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Imprinting

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ABSTRACT Imprinting provides a fascinating mechanism of control of gene expression so that the maternal and paternal alleles of some genes are unequally expressed. Imprinting is most likely established during gametogenesis by a mechanism not completely clear, though DNA methylation probably plays a certain role. Expression of imprinted gene significantly affects mammalian development so that only the maternal or only the paternal diploid genomes cannot support normal development. Since imprinting results in functional hemizygocity, mutation of the expressed allele can have the drastic consequences of a null mutation. For this reason identification of imprinted genes and further understanding of the imprinting mechanism represent an important change for human medical genetics.

KEY WORDS: *imprinting, epigenetics, mammalian development*

The fascination with imprinting and its umbrella organization – epigenetics – continues unabated as witnessed by the number of recent books (Ohlsson *et al.*, 1995; Russo *et al.*, 1996; Reik and Surani, 1997; Chawick and Cardew, 1998) and reviews (Latham *et al.*, 1995; Lalande, 1997; Bartolomei and Tilghman, 1997). Imprinting seems to disprove Thomas Kuhn's statement that textbooks are produced in the aftermath of scientific revolution. Actually from the very beginning and regardless of how little we know about imprinting, we have always tried to summarize and synthesize it, as if this would help our ignorance. This brief text has no such ambition and the interested reader is directed to the references mentioned above. All I will try to do is to list a few questions and suggest what may be necessary to do in order to answer them.

The nature and extent of imprinting

At present the majority would agree with the suggestion extensively discussed by Efstratiadis (1994) that imprinting results in (and is necessary for) differential transcription from paternal and maternal alleles which otherwise have an absolutely identical sequence. This differential expression can be developmental stage, tissue and cell type-limited, i.e., a gene may show its imprinted character only in certain cells during a specific time in development. The fact that a gene can behave as imprinted in one tissue and be biallelically expressed in another must be kept in mind when discussing possible mechanisms of imprinting. At present we are not sure how widespread imprinting is in the animal and plant kingdom. In functional terms, imprinting should prevent parthenogenetic/gynogenetic and androgenetic development (Solter, 1988), so any species in which these can be observed should not possess imprinting. This may be an oversimplification and we can imagine conditions in which imprinting can exist, but it does not affect development in such a dramatic way. Nevertheless (again by majority vote), we believe that imprinting is restricted to mammals and possibly some flowering plants. It is remarkable how little has been done so far to prove or disprove this assumption. The extent and modes of imprinting will have to be established before we can hope to understand the mechanisms. For example, is the imprinting observed in flowering plants restricted to *triploid* endosperm (Kermicle and Alleman, 1990)? This is important because, if we are to disregard endosperm, all examples of imprinting as differential allele expression will be restricted to mice and men.

Developmental consequence of imprinting

The hint that something like imprinting exists was provided by nuclear transfer experiments in mice (Barton *et al.*, 1984; McGrath and Solter, 1984a; Surani *et al.*, 1984) and confirmed by the genetic analysis of various chromosomal abnormalities resulting in uniparental disomies–monosomies (Cattanach and Kirk, 1985). In simplest terms, embryos with only a paternal or only a maternal diploid genome die with specific and diagnostic morphologies (McGrath and Solter, 1984a; Barton *et al.*, 1985; Surani, 1986), so that gynogenetic embryos fail to develop extraembryonic tissues and derivatives of the inner cell mass are absent in androgenetic embryos. One would anticipate that as the identification of imprinted genes progresses, we will encounter the earliest acting ones whose absence results in the above-mentioned phenotypes. So far this has not been the case and we do not know exactly why

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uniparental embryos fail in development. One possibility is that the imprinted X-inactivation is responsible (Latham, 1996; Solter and Wei, 1997). The analysis of X-inactivation in mice following the elimination of *Xist* (Marahrens *et al.*, 1997) showed that the maternal X chromosome cannot be inactivated in extraembryonic membranes, thus embryos which inherit the paternal X chromosome with *Xist* deletion have two active X chromosomes in extraembryonic membranes, which results in postimplantation lethality with a phenotype very similar to the loss of gynogenetic embryos. One can therefore assume that all parthenogenetic and gynogenetic embryos die because of two active X chromosomes in extraembryonic membranes. The situation with androgenetic embryos may be more complicated. The simplest explanation would be if we were to assume that the paternal X chromosome is always inactivated in extraembryonic membranes regardless of how many X chromosomes are present. In this case obviously all three classes of androgenetic embryos –YY, XY and XX– would die due to the absence of an active X in extraembryonic membranes. The problem with this explanation is that XO mice with a paternal X develop and live (reviewed in Solter and Wei, 1997). So either the XO and XY situations differently affect X-inactivation or there is an X-independent mechanism of androgenetic embryo lethality. As the search of chromosomal regions for imprinted genes approaches its final stage (Cattanach and Beechey, 1997) – or it may have already reached it (Cattanach, personal communication) – without finding the gene responsible for the death of androgenetic embryos, we may have to postulate the combined effect of several genes as a rather unsatisfactory explanation.

Establishment and erasure of imprinting

Before we can discuss when the imprint is established and when it is erased, we have to be clear about one basic principle, namely that the expression (biallelic or monoallelic) of an imprinted gene cannot be the indicator of the imprinting status. It is now obvious that in contrast to "normal" genes whose expression status can be off or on, the "imprinted" gene can be on, off and monoallelically on. Once we know that the gene is imprinted (by detecting monoallelic expression at least sometime in some cells), the fact that the same gene is expressed biallelically does not mean that the imprint is erased. It may only mean that, whatever mechanism is recognizing the imprint, this mechanism is not operational. Thus, otherwise very detailed and informative studies addressing the expression of several imprinted genes during gametogenesis and early development (Szabó and Mann, 1995a,b) do not address this specific point. However, we can make some tentative (mostly negative) assumptions about the timing of imprint establishment and erasure. The most stringent test of the completion of imprinting is the ability of the haploid genome to support development in a parentally-specific fashion, i.e., testing if a specific haploid genome can substitute for a male or female pronucleus. Several studies have analyzed the ability of cells in different stages of spermatogenesis to substitute for mature sperm. Round spermatids injected into mature oocytes support normal development (Kimura and Yanagimachi, 1995b), which is not too surprising (although it demonstrates that all events occurring during spermiogenesis – histones being replaced by protamines and reversal of this process after fertilization – are essentially irrelevant). Interestingly, nuclei from secondary spermatocytes injected into ovulated oocytes also support development following completion of meiosis II (Kimura and Yanagimachi, 1995a). The attempt to test nuclei from primary spermatocytes using similar approaches failed (Ng and Solter, 1992; Ogura *et al.*, 1997), though the reasons are not clear. Ogura *et al.* (1997) fused the primary spermatocytes with GV (germinal vesicle) or GVB (germinal vesicle breakdown) oocytes. The oocytes underwent maturation and arrested in meiosis II, and the metaphase chromosomes were transferred to freshly isolated enucleated oocytes in meiosis II, which was in turn activated and meiosis II was completed. The resulting structures were diploid and contained presumably both the maternal and paternal haploid chromosome set. These "zygotes" developed to blastocyst stage but no live births were observed. It is difficult to be certain if the developmental failure was caused by the incompleteness of imprinting in the primary spermatocyte or by some chromosomal imbalance due to very unorthodox meiosis or by technical problems. It should be possible (though rather difficult) to enucleate the GV oocyte (Latham and Solter, 1993) and fuse the resulting cytoplast with primary spermatocytes. Provided that the spermatocyte nucleus can complete the entire meiosis in oocyte cytoplasm, the resulting haploid genome can be tested, i.e., combined with the male or female pronucleus in the partially enucleated zygote (McGrath and Solter, 1984a) in order to determine the nature of its imprinting. In summary, we know that imprinting of the male genome is completed before meiosis II and probably before meiosis I. Similar experiments addressing the timing of imprinting of the female genome have not been done and will be technically much more difficult.

Until recently the only information as to the erasure of imprinting came from the analysis of expression of imprinted genes in the tissues of newborn and adult mice and, as discussed before, these data are not really informative. We assumed that the imprint has to be erased in cells destined to become germ cells, since each mammal? mouse? human? inherits two differently imprinted haploid genomes but transmits only one imprinting status (Solter, 1987). Whether the imprint was erased or maintained in somatic cells was not known, and the importance of either for maintaining the homeostasis of the organism was not clear. The assumption that the imprint may be erased led us to suggest that cloning from adult cells may be impossible (McGrath and Solter, 1984b), an assumption which proved spectacularly wrong (Wilmut *et al.*, 1997) and for which I was deservedly reproved (Kolata, 1998). What does successful cloning of sheep from adult cells (and I anticipate that mice, though maybe more difficult, will eventually prove clonable) tell us about imprinting? The simplest explanation is that the imprint is retained completely and that erasure requires passage through gametogenesis. It is, however, equally possible that the low incidence of successful cloning (the incidence decreases with developmental age) indicates a random, progressive loss of imprinting and that only cells in which essential imprints are accidentally retained can support cloning. In order to answer this question we will have to know more about the mechanisms of imprinting.

Mechanisms of imprinting

We always assumed that with the isolation and characterization of several imprinted genes the mechanisms which mediate imprinting would become obvious. After a score or more of imprinted

genes have been described (Bartolomei and Tilghman, 1997), the presence of unified imprinting mechanisms is by no means an established fact. Nevertheless, some hints are obvious. It seems that imprinting genes do come in clusters (Ohlsson *et al*., 1995; Russo *et al*., 1996; Reik and Surani, 1997; Chawick and Cardew, 1998) and that methylation is directly or indirectly involved (Jaenisch, 1997). It is interesting that in the case of the genes studied in most detail – *Igf2*, *H19*, *Igf2r* (Bartolomei and Tilghman, 1997; Reik and Constancia, 1997; Wutz *et al*., 1997) – the genes which are responsible for the imprinted phenotype *(Igf2, Igf2r)* may actually not be imprinted, but that the genes which reciprocally regulate their expression (H19, antisense transcript) are. Moreover, these particular imprinted genes are not likely to have any other but a regulatory function, so the effect of imprinting on the phenotype is achieved indirectly. It is too early to say if other imprinted genes will be regulated in a similar manner. It is also likely that these models of control will prove to be more complex upon further analysis. It is obvious that we have to analyze more genes to determine if imprinted genes tend to cluster, if most or only some imprinted genes have an imprinted silent partner, and which sequences are instrumental in controlling imprinting. It has not been possible so far to impose imprinting on a normal gene by transferring a specific DNA sequence, and even transgenic imprinted genes retained their imprinting character only if they were injected as YAC clones suggesting the need for a very large domain in order to preserve and protect the imprint (Ainscough *et al*., 1997; Wutz *et al*., 1997). Even in YAC transgenics a partial loss of correct imprinting in some transgenic lineages was observed, indicating the complexity of regulatory mechanisms.

Finally, as always we return to the functional role and origin of imprinting. The theory that imprinting exists (or it evolved) in order to mediate the parent-offspring conflict (Haig, 1992) is gaining in popularity (Bartolomei and Tilghman, 1997; Jaenisch, 1997). This theory essentially states that the genes expressed from the paternal genome will favor the growth of the fetus at the expense of the mother and vice versa. It is easy to understand why the selfish interest of each parental genome would result in such a mechanism. The problem is that the theory is largely unfalsifiable in Popperian terms. Strictly speaking, if this theory is correct, all genes which are imprinted should be involved in regulating fetal growth and all genes which affect fetal growth should be imprinted. Neither of these presumptions is correct (Hurst and McVean, 1997); however, this does not necessarily invalidate the hypothesis, and the parent-offspring conflict could have been the initial impetus for imprinting which later expanded to other genes which needed to be controlled in this particular way (Gilligan and Solter, 1995). The theory of the parent-offspring conflict is presently being further expanded to accommodate such complex social structures as monogamy and polygamy (Bartolomei and Tilghman, 1997; Jaenisch, 1997) which are very likely regulated by multiple genetic and environmental factors (Baker and Bellis, 1995). This may be indeed reaching too far, as we may end up explaining the fact that genes can be differently imprinted in mice and humans [the same gene imprinted maternally in mice and paternally in humans (Williamson *et al*., 1996)] by the subtle differences in their mating habits! Again, examination of imprinting in mammals other than mice and humans, paying attention to the subtleties of their embryonic development and evolutionary relationship, will undoubtedly prove rewarding in unraveling this fascinating biological phenomenon.

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