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Review article

Genomic imprinting in mammals

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Abstract. In contrast to the biallelic expression of most genes, expression of imprinted genes is monoallelic and depends on the sex of the transmitting parents. In humans it has been implicated in some developmental failures, neurodevelopmental and neurobehavioral disorders (such as Prader-Willi/Angelman, Silver-Russel or Beckwith-Wiedemann syndromes). The aim of this review is to present the phenomenon of parental imprinting as well as its molecular mechanism in various mammals. Several maternal and paternal imprinted genes and gene clusters are described.

Key words: gametic conflict, imprinting, *Igf2* gene, mammals, methylation.

Introduction

Genomic (or parental) imprinting is a phenomenon in which alleles of a gene are expressed differentially depending on their parental origin. Several dozen genes are currently known to be imprinted in various organisms.

In humans, the process of genomic imprinting has been intensively studied mainly because of the association of imprinted genes with severe genetic aberrations, such as Angelman, Prader-Willi, Beckwith-Wiedemann, and Silver-Russell syndromes (Henry et al.1991; Weksberg et al. 1993).

Imprinting is also associated with the Large Offspring Syndrome (LOS) – congenital abnormalities observed in animals produced by IVF/IVM and/or embryo culture (YOUNG and FAIRBURN, 2000).

Identification of imprinted genes is the focus of interest for both geneticists and livestock breeders. Imprinting analysis is increasingly accounted for by studies on mapping of quantitative trait loci (QTLs) of farm animals (De Konning et al. 2000). Benefits coming from learning about the manners of inheriting QTLs are obvious if we take into consideration the more and more common usage of marker-assisted selection (MAS). Due to intensive researches, several imprinted QTLs have been identified in the porcine genome (Jeon et al. 1999; Nezer et al. 1999; Thomsen et al. 2004). One of them is a paternally expressed QTL on chromosome 2 (near the *IGF2* gene) in pigs, affecting muscle growth, fat deposition and heart muscle size (Jeon et al. 1999; Nezer et al. 1999). The whole *IGF2* gene region has been sequenced in various commercial pig breeds and wild boars, and the causative mutation (nucleotide substitution G>A in intron 3) has been found (Van Laere et al. 2003). This mutation increases *IGF2* mRNA expression in postnatal muscles and is responsible for phenotypic effects. Another example is the muscle hypertrophy gene (*CLPG*) in sheep. This gene is maternally imprinted; however, only individuals with a mutant allele (C) inherited from the sire and a normal allele (N) from the dam (ge-

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notype CN) express muscle hypertrophy. Interestingly, sheep with two copies of the mutant allele (genotype CC) express the normal phenotype. The causative mutation is a single nucleotide substitution A>G in the coding region of the gene (Freking et al. 2002).

Genomic imprinting was first observed in mice (De Chiara et al. 1991). Soon afterwards, a search was started to find the reasons for this essentially unfavourable phenomenon, which exposes animals to the risk of recessive mutations. Various hypotheses have been advanced to suggest the adaptive role of imprinting (e.g. prevention of female reproductive diseases and the silencing of parasitic DNA) (Barlow 1993; Varmuza and Mann 1994). The best-documented hypothesis is based on the idea of a gametic conflict (Moore and Haig 1991) between parental alleles in the offspring. The conflict results from unequal maternal and paternal investment in the growth of the offspring because the fetus is nourished directly from maternal tissues.

This theory assumes that certain conditions need to be satisfied for imprinting to occur. Above all, the paternal allele must be able to influence the level of maternal investment in the offspring. This means that imprinting should only occur in organisms in which nutrients are passed directly from mother to fetus and in genes that control fetal development. Moreover, imprinting should be limited to polyandrous species.

In principle, the experimental data on the groups of organisms subject to parental imprinting are in agreement with the gametic conflict hypothesis. To determine the exact phylogenetic extent of imprinting, *Igf2* and *M6p/Igf2r* genes have been investigated in various organisms, including relict groups (Killian et al. 2001a; Nollan et al. 2001).

Studies by Killian et al. (2001a) and Nollan et al. (2001) prove that imprinting is characteristic of viviparous mammals. Basing on an analysis of *Igf2* gene transcripts, they conclude that this gene is not imprinted in birds or monotremes. The evolutionarily youngest animal in which imprinted genes have been found, is the opossum, which is a marsupial. Although gestation in this species is only 11-13 days, the fetus is nourished by the mother during that period. In the other mammals studied (rodents, artiodactyls, primates), the *Igf2* gene is maternally imprinted. On this basis, it is estimated that imprinting evolved about 150 million years ago, together with the appearance of intrauterine pregnancy.

For another locus, *M6p/Igf2R*, imprinting is not observed in birds and monotremes, but occurs in marsupials, rodents and artiodactyls (Killian et al. 2001a; Nollan et al. 2001). In the animals that rank higher in the evolutionary hierarchy – Scandentia (tree shrew), Dermoptera (colugo) and Primates (ringtail lemur) – no parental imprinting has been found at *M6p/Igf2R*. The above research may suggest the loss of imprinting at this locus some 75 million years ago (Killian et al. 2001b; Nollan et al. 2001).

In humans, just like in other primates, biallelic expression at the *M6P/IGF2R* locus was initially observed (Kalsheuer et al. 1993; Ogawa et al. 1993). However, today it is argued that in humans, imprinting at this locus may be a polymorphic trait (Xu 1993; Oudejans 2001).

Most experimental data concur with the gametic conflict theory, which states that growth-stimulating genes should be subject to maternal imprinting, and, conversely, in growth-suppressing genes only the maternal allele should be expressed. The expression pattern of the imprinted gene that has been studied the most intensively (*IGF2*) meets these criteria exactly. Heterozygous fetuses of mice, which received the mutated allele of the *Igf2* gene from their fathers, exhibited excessive growth, and conversely, when the mutated allele was transferred by the mother, the offspring showed a normal phenotype. After analysis of the transcripts from both alleles in the fetal tissues studied, it was found that only the paternal allele was imprinted (De Chiara et al. 1991). Nevertheless, with some genes the mode of imprinting is contrary to expectations, e.g. the *Mash2* gene supports placental growth, but only the maternal allele is expressed (Guillemot et al. 1995). Also imprinted genes whose products do not control growth were described, e.g. *Snrpn* (Leff et al. 1992) or *UBE3A* (Rougeulle et al. 1997), and conversely, there are loci (e.g. the *Igf1* gene) that regulate growth but are not imprinted.

Another assumption of the gametic conflict theory – the polyandry of the species subject to imprinting – was difficult to prove. This was due to a rather infrequent occurrence of true monogamy in mammals. Nevertheless, such an attempt was made by Vrana et al. (1998), who analysed the imprinting pattern in two species of rat: the monogamous *Peromyscus polionotus* and the polygamous *P. maniculatus*, and a hybrid of the two. Although these species are of similar size, the offspring of a *P. maniculatus* female with a *P. polionotus* male are 40% smaller than their parents, while the re-

verse mating results in low survival rates, and the offspring that survive are considerably greater than their parents. These observations indicated abnormal imprinting in a monogamous species, but analysis of the *Igf2*, *H19* and *Igf2R* gene transcripts in both monogamous and polygamous species showed a normal pattern of genomic imprinting. However, these results were not considered to contradict the gametic conflict theory, because the small evolutionary distance between these species may be the reason for no difference in the imprinting pattern. Interspecific hybrids of *Peromyscus* were also studied at the loci *Igf2*, *Peg3*, *Mest*, *Snrpn*, *Igf2r*, *H19*, *Mash2* and *Grb10*. In the offspring of a *P. polionotus* male with a *P. maniculatus* female, the analysed loci showed no abnormalities in the imprinting pattern. Only *Igf2r* was biallelically expressed in the placenta, *Grb10* in the placenta and in the heart, and *Mash2* in all the tissues analysed. In the offspring of a *P. maniculatus* male with a *P. polionotus* female, the imprinting pattern was disrupted at almost all loci by the presence of biallelic expression. The normal imprinting pattern has only been preserved at the *Igf2* and *Igf2R* loci. These results point to an association between changes in the imprinting pattern and growth defects that are common to hybrids in many mammal species (goats, cats, foxes, horses) and indicate the role of these changes in the evolution of mammals (Vrana et al. 1998).

Imprinting shares many characteristics with inactivation of the X chromosome in female mammals. Both processes are related to asynchronous DNA replication, DNA methylation, and hypoacetylation of histones (Lyon 1998). In essence, X inactivation in mammalian inner cell mass (ICM) occurs at random, whereas in trophoectoderm and marsupial embryos the paternal X chromosome becomes inactivated (Cooper et al. 1993). This suggests that the aetiology of the molecular mechanism of imprinting on autosomes and of the mechanism that controls the inactivation of the X chromosome, may be common to both these phenomena.

The evolution of genomic imprinting from the molecular standpoint was investigated due to comparative analysis of the physical location of imprinted genes in different organisms. Rather than being evenly distributed throughout the genome, these genes are organized in linkage groups or clusters. Often whole domains of imprinted genes are characterized by high genetic conservatism they have their counterparts in taxonomically distant genomes. In mice, this type of genes is most numerous on pair 7 chromosomes, where some of them were found to have paralogues – genes that have similar functions and arise from duplication – in their vicinity. On this basis it was concluded that gene duplication may have played a decisive role in the formation of imprinting. The excess of alleles could have silenced additional copies, concurring with the dosage compensation theory (Lyon 1998). Imprinted genes could then spread in the genome by means of translocation. A different sequence of events is also possible. The regulatory mechanism of imprinted genes could have functioned earlier on one or several ancestral chromosomes. Due to duplication and translocation, regulatory elements that control imprinting could have spread all over the genome (Walter and Paulsen 2003).

Imprinting in human genetic diseases

Uniparental disomies (UPDs) refer to a situation in which both chromosomes of a pair come from the same parent. Although the genome of humans with UPDs has a complete set of chromosomes, UPDs lead to serious phenotypic consequences. A paternal UPD of chromosome 11 has been found in some patients with the Beckwith-Wiedemann syndrome (Koufos 1989).

This disease is manifested by fetal and postnatal overgrowth, macroglossia, macrosomia, neonatal hyperinsulinism, abdominal wall defects and a high risk of embryonal tumours, mostly Wilms tumour. The aetiology of the Beckwith-Wiedemann syndrome is not entirely clear, but *IGF2* is the best candidate gene for this effect (Weksberg et al. 1993). This maternally imprinted gene is localized in the 11p15.5 region, and its product – the insulin-like growth factor – plays a major role in pre- and postnatal growth.

The Prader-Willi and Angelman syndromes are associated with deletion on chromosome 15. The deletion concerns the same chromosome fragment (15q11-q13), but different phenotypic effects are observed depending on whether the maternal or paternal chromosome is deleted (Knoll 1989). In the Prader-Willi syndrome, the deletion always concerns the paternal chromosome. 15q11-15q13 deletions were observed in 70% of the patients and a maternal UPD was present in the other 30%. The main phenotypic symptoms of the Prader-Willi syndrome are hypothalamic dysfunction, obesity, hypo-

gonadism, characteristic facial features, and behavioural disorders. In this case, the candidate gene is the maternally imprinted *SNRPN*, which encodes ribonucleoprotein involved in mRNA processing in the brain (Leff et al. 1992; Carrel et al. 1999). Symptoms of Angelman syndrome (mental retardation, speech disorders, ataxic gait, and behavioural disorders, such as jerky movements and sudden fits of laughter) are considerably different from symptoms of Prader-Willi syndrome and the deletion concerns the maternal chromosome 15. The candidate gene is the paternally imprinted *UBE3A*, which encodes E6–AP ubiquitin ligase (Rougeulle and Lalande 1998).

Patients with the Silver-Russell syndrome (SRS) show pre- and postnatal growth abnormalities and a characteristic small, triangular face. The aetiology of SRS varies, but some cases (about 10%) are related to maternal UPDs of chromosome 7. Probably, the gene responsible for SRS is located in the region 7p11.2-p13 (Hannula 2001). The imprinted gene *GRB10* was expressed in muscles and the brain, the encoding protein involved in the transduction pathway of insulin and the insulin-like growth factor (Monk 2000). However, it was found that *GRB10* is not imprinted in the growth plate cartilage (an area of cartilage where bone growth occurs). This organ is directly related to growth abnormalities. No mutations associated with different phenotypes were found in this gene, so *GRB10* is probably not responsible for the defects observed in the Silver-Russell syndrome (Mc Cann et al. 2001). Attempts to find another candidate gene for SRS have been unsuccessful.

The imprinting mechanism of the IGF2/H19 locus

Studies on the molecular mechanism of imprinting have proved that DNA methylation, i.e. the addition of a methyl group to cytosine, plays a key role during the acquisition and transfer of genomic imprinting. The presence of differential methylated regions (DMRs) the areas in which the degree of DNA methylation in parental alleles differs has been found in the vicinity of almost all imprinted genes (Stoger et al. 1993; Feil et al. 1994; Tremblay et al. 1995; Shemer et al. 1997; Weber et al. 2001). It is still unclear, however, if the mechanism of imprinting regulation is universal to all imprinted genes. The best-known imprinted genes are adjacent to *IGF2* and *H19*. Probably, their expression is regulated by a common mechanism, although *IGF2* is maternally imprinted and *H19* is paternally imprinted. Intense studies analysing the effects of deletion of different portions of the regulator region made it possible to present a model (Figure 1) for regulation of monoallelic expression of *IGF2/H19* (Thorvaldsen et al. 1998; Bell and Felsenfeld 2000; Pant et al. 2003). The model assumes that both genes are stimulated by a common enhancer element located downstream of the *H19* gene. Between *H19* and *IGF2* is a GC-rich DNA sequence known as the imprinting control region (ICR). This region plays a crucial role in maintaining the imprinting pattern, as deletion of this region results in the loss of imprinting of both genes (*IGF2*, *H19*) (Thorvaldsen et al. 1998). Differences have also been found in the ICR region in the chromatin structure between maternal and paternal alleles (Khosla et al. 1999) and in the degree of methylation, where only the paternal allele is methylated (Trembley et al. 1997). The CTCF protein can only bind to the unmethylated form of ICR. As a consequence of this binding, a barrier forms between the *IGF2* gene and the enhancer located downstream of the *H19* gene, making the expression of *IGF2* impossible. This situation has been observed on the maternal chromosome (Figure 1). According to the model, ICR and adjacent promoter sequences of *H19* on the paternal chromosome are methylated – the CTCF protein cannot be bounded, so the enhancer may stimulate *IGF2* expression (Bell, Felsenfeld 2000).

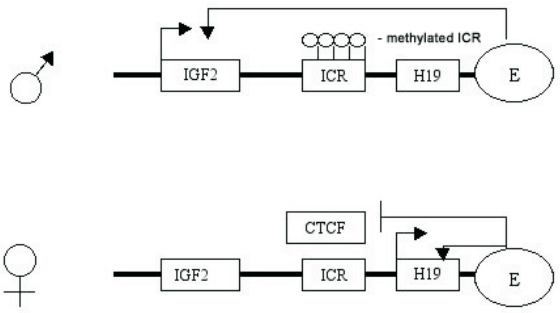

Figure 1. Scheme of the *IGF2/H19* imprinting
E = enhancer, ICR = imprinting control region, CTCF = CTCF protein

Birth of the parthenogenetic mouse and disrupted imprinted gene expression in IVP embryos

Knowledge of the molecular mechanism of imprinting at the *IGF2/H19* locus has allowed Japanese scientists to create the first parthenogenetic

mouse (Kono et al. 2004). Previous attempts to develop parthenogenetic mice failed, as parthenogenetic embryos died by day 10 of gestation. Recently, a parthenogenetic mouse has been created from a reconstructed oocyte containing two haploid sets of the maternal genome, obtained from non-growing and fully-grown oocytes. Appropriate expression of the *Igf2* and *H19* genes was possible thanks to the use of mutant mice with a 13-kb deletion in the *H19* gene as non-growing oocyte donors. A non-growing oocyte was obtained from newborn mouse. Such an oocyte does not have the maternal imprint, as maternal-specific *de novo* methylation occurs later during oocyte growth (Kono et al. 2004).

Genomic imprinting is one of the main barriers to the normal development of parthenogenetic individuals and those produced by other *in vitro* manipulations. A number of disruptions in the methylation pattern and expression of imprinted genes in embryos cultured *in vitro* has been reported. The incidence of these disruptions largely depends on the type of the medium used and the length of *in vitro* culture. Biallelic expression of the paternally imprinted *H19* gene was observed in murine embryos cultured in a deficient medium (Whitten's medium), while in an optimized medium (KSOM), the normal pattern was preserved (Doherty et al. 2002). Other investigations have revealed that expression of imprinted genes is disrupted in murine ES cells that are cultured in the presence of serum. Most of these alterations are not corrected in later development and can lead to aberrant phenotypes (Dean et al. 1998). Nevertheless, conditions of *in vitro* culture are not the sole factor influencing imprinting disruption. An abnormal pattern of expression of imprinted genes has been observed in neonatal cloned mice, derived by nuclear transfer from freshly isolated cumulus cells (Humpherys et al. 2002).

Conclusions

Imprinting studies are very important, as the knowledge about the molecular mechanism of imprinting and imprinting evolution can be utilized in many branches of biological sciences. Moreover, learning about the influence of *in vitro* culture conditions on appropriate expression of imprinted genes and appearance of abnormal phenotypes has important implications for medical science in ART (assisted reproductive technology).

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