

Basics of Gametic Imprinting

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ABSTRACT: The fundamental assumption of Mendelian genetics is that behavior of an allele is identical whether it arrives to a zygote through paternal or maternal germline pathway. Gametic imprinting phenomena discovered and studied in mammals show limitations of the classical view in special cases. Two sources of evidence were essential to describe gametic imprinting. The first approach based on genetic evidence demonstrated that some maternally and paternally derived regions of certain chromosomes were not equivalent. Paternal or maternal disomy of the regions containing particular genes caused significant effects on viability and development of progeny. The second set of data was obtained by nuclear transplantations and parthenogenetic activation of mammalian oocytes. These data suggested that the contribution of parental genomes was not equivalent and differential imprinting of nuclear genes during gametogenesis was very likely. The number of loci found in mice, which show gametic imprinting, is 34 and continues to grow. It is generally accepted that gametic imprinting is a mammalian invention and there

are differences in imprinting pattern between species. Most hypotheses propose involvement of imprinted genes in the control of fetal growth and fetal-maternal interactions, thus keeping a balance between contradictory fetal and maternal requirements. Molecular mechanisms responsible for gametic imprinting still remain to be studied, but for several genes it was shown that imprinting marks are imposed by a parent-specific methylation process during gametogenesis. These marks are resistant to global demethylation during cleavage and to global *de novo* methylation after implantation and maintain different methylation patterns in paternal and maternal alleles of imprinted genes. About 20 to 25% of all transgene loci studied demonstrate similarities with imprinted genes. For instance, methylation of some transgenes is dependent on parental gametic pathway and reversible in the next generation. There are data indicating that selection of modifier genes may change the effects of gametic imprinting. It is possible that future selection and crossbreeding programs may take gametic imprinting into consideration.

Key Words: Gametes, Epigenetics, Methylation, Development, Cloning

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Introduction

The term *gametic* (or *genomic*) *imprinting* has been widely used in the literature during the last decade. There is some degree of uncertainty in terminology and definition of the phenomenon (Barlow, 1994). However, a commonly used interpretation—an allele-specific reversible epigenetic modification dependent on the parental origin of the allele, which can cause functional differences in development—seems acceptable for the description of the phenomena for some autosomal genes in mammals. Three consequences of gametic imprinting are obvious: 1) a deviation from the Mendelian form of inheritance; 2) although genetically identical, the input of maternal and

paternal alleles in development is quite different in some cases; and 3) the expression pattern of maternal and paternal alleles may cause significant changes in ontogenesis. For these reasons, gametic imprinting became the subject of extensive investigations. The results are valuable not only from theoretical point of view, but also for their potential importance for medical and possibly agricultural applications.

Gametic Imprinting: Experimental Evidence

In the mouse, the potential opportunity to form balanced zygotes from unbalanced gametes has been used intensively (Lyon and Glenister, 1977; Cat-

tanach, 1986). Both Robertsonian and reciprocal translocations have been used in these studies, which investigated the effects of paternal or maternal disomy (full or partial) on viability and development of progeny. It was found that maternally and paternally derived regions of certain chromosomes are not equivalent and act differently during embryogenesis. The most plausible explanation of these phenomena is imprinting of the gametes. The most recent mouse genetic imprinting map reveals $\sqrt{34}$ genes located on ten chromosomes (Beechey and Cattanaach, 1998), which show the gametic imprinting phenomena.

Two embryological approaches: parthenogenetic activation of mammalian oocytes (Markert, 1982) and nuclear transplantations (McGrath and Solter, 1983) also provided convincing data in support of gametic imprinting. Investigation of early development of parthenogenetic, biparental diploid gynogenetic (2m:0p) and biparental androgenetic (0m:2p) embryos has shown that none of these types of embryos were viable, dying either at preimplantation or in early-postimplantation stages (McGrath and Solter, 1984; Surani et al., 1984). The development of the gynogenote is mostly embryonic, whereas the androgenote displays mostly extraembryonic development. All these data strongly suggest that the contribution of parental genomes is not equivalent and that differential imprinting of nuclear genes in mammals during gametogenesis is very likely (Surani, 1986; Solter, 1988).

Imprinting-Like Events: Transgenes

From the very start of experiments with transgenic mice, it has been found that many demonstrate a pattern of expression that correlated with a parentally imprinted methylation state (Hadchouel et al., 1987; Reik et al., 1987; Sapienza et al., 1987; Swain et al., 1987). In some cases, the transgene became hypermethylated when maternally inherited and hypomethylated when it came from the paternal line (Sapienza et al., 1987), but in the several other cases the situation was the opposite (Reik et al., 1987). A direct correlation between transgene expression and methylation status was established (Swain et al., 1987). The reversibility of methylation status has been observed in a majority of studies. However, in several cases, one of which was described by Hadchouel et al. (1987), the reversibility was lost. Approximately 20 to 25% of all transgene loci studied show a similar gamete-of-origin effect in their DNA methylation phenotype (Peterson and Sapienza,

1993). It has been concluded that methylation might be a necessary but not a sufficient factor in the process (Solter, 1988). What is essential to highlight here is that the integration sites of many transgenes prone to imprinting are beyond the chromosomal regions that are known to be imprinted according to the genetic imprinting map mentioned above (DeLoia and Solter, 1990). For example, transgene *Gtl2lacZ* (Gene trap Locus 2) in mice shows parental origin-dependent phenotype. This phenomenon may be a likely result of interaction between this transgene and an endogenous gene(s) required for fetal and postnatal growth (Schuster-Gossler et al., 1996).

Gametic Imprinting: Essential Questions

Why are some genes imprinted, and what are the developmental consequences? The final answer is still absent, but there are interesting hypotheses. One is based on the idea of genetic conflicts during pregnancy between maternally and paternally inherited genes (Moore and Haig, 1991; Haig, 1993; Haig, 1997). It has been proposed that imprinting evolved in mammals because they are viviparous and because their offspring are nourished directly from maternal tissues. For paternal genes, it may be selectively advantageous to promote the growth of the fetus increasing the chance for survival. Maternal genes may favor keeping the fetus smaller in order to increase the chances of successful delivery. Thus, gametic imprinting may be considered as a compromise between mother and fetus as well as between paternal and maternal genes. Deviations from the normal imprinting pattern of specific genes can decrease the viability of progeny and may change their growth parameters.

One of the predictions of the conflict hypothesis is the possible effect on fetal and early postnatal growth. This aspect was recently reviewed, and, surprisingly, much of the data do not directly support the hypothesis (Hurst and McVean, 1997). However, the mouse imprinting map has at least five regions with genes directly affecting prenatal or postnatal growth. In the mouse, a proper balance of two imprinted genes *Igf2* and *Igf2r* (located, respectively, at the chromosomes 7 and 17) seems to be essential for the embryonic growth (Table 1).

Direct proofs of gametic imprinting in mammalian species other than the mouse and humans are still rare. Nevertheless, there are interesting data that may indicate involvement of gametic imprinting in embryonic growth and placental development. For instance, it was proposed that differences between

Table 1. *Igf2/Igf2r* allelic ratio and embryonic growth in mice^a

Genetic constitution	Number of active alleles in <i>Igf2</i> and <i>Igf2r</i> loci	Growth
<i>Igf2</i> ⁻ (M. imprint)/ <i>Igf2</i> ⁺ (P. active)	1	Normal
<i>Igf2r</i> ⁺ (M. active)/ <i>Igf2r</i> ⁻ (P. imprint)	1	
<i>Igf2</i> ⁻ (M. imprint)/ <i>Igf2</i> ⁻ (P. mutation)	0	Deficient
<i>Igf2r</i> ⁺ (M. active)/ <i>Igf2r</i> ⁻ (P. imprint)	1	Filson et al. (1993)
<i>Igf2</i> ⁻ (M. imprint)/ <i>Igf2</i> ⁻ (P. mutation)	0	Deficient
M. deletion / <i>Igf2r</i> ⁻ (P. imprint)	0	Filson et al. (1993)
<i>Igf2</i> ⁻ (M. imprint)/ <i>Igf2</i> ⁺ (P. active)	1	Deficient
2 × <i>Igf2r</i> ⁺ (M. active)/ <i>Igf2r</i> ⁻ (P. imprint)	2	Ruvinsky et al. (1991)
<i>Igf2</i> ⁻ (M. imprint)/ <i>Igf2</i> ⁺ (P. active)	1	Intensive
M. deletion / <i>Igf2r</i> ^{-?} (P. imprint?)	0	Forejt and Gregorova (1992)

^a*Igf2* = insulin-like growth factor-II gene; *Igf2r* insulin-like growth factor-II receptor gene; M. = maternal; P. = paternal.

hinnies (female donkey × male horse) and mules (female horse × male donkey) may be attributed to the imprinting (Allen et al., 1993). The growth differences in interspecies reciprocal hybrids of two close species of *Peromyscus* may have similar explanation (Bartolomei and Tilghman, 1997). There are other examples of heterosis and retardation of growth in different reciprocal crosses. These cases may have similar explanation, but all of them require experimental verification.

How do genes become imprinted? To answer this question we have to understand how and when the imprinting marks are established. It is well known that methylation of cytosine in CpG islands plays an important role during development and is essential for gametic imprinting (see reviews: Bartolomei and Tilghman, 1997; Jaenisch, 1997). Typical for somatic and fetal cells, monoallelic (paternal or maternal) expression of imprinted genes disappears in germ cells by the time of genital ridge colonization (Szabo and Mann, 1995). These data likely indicate that parental-specific signals were erased during migration of primordial germ cells into the genital ridge. New imprinting signals reestablish in late gametes. It is still unknown why and how different allele-specific marks are imposed in maternal and paternal lines. Although the evidence for an essential role of methylation in regulating imprinted genes is strong, the simplistic interpretation of the relations between both processes is controversial (Allen and Mooslehner, 1992; Surani, 1993; Barlow, 1994; Reik and Allen, 1994). A possibility that additional marks other than methylation can be put on the imprinted genes during gametogenesis exists.

How does genetic background affect imprinting? The data elucidating this question come from both transgenic studies and investigation of imprinted genes. Study of transgenic line TKZ 751 in mice, for instance, revealed a significant variation and dependence of the transgene methylation from genetic background and reciprocal crosses. Observed difference in reciprocal crosses indicates that parental transmission of a modifier locus controls methylation phenotype at the transgene locus (Reik et al., 1990). Imprinted genes, as all other genes, can be influenced by modifier genes. The *Tme* locus in *Mus m. domesticus* is paternally imprinted and controlled by an allele in the unlinked modifier gene. In *Mus m. musculus*, the *Tme* locus is not imprinted, and this phenomenon is described as a consequence of another allelic variant in the modifier gene (Forejt and Gregorova, 1992). It seems likely that modifier genes affect the specificity of DNA methylation, chromatin compaction, and nucleosome positioning in the imprinted genes and transgenes (Feil et al., 1995).

Is it possible to escape imprinting? The investigations of the *Tme* locus in mice has shown that two imprinted paternal doses of *Tme* failed to compensate the deleted maternal allele (Agulnik et al., 1991). However, a rearranged paternally derived chromosome duplicated for the *Tme* locus can act to rescue animals that have not received a maternal copy of the *Tme* locus (Tsai and Silver, 1991). It was also found that paternally nonimprinted *Tme* existing in some natural populations of *M. m. musculus* rescue mice with maternal *Tme* deletion (Forejt and Gregorova, 1992). Embryos with *Tme*^m deletion can be rescued also, if the paternal *Igf2* allele (*Chr 7*) is inactive (Filson et al., 1993). Thus, at least in the case with

the *Tme* locus, the possibility to escape the consequences of imprinting exists.

Common Features of Imprinted Genes

It is still not easy to figure out common features of the 34 imprinted genes studied so far. Bartolomei and Tilghman (1997) recently made an attempt to reveal these common features. Clustering of imprinted genes is one of them. The distribution of the imprinted genes on the genome map supports this statement. Interestingly, in most clusters, both paternally and maternally imprinted genes are found. Imprinted genes are located in clusters over a large distance (many tens of kilobases of DNA). The data indicate that this clustering occurred long ago, before divergence of marsupial and eutherian mammals (>180 million years ago) and most likely before the imprinting phenomenon arose (Toder et al., 1996). There is no clear explanation yet concerning the reasons of such clustering, but one can suggest that the distribution of imprinted genes is not random and that the interactions between these genes during development seems possible.

Asynchrony of DNA replication is another common feature of imprinted genes, which has been observed in several cases and usually reflects a different level of gene activity (Bartolomei and Tilghman, 1997).

The majority of imprinted genes, as can be anticipated, code proteins; however, some of them, like *H19*, code untranslated RNA (Brannan et al., 1990). It can be noted that in mice and humans more than a half of imprinted genes are conserved, but the rest behave differently. For instance, *Igf2r* is imprinted in the mouse, but not in humans (Kalscheuer et al., 1993). This can be an indication of independent evolutionary acquisition of imprinting by different genes during mammalian evolution.

Reversibility of imprinting is also a common feature, at least in the germ cell line. A new cycle of development usually starts with the erasure of previous imprints and other epigenetic signals on chromosomes; thereafter, new gametic imprinting becomes possible.

In eutherian mammals, X chromosome inactivation shows a number of similarities with imprinting, but there is no clear-cut evidence of their common origin. However, the *Xist* gene, which is required for X chromosome inactivation, likely uses similar molecular mechanisms for inactivation. It is essential to remember that gametic imprinting is a phenomenon applicable to autosomal genes.

Imprinting and Methylation

The molecular and chromosomal mechanisms of gametic imprinting are still enigmatic, as well as the essential mechanisms of cell differentiation. Nevertheless, the significant amount of experimental data and interesting hypotheses are worth a short discussion. A stable and heritable differentiation pattern of methylation was considered as a possible mechanism of gene regulation in development (Holliday and Pugh, 1975; Holliday, 1990).

A difference in the methyl cytosine content in the DNA of sperm and egg was interpreted as an indirect indication of possible involvement of methylation in gametic imprinting (Monk, 1988). A correlation of transgene expression with the level of its methylation and gamete origin was another reason to connect both processes (Hadchouel et al., 1987; Reik et al. 1987; Sapienza et al., 1987; Swain et al., 1987).

It was shown that region 1 of the *Igf2r* gene contains the transcription start region and is methylated only on the silent paternal chromosome; region 2 is located in an intron and is methylated only on the expressed maternal chromosome. Methylation of region 1 is acquired after fertilization, in contrast with the methylation of region 2, which is inherited from the female gamete (Stoger et al., 1993). A normal level of DNA methylation is required for controlling differential expression of the paternal and maternal alleles of imprinted genes (Li et al., 1993). A decrease in the level of DNA methylation is observed during sperm development from meiotic cells to elongated spermatids, and supports an idea that chromosomes are erased of epigenetic signals coming from the previous ontogenetic cycle (del Mazo et al., 1994). Methylation is important for somatic maintenance of imprinting after the global wave of demethylation in the blastocyst (Bartolomei and Tilghman, 1997; Jaenisch, 1997). However, the question of how maternal and paternal alleles can be distinguished after the global demethylation arises (Barlow, 1995; Jaenisch, 1997). It has been found that different methylation sites within imprinted genes may demonstrate significant temporal differences in methylation pattern and that the establishment of the final methylation pattern is a dynamic process (Shemer et al., 1996).

Elegant experiments with mice in which the DNA methyltransferase gene was deleted demonstrated an importance of methylation for gametic imprinting (Tucker et al., 1996). In the mouse, the *Igf2r* gene acquires a maternal-specific methylation mark during oogenesis (Stoger et al., 1993), whereas methylation marks appear in the *H19* gene during spermatogene-

sis (Tremblay et al., 1995, 1997). These so-called imprinting boxes resist not only demethylation during blastocyst stage, but also resist remethylation during later development, a necessary condition if the unmethylated allele is to retain its identity in somatic cells (Bartolomei and Tilghman, 1997; Jaenisch, 1997). However, these imprinting marks have to be removed during the next developmental cycle occurring most likely in germ cells. It is interesting that, in meiotic pachytene spermatocytes, the normal form of DNA methyltransferase is undetectable and a novel form of the enzyme is present instead (Jue et al., 1995).

A key role for specific DNA methylation in maintaining the imprinting in somatic cells is clear. Methylation also seems to be the best candidate for establishment of primary imprints in germline DNA, but direct evidence remains to be seen. However, methylation is not necessarily the only possible mechanism involved in establishment and maintenance of gametic imprinting. For instance, there are data indicating possible links between chromatin structure and imprinting (Feil et al., 1995).

Modulation of *Igf2* gametic imprinting in mice induced by 5-azacydine, an inhibitor of DNA methylation, is the first example of a pharmacological manipulation with imprinting (Hu et al., 1997).

Cloning, Totipotency, and Imprinting

Recent success in cloning sheep using somatic cell nuclei (Wilmut et al., 1997) raised several profound questions, including the question about involvement of imprinted genes in development. It was presumed for some time that egg cytoplasm is able to erase somatic methylation signals from transplanted nuclei. The latest experiments have shown that bovine oocytes have a capacity for reprogramming by removing the methylation signals from nuclei of different mammalian species (First and Dominko, 1998). However, as mentioned above, successful development in mammals does not happen when only maternal or paternal genomes are involved. Thus, neither maternal nor paternal genomes alone are able to support normal development, but nuclei from somatic cells can do that. Hence, at least some nuclei from somatic cells are totipotent and maintain both paternal and maternal epigenetic (imprinting) signals. There are two possible explanations as to how these signals can be maintained: 1) existence of allele-specific methylation of the "imprinting boxes" occurring during gametogenesis, which are resistant to early embryonic demethylation, and 2) marks other than methylation can be

put on the imprinted genes during gametogenesis and early embryonic demethylation is not able to affect them.

In any case, the initial marks imposed on imprinted genes in germ cells are stable and can be erased or replaced only during the next cycle of gametogenesis. The phenomenon of gametic imprinting allows us to distinguish two phases in development, gametic and zygotic. This raises the questions of the onset timing of the gametic phases and how the developmental cycle is organized in mammals. It seems plausible that the new cycle of development starts before the meiotic prophase (Figure 1 from Ruvinsky, 1997). Remarkably, recent findings provide direct evidence that the nuclei of male germ cells acquire the ability to fertilize before the first meiotic division (Ogura et al., 1998). The latest results support the view that the imprint initiation complex is confined to the germ cells (Suirani, 1998). Specific methyl-CpG-binding proteins attached to the short imprinting boxes play an essential role in the propagation of the imprinting signals (Birger et al., 1999).

The inevitable loss and restoration of totipotency during development is a problem, and it perhaps can be resolved in terms of nucleocytoplasmic interactions. Differentiation and the attendant loss of cellular totipotency eventually result in formation of highly specialized germ cells in which the nucleus "returns" to a totipotent state and the cytoplasm remains differentiated for some time. This restoration of totipotency could be described as initialization, the main function of which is to erase epigenetic information imposed by the preceding developmental cycle, including gametic imprinting. It seems possible that specific maternal and paternal signals are imposed on imprinted genes soon after initialization.

Inheritance of Epigenetic Signals: Creep into the Next Generation?

Observations indicate that some epigenetic variations may creep into the next generation and become inherited (Ruvinsky, 1987; Jablonka and Lamb, 1998). The same can be applicable to genomic imprinting, if erasure is not complete (Monk, 1988). For instance, transgenes prone to gametic imprinting usually demonstrate reversibility of their status depending on parental origin. The expression of hepatitis B surface antigen is an exception. It was irreversibly inactivated in some offspring after passage through the female line. The repression is associated with the methylation of all the *HpaII* and *HpaI* restriction sites within the transgene (Hadchouel et al., 1987).

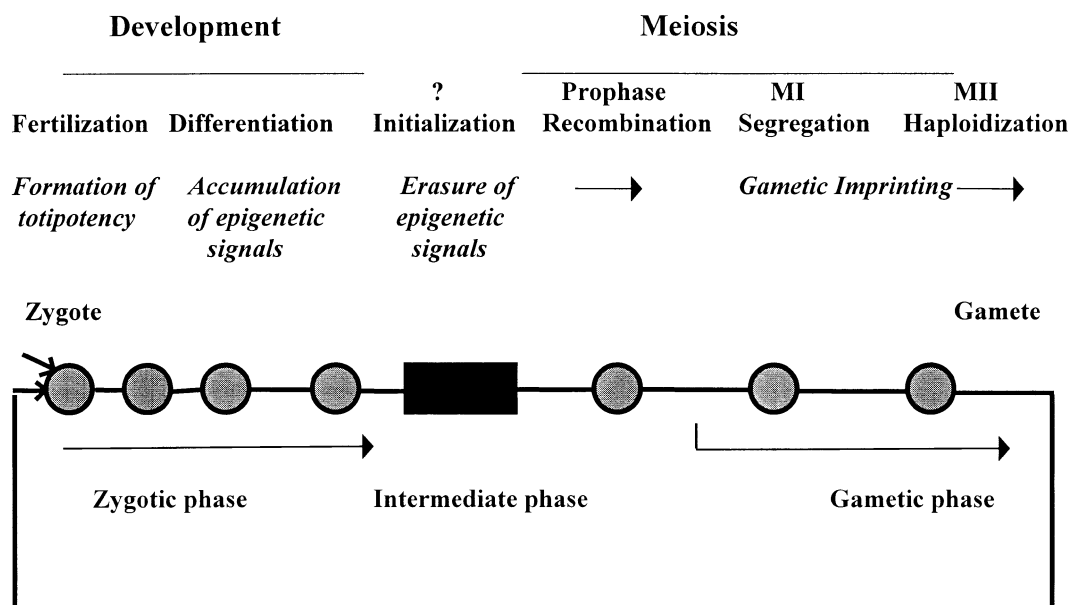


Figure 1. Schematic representation of developmental cycle in mammals.

Allen et al. (1990) also have shown that epigenetic modifications of the transgene locus TKZ751 was cumulative over successive generations, which in BALB/c mice resulted in an irreversible methylation after three consecutive germline passages. This is an interesting example of a failure by the germline to reverse previously acquired epigenetic modifications.

It was found in several cases that transgenes may lose transcription activity and become silent. The molecular basis of this is not yet known, but the phenomenon can be attributed to inherited epigenetic changes. Such events have been designated as epimutations to distinguish them from classical mutations (Holliday, 1987).

Imprinting and Diseases

Interest in gametic imprinting has been strongly accelerated by the discovery of connections with cancer and several other human syndromes (Erickson, 1985; Nicholls et al., 1989; Ferguson-Smith et al., 1990; Hall, 1990). Gametic imprinting may explain a number of human (and animal) disorders. Peterson and Sapienza (1993) reviewed a series of diseases that are relevant to genomic imprinting. Human chromosome region 15q11–13, for example, is involved in two clinically different syndromes: Prader-Willi and Angelman. In the first case, both chromosomes 15 are maternally inherited, in the second, paternally in-

herited. The candidate genes have been proposed (Ozcelik et al., 1992).

A locus on human chromosome 11p15.4-pter has been found to be consistently involved in many tumors, including the Beckwith-Wiedemann-associated tumors, such as Wilms' tumor; rhabdomyosarcoma; hepatoblastoma; adrenocortical carcinoma; and breast, bladder, and pancreatic cancers. The imprinting model of the Beckwith-Wiedemann syndrome shown in Figure 2 demonstrates different genetic changes leading to the development of the syndrome. Insulin-like growth factor-II plays a key role in the development of these pathologies (Mannens, 1991). The same is true for the homologous region on murine chromosome 17 (Sun et al., 1997). This homology certainly is much wider: 214 loci are known to be associated with human hereditary disease, which have been mapped on both human and mouse chromosomes. In 18 cases, the manifestation or severity of pathological effects is thought to be the result of imprinting (Searle et al., 1994). Given the homologies between mammalian genomes, similar patterns are likely to emerge in domestic animals.

Imprinting and Livestock?

Current information on gametic imprinting effects in domestic mammals is scanty. However, the idea of gametic imprinting stimulated a few research projects.

For example, gametic imprinting may likely influence the rate and composition of pig growth (de Vries et al., 1994). A significant proportion of the phenotypic variance in backfat thickness (5 to 7%) has been explained by genes subject to paternal imprinting. The paternally imprinted effects also explain 1 to 4% of the phenotypic variation of growth rate. The maternal imprinting effects can explain 2 to 3% and 3 to 4% of the phenotypic variance in backfat thickness and growth rate, respectively. To separate these effects, specific methods of quantitative genetics have been developed (Tier and Sölkner, 1993). The data above have been derived from the pure porcine breeds: Yorkshire, Large White, and Landrace. The latest data prove that in the pig a paternally expressed QTL affects skeletal and cardiac muscle mass and fat deposition. The gene(s) involved in regulation of these traits map to the *IGF2* locus (Jeon et al., 1999; Nezer et al., 1999).

Reciprocal crosses between different sheep breeds also provided valuable information. Pitchford (1993) found a number of differences in birth weight, growth rate and other traits. For example, comparison of the growth parameters in offspring from Dorset Horn \times Corriedale reciprocal crosses indicates that "it is not only maternal size that affects the growth of the offspring; there must also be effects of the dam's ability to supply nutrients across the placenta and of the dam's mothering ability." In sheep, as in humans and mice, the growth-related *PEG1/MEST* and *IGF2* genes expressed from the paternal chromosome give clear evidence of gametic imprinting (Feil et al., 1998).

The *callipyge* mutant allele in heterozygous sheep causes pronounced muscle hypertrophy in the hind-quarters and reveals a hitherto undescribed form of inheritance referred to as polar overdominance (Cockett et al., 1996). This type of inheritance looks similar

Imprinting model Beckwith-Wiedemann syndrome

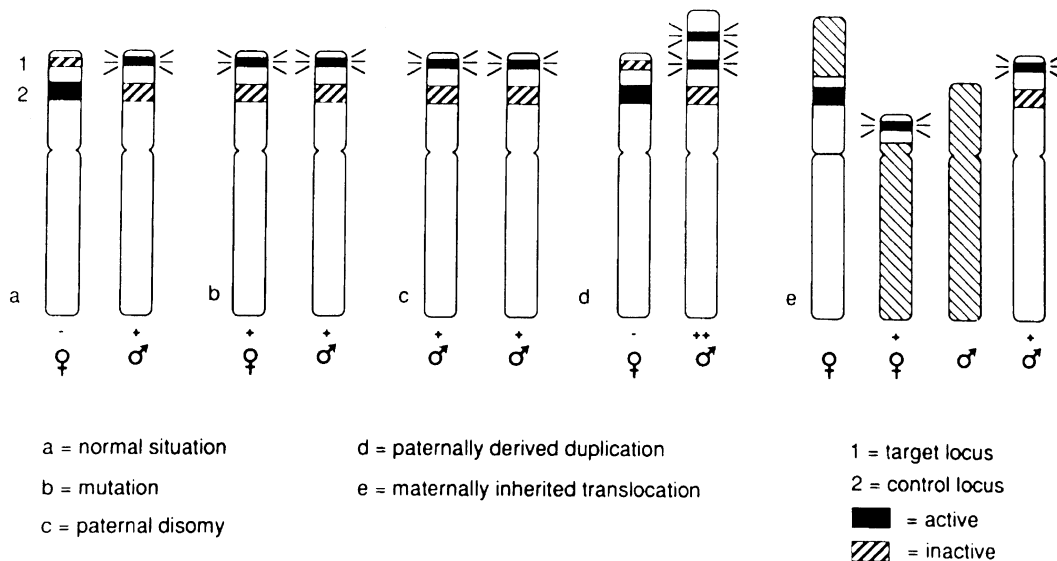


Figure 2. Imprinting model for the Beckwith-Wiedemann (BWS) syndrome involving two loci in 11p15.4-pter. (Reproduced, with permission, from Mannens, 1991). a) Proposed normal situation with inactivation of the maternal locus one. The expression of the *IGF2* gene in this region (marked with +) is controlled by locus 2 that contains a maternally expressed suppressor gene, which could also be involved in the development of tumors; b) Mutation in familial BWS in locus 1 or 2 can lead to increased expression of the *IGF2* gene. Maternal mutations can activate locus 1 or inactivate locus 2 or both (as shown); c) Paternal disomy with two copies of paternal locus 1 and 2 (as shown) or only two paternal copies of either locus; d) Paternally derived duplication with two active copies of locus 1; e) Maternally inherited translocation involving 11p15 and another chromosome leading to loss of the existing maternal imprint and consequently an active region 1. The translocation chromosomes are shown to the left, the normal chromosomes to the right.

to the gametic imprinting. Offspring of heterozygous *CLPG/clpg* males do not deviate from the Mendelian ratio (1 callipyge:1 normal) in test crosses, and all offspring from heterozygous females are normal. Clearly, the mutant allele is suppressed when it comes from the dam. Involvement of epigenetic events close to those typical for gametic imprinting is likely. This gene is located at the telomeric region of the ovine chromosome 18 (Cockett et al., 1994; Freking et al., 1998). Further studies of the gene may bring information useful for theory and practical applications.

In cattle, growth abnormalities resulting from the manipulation of preimplantation embryos in vitro (large calf syndrome) may reflect shifts of a balance between paternal and maternal contributions (Moore and Reik, 1996).

It is likely that crosses between distant breeds or species may give stronger differences. A classic example of such differences comes from the reciprocal horse-donkey crosses, mentioned earlier. It is well known that the hinny pregnancies (female donkey × male horse) are very disturbed in comparison with mule pregnancies (female horse × male donkey). There are many indications that the gametic imprinting phenomena may be contributing factors (Chandley, 1989; Allen et al., 1993; Haig, 1993). Measurement of the concentration of equine chorionic gonadotropin in the serum of pregnant mares and jennies carrying normal intraspecies and hybrid interspecies pregnancies suggested that the production of this hormone may be influenced by the parental imprinting. The striking differences in morphology and uterine function between horses and donkeys are in good agreement with the differences between those of the mule and hinny (Allen et al., 1993).

It seems possible that knowledge about the influence of pathway (paternal or maternal) used by an allele to enter the next generation can be adopted in some selection programs. Selection of modifier genes, as has been described in this article, can significantly change the effect of gametic imprinting. Crossbreeding programs perhaps may also take gametic imprinting phenomena into consideration.

Implications

Gametic (genomic) imprinting is a genetic phenomenon that was discovered in mice, but it has been confirmed in humans and several other mammals, including sheep, cattle, pigs, and horses. Gametic imprinting reveals that the expression of some alleles can be different depending on the paternal or

maternal germline pathway used for the allele to enter the zygote. Growth and development are among the traits affected by gametic imprinting. Selection of modifier genes can significantly change the effect of gametic imprinting. It seems likely that knowledge about gametic imprinting may be adopted in some selection and crossbreeding programs.

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