# FOUR

# Genomic Imprinting

**GENOMIC IMPRINTING RESULTS** in parent-specific gene expression, that is, in a difference in gene expression depending on which parent contributed the gene. In the usual case, an allele is silent when inherited from one parent while the identical stretch of DNA is active if inherited from the other; but often this effect is seen in some tissues and not others, or there is only a quantitative difference in gene expression, depending on the parent of origin. To give but one example, in the mouse *Meg1/Grb10* shows maternal expression in most tissues, but paternal expression in the brain; in humans it also shows paternal expression in the brain, but biallelic expression in other tissues (Kaneko-Ishino et al. 2003). Because the difference in expression occurs when DNA is identical, the effect is said to be "epigenetic," that is, the difference is caused by some aspect of the chemistry of the proteins binding DNA or of the DNA itself, but unrelated to nucleotide sequence. Such genes are said to be imprinted.

This ability to be expressed according to parent-of-origin has striking implications for a gene's degree of relatedness to its parents–and, thus, to all other relatives differentially related through them. Consider an autosomal gene's relatedness to its mother. An unimprinted gene has no information where it came from and computes only the average chance of one-half. An imprinted gene calculates exact relatedness, maternal = 1, paternal = 0. These sharply divergent kinship coefficients create 2 kinds of conflict. One occurs over evolutionary time in which the spread of selfish paternally ac-

tive genes naturally selects for opposing maternally active genes (and vice versa), while both select (albeit less strongly) for opposing unimprinted genes. The second kind of conflict is imagined to occur within an individual whenever the opposing genes act against each other. Unlike most forms of conflict described in this book, the conflict here is not over which genes to transmit at what rates but rather over how much an individual should help or hinder a given relative.

The major focus of conflict between maternally and paternally active genes is parental investment. Degrees of relatedness are maximally divergent and a critical resource is at issue. Consider a fetus developing and growing within its mother's womb. Because they are not genetically identical, there will inevitably be some conflict between the fetus and the mother over how much investment should be transferred from the mother during the pregnancy, with the fetus selected to acquire more than the mother is selected to provide (Trivers 1974). A similar conflict also exists between maternally and paternally derived genes in the fetus: selection on maternally derived alleles is influenced by the fact that a copy of the gene is also found in the mother and will be passed on to half of her other offspring (Haig 1993b). This will tend to dampen selection to be ever more demanding. However, the paternally derived allele is (barring inbreeding) not found in the mother, and it will only be selected to reduce demand insofar as the mother has (or will have) other offspring with the same father. In the extreme case, in which each of a female's offspring is fathered by a different male, paternally derived genes in each offspring are selected to take as much as possible from the mother, without regard for her future survival or reproduction. At the opposite extreme, if females only mate with a single male in their lifetime and vice versa, there is no conflict because the father suffers just as much as the mother from any decrement in maternal fitness. Conflict–though less intense–is also expected over actions affecting other relatives, whenever these are differentially related through mother or father (see Fig. 4.1).

This general approach to the meaning of genomic imprinting may be called the kinship theory of imprinting and is due primarily to the work of David Haig, who first introduced the approach (Haig and Westoby 1989, Haig and Graham 1991, Moore and Haig 1991) and who has been most active in developing it further (reviewed in Haig 2000a, 2002). Other theories have been advanced to explain the adaptive significance of imprinting (reviewed in Hurst 1997), but those to date seem to lack both supporting logic and evidence (Haig and Trivers 1995, Wilkins and Haig 2003a), while Haig's

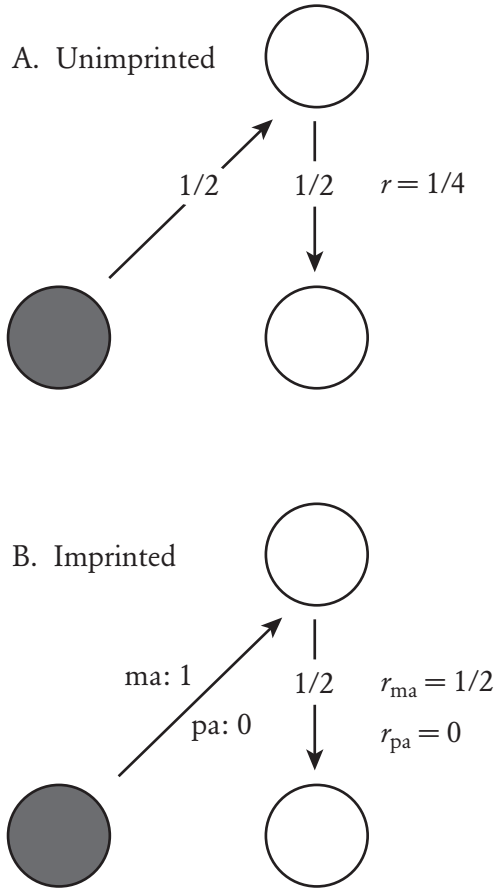


**Figure 4.1** Degrees of relatedness (*r*'s) under imprinting. A. *r*'s to mother and mother's offspring for an unimprinted gene (1/2 to each, *r* to maternal half-sib = 1/4). B. *r*'s to mother (1 and 0) and mother's offspring (1/2) for a maternally (ma) and paternally (pa) active gene, respectively (*r* to maternal half-sib is 1/2 and 0).

theory explains a broad pattern of evidence, with no facts clearly in contradiction. One need assume no more than Mendelian genetics and the action of natural selection. Once parent-specific gene expression appears, it must by logic evolve the biases Haig describes, with paternally active genes acting to further paternal relatives–in other words, patrilines–and maternally active genes, matrilines. If there are additional selection pressures we need to take into account to understand imprinting, we do not yet know what they

are. (We exclude here, and throughout this chapter, "imprinting" in the sense of paternal genome loss in scale insects and others, which we explain according to a different logic in Chapter 10.)

Because our own genes are imprinted and a number of developmental disorders are associated with failures of imprinting, including cancers (reviewed in Hall 1999, Tycko 1999, Walter and Paulsen 2003), this category of selfish gene has been studied with unusual intensity. In addition, because the mouse, with most of the same imprinted genes, is a model genetic organism, there is a rich experimental and genetic literature regarding the mechanisms (and effects) of imprinting, especially early in development.

In this chapter, we begin our account with a review of the role of imprinting in mother-offspring interactions over parental investment, especially fetal growth. We then review the molecular mechanisms underlying imprinting, focusing in particular on the idea that conflict is expected not only between maternally and paternally active genes but also between the various components of the imprinting machinery itself–and that this may help explain much of the molecular complexity. We then expand the discussion to other sorts of social interactions and to the special factors that apply to imprinted genes on sex chromosomes. Finally, we review what is known, and what might be predicted, about the distribution of imprinting outside of mammals.

## Imprinting and Parental Investment in Mammals

At least 100 genes are thought to be imprinted in the mammalian genome (out of a total of about 30,000; Reik and Walter 2001b, www.mgu.har.mrc.ac.uk/imprinting/imprinting.html, www.otago.ac.nz/IGC). Those that have been described are disproportionately expressed in the placenta and involved in fetal growth. We start with a detailed look at 2 well-studied loci in mice, oppositely imprinted and with strong opposite effects on early growth.

### *Igf2* and *Igf2r:* Oppositely Imprinted, Oppositely Acting Growth Factors in Mice

*Igf2* and *Igf2r* are oppositely imprinted genes with strong contrary effects on fetal growth in mice. They were the first 2 imprinted genes discovered in mammals (Barlow et al. 1991, DeChiara et al. 1991), and they provided a

paradigm for the underlying kinship logic of imprinting (Haig and Graham 1991). *Igf2* is imprinted in both mice and humans. It acts as if it has a complex, conditional strategy (reviewed in Stewart and Rotwein 1996). It is inactive in almost all adult tissue, save the choroid plexus and leptomeninges, in which both alleles (paternal and maternal) are active. By contrast, it is very active in almost all tissues of the fetus, including extraembryonic; but only the paternal allele is active, with the maternal being transcriptionally silent. Inactive copies can be generated by homologous recombination and when such a copy is inherited from the father–so no *Igf2* is expressed in the fetus–the individual mouse is 40% smaller at birth and throughout life but is otherwise properly proportioned (DeChiara et al. 1991). *Igf2* produces insulin-like growth factor 2, which is a growth promoter. It stimulates cells to divide. When found in 2 active copies in humans, it is often associated with Beckwith-Wiedemann syndrome, a fetal overgrowth disorder with frequent tumors (Rainier et al. 1993, Ogawa et al. 1993).

In mice and humans, the action of *Igf2* is opposed by the action of *Igf2r,* or mannose-6-phosphase-receptor, except that this gene is maternally active in mice but biallelic (active from both alleles) in humans, in which there is also no good evidence that *IGF2R* inhibits prenatal growth (for a recent review, see Dahms and Hancock 2002). In vertebrates it targets proteins tagged with mannose 6-phosphates (as well as the digestive enzymes) to the lysosomes, where they are degraded. In mammals, it has evolved a secondary binding site for *Igf2,* which it then degrades. Its deletion in mice causes offspring to be born that are 125–130% of normal birth weight (Ludwig et al. 1996). Its evolution, along with that of *Igf2,* is summarized in Table 4.1. Neither is imprinted in monotremes and both are in marsupials, the secondary binding site in *Igf2r* having also evolved in the interval. It is reasonable to suppose that *Igf2* became imprinted first, in response to the appearance of intrauterine life, and that *Igf2r* evolved its secondary binding site next and became imprinted, but there is no evidence on this (Wilkins and Haig 2001, 2003a).

*Igf2*'s imprinting status does not change in placentals, but for unknown reasons *Igf2r* becomes biallelic in tree shrews, flying lemurs, and other primates, including ourselves. It is not at all clear why imprinting of these genes is not also found in monotremes. They do lay eggs, but these develop within the mother for up to 4 weeks, with maternal nourishment, and they increase in size from 4mm to 20mm in diameter (John and Surani 2000).

*Table 4.1* *Igf2* and *Igf2r* in mammals

| Gene | Imprinting Status |
|---|---|
| Monotremes | |
| *Igf2* | Not imprinted |
| *Igf2r* | Igf2-binding site absent |
| Marsupials | |
| *Igf2* | Imprinted |
| *Igf2r* | Binding site present and imprinted |
| Placentals | |
| *Igf2* | Imprinted in rodents, artiodactyls, primates |
| *Igf2r* | Imprinted in rodents, artiodactyls, not in primates |

References in Wilkins and Haig (2003a).

Perhaps lacking a placenta, the egg has few ways to induce greater investment. After birth, offspring lick milk provided by the mother; so all during this time, increased growth rate associated with paternal genes would seem to give an advantage, assuming multiple paternity of a female's lifetime reproductive output. As expected, neither *Igf2* nor *Igf2r* is imprinted in chickens (Nolan et al. 2001; Yokomine et al. 2001).

Here are 2 oppositely imprinted genes in mice with strong counteracting effects on growth that cancel out to give little or no net effect–precisely what is expected of internal genetic conflict based on evenly matched paternal and maternal alleles. There are additional complexities we shall consider shortly. For example, *Igf2* is located on mouse and human chromosomes in a cluster of at least 10 imprinted genes and there are complex interactions between them. One of these, the oppositely imprinted and nearby *H19,* shares many control elements in common with *Igf2,* its mRNA interacts with that of *Igf2,* and some knockouts cause *Igf2* to become biallelic while others do not (reviewed in Runge et al. 2000, Arney 2003).

## Growth Effects of Imprinted Genes in Mice and Humans

Detailed physiological and genetic evidence is now available for at least 27 imprinted genes in mice (most of which are also imprinted in humans), and this evidence shows a striking pattern (comprehensively reviewed by Tycko and Morison 2002; Table 4.2). Most of the genes have early effects on

You can ignore this Table.



*Table 4.2* Imprinted genes in mice, their effects, and whether these effects support the kinship theory of imprinting

| Imprinted Gene | Effect | Support kinship theory? |
|---|---|---|
| PATERNALLY ACTIVE | | |
| *Igf2* | Increases size at birth (40%). | + |
| *Ins1, Ins2* | Imprinted expression in yolk sac, biallelic in other tissues (including pancreas). *Ins* known to affect early growth positively, but parent-of-offspring effect not yet demonstrated. | 0 |
| **Kcnq1ot1* | Antagonizes expression of growth inhibitor $p57^{KIP2}$. | + |
| *Dlk1* | Same gene causes skeletal muscle overgrowth in sheep but time of action and cost to mother uncertain. | (+) |
| *Sgce* | Absence of gene in humans associated with obsessive-compulsive behavior and panic attacks. | 0 |
| *Rasgrf1* | Affects postnatal growth positively, with maximum effect at weaning (Itier et al. 1998). | + |
| *Peg1/Mest* | Increases placental and embryonic growth, which is associated with greater postnatal growth and survival. Positive effect on maternal behavior, such as pup retrieval (see text). | + |
| *Peg3* | Increases placental size and weight at birth (20%) and nursing by pups. Positive effect on nursing by mothers and on oxytocin-positive neurons in the hypothalamus (see text). | + |
| *Nnat* | Appears to have positive effect on embryonic growth, but data only from uniparental disomy. Overexpressed in embryonic cancers. Produces a neuronal protein with strongest expression during perinatal period. | (+) |
| *Ndn* | Absence associated with early postnatal lethality in mice and failure to thrive in humans (Prader-Willi syndrome) including hypotonia and failure to nurse; obsessive food-seeking behavior later in life (for this and other references to Prader-Willi, see Haig and Wharton 2003). | (+) |
| *Snrpn* | Active in many fetal and adult tissues, encodes a splicing factor component expressed at highest levels in neurons. Knockout has no obvious phenotype. | 0 |
| **Pwcr1* | Absence associated with failure to thrive, early postnatal lethality, and Prader-Willi syndrome in humans. Expressed predominantly in brain, encodes a small nucleolar RNA that may affect processing of serotonin. | (+) |

*Table 4.2* (continued)

| | | |
|---|---|---|
| **Ipw* | No obvious effect on phenotype at birth. Encodes an abundant spliced RNA that builds up in cytoplasm and shows high expression in brain and other tissues. | 0 |
| **Xist* | Causes paternal X-chromosome inactivation in the placentae of mice, either by direct action or via its oppositely imprinted antisense transcript *Tsix*. | 0 |
| MATERNALLY ACTIVE | | |
| *Igf2r* | Decreases placental size and size at birth (25%) by binding to and degrading *Igf2*. In humans, not imprinted (or only in some individuals) and affects cognitive ability in children (Chorney et al. 1998). | + |
| **Meg3* | Encodes an abundant, nontranscribed, spliced RNA, found in many fetal and adult tissues, esp. adult skeletal muscle; closely linked to *Dlk1* and appears to down-regulate overgrowth of skeletal muscle associated with *Dlk1,* but cost of overgrowth to mother unknown. | (+) |
| *Gnas* | Encodes subunit of signaling protein that decreases insulin sensitivity and fat cell growth. | + |
| *Ube3a* | Imprinted in hippocampal and cerebeller neurons, increases learning and long-term potentiation of synaptic transmission. Absence contributes to Angelman syndrome in humans, which includes neonatal hyperactivity, opposite of the oppositely imprinted Prader-Willi syndrome. Imprinted in fibroblasts, lymphoblasts, and neural-precursor cells (Herzing et al. 2002). | 0 |
| **H19* | Closely linked to the oppositely imprinted *Igf2,* it encodes an abundant, spliced RNA and acts in *cis* to down-regulate *Igf2* expression via shared insulator and enhancer sequences. Silenced in most Wilms' and other embryonic tumors in humans. | + |
| *Ascl2/Mash2* | Expression limited to placenta; increases spongiotrophoblast growth at the expense of giant cells, which secrete placental lactogens (which actively solicit maternal investment; Haig 1993b). Giant cells penetrate farthest into maternal decidua; limitation on giant cells suggests limitation on maternal investment. Absence causes death in midgestation. | (+) |
| *Cd81* | Weakly imprinted only in extraembryonic tissues, biallelic in others, codes for a membrane protein, expressed in many tissues; no evidence on imprinted phenotype. | 0 |
| *Kcnq1* | Imprinted only in fetal tissues of mice and humans, biallelic in adults, encodes a voltage-sensitive potassium channel. Absence increases stomach size 3-fold but no evidence of an imprinted phenotype. | 0 |

*Table 4.2* (continued)

| | | |
|---|---|---|
| *p57$^{Kip2}$/Cdkn1c* | Imprinted in multiple fetal tissues, encodes a kinase inhibitor, evidence from *in vitro* and *in vivo* suggests that it inhibits cell proliferation in various tissues. | + |
| *Tssc3* | Strongly imprinted in placenta (and liver) of mice and humans and weakly imprinted in other fetal and adult tissues; encodes a small cytoplasmic protein, restrains placental growth but without reducing fetal growth. | (+) |
| *Esx1* | Imprinted in mouse placenta, X-linked so probably imprinted as a result of general paternal X-inactivation (see *Xist*). Absence of gene increases size of early placenta but, at later stages, depresses fetal growth, probably due to placental pathology. | (+) |
| *Gatm* | Catalyzes the rate-limiting step in the synthesis of creatine, an important molecule in energy metabolism. Imprinted in placenta and yolk sac but not embryo; effect on offspring and maternal resources unknown (Sandell et al. 2003). | 0 |
| *Grb10* | Potent growth inhibitor: deleting maternal copy leads to placental and fetal overgrowth, especially liver but not brain, and offspring are 30–40% larger at birth, independent of *Igf2* pathway (Charalambous et al. 2003); associated with Silver-Russell syndrome in which doubled maternal copy gives growth retardation. | + |

* codes for untranslated RNA.

Except where otherwise noted, all information (including almost every evaluation regarding kinship theory) is from Tycko and Morison (2002).

\+ strongly supports kinship theory; N=10

(+) weakly supports kinship theory; N=8

0 neutral; N=9

No cases contradict theory.

Total: N=27

growth (18 of 27). When organized according to whether the genes are paternally active or maternally active and whether they affect growth positively or negatively, 10 strongly support the kinship theory, 8 weakly (at least 1 piece of evidence absent), and none is clearly opposed. In one case *(Rasgrf1),* the expected growth effect occurs after birth but before the end of weaning (Itier et al. 1998). In addition, some of the neutral cases are at least suggestive–for example, early growth effects not yet shown to be imprinted. In any case, the pattern is so strong as to be impervious to minor decisions regarding evaluation of individual cases (for detailed analysis of selected cases, see also Haig 2004b).

Additional supporting evidence regarding growth effects comes from mice chimeras (individuals produced in the lab that consist of a mixture of wildtype cells and others). Mice chimeras in which the added cells are androgenetic (double dose of paternal) are, as expected, usually larger than pure wildtype individuals, which in turn are larger than chimeras in which the added cells are parthenogenetic (Fundele et al. 1997). These differences tend to disappear after weaning, again as expected because there is little scope for internal genetic conflict over growth when the resources to support growth are supplied from outside the family.

Humans are very unusual in the length of the period of parental investment–well past weaning and usually into young adulthood. Thus, important imprinted effects concerning parental investment may concern post-weaning life. With this in mind, Haig and Wharton (2003) have recently reinterpreted the symptoms of Prader-Willi syndrome, which results from the absence of the paternal copy of a segment of chromosome 15. For the first few years of life, children suffer lassitude and low appetite, as expected from loss of paternally active genes during nursing; but then appetite becomes voracious and undifferentiated, that is, the children eat anything and even forage afield for new foods, traits that would tend to reduce cost to the mother. For a detailed analysis of imprinted effects on calcium metabolism in pregnancy, see Haig (2004a).

Though the data summarized in Table 4.2 provide compelling evidence that kinship conflicts have played an important role in the evolution of genomic imprinting, this evidence does not mean that every imprinted gene must have been selected for its kin effects. Some genes may be imprinted as a pleiotropic effect of imprinting at a neighboring locus and some because in the past there was selection for a change in expression level, and the first appropriate mutation happened to work in a parent-specific manner. Once the imprinting machinery has evolved, we should expect non-kin effects to occur at least some fraction of the time.

## Evolution of the Imprinting Apparatus

### The Mechanisms of Imprinting Involve Methylation and Are Complex

In order for maternal and paternal alleles to have different expression levels despite having the same nucleotide sequence, there must be some "epigenetic" difference between them. Considerable progress has been made in