2005; Varrault et al., 2006). The full extent of the co-regulation of these genes and the mechanism of co-regulation are still unknown. The transcription factor *Zac1* (*Plagl1*), which is a paternally expressed imprinted gene, has been suggested to alter the expression of genes in the imprinted gene network (Varrault et al., 2006). Deletion of this gene in mice results in intrauterine growth restriction and neonatal lethality. Moreover ZAC1 alters the expression of several imprinted genes, including *Cdkn1c* and *Dlk1*, and it directly regulates the *H19*/*Igf2* locus through binding of its shared enhancer (Varrault et al., 2006). Additionally, *H19* has been proposed as a possible regulator of the imprinted gene network in *trans* by recruiting MBD1 (Gabory et al., 2009; Monnier et al., 2013). Finally, BMI1, a member of the Polycomb Repressive Complex 1 (PRC1) has also been implicated in the coordinated expression of multiple imprinted genes within this network (Zacharek et al., 2011).

Imprinting and placental development

Some imprinted genes also have key functions in placental development (Fig. 3). These genes control embryonic growth, as the placenta acts as the singular point of regulation between maternal and embryonic tissues, and is the source of many hormones and growth factors (Abu-Amero et al., 2006). A large number of imprinted genes (~80) are reported to be highly expressed in the placenta, although recent work has highlighted the prevalence of confounding maternal contamination and has questioned whether some of these genes are in fact imprinted (Okae et al., 2012). The deletion of some imprinted genes, including the paternally expressed *Peg3* and *Peg1*, in mouse knockout models causes growth restriction of the entire placenta (Curley et al., 2004; Lefebvre et al., 1998). miR-675, which is processed from the first exon of *H19*, is also highly expressed in the placenta and is important for signaling the end of placental growth by downregulating *Igf1r* (Keniry et al., 2012).

Other imprinted genes are essential for the proper development of placental tissues. For example, *Ascl2* and *Peg10* are required for the development of the spongiotrophoblast, one of the major endocrine factor-producing regions of the placenta (Guillemot et al., 1994; Ono et al., 2006). Conversely, *Phlda2* and *Cdkn1c* are maternally expressed imprinted genes that, when deleted, cause improper spongiotrophoblast expansion (Frank et al., 2002; Zhang et al., 1998). *Rtl1*, a paternally expressed imprinted gene, is crucial for the maintenance of placental capillaries (Sekita et al., 2008). Other imprinted genes are central to placental function; mono-amine uptake to the embryo through the placenta is inhibited by deletion of *Slc22a3* and nutrient uptake is inhibited by the deletion of the placental-specific isoform of *Igf2*, *Igf2 P0* (Constância et al., 2002; Zwart et al., 2001). Given the roles of imprinted genes in the placenta, it is unsurprising that deletion of *Dnmt3l*, a member of the DNA methyltransferase family that acts with DNMT3A to establish DNA methylation imprints in germ cells, results in extensive abnormalities in placental development (Arima et al., 2006).

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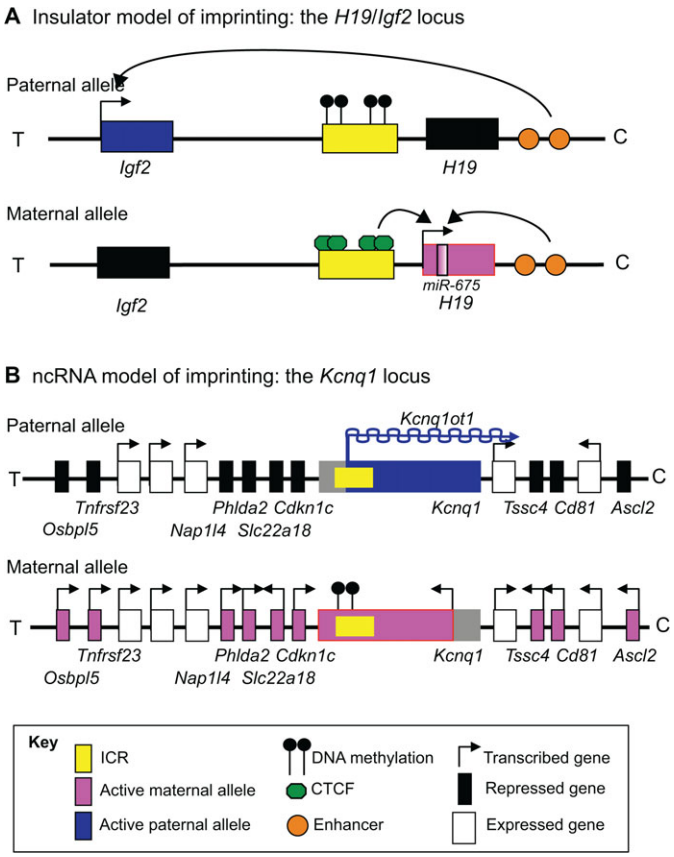

Fig. 2. Imprinting mechanisms. (A) The insulator model is best represented by the *H19*/*Igf2* locus. The ICR on the paternal allele of this locus is DNA methylated. By contrast, the ICR on the maternal allele is unmethylated, which allows binding of the insulator protein CTCF and prevents enhancers from interacting with the insulin-like growth factor 2 (*Igf2*) promoter. Instead, the enhancers activate *H19* expression. On the paternal allele, DNA methylation prevents CTCF from binding to the ICR, allowing the enhancers to activate *Igf2* expression. (B) The ncRNA model is best illustrated by the *Kcnq1* locus. Here, the ICR contains the promoter of the *Kcnq1ot1* long ncRNA. On the paternal allele, the ICR is unmethylated, allowing the expression of *Kcnq1ot1*, which in turn silences the paternal alleles of the adjacent genes. On the maternal allele, *Kcnq1ot1* is not expressed owing to DNA methylation of the ICR, and the adjacent imprinted genes are expressed. All imprinted domains are depicted for the mouse, although the human regions are largely conserved. T refers to the telomeric end of the cluster and C the proximal end of the chromosome. Not drawn to scale.

Imprinting and human growth disorders

Although the previously described work on imprinted genes was performed using mouse models, the consequence of misregulated imprinting on embryonic growth is obvious in humans where it manifests in multiple clinical phenotypes (Table 1). For example, Beckwith–Wiedemann syndrome (BWS) is an overgrowth disorder associated with genetic defects in two adjacent clusters of imprinted genes on chromosome 11. Individuals with BWS often present as large for gestational age at birth, with large tongues (macroglossia), large bodies and placental overgrowth. This syndrome most commonly arises owing to the loss of methylation at the ICR for the long ncRNA gene *KCNQ1OT1* (Higashimoto et al., 2006). This loss of methylation results in biallelic expression of the ncRNA and consequently *cis*-acting repression of the protein-coding genes regulated by KCNQ1OT1 (Fig. 2B). One protein-coding gene from this cluster that drives the BWS phenotype is *CDKN1C*, which makes a protein product that acts as a cell cycle inhibitor and growth restrictor; absence or mutation of CDKN1C promotes overgrowth (Andrews et al., 2007; Matsuoka et al., 1996). BWS is less frequently caused by activation of *IGF2* and reduced *H19* expression (typically through ICR deletions and an increase in methylation at the *H19* promoter), although in these cases it is often accompanied by Wilms and other tumors (Choufani et al., 2013). In contrast to BWS, Silver–Russell syndrome (SRS) is a genetic disorder in which babies are born small for their gestational age and later exhibit dwarfism. SRS is highly associated with hypomethylation at the *H19*/*IGF2* ICR (designated IC1 at the human locus), resulting in biallelic expression of *H19* and biallelic repression of *IGF2* (Gicquel et al., 2005). Both BWS and SRS are also associated with asymmetrical growth and a variety of deleterious phenotypes, suggesting that the role these imprinted genes play in growth has fundamental importance in many biological processes.

Mouse mutants in the BWS and SRS orthologous regions have been instrumental in uncovering the mechanism of imprinting regulation at the *H19*/*Igf2* and *Kcnq1*/*Kcnq1ot1* loci. Most of these loss-of-imprinting (LOI) mouse models for BWS and SRS mimic patient cases, although curiously they do not fully recapitulate the phenotypes observed in these human syndromes. Mice in which *Igf2* is overexpressed and *Cdkn1c* or *Igf2r* is deleted display some but not all of the BWS phenotypes (Caspary et al., 1999; Eggenschwiler et al., 1997). It has been suggested that mice cannot fully recapitulate the BWS phenotype at least in part because of differences in proliferation rates between mouse and human (Caspary et al., 1999). With respect to SRS, an engineered mouse strain in which CpG mutations prevented maintenance methylation of the paternally transmitted *H19*/*Igf2* ICR exhibited diminished *Igf2* expression and overexpression of *H19* (Engel et al., 2004). Although these mice are small, they do not appear to exhibit the other features characteristic of SRS, such as asymmetry.

Imprinted genes and neural development and function

In addition to their roles in the general growth and health of the embryo, imprinted genes also play numerous, highly specialized and cell type-specific functions during development. Although this is true in a variety of tissues and contexts, imprinted genes have a particularly important and complex role in the development of the mammalian brain. This was first highlighted by foundational work examining the contribution of parthenogenetic (PG, similar to gynogenetic embryos but exclusively derived from an egg) and androgenetic (AG, paternal only) cells to the nervous system in a developing chimeric embryo (Keverne et al., 1996). Although both PG and AG cells exhibited low levels of contribution to the developing brain in chimeras, PG chimeras had a larger brain and a smaller body, whereas AG chimeras had a smaller brain, but a larger body. Furthermore, PG and AG cells contributed to distinct sub-regions of the brain, with PG cells being more prevalent in the neocortex and AG cells more prevalent in the pre-optic area and hypothalamus. This work was the first to suggest that the maternal and paternal genomes may have distinct roles in neuronal development, and has subsequently been tied to several key imprinted loci and their proper regulation.

Imprinted gene expression in the brain

It is interesting to note that a number of imprinted genes have expression patterns and functions in the brain that are distinct from those seen in other tissues (Fig. 3). *Ube3a* is the most well studied example, being biallelically expressed in most tissues but maternally expressed within certain neuronal subtypes (Albrecht et al., 1997).

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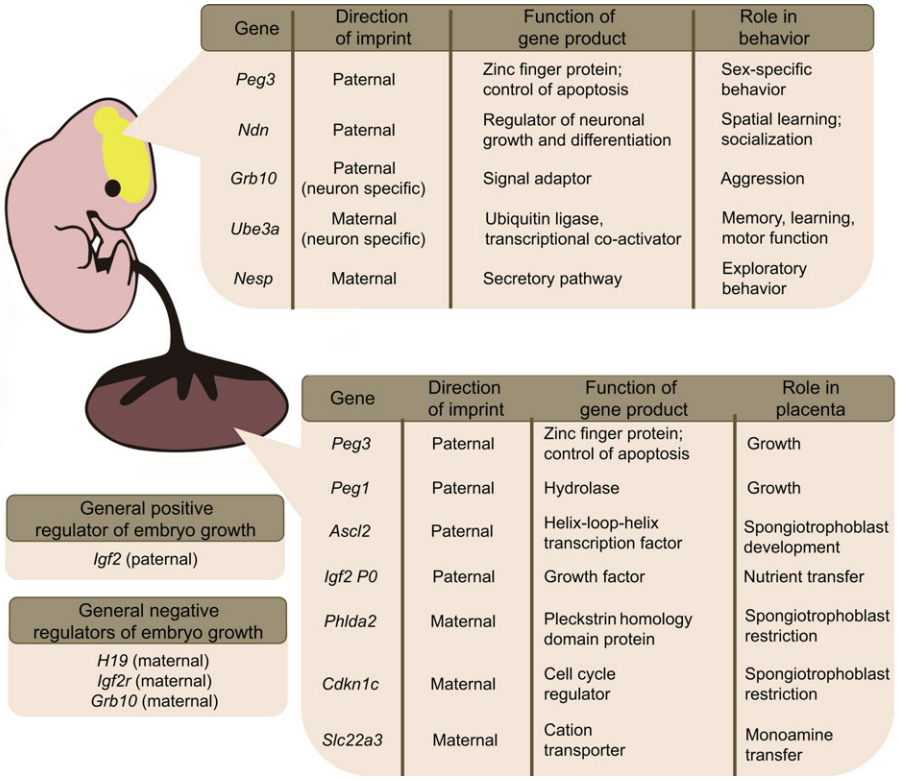

Fig. 3. A summary of imprinted gene functions during embryogenesis. Examples of imprinted genes and their functions in the brain (top box), in the placenta (lower box) and in general growth (left-hand boxes) during embryonic development are listed.

Maternal deficiency of *UBE3A* causes Angelman syndrome (AS; Table 1), a neurodevelopmental disorder associated with cognitive and motor impairment (Kishino et al., 1997). UBE3A is an ubiquitin ligase that can target proteins for degradation. *In vitro*, UBE3A has been shown to ubiquitylate proteins important for cell cycle regulation, such as p53 (TRP53), HHR23A (RAD23A) and MGMT (Kumar et al., 1999; Scheffner et al., 1993; Srivenugopal et al., 1996). UBE3A has similarly been shown to ubiquitylate ARC, a protein localized to neuronal synapses that promotes internalization of specific glutamate receptors important for neural plasticity (Greer et al., 2010). Such results should be taken with caution, however, as ubiquitylation of these proteins by UBE3A *in vivo* is largely unproven, or, in the case of ARC, has recently been contested (Kühnle et al., 2013). Nevertheless, *Ube3a* knockout in the mouse is associated with increased levels of ARC and p53 in brain lysates, suggesting at least a genetic role for *Ube3a* in regulating protein levels in neurons. In addition, Ube3a knockdown in primary hippocampal neurons results in decreased synaptic localization of AMPA receptors, which are important for the plasticity of neuronal connections and which can be sequestered by ARC (Greer et al., 2010). As mentioned above, very recent work argues that UBE3A does not directly ubiquitylate ARC, but rather that UBE3A acts as a negative regulator of estrogen-mediated *Arc* transcription (Kühnle et al., 2013). Consistent with this model, UBE3A has been shown to act as a transcriptional co-regulator by interacting with steroid hormone receptors, including the estrogen receptor (Ramamoorthy and Nawaz, 2008). However, biochemical analysis of the *UBE3A* point mutations in found in AS patients shows very frequent loss of ubiquitin ligase activity and a general preservation of co-activator activity (Cooper et al., 2004). Taken together, it is possible that defects in both mechanisms (transcription and ubiquitylation) may contribute to the AS phenotype.

Other imprinted genes that show brain-specific expression patterns contribute to the proper establishment of the highly complex cell types that make up the brain. *Peg3* is a paternally expressed gene that encodes a zinc finger protein that exhibits high expression levels in certain brain regions. Importantly, *Peg3* has been implicated in the control of apoptosis in neurons through its interaction with p53 and the pro-apoptotic factor BAX (Johnson et al., 2002). Deletions of paternal *Peg3* result in increased neonatal apoptosis in specific brain regions. This apoptosis ultimately reduces the total number of oxytocin-secreting neurons and masks normal sex-specific differences in apoptosis in brain regions involved in sexual behavior, olfaction and pheromone processing (Broad et al., 2009; Li, 1999).

Another example of an imprinted gene that affects specific neuronal subtypes is the paternally expressed *Ndn* gene, which encodes a protein that interacts with p53 and a variety of growth factors to influence neuronal differentiation and growth (Kuwajima et al., 2006; Salehi et al., 2002; Taniura et al., 1999). *Ndn* mutant mice exhibit reduced neuronal density in the hypothalamus and morphological abnormalities in axonal outgrowths (Muscatelli et al., 2000; Pagliardini et al., 2005). Interestingly, a variety of hypothalamic dysfunctions are evident in Prader–Willi Syndrome (PWS; Table 1) (Swaab, 1997). Accordingly, mutations in *NDN*, as well as in other genes within the same imprinting cluster including *SNRPN* and several small nucleolar RNAs (snoRNAs), are associated with PWS.

Imprinted genes in the brain: human disorders and effects on behavior

The importance of imprinted genes in neurodevelopment is probably best highlighted by the wide variety of behavioral phenotypes associated with their misexpression. Mice deficient in maternal *Ube3a* exhibit defects in hippocampal-related memory and learning, along with a variety of abnormalities in motor system behaviors (Heck et al., 2008). These phenotypes are partially mirrored in patients with AS, a disorder characterized by attention deficits and delayed motor development (Pelc et al., 2008). Furthermore, *Ndn* null

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mice show an abnormal skin scraping tendency and modified spatial learning, both of which are reminiscent of some behaviors associated with PWS (Muscatelli et al., 2000). It is important to note, however, that mouse model systems do not recapitulate the full gamut of behavioral phenotypes exhibited by AS and PWS patients. For example, some, but not all, mice models for PWS exhibit increased appetite, which is also observed in human PWS patients (Rieusset et al., 2013), but this may not be from the lack of satiation that underlies these phenotypes in humans.

A variety of other imprinted genes are also associated with the regulation of behavior. Two imprinted genes, *Peg1* and *Peg3*, are associated with an important role in maternal care behaviors. *Peg3* null female mice exhibit deficiencies in sexual behavior and maternal care actions, such as milk-letdown and nest building. Additionally, *Peg3* null neonates have reduced suckling behavior (Champagne et al., 2009). Similarly, *Peg1* null females display abnormal maternal care and impaired placentophagia, which both are behaviors associated with the successful rearing of young (Lefebvre et al., 1998). Recent work has also shown that paternal deletion of *Grb10*, which is expressed from the paternal allele in a subset of neurons from alternative promoter(s), is associated with hyper-aggression and increased social dominance in mice (Garfield et al., 2011). Finally, a maternally expressed gene, *Nesp* (*Gnas*), which encodes a protein involved in neuro-excretory function, is associated with novel exploration behavior and has been observed to have striking overlap in expression with *Grb10* in the brain (Dent and Isles, 2014; Plagge et al., 2005).

Roles for imprinted genes in stem cells and reprogramming

A crucial function for genomic imprinting in stem cells, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and adult stem cells, has recently garnered much attention (Papp and Plath, 2013; Stadtfeld and Hochedlinger, 2010). As discussed above, genomic imprints are established in the male and female germline when the parental alleles can be independently marked. This establishment occurs after imprint erasure and is part of the widespread epigenetic reprogramming and genome-wide demethylation that is essential for totipotency (Hajkova et al., 2008; Surani et al., 2007). However, it is now known that at least some of the germline-specific reprogramming events can be bypassed during reprogramming, when iPSCs are derived from differentiated somatic cells. An important question for practitioners of iPSC technology during its early development was whether imprints would be appropriately maintained during the reprogramming process. For example, nuclear transfer (NT)-derived mouse clones exhibited general epigenetic instability and LOI, and suffered from a variety of defects often associated with imprinting disorders (Humpherys, 2001).

Regardless of whether these cells will be used to study basic developmental processes or employed for human therapeutics, proper imprinting is an essential benchmark. LOI not only can result in the previously mentioned errors in early growth and development, but additionally is highly correlated with cell transformation and cancer. Many imprinted genes, including *H19*, *Peg1* and *Peg3*, are known tumor suppressors (Feinberg, 1999). Additionally, 'imprint-free' mouse ESCs that have global LOI effectively contribute to chimeras, but these mice develop multiple types of cancer by one year of age (Holm et al., 2005). Thus, the careful study of imprinted gene expression and function in iPSCs is required for full confidence in their application. Such studies, together with analyses of imprinting in embryonic and adult stem cells, highlight the functional importance of imprinted genes in pluripotent cell populations.

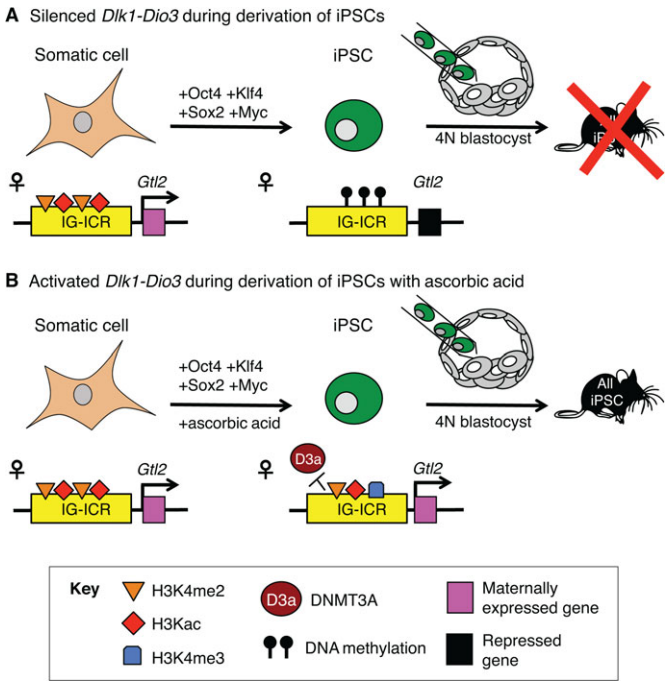

Fig. 4. *Dlk1-Dio3* expression during iPSC induction. (A) *Gtl2*, a non-coding RNA in the *Dlk1-Dio3* imprinted cluster, is expressed from the maternal allele in most somatic tissues. Within these tissues, the ICR (IG-ICR) within the cluster exhibits activating histone marks (H3K4me2 and H3Kac). Induction of pluripotency in somatic cells via exogenous expression of *Oct4* (*Pou5f1*), *Klf4*, *Sox2* and *Myc* frequently results in the aberrant silencing of maternal transcripts within the *Dlk1-Dio3* cluster and DNA methylation of the ICR by DNMT3A (not pictured). The subsequent misexpression of *Gtl2* and other *Dlk1-Dio3* transcripts results in poor incorporation of these iPSCs into chimeric mice made using the tetraploid (4N) complementation method. No 'all iPSC' mice have been made from iPSCs with silenced *Dlk1-Dio3*. (B) The addition of ascorbic acid (vitamin C) during the iPSC reprogramming process results in activating histone marks at the IG-ICR, including H3K4me3, and the expression of *Gtl2*. The addition of ascorbic acid prevents the recruitment of DNMT3A (D3a) by an unknown mechanism. These iPSCs can give rise to 'all iPSC' mice.

Imprinting and reprogramming

An initial report describing the reprogramming of mouse embryonic fibroblasts to a pluripotent state showed that several imprinted genes (*H19*, *Peg1*, *Peg3* and *Snrpn*) maintained proper allele-specific DNA methylation after reprogramming (Wernig et al., 2007). Two subsequent studies characterizing imprinting during the induction of human iPSCs showed that LOI is an exceedingly rare but observable event that is evident at early stages in the reprogramming process, is highly cell-line specific, and is maintained through multiple passages (Hiura et al., 2013; Pick et al., 2009). Interestingly, maintenance of the state of imprinting is also evident in iPSCs generated from AS and PWS patient fibroblasts, with pathological errors in imprinting and expression being retained through reprogramming and subsequent culture (Chamberlain et al., 2010). Thus, it appears that imprints present in the somatic cell of origin are, for the most part, faithfully retained in iPSCs after reprogramming.

A significant and functionally crucial exception to these trends involves errors during iPSC reprogramming at the *Dlk1-Dio3* imprinted cluster (Fig. 4A). In a genome-wide comparison of expression between genetically identical mouse ESCs and iPSCs, the only two significantly downregulated transcripts in iPSCs were the maternally expressed ncRNA *Gtl2* and the long ncRNA *Rian*, both of which are found within the *Dlk1-Dio3* imprinting region (Stadtfeld

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et al., 2010). Analysis of 62 additional iPSC lines showed that only ~6% of these lines exhibited normal expression of *Gtl2*, and that lines that misexpress genes in this cluster demonstrated a greatly reduced ability to contribute to chimeras. Further work corroborated that repression of genes in the *Dlk1-Dio3* cluster correlated with reduction in pluripotency hallmarks, specifically the generation of 'all iPSC' mice (Carey et al., 2011; Liu et al., 2010). Mechanistically, it was found that hypermethylation across *Dlk1-Dio3* causes aberrant repression at the cluster and is dependent on the inappropriate recruitment of the *de novo* DNA-methyltransferase DNMT3A. Surprisingly, recent follow-up work has shown that treatment of iPSCs with ascorbic acid (vitamin C) during passage and reprogramming ensures the maintenance of euchromatic marks across *Dlk1-Dio3*; ascorbic acid treatment inhibits the recruitment of DNMT3A by a highly specific but unknown mechanism and increases iPSC pluripotency and reprogramming efficiency (Stadtfeld et al., 2012).

Imprinting in adult stem cells

Imprinted genes have been recently implicated in the maintenance and function of adult stem cell populations. A transgenic reporter mouse line for the paternally expressed *Peg3* gene has shown that *Peg3* expression in adults is restricted to stem cell/progenitor populations in a variety of tissues, including the brain, gut, bone, muscle and skin (Besson et al., 2011). The generation of neurospheres, via an *in vitro* technique used to isolate and amplify neuronal progenitors, resulted in ~100% *Peg3*-positive cells after a single passage. Additionally, engrafting experiments in the epidermis revealed that transferred *Peg3*-positive cells have the ability to self-renew within the follicular stem cell niche and differentiate effectively. These experiments suggest that *Peg3* plays a functional role in adult stem cells, although it is currently unclear whether this role is different from that observed in early development.

The maternally expressed *H19* gene is also involved in the maintenance of adult hematopoietic stem cell (HSC) populations in the mouse (Venkatraman et al., 2013). Conditional maternal deletion of the *H19/Igf2* ICR in HSCs caused reduced expression of *H19* and increased expression of *Igf2*, accompanied by a reduction in the number of long-term HSCs, an increase in short-term HSCs, and overall compromised hematopoietic potential and function. In addition, maternal deletion of the *H19/Igf2* ICR caused inappropriate activation of the *Igf2-Igf1r* pathway via increased expression of *Igf2* and decreased repression of *Igf1r*, which is a target of *H19*-derived miR-675. This led to inhibited quiescence-associated cell cycle arrest mediated by FOXO3, ultimately resulting in the activation and exhaustion of long-term HSCs.

Additionally, selective loss of *Dlk1* imprinting within mouse neural stem cells (NSCs) and their niche has been shown to be crucial for postnatal neurogenesis (Ferrón et al., 2011). *Dlk1* is a membrane-bound receptor for Notch signaling that is downregulated postnatally in most tissues. *Dlk1*-deficient mice show decreased pools of slow-dividing NSCs, resulting in depletion of neurons in the adult olfactory bulb. Interestingly, the NSCs and the surrounding astrocytes that make up their niche express *Dlk1* from both alleles, whereas *Dlk1* is otherwise expressed exclusively from the paternal allele. This coordinated biallelic expression of *Dlk1* highlights the importance of specific contexts in imprinted gene regulation and underscores the significance of gene dosage for imprinted genes.

Conclusions

Although initial work suggested that imprinted gene expression is crucial for early embryonic growth and differentiation, it is now clear that genomic imprinting exhibits a much more varied role in mammalian development. It is important to note that this Review is intended to highlight just some of the functions of genomic imprinting and imprinted genes, but is not comprehensive; various roles for many imprinted genes have been documented. For example, LOI at numerous imprinted genes has been associated with cancer and oncogenic phenotypes (Baylin and Jones, 2011); a functional importance not detailed at length in this Review. Additionally, imprinted expression plays a variety of other tissue-specific roles in many other organs and cell types not elaborated here (Prickett and Oakey, 2012). It is almost certain that a full understanding of tissue-specific imprinting is incomplete. Further exploration of the prevalence of imprinting in complex tissues such as the brain and in novel contexts such as adult stem cell populations will undoubtedly lead to exciting discoveries regarding the expression and functions of imprinted genes.

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Competing interests

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