The silence of genes

Is genomic imprinting the software of evolution or just a battleground for gender conflict?

Although the discovery of genomic imprinting dates back more than two decades, its significance for both disease pathogenesis and evolutionary theory is only now emerging. However, its effects had been observed for much longer, and if biologists had been influenced less by prevailing orthodoxies, they might have stumbled on it sooner. Mule breeders encountered the phenomenon three millennia ago, when they observed that a female horse crossed with a male donkey yielded a mule, whereas a male horse crossed with a female donkey gave a 'hinny'. The mule has longer ears, whereas the hinny has stronger legs and a thicker mane.

The differences between the mule and the hinny are now known to be caused by genomic imprinting, whereby the expression of a gene is determined by its origin rather than its DNA sequence. This phenomenon had previously been largely discounted because of the powerful influence exerted by Gregor Mendel's rules of inheritance. Mendel asserted that phenotype was determined entirely by the underlying alleles and was independent of any other parental or environmental factors. This in turn led to the complete dismissal of alternative theories, notably from Jean-Baptiste Lamarck, who proposed that acquired characteristics could be inherited. The discovery of genomic imprinting neither overturns Mendelian inheritance nor restores Lamarckism. However, it does muddy the waters, and identifies an important mechanism of inheritance and evolution that Mendel missed.

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Some researchers claim that imprinting provides an additional mechanism for mammalian inheritance, which allowed these animals to evolve more quickly than other species. One could even speculate that humans owe their existence to imprinting, although it involves only around 1% of the total genome—so far, about 80 imprinted genes have been identified in humans and the mouse.

Imprinting is also implicated in many serious diseases, including some cancers. Its medical importance has created huge interest in the field in recent years, which has led to the discovery of more diseases associated with imprinting and new ideas about how and why it evolved, along with a greater understanding of the underlying molecular mechanisms.

The first molecular evidence for imprinting in mammals came in the mid-1980s, when two studies showed that functional differences between maternal and paternal gametes are carried through to the embryo and survive activation of the embryonic genome in the two-cell phase (McGrath & Solter, 1985; Surani *et al*, 1986). Since these publications, the phenomenon has been studied in other organisms and has been found to occur whenever sexual reproduction occurs.

Sexually reproducing, diploid organisms normally inherit one copy of each gene from each parent. In most cases, the expression of the gene is determined by its primary DNA sequence, which explains why some alleles are dominant and some recessive. In the case of genomic imprinting, however, some alleles are actively suppressed by chemical modification of the DNA, irrespective of their sequence. Imprinting not only shuts down affected alleles but also can increase the expression of other genes—for example, by suppressing an inhibitor. In this way, imprinting provides another heritable factor of gene regulation that is not encoded in the primary DNA sequence of either gamete.

Recent evidence from mice and *Arabidopsis* shows that the imprinting mechanism—which occurs during the maturation of sperm and oocytes—is remarkably similar in some plants and mammals even though it emerged independently, suggesting that it is an example of convergent evolution. In both cases, imprinting is mediated by the methylation of cytosine–phosphate–guanine (CpG) sites in the DNA sequence. Methyl groups inhibit expression by preventing the transcription machinery from attaching to the DNA. These sites, comprising alternating C and G without any adenine or thymine, are relatively sparse, but more frequent in the vicinity of gene promoters where they form so-called CpG islands. This provides a relatively efficient mechanism because silencing a promoter region can influence the activity of several genes.

Although flowering plants and mammals share this mechanism, they differ significantly in the structure and distribution of the imprinted genes themselves. This might reflect different selective pressures during the reproductive stage: plants have a variety of mating strategies to reproduce sexually, but imprinting crucially affects only the cells of the endosperm—the tissue in the seed that provides nutrients for the developing embryo. Once the seed germinates, the endosperm is discarded and imprinting has no effect on the growing plant. In *Arabidopsis*, for example, the paternal *MEDEA* gene, which suppresses endosperm growth, is imprinted in the endosperm but not in the embryo or adult tissue.

The situation differs in mammals, as both maternal and paternal imprinting are carried through to the embryo and in some cases to adulthood, although they are usually removed through epigenetic reprogramming early in embryonic development. Despite these differences, imprinting in plants and mammals affects genes involved mostly in growth and the transmission of nutrients from the endosperm or placenta to the embryo during early development.

Intriguingly, there seems to be no obvious evolutionary reason for imprinting, as it removes the protective shield of diploidy that guards against lethal alleles and mutations. Several theories for its existence have therefore been proposed, such as the idea that imprinting prevents parthenogenesis—the mechanism by which offspring

are produced from unfertilized oocytes and contain two sets of maternal genes. As more genes are imprinted on the maternal side, the offspring must inherit an active copy from the father if the gene performs a vital function. For example, the embryo needs the father's insulin-like growth factor 2 (*IGF2*) gene because the mother's copy has been imprinted and is inactive. But this theory has not gained much credibility because there is little evidence that selective pressures would be sufficiently strong; for instance, parthenogenesis has re-emerged and operates in parallel with sexual reproduction in some plants.

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The current favourite is the sexual conflict theory, which was first suggested by David Haig, an evolutionary biologist and geneticist at Harvard University (Cambridge, MA, USA). He proposed that imprinting specific genes is the result of a conflict of interest between the father and mother, if the latter must invest proportionately greater resources to produce and rear offspring (Haig, 1993). It is in the best interests of the father for the offspring to extract maximum resources from the mammalian placenta or the endosperm to grow as quickly and as large as possible. In the case of mammals, this would extend beyond the womb in the case of multiple births, as stronger individuals would outcompete weaker siblings for milk. However, the mother has only a limited capacity to produce offspring during her lifetime and it is therefore in her best interests to spread her resources more evenly. In addition, there are also health risks associated with giving birth to large offspring. Maternal imprinting would therefore shut down genes that enhance the embryo's growth whereas paternal imprinting would silence genes that limit growth and development.

There are some caveats though, the obvious one being that sexual conflict only makes sense if females give birth to offspring from multiple fathers. If all relationships were monogamous, the interests of both parents would coincide, although there would still be conflict between a mother and her offspring. However, polygamy is common in mammals, which explains the prevalence of the sexual conflict theory. "It makes a number of predictions that have been shown to be correct," said Randy Jirtle, who studies genomic imprinting in carcinogenesis, embryogenesis and toxicology at Duke University (Durham, NC, USA). "Furthermore, since the accuracy of other postulates can never be confirmed experimentally, I prefer to discuss the theory that not only makes interesting predictions that can be tested, but also is the most interesting to talk about."

Nevertheless, some researchers contend that the sexual conflict theory is far from proven and that it cannot fully explain how imprinting arose. "Although most of the known imprinted genes fit into this theory, some don't," said Deborah Bourc'his, from the European Epigenome Centre of Excellence in Paris, France. In particular, she points to two maternally expressed genes that facilitate transport of nutrients from mother to daughter in mice—according to the theory, only fathers would have an interest in actively stimulating growth and thus expressing these genes.

There is therefore a broader theory, namely that all imprinted genes in mammals are involved in exchanging nutrients between mother and fetus. This alternative theory, although it does not preclude sexual conflict, is based on the idea that parents and offspring have colluded in evolving genes that optimize development. "There is a possibility that maternal trait selection also plays a role, with a recent theoretical analysis supporting this," noted Wolf Reik, assistant director at the Babraham Institute Laboratory of Genetics and Imprinting near Cambridge in the UK, referring to his own work (Wolf & Hager, 2006).

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According to this theory, it is in the interests of both parents to maintain efficient nutrient transfer from mother to child while the two are either physically attached through the placenta or endosperm, or closely coupled, as when suckling. Indeed, there is evidence that some maternal genes imprinted in the placenta have co-evolved in this way (Zechner *et al*, 2004). This theory would also explain the predominance of genes imprinted by the maternal rather than the paternal side.

Still, this theory is unlikely to explain all instances of imprinting, and so maternal trait selection may well operate in conjunction with sexual conflict. Genes that favour all offspring while minimizing expense by the mother might be subject to maternal selection. Conversely, genes that maximize the supply of nutrients to one particular offspring might be expressed paternally, and in some cases countered maternally—that is, governed by sexual conflict.

Regardless of which theory is correct, there is a growing consensus on one aspect of imprinting: its role in speciation. "This is not known in great detail, but theoretical considerations, and a couple of studies in *Peromyscus*, support the idea that imprinting could have an important role in post-zygotic isolation mechanisms in speciation," said Weik. Jirtle is also convinced that imprinting has a major role in mammalian speciation. "This is maybe why the repertoire of imprinted genes, and maybe even the diseases that result from their dysregulation, are species-dependent," he said. If members of two diverging species mate, the distribution of some imprinted genes would therefore lead to dysregulation. "This can lead to either death or sterility," Jirtle explained. "Therefore, imprinting acts somewhat like a ratchet, ultimately restricting the ability of newly evolved mammalian species to mate successfully with other similar species."

The first case demonstrating how imprinting enforces separation between two closely related species, namely the North American rodent species *Peromyscus polionotus* (PO) and *P. maniculatus* (BW), was published earlier this decade (Vrana *et al*, 2000). The two species are about the same size, but whereas a female BW crossed with a male PO produces offspring that are smaller than either parent, the reciprocal cross produces offspring that are oversized and typically die before birth. The oversized crosses in particular showed disrupted imprinting in several growth-related genes, which led the authors to conclude that elements regulating epigenetic gene expression must have evolved rapidly. This in turn implies that a relatively short period of separation between two closely related species can be sufficient for them to become reproductively isolated.

Unfortunately, dysregulation can also lead to disease, particularly if the expressed gene is mutated or if the process goes wrong. One recently discovered example is Silver–Russell syndrome, in which the loss of methylation in a region that controls imprinting of the *H19* gene results in babies being underweight at birth and having other growth-related abnormalities (Bliek *et al*, 2006). The region involved—called *H19* differentially methylated region (DMR)—controls the expression of the imprinted *H19* tumour suppressor gene and *IGF2*. *IGF2* was one of the first imprinted genes to be discovered and provided the first molecular evidence for the sexual conflict theory.

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The key finding was that the methylation pattern in *H19* DMR is differential, meaning that it varies between individuals and is imprinted by either parent—hence the potential for sexual conflict. In the case of Silver–Russell syndrome, reduced methylation (hypomethylation) in this region leads to a loss of imprinting of the *H19* gene, which is de-repressed as a result. *H19* encodes a non-coding RNA, which in turn retards the expression of IGF2, thus diminishing growth. By contrast, hypermethylation of *H19* DMR—that is, an increase in methylation within this control region—has the opposite effect, and is a major cause of gigantism, as seen in patients with Beckwith–Wiedemann syndrome.

Given the link between imprinting and growth control, it is not surprising that the mechanism is also implicated in several cancers. According to Andrew Hoffman, a professor of endocrinology at Stanford University (Palo Alto, CA, USA), an important discovery was a link between the imprinting of *IGF2* and colon cancer (Cui *et al*, 2003). This has also raised hopes of treating several cancers through epigenetic reprogramming to restore normal gene expression, which is the focus of Hoffman's research (Chen *et al*, 2006). "We have been using epigenetic reprogramming of human tumour cells to alter epigenetic marks, and have developed evidence that we can restore normal imprinting in tumour cells in which *IGF2* imprinting has been lost," said Hoffman. "Our data suggest that there is an imprinting maintenance factor made by normal cells that has been inactivated […] in cancer. Transplanting nuclei from tumour cells into the cytoplasm of normal cells leads to restoration of *IGF2* imprinting."

Research into the mechanisms of imprinting might therefore not only shed further light on this complex phenomenon, but also yield benefits for human health. Meanwhile, some researchers such as Jirtle believe that it is already clear that imprinting has had an important role in evolution, certainly of therians—marsupials and placental mammals—by providing a faster mechanism of adaptation than gene mutation.

"It is my view that evolution could occur in therian mammals by rewriting the software of the computer rather than by changing its hardware wiring," said Jirtle, pointing out that methylation to inhibit allele expression is reversible and therefore reprogrammable, just as if it were a piece of software. Indeed, many imprinted genes are reprogrammed during embryonic development or just after birth. This not only enables gene expression to be modified, but also makes imprinting specific in space and time such that it applies only in some tissues or organs and over a certain period of time.

But, Jirtle notes, despite its positive effect on evolution and speciation, imprinting has its downsides. Just as changing software can create bugs, environmentally induced dysregulation of imprinting can create developmental and neurological disorders. This might well be the price that therian mammals had to pay to accelerate their evolution.

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