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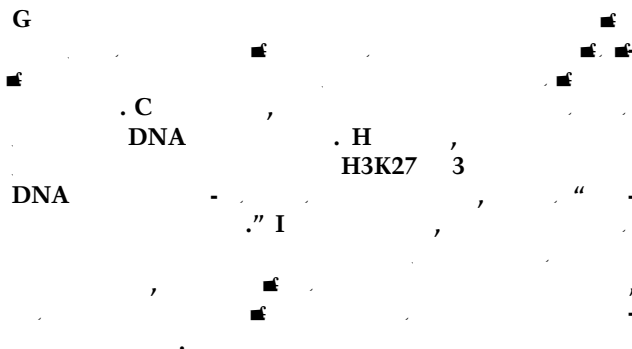
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Supplemental material is available for this article.

I

Genomic imprinting is the monoallelic expression of a gene based on parent of origin. Imprinted genes are essential for fetal and placental growth and development. It is hypothesized that imprinting arose in placental mammals due to the conflict between maternal and paternal genomes in the fetus to regulate maternal resources during and immediately after pregnancy, with maternal imprints repressing fetal growth while paternal imprints promote it (Moore and Haig 1991). To date, there are several examples of imprinted genes that fit this model, including key regulators of fetal growth such as the insulin growth factor 2 (IGF2) and its receptor IGF2R, which are reciprocally imprinted (DeChiara et al. 1990; Barlow et al. 1991; DeChiara et al. 1991; Weksberg et al. 1993).

Shortly after the discovery of the first imprinted genes, it was shown that imprinted gene expression was regulated by allelic epigenetic marks, in particular repressive DNA methylation, inherited from the parental germline (Bartolomei et al. 1993; Brandeis et al. 1993; Ferguson-Smith et al. 1993; Li et al. 1993). This canonical form of imprint-

ing has since been characterized across mammals and is highly conserved at a number of imprinted gene clusters. Imprinted genes and their regulatory features have been most extensively characterized in the mouse and human genomes, and genome-wide screens have identified not only species-specific but also tissue-specific imprinting. It was recently demons.4(tglso)-33(r)131.222T355.2177.1 humcs84rint

underlying histone modification landscape. In the oocyte, DNA methylation is almost exclusively restricted to transcribed gene bodies (Kobayashi et al. 2012). The widespread use of oocyte-specific alternative transcription start sites means that the majority of maternal ICRs are spanned by transcription (Fig. 1; Chotalia et al. 2009; Veselovska et al. 2015; Singh et al. 2017). The establishment of DNA methylation at maternal ICRs is a consequence of acquiring a permissive chromatin state for the recruitment of DNMTs. Loss of histone 3 lysine 4 dimethylation (H3K4me2) at intragenic CpG islands is catalyzed by the transcription-coupled lysine demethylase KDM1B (Ciccone et al. 2009; Stewart et al. 2015; Veselovska et al. 2015), and deposition of H3K36me2 and H3K36me3 over transcribed regions by the histone lysine methyltransferase SETD2 (Xu et al. 2019; Shirane et al. 2020). Conversely, sperm is highly methylated throughout much of the genome, a pattern that is conferred by DNMT3A and DNMT3L (Bourc'his and Bestor 2004; Kaneda et al. 2004), with the addition of DNMT3C in rodents (Barau et al. 2016). Unlike the oocyte, the deposition of DNA methylation in spermatogenesis is not dependent on H3K36me3, but rather H3K36me2, which shows broad genomic distribution through the activity of methyltransferase NSD1 (Shirane et al. 2020).

Thus, it appears that there is no mechanism of de novo methylation in the germline specifically targeted to imprinted loci per se. Rather, the distinctive dependence of the de novo DNMTs on H3K36 methylation in the oocyte and sperm results in dimorphic DNA methylation landscapes, providing the opportunity for imprinting to emerge at gDMRs. Consequently, locus-specific differences in gamete methylation between species is one mechanism that enables species-specific imprinting to arise (Brind'Amour et al. 2018).

Postfertilization maintenance mechanisms

While distinct patterns of DNA methylation in the egg and sperm are the prerequisite for imprinting, gametic methylation differences are far more extensive than the number of imprinted loci; for example, there are ~2000 CpG islands highly methylated in oocytes but not sperm (Kobayashi et al. 2012). Therefore, the maintenance of gamete-derived methylation in the embryo is critical in specifying the number of persistent gDMRs and, consequently, the number of imprinted loci. The discovery of the involvement of the zinc finger protein ZFP57 demonstrated that imprint maintenance relies on sequence-specific factors (Li et al. 2008; Mackay et al. 2008). ZFP57 is a member of the large family of Krüppel-associated box (KRAB)-containing zinc finger proteins (ZFPs) that provide DNA sequence binding specificity to the KRAB repressor complex. ZFP57 binds a CpG-containing hexanucleotide motif present in multiple copies in most ICRs (Quenneville et al. 2011; Strogantsev et al. 2015; Anvar et al. 2016) and, critically, binds the motif when the central CpG.5(C)1y(ogin)18.9nxaylaalpG(seet)224TJ3.6(ep)18.9(c)

possibility is that monoallelic Airn transcription through essential placenta-specific enhancers represses the distal genes that depend on these enhancers. However, this model has been discounted by genetic experiments deleting the entire Airn transcribed region (Andergassen et al. 2019). This finding returns to the frame a long-established model that lncRNAs bind and recruit repressive chromatin modifiers, such as G9a (EHMT2) or polycomb repressor complexes (PRCs), to imprinted domains (Nagano et al. 2008; Terranova et al. 2008). For the megabase-scale imprinted domains, parallels with the lncRNA Xist and X-chromosome inactivation re-emerge (Khamlichi and Feil 2018). Molecular investigations in trophoblast and embryonic stem cells have demonstrated that 3D folding is essential to bring CpG islands within close proximity to ICRs at the Airn and Kcnq1ot1 loci in cis, enabling PRCs to facilitate allelic silencing (Schertzer et al. 2019). Nevertheless, what features are critical for determining the extent of these imprinted domains and how exactly imprinted lncRNAs function remain to be fully elucidated.

N

Discovery and properties

For many years, we have understood that DNA methylation is central to regulating imprinting; however, there have been examples of imprinted loci that appeared not to be controlled by DNA methylation, which compelled us to entertain alternative mechanisms of imprinting. For example, there were no detectable promoter DMRs at the placenta-specifically imprinted genes Gab1 and Sfmbt2; in addition, their imprinting is retained even in conceptuses lacking oocyte-derived DNA methylation (Okae et al. 2012). An explanation for these anomalies has emerged with the discovery of a parallel mechanism of imprinting, which has been termed "noncanonical" imprinting.

Work that profiled DNase I-hypersensitive sites (DHSs) separately in isolated maternal and paternal pronuclei of mouse zygotes found that a subset of paternal-specific DHSs was not associated with known imprinted genes but with genes with paternal allele-biased expression (Inoue et al. 2017a). This was further evidenced from analysis of gynogenetic or androgenetic preimplantation embryos, as well as reciprocal hybrids. These genes do not map into regions of DNA methylation in oocytes, and their imprinting is maintained when oocytes are deprived of DNA methylation (Chen et al. 2019; Hanna et al. 2019). Critically, forced expression of the H3K27me3 demethylase KDM6B in zygotes abrogates their imprinted status (Inoue et al. 2017a). Genetic confirmation of the role of H3K27me3 has subsequently been obtained by conditional deletion of Eed, which encodes an essential component of the PRC2, in oocytes (Inoue et al. 2018).

An intriguing property of noncanonical imprinting is its tissue specificity. Although multiple genes with paternal

embryonic or adult origin should have normal canonical imprints, they lack imprinting of noncanonical imprinted

complementary flanking LTRs and thus remain as "solo-LTRs" (Belshaw et al. 2007). LTRs have been frequently commandeered as cis-regulatory elements and significantly contribute to the gene regulatory landscape (Faulkner et al. 2009). LTR sequences can contain, or acquire through mutagenesis, sites for transcription factor binding, transcription initiation, splicing, and/or polyadenylation and thus can impact gene regulation in a multitude of

in its premature truncation and polyadenylation (Fig. 2C; Wood et al. 2008). At a number of noncanonically imprinted loci, LTR-initiated transcripts are spliced on to nearby protein-coding or noncoding RNA genes, resulting in imprinted chimeric transcripts (Fig. 2D; Hanna et al. 2019). Consistent with LTRs demonstrating tissue-specific activity, noncanonically imprinted LTRs are exclusively expressed in extraembryonic tissues, including the placenta and visceral endoderm (Hanna et al. 2019).
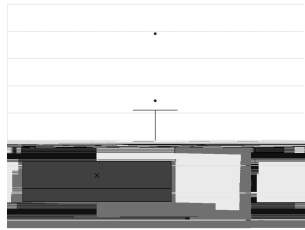
Imprinted gDMRs have also co-opted the ERV silencing machinery, KRAB-ZFPs, to enable the protection and maintenance of monoallelic DNA methylation during developmental reprogramming (Li et al. 2008; Pathak and Feil 2018), as previously discussed. While the vast majority of gDMRs do not contain an identifiable ERV, each (with the exception of one) contains motifs that are recognized by ZFP57 and/or ZNF445 (Quenneville et al. 2011; Takahashi et al. 2019). Notably, ZFP57 binds not only imprinted gDMRs but also a number of ERVs throughout the genome (Shi et al. 2019). Despite using a common KRAB-ZFP, the underlying mechanisms silencing imprinted gDMRs and ERVs appear to be distinct; in the absence of ZFP57, imprinted gDMRs become derepressed, while ERVs remain silenced (Shi et al. 2019). Beyond ZFP57 and ZNF445, there are also KRAB-ZFPs that act in a locus-specific manner. ZFP568 is essential for the establishment of DNA methylation at a sDMR at the placental-specific promoter of Igf2 (Yang et al. 2017). Deletion of Zfp568 results in up-regulation of Igf2 and embryonic lethality, a phenotype that was partially rescued by deletion of Igf2 (Yang et al. 2017). As the mechanisms regulating the establishment of sDMRs are investigated further, we may discover additional roles for KRAB-ZFPs in targeting allelic de novo DNA methylation in the postimplantation embryo.

Overall, ERVs have contributed to the evolution of genomic imprinting in mammals by several distinct mecha-

(2019) identified regions of pref
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common SNP annotation (http://www.ncbi.nlm.nih.gov/SNP) and the SNPsplit mapping program (Krueger and Andrews 2016). Allelic analyses confirmed a >10% allelic difference in DNA methylation at 16/26 of informative DMRs (Fig. 3C), although parent of origin was not assessed (Supplemental Table S1). Together, these data provide preliminary evidence that noncanonical imprinting may also exist in the human genome, while further work will be needed to validate candidate loci and demonstrate whether these DMRs regulate allelic expression. Notably, the putative noncanonical imprinted DMRs, compared with the Illumina 450K array probes, were significantly enriched for CpG islands and SINEs (Fig. 3D), rather than LTRs as in mice. This suggests that while the mechanism may be conserved between species, the underlying regulatory features are likely not. This difference may reflect the dissimilarities in the prevalence of repetitive elements between the mouse and human genomes (Thomas et al. 2003). However, it is important to highlight that repetitive elements in general are underrepresented on the Illumina 450K array (Fig. 3D); therefore, it is likely that loci have been missed by this approach.

The pursuit of comprehensively identifying human imprinted domains continues to present challenges, including the necessity for deep sequencing of genomics data sets, the scarcity of informative SNPs, obtaining parent of origin information for relevant SNPs, and the cellular heterogeneity of human samples, as previously discussed. The initial identification and characterization of noncanonical imprinting in mice emphasizes the value in using animal models to direct our approaches for investigating molecular and epigenetic phenomena in human development.

C

The authors declare no competing interests.

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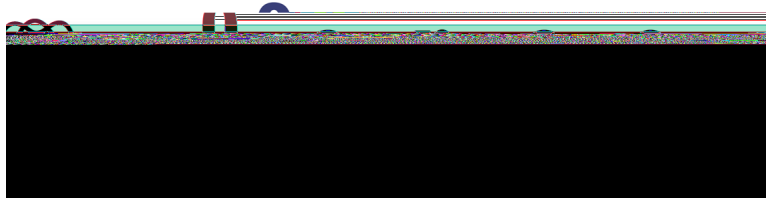
Features and mechanisms of canonical and noncanonical genomic imprinting

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