REGULATION OF IGF2 IMPRINTING IN DEVELOPMENT AND DISEASE

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Imprinted genes are those genes in the mammalian genome which are expressed or repressed depending on which parent they are inherited from. Such imprinted genes probably constitute 0.1 - 1% of all genes in mammals and have key roles in fetal and placental development, and the control of aspects of behaviour after birth. When there are defects in the imprinting mechanism by mutation or imprinting errors, there are detrimental consequences for development in the form of many genetic diseases and cancers. Indeed, of the 15 or so imprinted genes identified so far, 9 have already been directly implicated in genetic diseases and cancers.

Imprinting is controlled by epigenetic inheritance which originates in the gametes. DNA methylation is one of the imprinting mechanisms, and all imprinted genes examined so far show differences in DNA methylation between the parental alleles. Interestingly, some imprinted genes, such as *H19*, show the classical pattern of DNA methylation and transcriptional inactivity (of the maternal allele) whereas others, like *Igf2*, are methylated on the active (paternal) allele, suggesting the presence of silencer sequences that are suppressible by DNA methylation. The function of cis-acting sequences in the control of some of these imprinted genes is currently being tested by transgenesis and gene targeting.

In addition to closely linked cis-acting sequences, longer-range regional controls are also likely to operate in imprinting. In this regard, it is intriguing to notice that imprinted genes tend to be clustered in the genome. For example, there is one major cluster on human chromosome 15q, associated with the Prader-Willi and Angelman syndromes, and another major cluster on chromosome 11p15.5, which is involved in the Beckwith-Wiedemann syndrome. The syntenic cluster in mouse is on distal chromosome 7. The imprinted genes in this cluster include the paternally expressed *Igf2* and *Ins2* genes, and the maternally expressed *H19*, $p57^{Kip2}$, and *Mash2* genes.

The fact that imprinted genes are clustered raises a number of important issues. First, is there a mechanistic reason for clustering? Indeed, enhancer sharing and co-ordinated imprinting has already been demonstrated for *Igf2* and *H19*. Regional control in the form of putative imprinting centres (IC) has been suggested in the Prader-Willi/Angelman region on 15q. Second, it is important to consider whether there are functional relationships between imprinted genes in a cluster. Already, *Igf2* has been shown to be required for fetal growth, whereas *H19* appears to suppress fetal growth. $p57^{Kip2}$ possibly also suppresses fetal growth. Hence, it is important to investigate whether there are paternally expressed growth enhancers balanced by maternally expressed growth suppressors in this region that interact in the same physiological pathways.

The Beckwith-Wiedemann syndrome (BWS) is a human fetal ongrowth syndrome characterised by multiple organ overgrowth and a propensity to develop childhood tumours. While most cases are sporadic, the rare familial cases are linked to chr 11p15.5. A number of observations implicate deregulated imprinting of this region in the disease (a) there are cases with paternal isodisomy of this region, suggesting overexpression of paternally expressed imprinted genes (such as *Igf2*) or deficiency of maternally expressed genes (such as *H19* or *Kip2*). (b) A large proportion (~ 70%) of all sporadic non-disomic cases express *Igf2* biallelically rather than monoallelically, suggesting loss of imprinting as a major cause of the disease. (c) Of these patients, a proportion have methylated *H19* and *Igf2* genes, suggesting that a regional imprinting switch can be involved in causing the disease. (d) A minority of familial cases have maternally inherited translocations which are located several hundred kb centromeric of *Igf2*; in a recent analysis we have shown that such a translocation can result in biallelic *Igf2* expression but *H19* imprinting was not affected.

These results suggest that derepression of *Igf2* (and perhaps other growth enhancers) is a major event in the molecular pathology of BWS, and that there are a number of different mechanisms that can lead to this derepression. In particular, mutations in maternal suppressors, or imprinting errors leading to a paternal epigenotype, seem to be important events.

In order to understand the molecular basis of these imprinting mutations, and to identify the whole cluster of imprinted genes, we are isolating a P1 clone contig of the region in the mouse. We found that the cluster of imprinted genes is flanked by non-

imprinted genes with structural features that are quite different from those of the imprinted genes. These experiments may begin to define the boundaries of the imprinted domain. We have also isolated additional cDNAs that may map into the cluster and their imprinting status is being assessed.

In order to assess the phenotypic effects of aberrant imprinting and expression of a number of genes in the cluster, especially in the BWS phenotype, transgenic and knockout experiments in the mouse are necessary. We have begun such experiments by trying to express single transgenic copies of the *Igf2* gene in fetuses. Expression constructs were introduced into ES cells, and chimeras were made that overexpress *Igf2* at levels ranging from 1.5 x to 3.0 x. These chimeras are overgrown from d13 of gestation and at birth (up to 160% of birth weight of controls). Fetal and postnatal lethality are also observed in high contribution and high level expressing chimeras. A number of additional phenotypes are also observed: cardiac and kidney hyperplasia, and skeletal defects are amongst the most conspicuous ones. Clearly, these results show very substantial overlap with the BWS symptoms and establish for the first time that *Igf2* overexpression can cause these major symptoms. Substantial phenotypic overlap is also observed with *Igf2* receptor deficient mice and with androgenetic chimeras. Precise analysis by RNase protection of levels of transgene expression in various tissues, and methylation analysis of the transgenes, is presently being carried out.