Imprinting Weaves Its Web

It has long been recognized that imprinted genes often act in common developmental or physiological pathways. A new knockout of the gene Zac1 reveals just how extensive the transcriptional network of imprinted genes may be.

Genomic imprinting is an intriguing form of epigenetic gene control in mammals, which affects a subset of our genes and results in the silencing of one allele, according to a strict parental bias. We have known of the existence of imprinting for over two decades and have accumulated a list of 80 or so imprinted genes. We understand something of the range of functions of these genes and the mechanisms that govern their monoallelic expression (Reik and Walter, 2001). But, there is still plenty of room for new insights into what these genes do and how they relate to one another.

The report of a knockout of an imprinted gene is not normally a headline event. In this issue of Developmental Cell, Varrault et al. (2006) describe the knockout in mice of the paternally expressed imprinted gene Zac1. Zac1 (also known as Plag1 and Lot1) is an interesting gene: it encodes a zinc finger protein originally identified through its role in promoting apoptosis and cell-cycle arrest (Spengler et al., 1997), and as a putative tumor-suppressor (Abdollahi et al., 1997). Overexpression of human ZAC1 appears to account for an uncommon and puzzling developmental syndrome, transient neonatal diabetes mellitus (Ma et al., 2004). In the present report, Varrault et al. show that inactivating the paternal copy of Zac1 leads to significant intrauterine growth restriction, impaired neonatal survival (attributed to delayed maturation of the lungs), and some morphological abnormalities with incomplete penetrance. Given the widespread expression of Zac1 in the embryo and the functional properties of the protein, further examination may well reveal additional developmental defects. At least in its action to control overall growth of the fetus, Zac1 conforms to current imprinting dogma (Wilkins and Haig, 2003), which anticipates that paternally expressed genes are growth promoting, although an expectation from its proapoptotic function may have been growth enhancement of Zac1 mutants. Whether Zac1 deficient mice are more prone to cancer awaits further investigation.

But the particular timeliness of this report is the attempt to link Zac1 to other imprinted genes as a means of throwing light on the Zac1 null phenotype. The authors have done this by conducting a meta-analysis of 116 freely available mouse microarray datasets to find genes frequently coexpressed with Zac1. The premise is that genes that cluster together share biological functions. The “transcriptional networks” that emerge have been very powerful tools in simple organisms, but rarely applied thus far in vertebrate systems. Varrault et al. identify 353 such genes (details of the methodology are found in Lee et al., 2004), including 13 imprinted genes, which is a statistically significant enrichment of imprinted genes. They then focus on the 60 imprinted genes present on the arrays and identify the 246 most strongly linked genes. They call this association an “imprinted gene network” (IGN).

What is the significance of this IGN and does it have any predictive power? As imprinted genes often function in common pathways (e.g., control of fetal growth, development and function of the placenta), one might anticipate a degree of coregulation. (One can anticipate that any “hub” in the IGN is the sum of smaller, distinct networks in different tissues.) The clustered organization of many imprinted genes also leads to sharing of some control elements. Changes in the network, e.g., loss of Zac1, may have knock-on effects on the expression of genes linked in the network, but the authors argue that the response of a robust network is to adapt to change by compensatory alterations in the expression of other genes. In this way, they suggest, the otherwise catastrophic loss of a potential key control gene such as Zac1 can be mitigated. The IGN is also likely to contain direct transcriptional interactions and, as a transcription factor, Zac1 could be directly involved in regulation of more strongly linked genes in the IGN, a possibility that the authors explore further.

The authors analyze the consequences of Zac1 deficiency (in liver) or forced overexpression (in transfected cells) on the expression of other imprinted genes. Among the effects observed, significant and parallel changes in the expression of that classical pair of imprinted genes, Igf2 and H19, are found in both experimental settings. The authors then go on to identify Zac1 binding sites in the endodermal enhancers 3’ of H19 and show that Zac1 can potenti ate Igf2 and H19 expression in vitro. We can then conclude that the IGN has, at least between Zac1 and Igf2-H19, a transcriptional basis. This finding may go some way to explain aspects of the Zac1 null phenotype, such as intrauterine growth restriction. But it is equally likely that nonimprinted targets of Zac1 make substantial contributions to the phenotype.

It is timely then to consider the present work of Varrault et al. alongside that of Zhao et al. (Zhao et al., 2006), who describe a “chromosomal IGN” which they have discovered by “4C” (capturing chromatin conformation in a fourth dimension). This methodology seeks to identify, on a genome-wide scale, genes in physical association, for example, because they occupy common transcription factories in the nucleus (Osborne et al., 2004). Looking for the chromosomal regions interacting with the Igf2-H19 imprinting control region (ICR), Zhao et al. found 114 interacting domains of which 21 correspond to 15 imprinted genes: one of
these sits very near Zac1. Significantly, it was found that the paternal and maternal Igf2-H19 ICR chromosomal networks were different (though unfortunately we do not know which Zac1 allele interacts with which parental allele of Igf2-H19 ICR). Interaction of the Igf2-H19 ICR with an unlinked locus was recently also found by another group (Ling et al., 2006). Both groups report that losing CTCF, a linchpin of chromatin insulator elements, abrogated trans-chromosomal interactions of the maternal Igf2-H19 ICR, with resultant dysregulation of genes in the putative chromosomal IGN (Ling et al., 2006; Zhao et al., 2006). By the same token, could it be that loss of Zac1 perturbs a hub of chromatin interactions and dysregulates Igf2-H19 and other associated genes? Maybe 4C analysis in the Zac1 knockout will tell.

It is too early to say how important a chromosomal IGN is in coordinating the expression of imprinted genes, but comparisons between chromosomal networks found by 4C and transcriptional networks as described in the present work should be illuminating. It will also be important to characterize IGNs at a tissue-specific level and, perhaps, in different species, to give a better understanding of the evolution of mechanisms regulating imprinting and the biological functions these genes serve. This fascinating class of genes continues to provide new models of epigenetic mechanisms and higher levels of gene control. The imprinting web is definitely a catching one.