Epigenetic Status of Genes on Imprinted Regions

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Genomic imprinting is a mammalian epigenetic mechanism marking gametic or zygotic genome leading to parent-of-origin-specific differential expression of the two alleles of a gene in somatic cells of the offspring. Such an epigenetic modification makes an animal functionally hemizygous for the imprinted genes and requires strict contribution of both parental genomes for the normal development of the progeny. The first convincing evidence for genomic imprinting came from nuclear transplantation experiments, which showed that development did not proceed properly in embryos derived from two maternal or two paternal nuclei, suggesting that the expression patterns of each parental genome are different and complementary (McGrath and Solter, 1984). Analysis of mice carrying partial uniparental disomies (UPDs) further demonstrated that there are unique chromosomal regions that can generate a parent-of-origin-specific phenotypes (Cattanach and Kirk, 1985). A certain part of chromosomes gave rise to embryonic lethality or abnormal development in the progeny when it was inherited exclusively from paternally or maternally. Partial UDPs were created by mating mice which carry an appropriate set of Robertsonian or reciprocal translocations in the chromosomes. Studies of UPDs also identified chromosomal regions which required both parental contributions in the offspring for the normal development. The regions identified by this manner were used as landmarks of candidate loci where an individual imprinted gene could be localized.

Imprinted genes were identified serendipitously (*Mash2*; Guillemot, 1995) or depending on the information including the map position of genes and their relevance to chromosomal region implicated in UPD studies (*Igf2r, Snrpn, Ipw, Znf127* etc.) in the early 1990s. Recently, several imprinted genes have been identified through genome-wide systematic searches for monoallelically expressed genes. Differential display and differential cDNA screens of RNAs expressed by androgenetic or parthenogenetic embryos have successfully resulted in the identification of new imprinted genes on chromosomes 6 and 7, *Peg1/Mest* and *Peg3*, respectively (Kaneko-Ishino *et al.*, 1995; Kuroiwa *et al.*, 1996). Alternative systematic search for the imprinted genes was devised to take advantage of the fact that imprinted genes have differentially methylated region in their CpG clusters. This approach exploiting restriction landmark genomic scanning, or RLGS, has identified at least two imprinted genes, *U2afbp-rs* and *Grf1* (Hayashizaki *et al.*, 1994; Plass *et al.*, 1996).

To date, about 30 imprinted genes have been identified in human and mouse. It is estimated that there are hundreds of imprinted genes to be identified. One of the major characteristics of imprinted genes is that they are clustered within small regions of chromosomes. Of the 27 imprinted

genes of mice, 11 exist in close proximity to imprinted genes. The largest cluster of imprinted genes is found at the distal end of mouse chromosome 7 (Caspary, 1998) and at the proximal end of human chromosome 11p15.5. Both regions show a clear synteny to each other. In the region at the distal end of mouse chromosome 7 spanning 1.5 Mb, 8 imprinted genes have been identified. It includes *Ipl, Imptl, p57*^{KIP2}, *Kvlqtl, Mash2, Ins2, Igf2* and *H19* (Dao *et al.*, 1998). It is also well known that cluster of imprinted genes are located at human chromosome 15, where both the Prader-Willi and Angelman syndromes have been mapped (LaSalle and Lalande, 1995). This region contains 3 genes expressed paternally.

These features of clustering suggest the possibility that the mechanism of imprinting is not local or gene specific, but rather functions over long distances. Based on this tendency of clustering, it is plausible that more imprinted genes can be identified in the region which was known to contain few imprinted gene to date, not a cluster of imprinted ones. An imprinted gene, *Peg1/Mest* is selected for this study because it was mapped to a locus which is known to be implicated in UPD-related embryonic lethality and information of many genes around the locus in mice and humans is available through public databases.

Peg1/Mest, a maternally imprinted gene isolated by the subtractive hybridization between parthenogenetic and normal embryos, is the first imprinted gene identified on mouse chromosome 6. It functions as the mouse mesoderm-specific transcript that shares 70% sequence homology between mouse and human (Nishita *et al.*, 1996). *Peg1/Mest* is mapped to the sub-proximal end of mouse chromosome 6 and its human homolog is localized in human chromosome 7q32, which is analogous to the mouse chromosomal region of *Peg1/Mest*. The sub-proximal region of mouse chromosome 6 has been known to be involved in UPD-related embryonic lethality in mice.

Thus, the region may have other imprinted genes close to *Peg1/Mest* and a search for new imprinted genes on this locus was tried in this study. Investigation of allele-specific expression was performed within the mouse chromosomal region homologous to human locus covering *PEG1*. Mouse genes and ESTs of which human homologs were mapped near the human *PEG1* locus were subject to the investigation of parent-of-origin specific expression. In addition, *Peg3* region on chromosome 7, *Meg1/Grb10* region on chromosome 11, and *Peg5/Nnat* region on chromosome 2 were also investigated for the possible presence of novel imprinted genes. Polymorphisms of the genes and ESTs mapped to this regions in mouse and human were identified and used to physically discriminate one of parental alleles from the other in the F1 hybrids between two inbred mouse strains, *Mus musculus molossinus* and *Mus musculus domesticus* (C57BL/6J). Restriction fragment length polymorphism analyses of RT-PCR products were carried out to investigate the allele-specific expression of genes and ESTs in the F1 hybrids. *Calu, Ube2h, Cappa2* genes, and a cDNA homologous to human STS Cda15a02 within *Peg1/Mest* region showed biallelic expression patterns without allelic expression bias. Other candidates, *A2a/Emap2, A2b, A2e, P3a* and *P3b* in the *Peg3* region, *Rbll* within *Peg5/Nnat* region, *M1a* and *M1b* within *Meg1/Grb10* region, were shown to be expressed biallelically. However, a cDNA derived from one of the mouse ESTs, tentatively named *CopG2*, was obviously shown to be expressed only from the paternal allele in the brain of F1 hybrids. Thus, *CopG2* is maternally imprinted in the adult mouse brain. Expression pattern in the reciprocal cross unambiguously confirmed the maternal imprinting of *CopG2* gene. Southern hybridization using the 3' sequence of *CopG2* cDNA as a

probe indicated that the *CopG2* gene is present as a single copy in the mouse genome. Imprinting of *CopG2* seems to be delicately regulated in a tissue-specific manner. In adult heart and lung in which *CopG2* is less transcribed than in brain as monitored by Northern hybridization, imprinting status of *CopG2* was relaxed. The extent of relaxation was quantitatively analyzed. It was estimated that the percentage of C57BL/6J allele was 93% for F1 hybrid of (M × C) in heart, 29% for hybrid of (C × M) in heart, 86% for hybrid of (M × C) in lung, and 50% for hybrid of (C × M) in lung. *CopG2* provides an example of endogenous gene that shows relaxation of imprinting in several tissues. Such a relaxed imprinting of *CopG2*, along with Northern data, indicates that the function of this gene is regulated in a tissue-specific imprinting manner.

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