OUTLOOK

# Folding makes an imprint

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**Imprinted gene clusters are confined genomic regions containing genes with parent-of-origin-dependent transcriptional activity. In this issue of *Genes & Development*, Loftus and colleagues (pp. 829–843) made use of an insightful combination of descriptive approaches, genetic manipulations, and epigenome-editing approaches to show that differences in nuclear topology precede the onset of imprinted expression at the *Peg13-Kcnk9* locus. Furthermore, the investigators provide data in line with a model suggesting that parent-of-origin-specific topological differences could be responsible for parent-of-origin-specific enhancer activity and thus imprinted expression.**

Genomic imprinting is a remarkable epigenetic phenomenon. It is based on the fact that some mammalian genes are not equally transcribed from both parental gene copies present in diploid cells. Instead, one gene copy is preferentially used, while the other is stably silenced. Importantly, this gene activity imbalance is independent of genetic variation and also is prevalent when both alleles are genetically identical, such as in inbred mouse strains. In contrast to random monoallelic gene expression, imprinted expression patterns are predictive, meaning that in all cells and in all individuals, the active copy stems from the same parent (e.g., the mother), while the other gene copy (e.g., the paternal one) is silenced. Since the discovery of the imprinting phenomenon in the 1980s and the first three associated genes (*Igf2r*, *Igf2* and *H19*) in the early 1990s, around more than a hundred genes have been attributed imprinted expression (Ferguson-Smith and Bourc'his 2018). Most of those are found in imprinted gene clusters, often several hundred kilobases long, usually containing not only genes expressed exclusively from one parental allele, but also some transcribed from the other parental allele and/or even biallelically expressed genes as well.

Studying imprinted genes, their evolution and function are clearly interesting in their own right. It turns out that the evolution of genomic imprinting could at least partially be explained by the functional role that imprinted genes play during embryonic and postnatal development (Reik and Walter 2001). Paternally expressed gene products are often those that increase the consumption of maternal resources—for example, leading to a large embryo size—and as such are transcribed in line with the evolutionary interest of the father. The genes exclusively expressed from the maternal allele often have opposing roles.

Moreover, research on the mechanistic regulation of genomic imprinting (and imprinted expression) has been proven to be even more insightful, far beyond the evolutionary and developmental perspective. This might be due to the fact that genomic imprinting allows analysis of an active and a repressed allele in the same individual cell or because imprinted gene clusters have been studied with more vigor than most other "normal" gene loci. Either way, pioneering research proved instrumental to the discovery and characterization of epigenetic silencing mechanisms that were later shown to be crucial for the regulation of nonimprinted genes as well. Examples include the regulation of larger domains by DNA methylation of small control elements (DMRs) (Li et al. 1993), parent-of-origin-specific binding of CTCF and chromatin folding (Kurukuti et al. 2006), silencing long noncoding RNAs (such as Kcnq1-ot1, Ube3a-ats, or Airn) (Lyle et al. 2000; Mancini-Dinardo et al. 2006), and chromatin remodeling through transcriptional interference (Latos et al. 2012).

Unfortunately, the recent age of epigenomic research, initiated by the availability of technology for mapping chromatin features epigenome-wide, attenuated interest in one-by-one locus dissection of gene-regulatory mechanisms. In other words, why would you go through the laborious mechanistic analysis of one (potentially exotic) gene locus if you can impute gene-regulatory mechanisms from descriptive data of all loci? Well, the answer is that as much as the last decade of epigenomic research has informed us on the prevalence of chromatin marks and other epigenomic features (such as topology), it seems that epigenetics still depend on focused mechanistic studies. Descriptive characterization of chromatin features, as

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comprehensive and precise as they may be, might never be able to solve the quintessential question of epigenetics: What causes gene activity states?

The availability of novel technology, in particular "epigenome editing," allows researchers now to revisit established model systems of epigenetic gene regulation, such as genomic imprinting. These days, it is possible to manipulate chromatin features on specific loci directly instead of manipulating the underlying DNA sequence (Breunig et al. 2020). The study by Loftus et al. (2023) in this issue of *Genes & Development* is an excellent example of the renaissance of dissecting single-locus gene regulation. The investigators studied the *Peg13-Kcnk9* locus, a brain-specific imprinted gene cluster containing several genes associated with disease and intellectual disability; most noteworthy, the potassium channel Kcnk9, but also the long ncRNA Peg13, whose role during brain development remains unclear. To shed more light onto the regulation of this interesting locus, Loftus et al. (2023) used a thoughtful combination of descriptive analysis, genetic manipulation, and dCas9 approaches. In this way, they were able to prove that nuclear topology differs between the parental alleles. Most noteworthy are paternal-specific contacts between the DMR and two enhancer elements. Using Ngn2-directed differentiation, the investigators showed that these epigenetic differences are established before imprinted expression of *Kcnk9* is even established. Gene targeting of CTCF binding motives in the DMR provided evidence that CTCF binding might be necessary for the parental differences. Furthermore, the investigators used epigenome-editing approaches to activate the enhancer elements prematurely in pluripotent cells and showed that this results in a maternal-specific activation of *Kcnk9*. This indicated that the topological structure might predetermine the consequences of enhancer activity on this locus. With this interesting result in hand, it is now tempting to ask how nonneural tissues remodel the locus for biallelic expression and whether the noncoding RNA Peg13 has any functional role in this.

The report by Loftus et al. (2023) blends into a series of recent studies using novel epigenome-editing approaches to re-evaluate the mechanisms of genomic imprinting (Monteagudo-Sanchez et al. 2020; Wei et al. 2022), suggesting that a deeper one-by-one functional analysis of imprinted expression will eventually reveal the general interconnection of epigenetic mechanisms causing gene expression states.

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