GENOMIC IMPRINTING: EPIGENETIC CONTROL OF GENE EXPRESSION, PHENOTYPIC VARIATIONS AND DEVELOPMENT

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SUMMARY

Genomic imprinting confers functional differences on parental chromosomes and alleles as a result of epigenetic inheritance through maternal and paternal germlines. Repressed and derepressed chromatin structures probably constitute the initial germline-dependent 'imprints'. The subsequent modifications, such as changes in DNA methylation during early development, will be affected by the initial epigenetic modifications. Hence, differences in epigenetic modifications of parental alleles can occur progressively which will ultimately affect their potential for expression; this may account for the functional differences between some chromosomal regions observed during mammalian development. Consequently, neither the male nor the female genome is by itself totipotential and both are essential for normal development. It appears that the parental origin of chromosomes is critical to maintain a balance between growth and differentiations of embryonic cells during development.

Genomic imprinting is also observed with respect to some transgene inserts and dominant traits. These can display both the parental origin effects as well as variable penetrance and expressivity. The response of transgenes is apparently influenced by the products of modifier genes whose presence is most readily detectable in different inbred backgrounds. The influence of modifier genes can in turn be affected by their parental origin, perhaps partly by the maternally inherited oocyte cytoplasmic factors, as well as by the more complex interactions between some parental alleles and oocyte cytoplasmic factors. Hence, genomic imprinting and the influence of modifier genes on parental alleles can produce substantial phenotypic variation. These effects are dependent on the parental origin of the genomes together with the particular activities of the modifier genes controlling expressivity of numerous alleles.

INTRODUCTION

Embryonic development and other phenotypic characteristics are influenced by the genetic constitution of zygotes as well as by epigenetic inheritance and modifications which will determine the timing and sequence of gene expression (Holliday, 1987, Surani et al. 1990). The genetic contributions made by the mother and the father are usually equivalent. However, there are possible differences in epigenetic modifications of some parental alleles which give rise to functional differences between certain homologous chromosomal regions (Cattanach, 1986). One of the categories of differences in the epigenetic modifications of parental chromosomes is determined by their parental origin; the process which gives rise to differential epigenetic modifications is called genomic imprinting. Another category of parental origin effects are apparently observed secondarily as a result of the activities of modifier genes which also influence the penetrance and expressivity of responding genes. Such activities of modifier genes is detected with respect to dominant traits and some transgene inserts which is most readily recognised in inbred backgrounds. (Sapienza et al. 1989; McGowan et al. 1989; Allen et al. 1990; Surani et al. 1990).

One of the most significant consequences of genomic imprinting is that neither the male nor the female genome is by itself totipotential since the presence of both a maternal and a paternal genome are necessary for normal development (Solter, 1988; Surani et al. 1990). Specific developmental abnormalities are observed in parthenogenetic embryos (with maternal chromosomes only) and androgenetic embryos (with paternal chromosomes only) which exhibit antipodal phenotypes with abnormalities affecting the embryo or the extraembryonic tissues, respectively. (Surani et al. 1990). It appears that the maternal chromosomes are essential when embryonic cells are totipotential or pluripotential while the paternal chromosomes sustain proliferation of progenitor cells of specific differentiated tissues, both in the embryonic and extraembryonic lineages. However, viable oocytes can be derived from cells which contain maternal chromosomes only. (Surani et al. 1990).

MECHANISMS OF EPIGENETIC CONTROL OF GENE EXPRESSION

The precise nature of the imprinting signals responsible for functional differences between parental alleles, and the manner in which they are propagated is not known. However, the inheritance of repressed and derepressed chromatin patterns from the maternal and paternal germlines by homologous chromosomes may constitute the primary imprinting signals. But, any distinct chromatin structures and modifications established in the parental germlines may not persist for very long. It seems likely that all the subsequent changes in chromatin structure will nevertheless be influenced by any initial germline-specific 'imprints' in the chromatin structure.

Derepressed chromatin structure can be detected by its DNAase-1 hypersensitive state. This modification can be both global and affect a large domain encompassing at least 40-50 kb, as well as by site-specific changes which will control the precise timing of gene expression in specific tissues. This type of modification can be highly stable and heritable over many cell generations (Weintraub, 1985). By contrast, repressed chromatin structure, which is also stable and heritable, is usually encountered with widespread DNA methylation involving CpG dinucleotides (Keshet et al. 1986). Furthermore, precise site-specific changes in DNA methylation within particular genes are known to accompany tissue and temporal specificity of gene expression (Doerfler, 1983; Cedar, 1988). It is particularly interesting that allele-specific differences with respect to methylation patterns have recently been detected in a number of tissues (Chandler et al. 1987; Silva & White, 1988). Although these particular allelic differences were not influenced by their parental origin, it is possible that such differences may occur in some endogenous genes.

Methylation of the CpG dinucleotide is the most widely studied of all the epigenetic modifications because it is particularly amenable to experimental analysis. Hence genomic imprinting has so far been examined primarily with respect to DNA methylation. However, it is essential to bear in mind that the control of gene expression is a multilevel process (Gilbert, 1985), and other forms of changes in chromatin structure and epigenetic modifications will undoubtedly be involved in controlling functional differences between the parental chromosomes.

The influence of strain-specific modifier genes on the imprinting process occurs after the new zygote is established (McGowan et al. 1989; Allen et al. 1990). The modifier genes will act both in cis and in trans to influence a

variety of loci and that different genic loci will be imprinted on different genetic backgrounds. These influences will be reflected in phenotypic differences resulting from variable penetrance and expressivity of various alleles on different genetic backgrounds. Nevertheless, it appears that the activity of some genes will be controlled strictly by germline-specific imprinting and these may not be subject to any marked influence by the genetic determinants. (Allen et al. 1990; Surani et al. 1990).

A number of studies have focussed on DNA methylation changes in gametes and embryos because of the global differences in DNA methylation between parental chromosomes; these could be propagated after fertilization as the primary imprinting signals (Sanford et al. 1987). However, it has been proposed previously that a de novo methylation event may occur following fertilization (Razin & Riggs, 1980; Jähner & Jaenisch, 1984) which may result in the initial repression of the embryonic genome, with derepression occurring during the course of development. Indeed, de novo methylation could perhaps obliterate the overall differences in DNA methylation observed in sperm and eggs. Despite such effects, crucial differences in DNA methylation patterns could still remain between some homologous regions of parental chromosomes. There is little information regarding the precise methylation differences between parental genomes during preimplantation development at present, except that there is a dramatic loss of overall DNA methylation by the blastocyst stage (Monk, 1988). The second de novo methylation event occurs at gastrulation but it is lineage-specific and it apparently acts primarily on the epiblast (primitive ectoderm) cells and not on the extraembryonic tissues (Chapman et al. 1984; Monk, 1988). Nevertheless, crucial differences in epigenetic modifications of some parental alleles could survive these genome-wide methylation changes.

IMPRINTING OF TRANSGENES

A number of studies have reported that the methylation patterns of transgenes can be influenced by their parental origin (Reik et al. 1987; Sapienza et al. 1987; Swain et al. 1987; Hadchouel et al. 1987; Surani et al.1988). Transgenes are useful as molecular probes to examine the mechanism of genomic imprinting. This is because transgenes integrate randomly in the genome and the hemizygous inserts can be analysed easily for epigenetic modifications and expression. Transgenes may be subjected to imprinting influence if they respond in a position-dependent fashion when they integrate into 'imprinted' chromosomal domains (Kothary et al. 1989). The transgene insertion process itself, however, may trigger an imprinting response because of the accompanying changes in the chromatin structure at the integration site. Finally, transgenes may arrest transient and widespread dynamic changes and differences between homologous loci and 'lock' the integration site in a permanent state which under normal circumstances would not retain the differences and these may therefore go undetected.

A wide category of transgenes are prone to imprinting which may suggest that it occurs irrespective of the sequence of the transgene, although sequences flanking the transgene insertion may serve as imprinting signals (Surani et al. 1988). Imprinted transgenes are almost invariably undermethylated when paternally derived with a potential for expression while maternal transmission causes them to be hypermethylated and less likely to be expressed. In all instances, bar one, the transgene methylation patterns are reversible when transmitted alternately between the male and female germlines. In the one

exceptional instance, transmission through the maternal germline apparently caused irreversible hypermethylation (Hadchouel et al. 1987). Indeed the insert remains active and capable of being expressed only provided it is repeatedly transmitted through the paternal germline. It also appears that the size of transgene insertions do not influence the capacity to be imprinted even though large tandem repeats of 50-200 kb will disrupt the chromatin structure more severely. Finally, all somatic tissues show a similar overall pattern of DNA methylation even though expression, when it occurs, is confined to the appropriate tissues, perhaps because certain specific sites within the imprinted transgene undergo demethylation in the appropriate tissues. Finally, methylation and expression of transgenes is apparently influenced by the modifier genes which act after fertilization to modulate penetrance and expressivity of such transgene inserts (McGowan et al. 1989; Allen et al. 1990; Surani et al. 199).

THE ROLE OF MODIFIER GENES ON GENE EXPRESSION AND IMPRINTING

Both the cis and transacting factors can induce differential epigenetic modifications of some parental alleles as well as produce variations in their penetrance and expressivity (Reik, 1989; Surani et al. 1990). In particular, transacting genotype-specific modifier genes are likely to interact with specific loci to modulate their expression. Modifiers may repress or derepress expression of certain loci by direct or indirect influences on transcription and chromatin structure, resulting in subsequent epigenetic changes such as DNA methylation (Surani et al. 1990). Both the transgenes and dominant mutations are prone to such influences. (Reik, 1989).

Studies on the penetrance of the gene fused (Fu) after transmission from mother or father are highly informative in this regard. In some inbred strains of mice, there is a suppressor gene not linked to the mutation which dramatically decreases penetrance of maternal Fu to 12-18%, whilst reducing that of the paternal Fu only slightly to 70% (Agulnik & Ruvinsky, 1988). The suppressor itself has this effect regardless of its parental origin, but this is not the case in all instances. The genes which respond to the effects of modifier genes may display all the attributes of being imprinted, but as a secondary consequence of the parental origin of the regulatory suppressor or enhancer modifier genes. Hence, phenotypic variations may result in different strains of mice because of the presence of specific modifier genes in these animals.

A number of observations show that the expression of transgenes can be coupled by the genotype-specific modifier genes. For instance, in one particular transgenic line with thymidine kinase promoter linked to a β-galactosidase reporter gene (TKZ751), expression was detected in postimplantation embryos (Allen et al. 1990). The founder male 751 was made on a mixed genetic background (C57BL/6♀ x CBA♂)F_1 x CFLP♂ , and transgenic embryos were analysed after backcrossing onto the (C57BL/6♀ x CBA♂)F_1 hybrid strain. Two types of fetuses were detected after maternal transmission; those with high expression (Hi) and those with low expression (Lo). The ratio of Hi and Lo fetuses was approximately 1:1. Furthermore, there was a correlation between the levels of expression and the degree of methylation of the transgene. It is possible that the segregation of Hi and Lo expressing transgenic lines is accompanied by the segregation of strain-specific enhancer or suppressor modifier genes. These lines could be segregated by breeding through to the F_3 generation so that both the Hi and Lo expression differences were enhanced which also correlated with DNA methylation levels of the transgene.

Further analysis of the genetic control of expression and methylation was examined by introducing the TKZ751 transgene insert into mice of defined genetic backgrounds. DBA/2 and 129 genetic backgrounds enhanced expression, while the Balb/c background suppressed expression, but only following maternal inheritance of the Balb/c modifier. Epigenetic modification of the transgene locus was cumulative over successive generations, which in Balb/c mice resulted in an irreversible methylation after three consecutive germline passages. Therefore in this exceptional case, the germline fails to reverse previously acquired epigenetic modifications which is normally necessary to restore genomic totipotency. Hence the genotype-specific modifier genes regulate penetrance and expressivity as well as parental imprinting of the transgene locus through epigenetic modifications. At the level of individual cells, variable expression could affect all the cells more or less equally, or such changes may occur due to fluctuations in the total number of expressing cells which are otherwise identical, resulting in true mosaicism with respect to transgene expression. Both types of expression patterns have been observed with different transgene inserts (McGowan et al. 1989; Allen et al. 1990).

The different methylation states of some transgene loci show remarkable similarities to the developmental regulation of the suppressor-mutator transposable element (Spm) in maize (Banks et al. 1988). This overall similarity is intriguing because of the extreme evolutionary divergence of mice and maize. It is possible that the modifiers of gene expression form part of a highly conserved group of regulatory proteins. Such a family of modifier genes may also include regulatory genes involved in position-effect variegation in Drosophila in which there is no role for CpG methylation. The phenotype observed is due to the variable spread of heterochromatin into a normally active gene. Both suppressors and enhancers of position-effect variegation have been identified (Locke et al. 1988). Interestingly, modifiers of variegation also show parental origin effects (Spofford, 1961).

It appears that different genic loci may be imprinted on different genetic backgrounds. Consequently, in an outbred population, the number and variety of imprinted genes may be quite diverse. Hence, genotype-specific variations in imprinting affecting certain genetic traits would be reflected in phenotypic differences resulting from variable penetrance and expressivity on different backgrounds. In this regard, genomic imprinting can be considered as an extreme example of modifier control of gene expression. This argues that a diverse number of genes are differentially imprinted depending upon their parental origin and genetic background. However, this does not exclude the possibility that there are some key regulatory genes which are strictly subject to germline-specific imprinting.

GENOMIC IMPRINTING AND DIFFERENTIAL ROLES OF PARENTAL CHROMOSOMES DURING DEVELOPMENT

The germline-specific imprinting of some parental chromosomes has a marked effect on mammalian development. This influence results in the differential roles of parental chromosomes during development probably because of the differences in the expression some parental alleles. Hence, studies show that neither the maternal nor the paternal genome is by itself totipotential, and that both are essential for normal development; these studies are documented comprehensively elsewhere (Solter et al. 1988; Surani et al. 1990). No endogenous 'imprinted' genes have yet been identified unequivocally so that it

is not possible to study their activities during development. For the present, imprinting of transgenes provides the best model to investigate the molecular mechanism of genomic imprinting as discussed above.

CONCLUSIONS

An imprinting process which results in functional differences between homologous chromosomal regions is widespread in plants, insects and animals (Monk, 1988; Solter, 1988; Surani et al. 1990). The process manifests itself in many different ways with important consequences for normal and abnormal development, genetic diseases (Reik, 1989) and phenotypic variations in plants and animals. In all instances where genomic imprinting is encountered, there is evidence for some form of epigenetic control of gene expression which results in the inactivation of one of the parental alleles, and this is in turn dependent on the parental origin of the alleles.

We must assume that the germline-specific imprinting of parental chromosomes provides better control over expression of parental alleles during development in mammals. Mammalian development is especially prone to deleterious effects of aneuploidy, which suggests a marked sensitivity to the levels of particular gene products (Baranov, 1983; Epstein, 1985; Solter, 1988). It is remarkable that even in the genetically balanced genotypes, aberrant development occurs if some chromosomal regions are derived exclusively from one parent (Cattanach, 1986). These chromosomal determinants apparently maintain a tight control over cell proliferation and differentiation during development (Surani et al. 1990). The marked global changes in epigenetic modifications, especially in DNA methylation, observed in germ cells and during early development may have some role in influencing differential functions of parental chromosomes (Monk, 1988). Such epigenetic changes also display cell lineage specificity during development. The marked epigenetic changes in the germline are also clearly needed to reverse any previously acquired epigenetic changes in order to restore genomic totipotency.

The additional imprinting influences observed after fertilization is controlled by genetic determinants through the activities of modifier genes. The influence by modifier genes is sometimes dependent on their parental origin. The activities of modifier genes will give rise to considerable phenotypic variations when different strains (and species) are involved. The fact that the phenotypic variations and nonreciprocal effects are encountered in interstrain, random and interspecific crosses and are subject to the parental origin of the genomes, can probably be accounted for by the preferential expression of parental alleles. This implies that it is imperative that not only the genetic factors, but also the epigenetic regulation of gene expression must be taken into consideration for the breeding of plants and animals. The effects are particularly marked when the genetic distance between the strains or the interspecific crosses increases (Monk, 1990).

It is possible that the germline-specific imprinting of parental chromosomes may have evolved at a later stage because of the significant shift in the control of development by cytoplasmic determinants in lower organisms, towards chromosomal determinants as observed in mammals. The imprinting process which occurs after fertilization may have been the original form of epigenetic

control of gene expression. Germline-specific imprinting may then have evolved with the increasing importance of the chromosomal determinants to control development.

How precisely the modifier genes bring about epigenetic changes in responding alleles remains to be determined. They may act primarily in trans and bring about changes in chromatin structure and subsequently in DNA methylation. It is also possible that some of these factors are maternally inherited as oocyte cytoplasmic factors whereas others may be generated as a result of nuclear-cytoplasmic interactions. It is noteworthy that even some modifier alleles which are implicated in the imprinting process themselves display parental origin effects. It appears that the germline-specific imprinting of some alleles has an important role in development. In this instance, genotype-specific modifier genes may have only a minor effect on their expression.

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