ORIGINAL ARTICLE

Mammalian viviparity: a complex niche in the evolution of genomic imprinting

EB Keverne

Evolution of mammalian reproductive success has witnessed a strong dependence on maternal resources through placental *in utero* development. Genomic imprinting, which has an active role in mammalian viviparity, also reveals a biased role for matrilineal DNA in its regulation. The co-existence of three matrilineal generations as one (mother, foetus and post-meiotic oocytes) has provided a maternal niche for transgenerational co-adaptive selection pressures to operate. *In utero* foetal growth has required increased maternal feeding in advance of foetal energetic demands; the mammary glands are primed for milk production in advance of birth, while the maternal hypothalamus is hormonally primed by the foetal placenta for nest building and post-natal care. Such biological forward planning resulted from maternal–foetal co-adaptation facilitated by co-expression of the same imprinted allele in the developing hypothalamus and placenta. This co-expression is concurrent with the placenta interacting with the adult maternal hypothalamus thereby providing a transgenerational template on which selection pressures may operate ensuring optimal maternalism in this and the next generation. Invasive placentation has further required the maternal immune system to adapt and positively respond to the foetal allotype. Pivotal to these mammalian evolutionary developments, genomic imprinting emerged as a monoallelic gene dosage regulatory mechanism of tightly interconnected gene networks providing developmental genetic stability for *in utero* development.

Heredity (2014) **113**, 138–144; doi:10.1038/hdy.2014.8; published online 26 February 2014

INTRODUCTION

Viviparity has had a major role in shaping mammalian evolution as characterised by invasive placentation, maintenance of body temperature (homeothermy), milk production and enlargement of the brain (encephalisation) relative to body size. Many of these evolutionary developments have incurred increased maternal investment ensuring reproductive success because of the substantial energetic contribution, which mother makes for offspring both pre- and post-natally (Curley *et al.*, 2005). Genomic imprinting has been integral to these evolutionary developments with a strong matrilineal bias for the mechanistic control of this kind of gene regulation.

The strong bias for maternal imprint control regions (ICRs) avoids the mutagenic environment of the paternal germline during the multiple divisions for spermatogenesis (Schultz *et al.*, 2010). Paternal ICRs are intergenic and tend to evolve neutrally while maternal ICRs coincide with CpG-rich promoters that are under selection pressure for conserving sequences that are linked to promoter functions. The rate of CpG loss has been considerably higher for paternal than for maternal ICRs. Methylation of cytosines in ICRs increases the rate of C-T transition mutations, higher in the male germline because of the longer germline passage and the greater number of cell divisions. Thus, two evolutionary drives tend to favour maternal ICRs over paternal. Meiotic recombination, which is high at ICRs, results in accumulation of CpG sites through biased conversions that favour AT-GC mutations, whereas C-T deamination in the paternal germline has failed to sustain paternal ICRs (Duret and Galtier, 2009). The strength of correlation between female recombination rate and local

GC content is more than doubled by controlling for replication timing (Pink and Hurst, 2011). Moreover, the molecular mechanisms governing replication timing are not only stable but also meet requirements to be readily reprogrammed in the next generation (Shufaro *et al.*, 2010).

Theories of genomic imprinting have focussed on the genes expressed as a consequence of imprinting, and referred to as the maternally expressed and paternally expressed genes. Most theories have failed to take into account the epigenetic mechanisms for genomic imprinting, which has itself become heritable and expressed primarily through the maternal germline. These ICRs, or differentially methylated regions (DMRs), are all reprogrammed and methylated in the female germline with the exception of H19, Rtl1 and Dlk1. Imprinting at the Rtl1 and Dlk1 genomic region is thought to have occurred after the marsupial–eutherian divergence with the accumulation of small nuclear RNAs (micro RNAs and small nucleolar RNAs) on the maternal chromosome, which have the active role in regulatory expression of these genes (Renfree *et al.*, 2009). H19 is a non-coding construct from the Igf2 genetic locus, which gave rise to the ‘conflict theory’ (Haig, 1992). In the relatively short-lived marsupial placenta, H19 is methylated on the paternal chromosome and Igf2 is also expressed from the paternal chromosome but there is no methylation imprinting of the Igf2 receptor (Renfree *et al.*, 2008). So although H19 can be linked, albeit indirectly to paternal expression of Igf2 in the marsupial placental, there is no evidence for genomic imprinting driven conflict as there is no imprinting of the Igf2 receptor. In eutherian mammals, H19 also undergoes DNA

methylation on the paternal allele. This prevents the binding of a maternal protein, CCCTC binding protein (CTCF), which is integral to paternal Igf2 expression. CTCF binding acts as an insulator for Igf2 expression but permits H19 expression on the maternal chromosome. H19 ICR itself contains an evolutionary conserved silencer element that functions autonomously even when inserted into *Drosophila* (Lyko *et al.*, 1997). H19 is not only responsible for regulatory methylation at the Igf2 locus, but also initiates asynchronous replication of parental chromosomes (Amey *et al.*, 2006). Asynchronous chromosomal replication is integral to the monoallelic silencing of genes seen in the immune system, olfactory receptors, the brain, X-inactivation and ICRs (Gimelbrant *et al.*, 2007).

CTCF has been described as a maternal effect gene and is more than just an insulator for Igf2 expression. CTCF serves as a master organiser of chromatin structure on the maternal chromosome along the H19 Igf2 domain (Singh *et al.*, 2012). CTCF binding at H19 provides this locus with pleiotropic influence over the epigenetic status of multiple genomic regions, even those on other chromosomes and identified by chromosomal differential replication timing events (Guilbert *et al.*, 2012). Maternal CTCF is critical to early embryogenesis and has also been described as a 'master weaver of the genome', particularly important to regulating developmental gene networks (Phillips and Corces, 2009).

Multiple levels of control for expression of paternal Igf2 thus involve the maternal genome. The action of Igf2 on the maternally expressed Igf2 receptor is also integral to the conflict theory, but when viewed over evolutionary time, matches better to explaining the balanced regulation of *in utero* growth early in pregnancy regulated primarily by the maternal genome. Late in pregnancy, this balanced regulation of growth is a function of the paternal H19 locus itself. The presence of a micro-RNA (Mir675) embedded in the first exon of paternal H19, and expressed exclusively in the placenta, has recently been identified (Keniry *et al.*, 2012). This non-coding RNA targets the growth-promoting Igf2 receptor and functions in limiting the growth of the eutherian placenta later in pregnancy. Although a balanced regulation of *in utero* foetal growth under control of Igf2 and its receptor still hold, the complexity of balanced paternal gene expression (H19 and Mir675) and the importance of maternal CTCF in enabling Igf2 expression are difficult to reconcile with conflict.

EVOLUTION OF GENOMIC IMPRINTING—MONOALLELIC GENE EXPRESSION

Monoallelic gene expression is more common than previously appreciated and has had an important role in the evolution of the immune system, the mammalian olfactory receptors, X-inactivation and genomic imprinting (Keverne, 2009). A question of some importance concerning monoallelic expression is the level of gene product and if this is affected by its transcription from one allele or both? One possibility is that the transcription of each allele is regulated independently such that having both alleles active leads to higher overall transcription levels. Assessments have shown that monoallelic expression of a given gene in cell clones has a lower level of expression than that of the biallelic expression of the same gene in other cell clones (Gimelbrant *et al.*, 2007). A disproportionate fraction of the monoallelic expressed genes encode cell surface proteins suggesting a role for monoallelic expression in each given cells interactions. The stability of allele-specific choice and clonal expansion to form tissues can lead to macroscopic patches of placenta (Gimelbrant *et al.*, 2007) or specific nuclear types in the brain (Gregg *et al.*, 2010). This would make these structures a mosaic with respect to monoallelic expression of such autosomal genes, as also occurs

with skewing of X-inactivation in the brain, providing an epigenetic basis for functional differences amongst individuals.

There are many more monoallelically expressed genes than previously recognised (Chess, 2012). The recent 'encode' of the human genome investigated the link between allele-specific binding and allele-specific expression, and have identified maternal-specific networks (Gerstein *et al.*, 2012). Some 4798 allele-specific transcription factors have been identified as mapping to maternally or paternally regulated target genes. As the degree of combinatorial regulation increases, so the relationship between expressed transcription factor and regulated alleles becomes stronger. Targets regulated by two or three maternally expressed transcription factors showed increasingly higher levels of maternal expression (60–92%), which was not the case for paternal expressed transcription factors (~50:50). Those target genes regulated by more than one transcription factor were found to be under stronger negative selection.

Although the mechanisms that operate for monoallelic expression are also active in genomic imprinting, the key to understanding the evolution of genomic imprinting is in the imprint regulation (ICR or DMR), those heritable epigenetic marks, which are germline reprogrammable. The important feature of the genes, which are imprinted, is their increased stability through purifying selection and the increased evolutionary recruitment of more genes to this mode of expression across mammalian evolution. Much of the theory behind genomic imprinting has focussed on the genes that are either maternally expressed or paternally expressed, rather than on the genetic process that brings this about. The imprinted genes are themselves remarkably stable and analysis of 34 orthologous imprinted genes demonstrates that the vast majority of mammalian imprinted genes have not undergone subsequent gene duplication, nor is there evidence for positive selection on these protein-coding genes in placental species (O'Connell *et al.*, 2010). The majority of orthologues of imprinted loci display high levels of micro-synteny conservation. However, many of these same genes, which are imprinted in mammals, are subject to positive selection in species where they are not imprinted. These results have thus provided little evidence for the protein-coding regions of imprinted genes evolving under positive selection. The authors concluded that these results do not provide genetic support for imprinted genes being involved in antagonistic co-evolution via a parental conflict. Others have also found that in rodent mammals there is no specific evolutionary pressure on protein-coding sequences of imprinted genes (Hutter *et al.*, 2010) and no evidence for antagonistic co-evolution (McVean and Hurst, 1997), but a strong purifying selection as suggested by the lack of SNPs.

IMPRINTED GENE NETWORKS

The evolutionary significance of genomic imprinting would appear to be in the progressive recruitment of genes to the imprinting locus producing ever larger networks of imprinted genes, expanded by the monoallelic control of downstream genes, which they regulate. There is no evidence for genomic imprinting in the egg-laying monotreme mammals and there are relatively few imprinted genes engaged in the development of the short-lived chorio-vitelline placenta (yolk-sac) of the Marsupial Wallaby (Renfree *et al.*, 2013). Only six genes have been identified as imprinted in the marsupials and all are expressed in the placenta, while a number of genes imprinted in the eutherian mammalian placenta are expressed but not imprinted in the marsupial placenta. In eutherian mammals, some 55 imprinted genes have been reported as expressed in the more invasive, longer-lived placenta (Tunster *et al.*, 2013). Therefore, it seems likely that as

placental complexity evolved with longer *in utero* development, the need for tighter control of gene networks resulted in the imprinting of regulatory genes already expressed in the metatherian placenta.

The ICRs appear to be gaining more genes from rodents to humans and are particularly important for regulation of gene dosage, as revealed from the complex disrupted phenotypes that are produced by perturbations to the ICR without any effects on the imprinted genes themselves in Prader–Willi syndrome and Angelman’s syndrome (Rabinovitz *et al.*, 2012) and Silver–Russell syndrome (Kagami *et al.*, 2007). Many of these imprinted genes are co-regulated as an ‘imprinting network’ that serves a similar functional goal, such as that of controlling growth and metabolic rates (Sandhu, 2010).

In addition to the networks across and between imprinted genes, each imprinted gene may itself regulate a network of downstream genes engaged in specific functions. The *Peg3* transcriptome has a network of 22 genes concerned with neural development and 21 genes that are regulating other transcription factors. *Peg3* also regulates a number of genes concerned with placental development (pregnancy-specific glycoproteins (PSGs), ceacams and prolactins (PRLs)). Many of these *Peg3*-regulated genes have undergone multiple duplications across mammalian species (1–23 PSG variant copies; 1–19 ceacam variant copies and 3–18 PRL variant copies) with the lowest copy number in metatherians and highest in eutherian primates. A large number of imprinted genes (11 recorded so far) are co-expressed in the developing hypothalamus and placenta, providing the potential for transgenerational co-adaptive functioning of these structures. *In utero* development cannot tolerate errors. The stability of imprinted genes and the robustness of the functional networks (growth, metabolism and apoptosis) means that interacting hubs provide for compensatory actions through their common downstream genes. Thus, errors may be absorbed and robustness prevails in such fundamental biological systems (Sandhu, 2010).

GENOMIC IMPRINTING AND MATERNAL FOETAL CO-ADAPTATION

Mammalian viviparity has to contend with a semi-allogenic foetus, especially during invasive haemochorial placentation, which characterises rodents and primates where foetal trophoblast cells intermingle with maternal tissue (Rossant and Cross, 2001). The earliest response to successful pregnancy has required a tolerance to be generated between the maternal immune system and the foetal placenta. Indeed, adaptation on both the foetal and maternal side, facilitated by genomic imprinting, has enabled this interaction to be both protected from immune rejection and provided with active support for successful pregnancy (Moffett and Loke, 2006).

There is no direct contact between mother and the embryo, only with the extra-embryonic placental trophoblast as it implants and develops in the uterus. No evidence for interaction of the mother’s immune response by immunological T-cell recognition has been found for the invasive placenta of humans and rodents, which may explain how the placenta evolved to avoid rejection. As trophoblast cells, which subsequently divide to form the placenta, take their origin from the zygote before activation of the paternal genome, it is possible that the early trophoblast cell line is closely related to the maternal haplotype at implantation. Some 13 genes have been reported as specifically expressed in the mouse placenta all of which are maternally expressed (Hudson *et al.*, 2010). Non-coding RNA is thought to be primarily responsible for paternal silencing of this particular form of placental-specific imprinting (Pauler *et al.*, 2012). The evolutionary clustering in the organisation of these imprinted genes enables the epigenetic initiator to extend imprinted

silencing of paternal alleles over broad chromosomal domains (Kulinski *et al.*, 2013). A further consideration in the avoidance of immune rejection is the large number of genes on the X chromosome that are expressed in the placenta, but again, studies on marsupial and mouse have shown X-inactivation to be imprinted, with only genes from the maternal X being expressed in the rodent placenta (Wagschal and Feil, 2006).

The human and mouse placenta have a trichorial structure with two multinucleated syncytiotrophoblast layers representing a barrier between the maternal and foetal vascular systems. This syncytiotrophoblast layer of the placenta is regulated by retroviral syncytin genes, including the imprinted *Peg10*, and lies in direct contact with maternal circulation (Henke *et al.*, 2013). Although exposed to the maternal immune system, this foeto-placental interface does not express leucocyte T-cell antigens. However, as pregnancy progresses, other invasive interstitial trophoblast cells do express polymorphic leucocyte antigens, but these facilitate both implantation (decidual natural killer (NK) cells) and invasive capacity (uterine NK cells). Decidualisation of the maternal uterus is a necessary prelude to embryonic implantation and formation of the placenta with some 11 imprinted genes now identified as expressed in the maternal decidua (Tunster *et al.*, 2013).

Although decidual NK cells are in close contact with foetal trophoblast, they do not exert cytolytic functions because of activation of inhibitory killer cell immunoglobulin-like receptors on the surface of these maternal immune cells. On the contrary, maternal NK cells react positively to epitopes on trophoctoderm at the maternal foetal interface. These create a pregnancy favourable environment by inducing angiogenesis, trophoblast invasion of the uterus and vascular remodelling (Moffett and Loke, 2006). Depletion of NK cells in the mouse decidua result in abnormal implantation and inadequate modelling of spiral arteries that are essential for the development of normal pregnancy. Uterine NK cells produce a variety of growth factors and angiogenic factors and are major regulators of vascular remodelling in the early stages of pregnancy, declining as pregnancy progresses (Zhang *et al.*, 2011). Thus, not only has the foetal placenta adapted to avoid the maternal immune system, but the maternal NK cells have adapted to activate growth and development of the foetal placenta.

In addition to functional co-adaptation between the foetal placenta and maternal immune system, there is also functional co-adaptation across the placenta and developing brain, most notably the developing hypothalamus. The placental genome directs the production of placental hormones, which regulate multiple aspects of maternal hypothalamic function (Keverne, 2006) (Figure 1). Placental hormones increase maternal feeding in advance of the energy requirements needed for foetal growth later in pregnancy. The placental hormones terminate fertility and suppress sexual behaviour in most female species, ensuring that maternal feeding time and energy expenditure are directed towards a successful pregnancy. These same placental hormones induce nest building in advance of birth and also prime the hypothalamus for post-partum maternal care and milk letdown (Figure 1). In short, the foetal placenta controls foetal destiny by forming an interface with mother, instructing successful maternalism via the hypothalamus. As the foetus is developing its own hypothalamus at this time period, a developmental template is available on which selection pressures may operate within a generation with functional consequences across two generations (Figure 2). Thus, an infant who has received optimal nourishment and care will itself be both energetically equipped and genetically predisposed to good mothering in the next generation (Keverne, 2012).

Placental regulated steroids - effects on maternal brain

Figure 1 The foetal placenta produces progesterone that acts on the maternal brain (bold arrows) to affect maternal fertility, maternal behaviour and oxytocin synthesis. As pregnancy approaches term, the foetal adrenal gland converts progesterone to oestrogen that acts on the maternal brain to induce oxytocin receptors, which promote parturition, enable milk letdown and activate maternal behaviour. Oestrogen in the maternal brain is converted to pregnenolone, which serves as a maternal anxiolytic. GnRH, gonadotrophin releasing hormone.

Co-adaptive evolution

Figure 2 Some 11 maternally imprinted genes have been identified that are co-expressed in the developing foetal placenta and foetal hypothalamus at the same time as the placenta itself is interacting with the hypothalamus of the mother. This concurrent co-activation provides a transgenerational template on which selection pressures have operated to ensure optimal maternalisation of the next generation.

Molecular genetic studies have identified several of the imprinted genes, which are co-expressed in the developing hypothalamus and placenta, to be concerned with regulation of maternal behaviour and physiology (Keverne, 2012). Detailed studies with mice carrying a mutation in the maternally imprinted gene (*Peg3*) have provided some insights into how the functioning of the hypothalamus, which develops *in utero*, is adapted to respond to the placental hormones that determine maternalism. When *Peg3* is mutated in the maternal hypothalamus, but not in the placenta or foetus, many aspects of the mutant phenotype are similar to the functional consequences of this same gene being mutated in the placenta, but not in the mother's hypothalamus (Curley *et al.*, 2005; Figure 3) The functional consequences of hypothalamic gene deletion are manifest through reduced cell numbers in the hypothalamus (paraventricular nucleus, supra-optic nucleus and medial preoptic area; Broad *et al.*, 2009), which result in impaired maternal care and impaired suckling, which in turn, delay growth and onset of puberty and adult reproduction. This same *Peg3* gene deleted in the placenta also impairs placental growth, which in turn impairs hormonal priming of the

Co-adapted functions

Figure 3 Co-adapted functions revealed by the mutation of *Peg3* in the placenta (but not maternal hypothalamus) and the future maternal consequences of this same gene inactivated in the foetal hypothalamus, (but not in the placenta) of this next generation.

hypothalamus for maternal care and milk letdown. Low birth weight also impairs postnatal growth and delays puberty onset. The convergent actions of *Peg3* in mother and foetus illustrate its functional co-adaption for the hypothalamus and placenta. This developmental period (E11-12-13, mouse) is important for hypothalamic development and represents a period for major changes in transcriptional synchrony for the imprinted genes co-expressed in the hypothalamus and placenta (Keverne, 2012). Inactivation of the imprinted *Peg3* genes both suppresses many downstream co-expressed genes, desynchronises placental and hypothalamic expression of others, and disrupts co-ordinated function of mother and foetus.

An increase in atmospheric oxygen appears to have had a significant role in early mammalian placenta evolution (Falkowski *et al.*, 2005) and hypoxic conditions are a notable signal for *Peg3* transcription in neurons (Yamaguchi *et al.*, 2002) and in the developing placenta for induction of vascularisation (Vaiman *et al.*, 2005). Interestingly, although the mammalian *Peg3* gene is structurally different from that of non-mammalian vertebrates, across mammalian lineages this gene and its promoter are highly conserved. However, the evolutionary significance of its imprinting is underpinned by its ability to regulate those large gene families (Prls, PsGs and ceacams), which have themselves undergone multiple duplications that are differentially represented across different mammalian species (Wildman, 2011).

Many of the genes that regulate placental development also regulate the developing hypothalamus under the coordinated expression of genes that are imprinted (E12-15, mouse). Challenging this genomic linkage of hypothalamic and placental expression with 24-h maternal food deprivation late in pregnancy (E13 mouse) results in disruption of co-expressed genes, primarily by affecting those expressed in the placenta (Broad and Keverne, 2011). Food deprivation also produces a significant decrease of *Peg3* expression in the placenta with consequences similar to many of the placental gene changes induced by the *Peg3* mutation. Such genomic dysregulation does not occur in the foetal hypothalamus where *Peg3* expression increases with maternal food deprivation. Such changes in gene expression brought about by food deprivation are consistent with the foetal genome maintaining hypothalamic development at a cost to its placenta. This biased change to gene dysregulation in the placenta is linked to autophagy and ribosomal turnover, which sustains, in the short term, nutrient supply for the developing hypothalamus. Thus, the foetus

controls its own destiny in times of acute starvation, especially in the last trimester of pregnancy, by short-term sacrifice of its placenta to preserve resources critical for brain development.

The co-adaptive development of the placenta and maternal hypothalamus has required a concomitant evolutionary development to ensure masculinisation of the male hypothalamus (Keverne, 2013). Males do not, of course, undergo pregnancy and parturition but they nevertheless benefit from viviparity and have been subject to the same placental hormones and selection pressures from viviparous *in utero* development. Indeed, the male brain is capable of maternalism in monogamous mammals and castration at birth enables the paternal care for offspring in many mammalian species (Lonstein *et al.*, 2002). Mammalian brain masculinisation has long been established to be a consequence of testosterone aromatisation to oestrogen that epigenetically regulates sex differences in the neuronal structure of certain hypothalamic nuclei (McCarthy, 2010) many of which are concerned with regulating maternalism. Thus, an important evolutionary step in masculinisation of the hypothalamus in placental mammals has been the evolution of male Sry sex-determining gene. Sry is not present in the earliest of mammals, the egg-laying monotremes, and is thought to have evolved in parallel with placentation. Interestingly Sry is a hybrid gene of Dgcr8 and Sox3 (Sato *et al.*, 2010), and serves as the master switch for testes development (Kashimada and Koopman, 2010). It provides the trigger for sex determination by activating Sox9 that not only brings about testes formation but blocks the genetic pathway to the differentiation of ovarian cells (Veitia, 2010).

Sox3, an X-linked maternally expressed gene from which Sry evolved, is also required for development of the hypothalamus and pituitary (Rizzoti *et al.*, 2004), while Dgcr8, which became inserted upstream of the ancestral Sox3 in Sry evolution, is important for regulating micro-RNAs of the Dgcr8-Drosha complex expressed in the placenta (Bortolin-Cavaillat *et al.*, 2009). Another member of the Sox gene family (Sox15) has 75% sequence in common with Sox3 and is also expressed in the placenta. Maternally expressed Sox3 is important for development of the placental giant cells that produce those hormones, which regulate the maternal hypothalamus. This hybridisation of placental and hypothalamic genes into a male Sry sex-determining gene has ensured critical *in utero* timing for the development of foetal leydig cells. Foetal leydig cell production of testosterone counteracts the maternalising influence of placental hormones and masculinises the male hypothalamus, an effect that continues into the early post-partum period when foetal leydig cells subsequently undergo apoptosis (Keverne, 2012).

DISCUSSION

Theory has been very helpful in conceptualising the evolutionary significance of genomic imprinting at a time when little empirical data were available. Very few publications on genomic imprinting had appeared at the time when parent–offspring conflict was formulated (Haig, 1992) or indeed the theory of parent–offspring co-adaptation (Curley *et al.*, 2004; Wolf and Hager, 2006). Haig (2014) now makes clear ‘parental conflict theory defines conditions for co-operation as well as conflict in mother–infant relationships’. With the wealth of knowledge now available for the molecular genetic control of genomic imprinting and the phenotypes this represents, I now feel these theories can be viewed with a more critical eye. Genomic imprinting made its earliest appearance in the marsupials with only two DMRs, H19 and Peg10. H19 may have tumour-suppressor activity when combined with Igf2, whereas Peg10 encodes a protein with homologies to retrotransposons. Recent analysis of the platypus genome when compared with that of the marsupial has found an

accumulation of repeat elements within imprinted regions of these prototherian mammals (Pask *et al.*, 2009). Genomic imprinting may thus have initially evolved from a defence mechanism against transposable elements that depended on DNA methylation in germ cells (Renfree *et al.*, 2013). Certainly, in the context of genomic imprinting, it is the heritable epigenetic non-coding ICR, which are of primary evolutionary significance. Across mammalian evolution, the ICRs have seen increasing numbers of genes recruited to these chromosomal regions. The genes themselves have remained remarkably stable, under purifying negative selection, while the ICRs have increased substantially in number from the original two found in marsupials.

In addition to providing resources from mother over lengthy pregnancies, the placenta has to hormonally prime the mother’s hypothalamus to ensure maternal care and provision of milk. This introduces a further tier of co-adaptive complexity aided by the co-expression of imprinted genes in the developing hypothalamus as well as the developing placenta. Each of these co-expressed genes can regulate complex networks engaging non-imprinted genes. The fidelity of the imprinted gene networks is primarily dependent for their regulation by maternal ICRs. Genomic imprinting thus becomes a co-ordinator of co-adapted gene expression, a viewpoint that has recently been given strong theoretical support (Wolf, 2013). These kinds of network interaction often favour the evolution of genetic co-adaptation where beneficially interacting alleles evolve to become co-inherited. An imprinting locus may thus select for interacting partners to match its pattern of imprinting and monoallelic expression. Thus, some genes may evolve imprinting status because selection favours parent-of-origin dependent status, other genes may evolve imprinting as a co-evolutionary response to match the expression pattern of their interacting neighbours (Wolf, 2013). Such matching may be important for coordination of downstream gene expression to specific cell types (placenta, neurons) and coordinate specific functions.

The strong matrilineal bias for the imprinting regulation of genes in eutherian mammals, and their reprogramming correlates with the increasing complexity of placentation and embryo development. Genomic imprinting has not been found in the non-placental monotremes, although genes expressed in monotremes became imprinted in marsupials. Marsupials have a relatively simple choriovitelline placenta, which is short-lived, most development occurring in the pouch. In marsupials, the yolk sac forms the definitive placenta being responsible for physiological exchange whereas eutherian mammalian placentae can be deeply invasive into the maternal endometrium (haemochorial), requiring increased maternal–foetal co-adaptation. Not only have more genes been recruited to ICRs, but many of these genes form extensive networks with other imprinted genes (Sumida and Ohlsson, 2010). The importance of the ICR for tight control over gene regulation is illustrated from complex human pathological syndromes, which develop when the bipartite ICR is dysregulated in Prader–Willi syndrome/Angelman’s syndrome. Such imprinting defects with the genes themselves remaining intact only represent 3% of cases of Prader–Willi syndrome, but they illustrate the importance of gene regulation control for this imprinted network.

There are clear evolutionary advantages that accrue from genomic imprinting, but there are also certain questions that arise. If stability and negative purifying selection are a feature of the imprinted genes, how has the negative selection survived the mutagenic environment of spermatogenesis? Although the control of imprints (ICR and DMR) is matrilineal, many imprinted genes are paternally expressed by virtue of this maternal silencing. Why does the maternal germline subject

certain genes to paternal expression, especially when mutations in the patriline are higher than matriline? Point mutations, expanded tandem repeats and structural chromosome mutations predominate through patrilineal transmission (Marchetti *et al.*, 2007). However, sperm DNA undergoes repair in zygotes after fusion with the oocyte (Derijck *et al.*, 2008). The zygote has several unique features influencing DNA repair depending on proteins and mRNAs of maternal origin stored in the oocyte. Male and female chromatin at this stage are epigenetically very dissimilar with male chromatin undergoing widespread demethylation. DNA methylation, particularly through loss of 5mC across the paternal DNA is linked to DNA repair mechanisms (Wossido *et al.*, 2010). Repair of double-strand breaks is executed by non-homologous end joining during homologous recombination. Both are functional in the zygotic cell cycle and both are operative in S-phase. Hence, DNA repair at the zygotic stage of development can be regarded as an important maternal trait for repairing paternally expressed gene errors.

Although the theories of conflict and kin selection are theoretically sound, the empirical data from genomic imprinting can no longer be shoe-horned into supporting these theories. The data do, however, lend support to the importance of genomic imprinting in underpinning the success of viviparous maternal foetal co-adaptive development with a very strong bias for maternal control in its evolutionary success.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The author declares no conflict of interest.

- Amey KL, Bae E, Olsen C, Drewell RA (2006). The human and mouse H19 imprinting control regions harbor an evolutionary conserved silencer element that functions on transgenes in *Drosophila*. *Dev Genes Evol* **216**: 811–819.
- Bortolin-Cavaille ML, Dance M, Weber M, Cavaille J (2009). C19MC microRNAs are processed from introns of large Pol-II non-protein-coding transcripts. *Nucleic Acids Res* **37**: 3464–3473.
- Broad KD, Curley JP, Keverne EB (2009). Increased apoptosis during neonatal brain development underlies the adult behavioral deficits seen in mice lacking a functional paternally expressed gene (Peg3). *Dev Neurobiol* **69**: 314–325.
- Broad KD, Keverne EB (2011). Placental protection of the fetal brain during short-term food deprivation. *Proc Natl Acad Sci USA* **108**: 15237–15241.
- Chess A (2012). Mechanisms and consequences of widespread random monoallelic expression. *Nat Rev Genet* **13**: 421–428.
- Curley JP, Barton SC, Surani AM, Keverne EB (2004). Co-adaptation in mother and infant regulated by a paternally expressed imprinted gene. *Proc R Soc Lond B Biol Sci* **271**: 1303–1309.
- Curley JP, Pinnock SB, Dickson SL, Thresher R, Miyoshi N, Surani MA *et al.* (2005). Increased body fat in mice with a targeted mutation of the paternally expressed imprinted gene Peg3. *FASEB J* **19**: 1302–1304.
- Derijck A, van der Heijden G, Giele M, Philippens M, de Boer P (2008). DNA double-strand break repair in parental chromatin of mouse zygotes, the first cell cycle as an origin of de novo mutation. *Hum Mol Genet* **17**: 1922–1937.
- Duret L, Galtier N (2009). Biased gene conversion and the evolution of mammalian genomic landscapes. *Annu Rev Genom Human Genet* **10**: 285–311.
- Falkowski PG, Katz ME, Milligan AJ, Fennel K, Cramer BS, Aubrey MP *et al.* (2005). The rise of oxygen over the past 205 million years and the evolution of large placental mammals. *Science* **309**: 2202–2204.
- Gerstein MB, Kundaje A, Hariharan M, Landt SG, Yan K-K, Cheng C *et al.* (2012). Architecture of the human gene regulatory network derived from ENCODE data. *Nature* **489**: 91–100.
- Gimelbrant A, Hutchinson JN, Thompson BR, Chess A (2007). Widespread monoallelic expression on human autosomes. *Science* **318**: 1136–1140.
- Gregg C, Zhang J, Butler JE, Haig D, Dulac C (2010). Sex-specific parent-of-origin allelic expression in the mouse brain. *Science* **329**: 682–685.
- Guilbert S, Zhou Z, Sjolinder M, Gondor A, Fernadex A, Pant V *et al.* (2012). CTCF-binding sites within the H19 ICR differentially regulate local chromatin structures and cis-acting functions. *Epigenetics* **7**: 361–369.
- Haig D (1992). Genomic imprinting and the theory of parent-offspring conflict. *Semin Devel Biol* **3**: 153–160.
- Haig D (2014). Coadaptation and conflict, misconception and muddle, in the evolution of genomic imprinting. *Heredity (Edinb)* **113**: 96–103.
- Henke C, Ruebner M, Faschingbauer F, Stolt CC, Schaefer N, Lang N *et al.* (2013). Regulation of murine placentogenesis by the retroviral genes Syncytin-A, Syncytin-B and Peg10. *Differentiation* **85**: 150–160.
- Hudson QJ, Kulinski TM, Huetter SP, Barlow DP (2010). Genomic imprinting mechanisms in embryonic and extraembryonic mouse tissue. *Heredity (Edinb)* **105**: 45–56.
- Hutter B, Bieg M, Helms V, Paulsen M (2010). Imprinted genes show unique patterns of sequence conservation. *BMC Genomics* **11**: 649.
- Kagami M, Nagai T, Fukami M, Yamazawa K, Ogata T (2007). Silver-Russell syndrome in a girl born after *in vitro* fertilization: partial hypermethylation at the differentially methylated region of PEG1/MEST. *J Assit Reprod Genet* **24**: 131–136.
- Kashimada K, Koopman P (2010). Sry: the master switch in mammalian sex determination. *Development* **137**: 3921–3930.
- Keniry A, Oxley D, Monnier P, Kyba M, Dondolo L, Smits G *et al.* (2012). The H19 lncRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. *Nat Cell Biol* **14**: 659–665.
- Keverne EB (2006). Trophoblast regulation of maternal endocrine function and behaviour. In: Moffett A, Loke C, McLaren A (eds) *Biology and Pathology of Trophoblast*. Cambridge University Press: New York, pp 148–163.
- Keverne EB (2009). Monoallelic gene expression and mammalian evolution. *BioEssays* **31**: 1318–1326.
- Keverne EB (2012). Importance of the matriline for genomic imprinting, brain development and behaviour. *Phil Trans Roy Soc B* **368**: 20110327.
- Keverne EB (2013). Importance of genomic imprinting in the evolution and development of the maternal brain. In: Pfaff D, Christen Y (eds) *Multiple Origins of Sex Differences in Brain. Neuroendocrine Functions and Their Pathologies*. Springer-Verlag: Berlin, pp 21–34.
- Kulinski TM, Barlow DP, Hudson QJ (2013). Imprinted silencing is extended over broad chromosomal domains in mouse extra-embryonic lineages. *Curr Opin Cell Biol* **25**: 297–304.
- Lonstein JS, Rood BD, De Vries GJ (2002). Parental responsiveness is feminized after neonatal castration in virgin male prairie voles, but is not masculinized by perinatal testosterone in virgin females. *Horm Behav* **41**: 80–87.
- Lyko F, Brenton JD, Surani MA, Paro R (1997). An imprinted element from the mouse H19 locus functions as a silencer in *Drosophila*. *Nat Genet* **16**: 171–173.
- Marchetti F, Essers J, Kanaar R, Wyrobek AJ (2007). Disruption of maternal DNA repair increases sperm-derived chromosomal aberrations. *Proc Natl Acad Sci USA* **104**: 17725–17729.
- McCarthy MM (2010). How it's made: organisational effects of hormones on the developing brain. *J Neuroendocrinol* **22**: 736–742.
- McVean GT, Hurst LD (1997). Molecular evolution of imprinted genes: no evidence for antagonistic coevolution. *Proc Biol Sci* **264**: 739–746.
- Moffett A, Loke C (2006). Immunology of placentation in eutherian mammals. *Nat Rev Immunol* **6**: 584–594.
- O'Connell MJ, Loughran NB, Walsh TA, Donoghue MT, Schmid KJ, Spillane C (2010). A phylogenetic approach to test for evidence of parental conflict or gene duplications associated with protein-encoding imprinted orthologous genes in placental mammals. *Mamm Genome* **21**: 486–498.
- Pask AJ, Papenfuss AT, Ager EL, McCall KA, Speed TP, Renfree MB (2009). Analysis of the platypus genome suggests a transposon origin for mammalian imprinting. *Genome Biol* **10**: R1.
- Pauler FM, Barlow DP, Hudson QJ (2012). Mechanisms of long range silencing by imprinted macro non-coding RNAs. *Curr Opin Genet Dev* **22**: 283–289.
- Phillips JE, Corces VG (2009). CTCF: master weaver of the genome. *Cell* **137**: 1194–1211.
- Pink CJ, Hurst LD (2011). Late replicating domains are highly recombining in females but have low male recombination rates: implications for isochore evolution. *PLoS One* **6**: e24480.
- Rabinovitz S, Kaufman Y, Ludwig G, Razin A, Shemer R (2012). Mechanisms of activation of the paternally expressed genes by the Prader-Willi imprinting center in the Prader-Willi/Angelman syndrome domains. *Proc Natl Acad Sci USA* **109**: 7403–7408.
- Renfree MB, Ager EI, Shaw G, Pask AJ (2008). Genomic imprinting in marsupial placentation. *Reproduction* **136**: 523–531.
- Renfree MB, Suzuki S, Kaneko-Ishino T (2013). The origin and evolution of genomic imprinting and viviparity in mammals. *Phil Trans Roy Soc B* **368**: 20120151.
- Renfree MB, Hore TA, Shaw G, Marshall-Graves JA, Pask AJ (2009). Evolution of genomic imprinting: insights from marsupials and monotremes. *Annu Rev Genomics Hum Genet* **10**: 11.11–11.22.
- Rizzoti K, Brunelli S, Carmignac D, Thomas PQ, Robinson IC, Lovell-Badge R (2004). SOX3 is required during the formation of the hypothalamo-pituitary axis. *Nat Genet* **36**: 247–255.
- Rossant J, Cross JC (2001). Placental development: lessons from mouse mutants. *Nat Rev Genet* **2**: 538–548.
- Sandhu KS (2010). Systems properties of proteins encoded by imprinted genes. *Epigenetics* **5**: 627–636.
- Sato Y, Shinka Y, Sakamoto K, Ewis AA, Nakahori Y (2010). The male-determining gene SRY is a hybrid of *DGCR8* and *SOX3*, and is regulated by the transcription of CP2. *Mol Cell Biochem* **337**: 267–275.

- Schultz RM, Proudhon C, Bestor TH, Woodfine K, Lin CS, Lin SP *et al.* (2010). The parental non-equivalence of imprinting control regions during mammalian development and evolution. *PLoS Genet* **6**: e1001214.
- Shufaro Y, Lacham-Kaplan O, Tzuberi BZ, McLaughlin J, Trounson A, Cedar H (2010). Reprogramming of DNA replication timing. *Stem Cells* **28**: 443–449.
- Singh P, Lee DH, Szabo PE (2012). More than an insulator: multiple roles of CTCF at the H19-Igf2 imprinted domain. *Front Genet* **3**: 214.
- Sumida N, Ohlsson R (2010). Chromosomal networks as mediators of epigenetic states. *Epigenetics* **5**: 297–300.
- Tunster SJ, Jensen AB, John RM (2013). Imprinted genes in mouse placental development and the regulation of fetal energy stores. *Reproduction* **145**: R117–R137.
- Vaiman D, Mondon F, Garces-Duran A, Mignot TM, Robert B, Rebourcet R *et al.* (2005). Hypoxia-activated genes from early placenta are elevated in preeclampsia, but not in intra-uterine growth retardation. *BMC Genomics* **6**: 111.
- Veitia RA (2010). FOXL2 versus SOX9: a lifelong 'battle of the sexes'. *Bio Essays* **32**: 375–380.
- Wagschal A, Feil R (2006). Genomic imprinting in the placenta. *Cytogenet Genome Res* **113**: 90–98.
- Wildman DE (2011). Review: toward an integrated evolutionary understanding of the mammalian placenta. *Placenta* **32**(Suppl 2): S142–S145.
- Wolf JB (2013). Evolution of genomic imprinting as a coordinator of coadapted gene expression. *Proc Natl Acad Sci USA* **110**: 5085–5090.
- Wolf JB, Hager R (2006). A maternal-offspring coadaptation theory for the evolution of genomic imprinting. *PLoS Biol* **4**: e380.
- Wossidlo M, Arand J, Sebastiano V, Lepikhov K, Boiani M, Reinhardt R *et al.* (2010). Dynamic link of DNA demethylation, DNA strand breaks and repair in mouse zygotes. *EMBO J* **29**: 1877–1888.
- Yamaguchi A, Taniguchi M, Hori O, Ogawa S, Tojo N, Matsuoka N *et al.* (2002). Peg3/Pw1 is involved in p53-mediated cell death pathway in brain ischemia/hypoxia. *J Biol Chem* **277**: 623–629.
- Zhang J, Chen Z, Smith GN, Croy BA (2011). Natural killer cell-triggered vascular transformation: maternal care before birth? *Cell Mo Immunol* **8**: 1–11.