A Custody Battle for the Mind: Evidence for Extensive Imprinting in the Brain

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Relatively few genes (~100) have previously been shown to be imprinted such that their expression in progeny derives from either the maternal or paternal copy. Two recent studies by Gregg et al. (2010a, 2010b) in *Science* expand this list by an order of magnitude, revealing complex patterns of parent-of-origin bias in gene expression in the brain that are developmentally and regionally restricted, and in many cases, sexually dimorphic.

Genomic imprinting is a phenomenon in which either the maternal or paternal copy of a gene is expressed preferentially in all progeny. This curious phenomenon, which violates classical Mendelian genetics, appears to occur only in mammals among vertebrates. The nonequivalence of the parental genomes was first revealed by observations that embryos derived exclusively from two male or female pronuclei failed to develop to term (Surani et al., 1987). This important result suggested that epigenetic control of gene expression plays an important role in development. Several research groups working over the past two decades have subsequently identified ~100 imprinted genetic loci in mammals (Efstratiadis, 1994; Tilghman, 1999). By examining gene expression in the brain, Dulac and colleagues have now expanded this list to over 1300 loci (Gregg et al., 2010a); in addition, they have identified 347 genes that are transcribed with a parent-of-origin allelic bias in a sex-specific manner (Gregg et al., 2010b). Work on previously identified imprinted loci that are transcribed in the brain has already shown that these genes can influence neuronal differentiation, behavior, or susceptibility to neurological disease (Butler, 2009; Keverne, 2009). Thus, the findings reported in these new studies are broadly relevant to neuroscience.

An imprinted gene renders the organism functionally haploid at that locus, and permits the expression of phenotypes from mutations that would normally be recessive. In other words, imprinting precludes the protection of a back-up copy afforded by a diploid genome. It has been postulated therefore that the existence of imprinting in mammals must confer a selective advantage. What this selective pressure might be remains to be settled, but the most widely accepted explanation is that imprinting is a consequence of parental conflict over resource allocation to the progeny (Haig, 2004; Hurst and McVean, 1998). Briefly, it is in the father's interest to maximize maternal resources devoted to his progeny, whereas the mother might wish to allocate resources more equitably to current and future progeny, who might conceivably result from matings with other males. This conflict is particularly acute in placental mammals, in whom the progeny develop in utero and often for prolonged gestational periods, requiring greater maternal investment. As applied to imprinting, the conflict theory predicts that paternally expressed genes should increase the use of maternal resources to produce more fit offspring. By contrast, maternally expressed genes should quell the effects of such paternally expressed genes. These expectations appear to be fulfilled by many imprinted loci, with some notable exceptions.

The molecular control of imprinting is best understood for the imprinted *H19-Igf2* locus, whose function fits the predictions of the parental conflict theory remarkably well (Tilghman, 1999). IGF2 enhances fetal growth and is paternally expressed, whereas *H19* encodes an untranslated RNA that is maternally expressed. Interposed between *H19* and *Igf2* in the genome is a differentially methylated CpG dinucleotide island (an imprinting control region, or ICR) that acts as an insulator to assure mutually exclusive expression of these two genes. In accord with the parental conflict model, loss of function of IGF2 leads to growth retardation whereas biallelic expression of IGF2 leads to overgrowth of progeny. Though the function of the H19 transcript is unknown, many imprinted loci encode noncoding RNAs (ncRNAs) that regulate the expression of other genes within the imprinted cluster. Despite the intense scrutiny to which the *H19-Igf2* locus has already been subjected, the studies by Dulac and colleagues reveal new twists in the expression of these genes.

Imprinted genes can also directly affect neuronal differentiation and behavior. Peg3, a zinc-finger protein, is imprinted and expressed from the paternal allele (Keverne, 2009). Pups carrying a paternal loss-of-function *Peg3* allele have growth retardation and suckling deficits. Adult females bearing a mutant paternal allele exhibit poor maternal care and males carrying such a mutation show impaired male sexual behavior. Peg3 is expressed in the developing and adult brain, indicating that these behavioral phenotypes likely arise from deficits in neuronal differentiation or function. In fact, there is a decrease in the number of oxytocin-expressing neurons in the hypothalamus

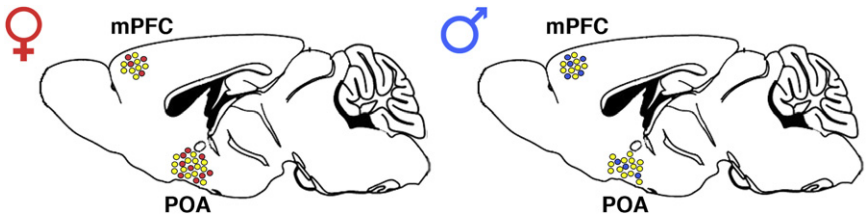

Figure 1. Schematic Illustrating Distribution of Imprinted Genes Identified by Dulac and Colleagues in the Adult Male and Female mPFC and POA
Filled yellow circles represent the relative number of imprinted genes expressed in a non-sex-biased manner; filled red and blue circles represent the relative number of female-specific and male-specific imprinted genes, respectively.

of Peg3 mutant females, although whether this particular cellular defect is responsible for poor maternal care is not known. Nevertheless, these results indicate that imprinted genes can substantially affect neuronal differentiation and behavioral outcome.

Dulac and colleagues first analyzed the expression patterns of known imprinted loci across multiple brain regions. This yielded a complex expression pattern, with some genes being transcribed in most regions whereas the expression of other loci was restricted to select areas. The authors reasoned that the number of known imprinted loci underestimates the total number of imprinted genes expressed in the brain. They used RNA sequencing technology (RNA-seq) to examine gene expression in the embryonic brain during a period of active neurogenesis (embryonic day 15, E15), and in the adult medial prefrontal cortex (mPFC) and preoptic area of the hypothalamus (POA). In order to distinguish the parental origin of expressed transcripts, the authors used mRNA from F1 progeny derived from reciprocal crosses of two mouse strains, CAST/EiJ and C57Bl/6J, whose genomes differ by many single nucleotide polymorphisms (SNPs). These genetic crosses allowed the authors to exclude the surprisingly large number of genes whose alleles are transcribed in a biased manner based solely on the strain of origin. The RNA-seq strategy combined with extensive sequence depth (23- to 29-fold coverage of the transcriptome) allowed Dulac and colleagues to use SNPs to reliably distinguish the parental origin of each transcript and to quantify its relative expression in a given brain region. This unbiased approach yielded spectacular dividends. The authors identified 824 genes, corresponding to ~3% of annotated genes in the database, and 424 putative ncRNAs, as being imprinted in the brain. Importantly, all mitochondrial transcripts were correctly tagged as being of maternal origin, indicating the validity of this strategy. These and other controls, including an independent approach to confirm the results obtained with RNA-seq, lend strong support to the accuracy and reproducibility of the data on imprinting.

These studies offer a detailed view of imprinting in the brain (Figure 1). The newly described imprinted loci are scattered across all autosomes, and two-thirds of these exist as clusters containing two or more loci, a feature also observed with previously described imprinted genes. Half of the newly described clusters contain imprinted genes and ncRNAs that could potentially influence imprinting within the cluster. Of the 72 previously identified imprinted loci that can be detected by RNA-seq in the brain, the authors find a third of these to be transcribed biallelically. H19 and Igf2 are maternally and paternally expressed in the prenatal brain, respectively, consistent with their imprinting pattern in the rest of the body; however, H19 is not expressed in the adult brain, and Igf2 is maternally expressed in the POA and mPFC. This unanticipated spatial and temporal complexity in imprinting is also observed in the newly identified imprinted loci. Over 90% of the 824 imprinted genes identified in these studies are expressed in all three tissues examined, but few (<10%) are imprinted in more than one of these three targets. Most imprinted genes in the E15 brain (60%) are maternally expressed, whereas there is a distinct paternal expression bias (70% of genes) in the POA and mPFC. Whether this distinction in parental bias is true for other developmental stages and other brain regions remains to be determined. The authors perform a similar analysis to detect X-linked imprinted loci that are expressed in the POA and mPFC. Analysis of all SNP reads from the two X chromosomes demonstrates a bias for preferential transcription from the maternal chromosome, a finding corroborated by the biased expression of an X-linked EGFP reporter when it resides on the maternal X chromosome. Normalizing the SNP reads for this maternal bias still yields 11 new candidate imprinted loci (with either a maternal or a paternal bias in allele expression), albeit at less stringent significance cutoff criteria than those utilized for the autosomal genes.

There are at least two features of the newly identified loci that distinguish them from most genes already known to be imprinted. First, the parental bias in expression for a majority of the newly discovered loci is not absolute in the tissue in which the gene is imprinted. Rather, both alleles are transcribed, albeit with a distinct parental preference. Such a biased expression pattern may reflect preferential, but not exclusive, transcription of one allele within single cells, or an unequal salt-and-pepper distribution of cells transcribing one or the other (or both) allele. Second, most loci (>90%) revealed by Dulac and colleagues are imprinted such that different SNPs within the same gene locus, and sometimes even within individual exons, reveal a distinct parental bias. These results suggest complex transcription units expressed with a distinct parent-of-origin preference; such isoforms could be coexpressed within individual cells or expressed in different cells commingled within the region. It may be possible to distinguish between these possibilities by allele (or isoform)-specific in situ hybridization or by tagging with genetic reporters. Regardless of the underlying mechanism, it is unlikely that genetic loci exhibiting these elaborate imprinting patterns could have been discovered by means other than the quantitative and unbiased approach used in these studies.

Dulac and colleagues find that 347 imprinted genes exhibit sexual dimorphism in parental bias in allele expression in the

POA and mPFC (Figure 1). As far as we can tell, this is the first demonstration of sexual dimorphism in imprinting on autosomal genes. An approximately equal number of genes (~75) is imprinted in a dimorphic manner in the mPFC of both sexes, whereas the female POA expresses three times the number of imprinted genes (150) compared to the male POA. Most of these genes (60%) are expressed from the paternal allele. The sexually dimorphic imprinting manifests as a preferential expression rather than an absolute choice of one of the two alleles, and as discussed earlier, biased allelic expression could result from one of several possibilities. In any event, several genes that are listed as being dimorphically imprinted will be of immediate interest to many groups, including the glucocorticoid receptor (*Nr3c1*), which modulates stress response and anxiety and depression-type behaviors in mouse models; Ncoa2 and Ncoa7 (*Ncoa2, Ncoa7*), cofactors in steroid-receptor-regulated transcription; Neurexin 2 (*Nrxn2*), which may modulate synapse function; Period 1 (*Per1*), which regulates circadian rhythms; and Trpc2 (*Trpc2*), a cation channel required for signal transduction in sensory neurons of the vomeronasal organ and essential for sex discrimination and aggressive behaviors (Dulac and Wagner, 2006). In future studies, it will be interesting to determine if such sex-specific imprinting results in an absolute dimorphism in the number of cells expressing that gene or in the resulting levels of transcription per cell. An absence of sexual dimorphism in such assays would indicate compensatory mechanisms that equalize the expression of genes imprinted in a sex-specific manner. There are only a few mechanisms that generate sexual dimorphism in mammals, including chromosomally based mechanisms and steroid hormones (Arnold, 2004; Juntti et al., 2010; McCarthy et al., 2009; Morris et al., 2004; Wu et al., 2009), and it will be important in future studies to determine whether (and how) these influence sex-specific imprints.

What are the implications of finding such large-scale imprinting in the brain? It seems reasonable to assume that similar analysis in other brain regions and perhaps elsewhere in the body will reveal many additional genes that are also imprinted. In other words, the selective pressure to imprint genes appears to be operating on a scale not previously appreciated. If imprinting in the brain is indeed a consequence of parental conflict, then Dulac and colleagues have uncovered a titanic custody battle to control the behavior of the progeny. It is therefore especially intriguing that the POA, previously shown to be important for sexual behavior and maternal care (Morris et al., 2004), expresses genes that are imprinted in a sex-specific manner. While gene ontology characterization suggests a preponderance of the newly identified imprinted genes as being involved in "metabolic processes" (E15 brain) and "cell adhesion" (POA and mPFC), further genetic characterization is likely to reveal additional functional themes.

As mentioned earlier, imprinting renders the organism haploid at a locus and increases the risk that otherwise recessive mutations will result in phenotypes. Dulac and colleagues find that most genes do not demonstrate absolute imprinting, suggesting a lowered risk for phenotypes resulting from recessive mutations if both alleles are coexpressed. The finding of sexually dimorphic imprinting patterns is intriguing, however, because it offers a possible mechanism underlying the sex differences in the incidence, prevalence, or outcome of many common neuropsychiatric conditions. Loss of imprinting of the Igf2 locus (biallelic expression) is found in peripheral tissues in a significant subset of humans, and it has been suggested to predict an increased risk of colorectal and other cancers (Feinberg, 2007). It is therefore conceivable that loss of imprinting, or altered imprinting patterns, for genes expressed in the brain will also be related to or predictive of mental illness. Many previously identified imprinted loci are associated with complex neurological phenotypes such as the Prader-Willi and Angelman syndromes. It is likely that the candidate imprinted genes identified in these studies will also be ultimately linked with other neuropsychiatric conditions.

The complex feature set of these genes, which includes sexually dimorphic, regionally restricted parental biases and opposite imprinting of different isoforms of single genes, suggests the presence of multiple specialized, and perhaps novel, mechanisms that govern these elaborate imprinting patterns. The relative parental bias rather than absolute imprinting, the complex nature of most imprinted loci (with different potential transcription units being imprinted by different parents), and the sex-specific imprinting described in these studies have the potential to increase neuronal diversity within a given brain region via previously unanticipated mechanisms. Recent studies with *agouti*^{vy}, which regulates coat color in mice, demonstrate that poorly understood physiological processes, as well as defined environmental factors, lead to a tremendous diversity in coat color phenotypes when this allele is maternally inherited (Morgan et al., 1999). If such a phenomenon can operate on any imprinted locus, then it has the potential to generate an enormous variability, within some physiological range perhaps, in the differentiation of the brain and behavior. Taken together, these studies highlight the complexity in the regulation of gene expression in the brain and suggest additional mechanisms that may increase neuronal diversity, modulate behavior, and confer susceptibility to neuropsychiatric illness.

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