Importance of the matriline for genomic imprinting, brain development and behaviour

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Mammalian brain development commences during foeto-placental development and is strongly influenced by the epigenetic regulation of imprinted genes. The foetal placenta exerts considerable influence over the functioning of the adult maternal hypothalamus, and this occurs at the same time as the foetus itself is developing a hypothalamus. Thus, the action and interaction of two genomes in one individual, the mother, has provided a template for co-adaptive functions across generations that are important for maternal care and resource transfer, while co-adaptively shaping the mothering capabilities of each subsequent generation. The neocortex is complex, enabling behavioural diversity and cultural learning such that human individuals are behaviourally unique. Retrotransposons may, in part, be epigenetic mediators of such brain diversity. Interestingly some imprinted genes are themselves retrotransposon-derived, and retrotransposon silencing by DNA methylation is thought to have contributed to the evolutionary origins of imprint control regions. The neocortex has evolved to be adaptable and sustain both short-term and long-term synaptic connections that underpin learning and memory. The adapted changes are not themselves inherited, but the predisposing mechanisms for such epigenetic changes are heritable. This provides each generation with the same ability to make new adaptations while constrained by a transgenerational knowledge-based predisposition to preserve others.

1. Introduction

The discovery of genomic imprinting provided the first specifiable genes which, at face value, seemed to provide a theoretical explanation for the manner by which mother–infant co-adaptation and conflict might have arisen [1,2]. Such theories have been recently expanded to explain psychological adaptations for dealing with kin relationships and the pathological consequences that may result. Crespi and colleagues [3] hypothesized that the conflict between maternal and paternal imprinted genes in offspring may be partly responsible for a wide spectrum of psychiatric disorders resulting from over-expression of genes underlying the maternal goal of greater social integration. They concluded that human psychiatric disorders may spring not from a failure of adaptation per se, but from conflict over what is the correct adaptation.

Attractive as such theories are, they fail to take account of the molecular mechanisms and evolutionary origins of the process for genomic imprinting, but rather they focus on the imprinted genes themselves. Surprisingly, few of the imprinted genes show significant sequence variation across different mammalian species [4]. This conservation is rather puzzling if we are to believe that these genes have played an important role in mammalian evolution. Analysis of 34 orthologous imprinted genes has demonstrated that the majority have not undergone any subsequent gene duplication within placental species, suggesting that selection pressures against duplication events are operating at imprinted loci [5]. The majority of imprinted genes also display high levels of micro-synteny and have undergone very few cis or transduplications across mammalian lineages. Although stability appears to be a common feature
across imprinted genes, the same does not apply for the imprint control regions (ICRs) or for the downstream genes that these imprinted genes regulate. Thus, for the pregnancy-specific glycoproteins, copy number expansion from 1 to 23 copies, ceacam expansion from 1 to 19 copies, and prolactin expansion from 3 to 18 copies occurred across mammalian phylogenies (Ensemble Genome Browser). The lowest expansion in copy number is found in metatherians, and the highest number in eutherian primates and rodents, both of which have invasive implantation. Different members of these families are regulated by the imprinted Peg1 and Peg3 genes. Moreover, many imprinted genes are members of a network of co-regulated genes comprising a hub for other imprinted genes, all of which are engaged in placental and embryonic growth. Thus, Zac1 alters the expression of several of these imprinted genes (H19, Cadkn1c, Dlk, Peg3) and directly regulates H19gf2 locus by binding to a shared enhancer [6]. Clearly then, imprinted genes are important, but their major contribution to mammalian evolution has been in providing a stable platform for the regulation of whole networks of downstream genes. Important to this stability has been the tight regulation of gene dosage that monoallelic expression provides, especially if this is always from the same parental allele in each individual.

Monoallelic expression is not unique to genomic imprinting, and it is becoming clear that many genes undergo monoallelic expression, especially in the brain [7]. However, the immune system and olfactory sensory systems also exhibit monoallelic expression, and here the mechanisms provide important clues to the advantages and mechanisms for monoallelic expression [8,9].

### 2. Monoallelic gene expression and the evolution of imprinting

In the immune system, there occur a number of epigenetic changes that result in monoallelic expression, including the receptors of the phylogenetically ancient natural killer cells, the first line of immune defence. T and B cell antigen receptors undergo developmentally regulated rearrangement, which is also enabled by epigenetic events that occur in a monoallelic manner, and by asynchronous DNA replication [10]. T and B cell antigen receptors constitute the adaptive immune system and undergo developmentally regulated rearrangement, providing the evolutionary advantage of generating diversity. This is brought about by a CTCF/cohesin complex that functions in S phase and regulates chromatin structure by creating chromatin loops and by insulating neighbouring genes from each other [11]. The CTCF/cohesin complex plays an important role in the process of V(D) recombination by enabling long-range interactions for rearrangement of chromatin structure. A similar mechanism is thought to provide the model for monoallelic olfactory receptor choice from a given olfactory receptor gene cluster [12]. Monoallelic expression has enabled the vast evolutionary expansion of olfactory receptor genes generated by tandem repeat duplications [13]. This diversity of receptor types is only functionally viable if each sensory neuron conveys only one message from each cluster of genes, because the same receptor protein contributes to the precision of axon targeting, which adds to the sensitivity and discriminatory power [14]. A conserved feature of olfactory gene clusters is their mapping to asynchronously replicating chromosome regions [15] and the flanking of CTCF/cohesin sites at intervals of 30–400 kb of the gene cluster. Olfactory receptor genes are marked with H3K9me3 and H4K20me3 silencing, which is reversed as a prelude to expression [16]. Two major advantages accrue from monoallelic gene expression, the potential for generating genetic diversity while maintaining stability through tighter control of gene dosage.

The differential replication timing of parental allele expression also appears to be a hallmark of imprinted domains. CTCF binding is a feature common to the imprinting of several genes and has been described as the ‘master weaver of the genome’ because of its ability to globally organize chromatin structure, enabling interchromosomal contacts at developmentally regulated genomic loci [17]. Thus, the maternal transmission of an H19 ICR that is unable to interact with CTCF disrupts the interactions not only between H19 ICR and its imprinted domain, but also between other imprinted domains [18]. The CTCF binding sites on the maternal H19 ICR appear to confer a later replicating feature on the entire chromosome 7 imprinted domain. As replication timing is an epigenetic mark that has been linked to the stable propagation of active and inactive domains, it may also confer or transfect epigenetic states to other members of the imprintome [19]. Because H19 ICR is the oldest known imprinting domain, which regulates only Igf2 in the marsupial, it is possible that this maternally expressed mark provided the means for initiating and implementing the evolution of imprinted states in gene clusters within and between chromosomes [20].

A recent study using high-resolution RNA sequence technology from mouse reciprocal cross hybrids has revealed more than 1000 new genetic loci with imprinted features and identified 824 candidate-imprinted genes expressed in the brain [21]. Hotspots for imprinted expression were the medial pre-optic area, arcuate nucleus, hypothalamus, all areas of the brain concerned with multiple aspects of reproduction and maternal behaviour. The nucleus accumbens and the dopaminergic substantia nigra, which engage behavioural reward, were also strongly represented for expression of these genes. The medial pre-optic area was highlighted for paternally expressed (maternally imprinted) genes at E15, an important period in placental production of hormones that act on the maternal brain. Moreover, this work of Gregg and colleagues indicated a 10-fold increase in chromosomal imprinting expressed loci than had previously been realized. The extent to which these loci represent imprints sculpted by germline epigenetic modifications (ICRs/differentially methylated regions (DMRs)) as well as their cell-type-specific allele expression bias probably needs further clarification. Nevertheless, this study confirms that imprinting appears to be a major mode of epigenetic regulation in the brain, especially for hypothalamic function.

### 3. The behavioural significance of maternal dominance for imprint control regions

The most significant biological innovation to have developed from mammalian viviparity was placentation, and the huge bias this provided for female investment in transfer of resources to and from the foetus during pregnancy, together with the subsequent commitment for maternal
care. The primary motivated behaviours of sex, feeding and aggression, regulated by the hypothalamus, were evolutionarily reorganized in the pregnant female, together with the subsequent post-partum provisioning of milk, warmth and maternal behaviour [22]. Indeed, maternalism became the major lifetime commitment of females and, in turn, the major drivers of mammalian reproductive success. Sexual activity is not unimportant, especially for males, but most female mammals are only in a sexually active mode for short periods of their oestrous, while males spend most of their adult life seeking and competing for fertile, sexually active oestrous females. Pregnancy in most mammals terminates female fertility and sexual behaviour, increases feeding behaviour and, following parturition, the female actively engages in maternal care and suckling of infants until weaning, following which the next pregnancy rapidly ensues. Because genomic imprinting has been integral to the evolutionary success of maternalism, it is logical that imprints are primarily under matrilineal control. Maternal imprinting with paternal gene expression has also ensured a more rapid propagation in the population because of the father’s capacity for multiple matings and, unless monogamous, no ‘time-out’ for pregnancy. Advantages accrued from placentation and good mothering apply to sons as well as daughters. However, advantaged males are likely to achieve more matings and thereby produce more offspring, whereas advantaged females achieve greater reproductive success (with few matings) and enhanced infant survival.

With so much of the mammalian reproductive biology being driven through the matriline, it is not surprising that there is a biased distribution of methylation-dependent ICRs applied in the maternal germline, and these maternal germline imprints play an overwhelming contribution to development compared with paternal imprints [23]. Maternal imprints have their most significant developmental effects for embryo survival before E8.5 through the impact of developmental pathways concerned with mother–foetus exchanges. Both sets of imprints, maternal and paternal, each regulate a similar number of genes, but paternal ICRs are sparse and have a dispersed and non-significant developmental effect with deletions being rarely lethal and then only at the later stages of development (E15.5) [24].

Monoallelic silencing according to parent of origin requires a heritable mechanism that both removes and reprograms the methylated ICRs in each successive generation. Reprogramming takes place in the developing foetal oocyte at the stage of meiotic arrest (E11.5) [25]. The male germline for this next generation does not mature until puberty when the final stages of mitotic cell divisions for spermatogenesis occur. The paternal imprinting marks are erased in the primordial germ cells and progressively re-established throughout male germline development, starting in prospermatogonia and continuing through the completion of meiosis of the arrested spermatocytes [26,27]. The consequences of female in utero early meiosis mean that by the time of birth, females have a full complement of reprogrammed oocytes. In males, mitosis is reactivated at puberty to produce spermatids; at this stage, the DNA becomes highly condensed as heterochromatin. The sequence of mitotic, meiotic and chromatin packaging events that occur at male puberty takes several weeks to complete and requires multiple divisions with incidence of mutations being higher than that which occurs in the development of oocytes. Indeed, it has been suggested that the paucity of paternal germline imprints may be explained by selection pressures acting to avoid the mutagenic environment of the paternal germline [24].

ICRs are, in general, rich in CpG sequences, but paternal ICRs are intergenic and tend to evolve neutrally while maternal ICRs coincide with CpG-rich promoters and are under selection pressures for conserving sequences that are linked to maintaining promoter function. The rate of CpG loss has been considerably higher for paternal than for maternal ICRs. Although methylation marks are established prior to birth, male germline spermatogonial stem cells undergo years of life and many hundreds of cell divisions after puberty while female methylation patterns are maintained for only a few days prior to meiotic arrest. Methylation of cytosines in ICRs increases the rate of C–T transition mutations, which in the male germline is likely to be higher owing to the longer passage and greater number of cell divisions that occur.

Thus, at the genomic level, there appear to be two evolutionary drives to maintain maternal versus paternal ICRs [24]. Meiotic recombination, which is high in ICRs resulting in accumulation of CpG sites through biased gene conversion that favour AT–GC mutations [28], while C-to-T deamination in the paternal ICRs increases the rate of C–T transition mutations, which in the male germline is likely to be higher owing to the longer passage and greater number of cell divisions that occur.

4. Hypothalamic/placental co-adaptation

That genomic imprinting is important for mammalian placental and hypothalamic development is well established both from human clinical disorders [29,30] and from mouse experimental studies [31]. This list of such imprinted genes that are expressed in both tissues continues to grow (table 1).

In the context of viviparity, the function of the placenta and hypothalamus are of crucial importance to reproductive success, as is the co-adaptive interface between them. Thus, hormones produced by the placenta influence the maternal hypothalamus determining endocrine function and behaviour. Progesterone, in particular, suppresses maternal sexual behaviour and fertility, increases maternal food intake in anticipation of subsequent foetal demands and promotes the synthesis of maternal hypothalamic oxytocin in anticipation of its requirements for parturition, maternal behaviour and milk ejection [32]. In short, the foetal genome determines its own destiny via the placenta, hormonally regulating the maternal hypothalamus to serve the interests of the foetus, which, at the same time, is developing its own hypothalamus. Conversely, maternal stress, glucocorticoids and insulin-like growth factors may induce changes in placental function, which determine foetal programming in the context of hypertension and metabolic disease [33,34].

The evolutionary selection pressures that have enabled the adult maternal hypothalamus to respond to the hormonal signals of the developing placenta have operated during hypothalamic development. Interestingly, the period of foetal hypothalamic development in the mouse coincides with the developmental maturation of foetal placental tissues (giant cells, spongiosrophoblast and vascular maturation) that are themselves responsible for producing the hormones that act on the maternal hypothalamus. Because the development of both tissues is regulated by the same imprinted genes (table 1), then the developing hypothalamus and placenta...
serve as the template on which selection pressures act, guided by the effectiveness of placental interactions with the adult hypothalamus (figure 1). Thus, viviparity has introduced a new environmental dimension to mammalian evolution in which two genomes function in one individual, the mother. The advantages gained from this mother–infant co-adaptation during pregnancy, directed by imprinted genes, extends to the post-partum period when epigenetic influences, especially on brain development, are brought about through maternal behavioural responses to the offspring. In this way, offspring that receive ‘optimal in utero’ nourishment and improved maternal care will themselves develop a hypothalamus both genetically and epigenetically predisposed to optimal mothering [35].

Detailed studies of mice carrying a mutation in the maternally imprinted genes (Peg3 and Peg1 (Mest)) have provided some insights as to how the functioning of the adult hypothalamus is adapted to respond to the placental hormones that determine maternalism. When these genes are silenced in the maternal hypothalamus, but not in the placenta or foetus, the phenotypes are characterized by impaired maternal care, milk production and milk let-down. Indeed, the phenotypic outcomes are so functionally convergent as to suggest the action of this gene is co-adapted in the hypothalamus and placenta [40].

Underpinning such convergent phenotypes suggests that these imprinted genes must not only be regulating gene expression in the placenta and hypothalamus, but also co-regulating this expression at critical times for development of the hypothalamus and placenta. If these gene expression changes are coincident with the timing of placental hormone production and development of the foetal hypothalamus (E11–12–13), then a temporal framework is available for selection pressures to shape the mothering capabilities of the next generations (figure 1). Between E11 and E12, most of the developmental changes in gene expression are unique to the placenta (57%) or hypothalamus (34%), with only 9 per cent of gene transcriptional changes being co-expressed (figure 2). Peg3 inactivation influences all of these co-expressed genes at this stage, and further increases a new set of co-expressed genes (now 38% of total). These induced gene changes to co-expression in the Peg3 mutant are, however, no longer in synchrony, with 90 per cent upregulated in the placenta, but downregulated in the brain. The Peg1 mutation is also disruptive to hypothalamic/placental co-expressed genes at this stage (figure 2) but has a much greater effect on genes expressed in the hypothalamus (figure 2).

Developmental days 12–13 show a marked increase in genes that are co-expressed in brain and placenta (fourfold increase from E11 to E12; figure 2), most of which are synchronized for direction of changes in expression. The Peg3 mutation suppresses approximately 20 per cent of these co-expressed genes and induces co-expression of a further 30 per cent. The Peg1 mutation leaves only 4 per cent of the co-expressed genes unchanged (figure 2). Days E12–13 thus represent a period for major changes for transcriptional synchrony in the hypothalamus and placenta and is also a period

### Table 1. Imprinted genes expressed in hypothalamus/pituitary and placenta. P, paternally expressed; M, maternally expressed.

<table>
<thead>
<tr>
<th>Imprinted Gene</th>
<th>Specialist Function</th>
<th>Generalized Function</th>
</tr>
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<tbody>
<tr>
<td>Necdin (P)</td>
<td>regulates Hif1α/Arnt</td>
<td>apoptosis</td>
</tr>
<tr>
<td>Nnat (P)</td>
<td>phosphorylates CREB</td>
<td>increase intracell Ca2+</td>
</tr>
<tr>
<td>Peg1 (P)</td>
<td>β3 hydrolase fold family</td>
<td>growth regulation</td>
</tr>
<tr>
<td>Magel2 (P)</td>
<td>MAGC family of proteins</td>
<td>apoptosis</td>
</tr>
<tr>
<td>Ghr10 (P) (M)</td>
<td>maternal expression—placenta; paternal expression—hypothalamus</td>
<td>apoptosis</td>
</tr>
<tr>
<td>Phlda2 (M)</td>
<td>placental growth/neuroendocrine tumours</td>
<td>apoptosis</td>
</tr>
<tr>
<td>P57 kip2/Cdkn1c (M)</td>
<td>tumour suppressor</td>
<td>cell-cycle regulation; Cdk inhibitor</td>
</tr>
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### Figure 1. Transcription factors triggered by important developmental changes to the tissue environment. E.g. hypoxia, activate imprinted gene expression simultaneously in the developing placenta and hypothalamus (grey arrows; ceg denotes co-expressed genes). The placenta via its hormonal changes to the tissue environment, e.g. hypoxia, activate imprinted gene expression in the developing placenta and hypothalamus. Some insights as to how the functioning of the adult hypothalamus is adapted to respond to the placental hormones regulating this expression at critical times for development of the foetal hypothalamus and is also a period.
Hif1 is crucial to trophectoderm differentiation to form the placenta. Oxygen-dependent hypoxia-inducible factors (Hifs) are also important for metabolically demanding tissues such as the brain. Effective placental oxygenation was a prerequisite of extended gestation in atmospheric oxygen from 15 to 20 per cent [41]. Oxygen for foetal growth, especially placental growth, is influenced by the Peg3 mutation. In the case of the Peg1 mutation E11–12, 138 genes (days E11–12) and 60 genes (E12–13) are co-expressed. Many of these co-expressed genes belong to the prolactin (8), the placental-specific glycoprotein (11) and ceacam families (6) with the principal functional groupings of cellular growth, differentiation and apoptosis.

Figure 2. Gene expression changes over developmental time. Only genes which are changing expression and therefore are developmentally important are included. Co-expressed genes are particularly notable over E11–12–13, and these genes are targeted by both Peg3 and Peg1 mutations. These data are based on 26 genes (days E11–12) and 41 genes (E12–13) that change expression over these time periods, are co-expressed in hypothalamus and placenta and are influenced by the Peg3 mutation. In the case of the Peg1 mutation, 138 genes (days E11–12) and 60 genes (E12–13) are co-expressed. Many of these co-expressed genes belong to the prolactin (8), the placental-specific glycoprotein (11) and ceacam families (6) with the principal functional groupings of cellular growth, differentiation and apoptosis.

The imprinted genes Peg3 and Necdin bind Hif1α and Arnt [44], the latter producing a HLH-PAS protein that dimerizes with Sim1 in the development of hypothalamic oxytocinergic neurons. Necdin-deficient mice have a reduction of oxytocin neurons [45] as does the Peg3 mutant mouse [37], and these play an integral role in parturition, milk let-down and maternal behaviour in the adult female. The Magel2 mutant also has a significant reduction in oxytocin, and a single injection of oxytocin production soon after birth can rescue the phenotype and the survival of Magel2 mutant pups [46]. The maternally imprinted gene Mest, like Peg3, also regulates hypothalamic development and maternal behaviour, both of which are impaired by its mutation. Both Peg3 and Mest are expressed in the placenta, and the mutants show impaired placental growth development. There are no reports on the function of Magel2 or Necdin in the placenta, but because they both code for the Mag family of proteins, which have roles in cell-cycle regulation, differentiation and apoptosis, it is likely that their expression in the placenta will have similar effects on growth as Mest and Peg3 [47]. Thus, all four maternally imprinted genes have common components to their mutant phenotype (milk let-down, sucking, feeding, maternal care) as well as determining growth and development of the placenta.

A feature that characterizes several imprinted genes is the formation of networks [6,18]. The maternally expressed Zac1 is a member of such a network of imprinted co-regulated genes expressed in the placenta and hypothalamus [48,49] and controlling embryonic growth. Zac1 directly regulates the Igf2/H19 locus through binding a shared enhancer. Gain of function of Zac1 in neuro-2A cells induces other imprinted gene expression (Igf2, Cdkn1c, H19, Dlk), while loss of function produces a downregulation of these genes [6]. Other imprinted genes significantly linked to Zac1 and over-represented in expression include Peg1, Gtl2, Dlk1, Peg3, Grb10, Gnas, Ndn, Gatm, Dtn and Slc38a4. Some of these imprinted genes that are co-expressed in the hypothalamus and placenta correspond to hub-like nodes, being interconnected to the largest number of network genes (Peg3, Ndn,Gnas). Zac1 is known to control neural cell-cycle arrest and apoptosis through different pathways and is expressed in the hypothalamus and pituitary [50]. A general feature of such gene networks is to provide robustness against perturbations during development. Thus, dysfunctional changes to a component gene can be compensated by homeostatic regulation of the imprinted gene network. This is illustrated by Grb10, a maternally expressed gene in the network that acts to restrict growth, while Igf2 (another imprinted gene in the network) promotes growth. Because Zac1 has a regulatory influence on both genes, this provides for a safeguard for the functional robustness of the network. The imprinted gene, Grb10, acts to limit placental size and hence efficiency [51]. Daughters inheriting a mutant Grb10 allele from their mother produce larger litters and smaller offspring than daughters inheriting the same mutation from their father. This suggests that Grb10 can also impact on reproductive success by differential allocation of maternal resources such that smaller litter size can be compensated by producing fewer but larger offspring, i.e. survival is offset against size.

Because the placenta and hypothalamus are developmentally synchronized by co-expressed imprinted genes, the placenta is in a pivotal position for providing the resources for foetal hypothalamic development at the same time as...
commandeering the adult hypothalamus to provide these resources. Challenging this developmental linkage of hypothalamus and placenta with 24 h food deprivation results in disruption of these co-expressed genes, primarily by affecting placental gene expression [35]. Food deprivation also produces a significant decrease in Peg3 gene expression in the placenta, with similar consequences for many of the placental gene changes induced by Peg3 mutation. Such genomic dysregulation does not occur at this late developmental time point (E18) in the hypothalamus, where Peg3 expression increases with food deprivation [35]. Thus, gene expression changes 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 authophagy and ribosomal turnover, which sustains, in the short-term, nutrient supply for the developing hypothalamus. Interestingly, gene pathway analysis reveals ‘neural disorders’ as a significant genetic component in placental but not hypothalamic function, which is protected by maintained Peg3 expression. What is clear is that the foetus controls its own destiny in times of acute starvation by short-term sacrifice of the placenta to preserve brain development. Placental stem cells from human first trimester placenta can be induced to differentiate into neurons [52], and a recent study has shown the placenta to be a source of the neurotransmitter serotonin (5HT) [53]. Whether or not serotonin can affect foetal brain development is not known, but serotonergic receptor signalling has been shown to increase placental aromatase activity [54,55]. Placental aromatase is a key enzyme controlling oestrogen synthesis, and this does have important actions on the maternal hypothalamus and placenta during pregnancy [32]. These findings illustrate the co-adapted hypothalamic/placental functional development, and suggest that the placenta may provide a genetic window on future brain dysfunction.

5. Neocortex: complexity, variability and matrilineal influence

The brain is a complex structure that has itself undergone radical changes in the life history of mammals. Placental mammals show a logarithmic increase in encephalization relative to non-mammalian vertebrates [56], and a major driver of mammalian brain evolution has been pregnancy and maternalism. While the fundamental hypothalamic influences on behavioural motivation associated with pregnancy have depended on prenatal developmental selection pressures, the more expansive neocortical brain regions undergo most of their development postnatally. Nevertheless, the mother–infant relationship still plays a primary role in shaping this postnatal development [57]. In the case of the large brain social primates that are living in groups, extensive social relationships in a post-weaning environment with matrilineal female kin have been hypothesized as an important selection factor for this further brain evolution [58]. Thus, there has been a very strong matrilineal bias for selection pressures to operate throughout all aspects of mammalian forebrain development.

At one level, the neocortex is a relatively simple structure made up of six layers, which develop as a result of radial migration of neurons from the neurogenetic cortical plate [59]. Formation of cortical columns provides the functional units of the neocortex formed by this radial migration of progenitor neurons. The number of columns depends on increased proliferation of cortical stem cells from the neurogenic pool produced by symmetrical divisions during development. Asymmetric divisions are responsible for producing neurons that differentiate to form the layers of the cortical columns and the number of cells in each column. Duration of neurogenesis varies across species from 8 days in mice to 80 days in humans. Although neurogenesis extends for longer with increasing cortex size, when expressed as a percentage of developmental time, there is remarkable congruence across mammalian species, suggesting conservation at the genetic level [59]. The major variance for cell-cycle duration is G1 phase, which, for cortical neurons, increases with the number of cycles. Imprinted genes contribute to this process by influencing neural cell-cycle arrest and apoptosis (Zac1, p57kip2, Necdin and Peg3).

In addition to the radially migrating cortical neurons, there is a second type of neuron, the GABAergic interneuron, which originates from the striatal ganglionic eminence [60]. Its developmental trajectory for migration is tangential to the cortical columns and faces a more challenging pathway without the guidance of radial glia. Because migration is tangential to the cortical plate, this presents problems for targeting and timing, especially at the extreme poles of GABAergic neural migration (pre-frontal and infra-temporal cortices). Neurons not reaching the right place at the right time undergo programmed cell death. Because there are billions of these neurons and trillions of connections to be made, errors involving programmed cell death have been integral to successful neocortical development and evolution. Indeed, it has been calculated, from comparative knowledge of the neurogenerative cortical pool in the mouse and rhesus monkey cortical plate, that the monkey neocortex should be three times larger than is actually achieved [59]!

During the early stages of cortical neurogenesis, GABAergic neurons are excitatory and play an important role in the organization of cortical architecture by increasing intracellular calcium, which is essential to neuronal growth and differentiation [61]. These same neurons switch from excitatory organizers of neural networks during development to hyperpolarizing inhibitory neurons in the adult cortex. As inhibitory network interneurons, these GABAergic neurons maintain network oscillations enabling widely distributed regions of the cortex to coordinate their firing [62]. This synchronization across large neural networks provides the means for establishing disambiguity of activity patterns for objects or events, disturbances of which can produce the disintegration of reality that characterizes human psychiatric problems. Hence, the massive expansion of the trajectory for these tangentially migrating neurons in the human brain render it especially susceptible to GABAergic dysfunction, producing a broad spectrum of disorders (epilepsy, schizophrenia, Tourette’s syndrome, bipolar disorders, mental retardation and autism) [63]. The imprinted gene Necdin promotes the tangential migration of GABAergic neocortical interneurons by forming a complex with the homeodomain protein Dlx2 [64]. In Necdin-deficient mice, the population of Dlx2 GABAergic progenitor neurons migrating from the medial ganglionic eminence are significantly reduced, making the mice susceptible to seizures [65]. Necdin is also co-expressed with and Dlx5 via Mage D-1, and when over-expressed by electroporation in cultured forebrain slices,
increases the population of cells expressing the GABAergic neuron marker, calbindin D-28k [64]. Neural stem cells derived from embryonic Dlx5 null mice have a severely reduced capacity to generate neurons [66]. Dlx2 is a marker for GABAergic cortical neurons migrating tangentially from the median ganglionic eminence, whereas Dlx5 promotes migration of GABAergic neurons from the lateral ganglionic eminence to the striatum and olfactory bulb [67]. Dlx5 is monoallelically expressed in the mouse brain [68] and monoallelically imprinted in the human brain [69,70], where failures in migration of these neurons to the striatum is associated with the motor dysfunction seen in Tourette’s syndrome [71]. Dlx5 loses its imprint and is biallelically expressed in Rett’s syndrome and may relate to the failure in chromatin looping, a failure of which has been shown to occur in the brains of MeCP2 null mice [69].

Dysfunction in GABA signalling is also known to mediate autism-like stereotypies and the Rett’s syndrome phenotype, which is an X-linked disorder caused by defects in the methyl-CpG-binding protein 2 (MeCP2) [72]. MeCP2 is engaged in DNA CpG methylation, chromatin remodelling, and in transcription by the recruitment of several key proteins to these processes. Recent studies have shown that neuronal progenitor cells derived from human tissue of patients with Rett’s syndrome and carrying MeCP2 mutations have increased susceptibility for L1 retrotransposition [73]. Active L1 retrotransposons are capable of mobilization in neuronal progenitor cells, but not other somatic tissues, and can impact the genome by creating insertions, deletions and fine-tuning of gene expression.

Neural L1 insertions could also be of advantage to the developing cortex, enabling neural diversity among the many billions of neurons and trillions of synapses they make. A mechanism that generates somatic variability during neurogenesis and permits greater adaptability to environmental changes could be favoured by natural selection. Selection would act on maintaining the genetic mechanisms but not the insertions themselves. Thus, somatic genomic mosaicisms driven by retrotransposition has the capacity to reshape the genetic circuitry of both the developing and the developed brain [74,75]. More than any other tissue, the neocortex has evolved to be adaptable and sustain both long-term and short-term synaptic connections that underpin learning and memory. Here, the adapted changes themselves are not inherited, but the predisposing epigenetic processes are, thus providing each generation with the same ability to create new adaptations while retaining a predisposition to preserve others. No two human brains are exactly alike, and even monozygotic twins show differences in behaviour and in psychiatric disorders [76].

Retrotransposon silencing by DNA methylation has also been proposed as a mechanism for driving genomic imprinting. Peg10 is an imprinted, gene-sharing homology with the sushi-ichi retrotransposon [77]. Peg10 is conserved in eutherian mammals but not in other vertebrates (birds and fish) and is essential for development of the placenta. It has not been found in the egg-laying monotremes and became inserted in the genome at the time when viviparity was evolving. Another retrotransposon-derived gene, Rtl1/Peg11, is essential for the development of the placenta, and both its loss of function or increased dosage causes late foetal or early neonatal lethality owing to a failure in the trophoblast endothelial cell invasion of foetal capillaries [78].

ICRs often include tandem arrays of transposition factor binding sites as exemplified by YY1 for the transcription of Peg3, Nespas, Gnas and Xist/Tsix domains [79]. A substantial number of L1 retrotransposed elements also contain the transcription factor YY1 binding sites on their promoter sequences, which may explain the success of retrotransposed copies to undergo autonomous retrotransposition in subsequent generations [80]. However, YY1-binding sites are not exclusive to imprinted or other retrotransposed genes, but they do seem to have an important role in neurogenesis and a widespread distribution in the brain. Retrotransposition has also resulted in the generation of new genes that are imprinted (Uppsf, Mcts2, Nap15 and U2af1-rsl), located within host transcripts, expressed in the developing brain and derived from genes on the X chromosome [81]. Imprinted retrotransposed genes share a common genome organization, including a promoter with a DMR. In the mouse, the methylated maternal allele is associated with repressive marks (H3K9me3, H4K20me3), while the unmethylated paternal allele is associated with the active mark (H3K4me2). The genes flanking host genes are biallelically expressed, making these retrotransposed loci distinct from large imprinted clusters [82]. These loci may also play a part in tissue-specific imprinting and may account for the exceptionally large number of imprinted genes recently identified in the brain [21].

6. Summary and conclusions

Genomic imprinting is brought about by epigenetic marks that are heritable and require germline reprogramming. This places genes that are ‘imprint’ regulated in a special developmental category of being monoallelically expressed according to parent of origin while the ‘imprint’ itself is primarily under matrilineal control. Imprinted genes provide a hub for robust networks of genes with a propensity to compensate dosage errors, and combining stability with flexibility for gene regulation. Genomic imprinting first appeared in marsupials, but the number of imprints and the genes they regulate have increased substantially in eutherian mammals. This is not surprising when we consider the increased complexity of structures such as the brain and placenta, for which genomic imprinting has an integral developmental role. The brain and placenta are not functionally independent structures, and have themselves provided a developmental framework governing many aspects of successful maternalism.

Mammalian viviparity and placentation have provided the matriline with the major commitment of time and energy to offspring development and have been major drivers for mammalian reproductive success. This success has required adaptations on multiple levels, including in utero maternalization of the developing hypothalamus occurring at the same time as the placenta is instructing the adult hypothalamus in the regulation of maternal care. The matriline also had a leading evolutionary role in the postnatal encephalization of the brain, because most of the primate neocortex and its connections develop under the primary care and provisioning of mother and female kin. Understanding this importance for the matriline and the significant developmental events that have determined the evolutionary success of mammals provides a logical framework for understanding genomic imprinting as well as providing insights into
the consequences of dysfunctioning. Thus, pathologies relating to point mutations or duplications in the protein coding genes that are imprinted are relatively rare, but their transcriptional regulation and dosage are susceptible to environmental perturbations. In vitro culture of mouse embryos prior to implantation and embryo transfer have been shown to produce aberrant expression of one or more imprinted genes in the placenta as well as in embryonic tissue.

Those parts of the brainstem concerned with maintaining physiological homeostasis over respiration, heart rate, digestion and autonomic control have changed very little over phylogenetic history. However, remarkable modifications have occurred in the basal parts of the mammalian forebrain. The presence of two genomes in one individual, the mother, has introduced a transgenerational dimension to the evolution of maternalism by foreshortening the outcome of selecting for optimal maternal care. Selection pressures on the developing hypothalamus at the same time as the placenta is instructing the adult’s maternal hypothalamus provides a template for selection of co-expressed genes that optimize maternal care in the next generation. The massive encephalization of the brain across mammalian phylogenies has resulted from most of its development occurring in the post natal period nurtured by the care and protection of the mother. The identification of retrotransposons and imprinted genes as regulators of transcriptional complexity have endowed this cortex with a capacity for random phenotypic variation on which developmental selection may act. This potential for variability in the developing brain contributes to the uniqueness of individual human brains as exemplified by the differences seen in monozygotic twins. Such developmental epigenetic modifications in the brain may also result in abnormal cortical connections as a consequence of early socio-emotional deprivation [83]. This has undoubtedly been facilitated by the guidance provided within the close relationship between mother and child, and the secure base this provides for developing broader social relationships.

References

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