KRABsody for embryo-placental development

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Many of the hundreds of KRAB zinc finger proteins encoded by human and mouse are involved in taming the transcriptional regulatory potential of transposable elements. Reporting recently in *Science*, Yang et al. (2017) reveal that one murine family member, ZFP568, controls igf2 expression for proper embryonic and placental development.

KRAB-ZFP genes emerged some 420 million years ago in a common ancestor of coelacanth, lung fish and tetrapods and amplified since then by gene and segment duplications to be present by hundreds in the genomes of most of today’s higher vertebrates (Imbeault et al., 2017). KRAB-ZFPs are characterized by an N-terminal KRAB (Kruppel-associated box) domain and a C-terminal array of zinc fingers (ZNF) with sequence-specific DNA binding potential. The KRAB region can recruit KAP1/TRIM28, which acts as a scaffold for heterochromatin-inducing factors, so that many KRAB-ZFPs function as transcriptional repressors (Friedman et al., 1996). Transposable elements (TEs) represent the preferential genomic targets of a majority of human and at least several examined murine KRAB-ZFPs, although recruitment at other types of genetic units has been noted for the most evolutionarily conserved family members (Ecco et al., 2016; Imbeault et al., 2017; Schmitges et al., 2016; Wolf and Goff, 2009).

In a recent report of *Science*, Yang et al. (2017) followed up on the curious previous observation that ZFP568, a mouse KRAB-ZFP, is essential for placental development in this species (Shibata and Garcia-Garcia, 2011) to provide evidence of direct KRAB-ZFP regulation of embryo-placental growth. Upon deleting zfp568 in murine embryonic and trophoblast stem cells (ESCs and TSCs, respectively), the authors found only two genes deregulated: zfp568 itself and igf2-P0, which encodes for insulin-like growth factor 2 from a placenta-specific promoter. IGF2 plays a key role in regulating early development, with deletion of igf2 or its receptor igf1r leading to placental and foetal growth restriction in mice, and misregulation of IGF2 in humans causing Russell-Silver and Beckwith-Wiedemann syndromes, two conditions linked to abnormal intrauterine development. In mice, igf2 is transcribed from three promoters (designated igf2-P1, P2, and P3) in both foetus and placenta and from the placenta-specific igf2-P0, which accounts for ~10% of the total expression of igf2 in this extra-embryonic tissue. Deletion of igf2-P0 results in reduced embryonic and placental growth due to a mismatch between placental supply and foetal needs for nutrients. However, igf2 is expressed at low levels in preimplantation development and in embryonic stem cells (ESCs), suggesting that repressors are required at implantation. The Yang et al. (2017) results identify ZFP568 as a key player in this process.
To support the functional evidence obtained in their knockout mice, the authors went on to demonstrate that ZFP568 recruits KAP1 and its partner histone methyltransferase SETDB1 at the Igf2 P0 promoter, inducing its repression. They further identified a 21-24 base pairs (bp) motif sufficient to confer ZFP568-repressibility to a heterologous promoter and to bind in vitro the 11 ZNF-long array of ZFP568. Upon ZFP568, KAP1 or SETDB1 depletion, or upon mutating the Igf2-P0 KZFP568-binding site by genome editing, the H3K9me3 (histone 3 trimethylated on lysine 9) repressive mark and DNA methylation were lost at the Igf2-P0 promoter, correlating with its transcriptional activation. Confirming that the embryonic lethal phenotype of zfp568-deleted mice was due an excess of Igf2 in the nascent embryo, the team could restore the viability of zfp568KO/KO embryos through mid-gestation by additionally knocking out Igf2, although only rare pups made it to birth, and these died immediately thereafter.

**FIGURE LEGEND**

_Igf2-controlling KRAB-ZFP duet in murine ESCs._

Maternal allele: CTCF recruitment on the unmethylated Igf2/H19 ICR blocks Igf2 promoters activation by distal enhancer.

Paternal allele, wild type ESC: ZFP57 is recruited at the methylated Igf2/H19 ICR, allowing activation of Igf2 promoters. However, P0 is silenced and P1-P3 are dampened by ZFP568-induced repression.

Paternal allele, ZFP568 KO ESC: P0 promoter is unleashed and P1-P3 further activated, resulting in increased IGF2 levels.
Several interesting questions are raised by this work. First of which, does it exemplify the ultimate evolutionary fate of TE-controlling KRAB-ZFPs or the accidental divergence of a family outlier? Indeed, even though the genomic targets of many KRAB-ZFPs reside in readily identifiable TEs, neither the Igf2-P0 locus nor any of the other sites identified by Yang et al. as capable of recruiting ZFP568 in murine ESCs or TSCs harbour a TE-reminiscent signature. Therefore, were these sequences seeded by an ancient and since decayed retrotransposon, or did they emerge by genetic drift? As a corollary, was ZFP568 initially selected for during evolution because it controlled this putative mobile element, or did the repressor act from the beginning on sequences unrelated to TEs? It is noteworthy that many evolutionarily older human KRAB-ZFPs associate with genomic features lacking recognizable TE signatures, notably promoters (Imbeault et al., 2017), and at least some of them do not recruit KAP1 and are thus not expected to act as repressors (Schmitges et al., 2016) (and our unpublished data). Yang et al. (2017) detected 137 and 86 ZFP568-binding sites in ESCs and TSCs, respectively, 29 of which were shared between the two cell lines. It seems unlikely that so many 21-24 bp-long sequences evolved independently, lending credence to a model of TE-mediated seeding of this binding sequence.

Another interesting question raised by the Yang et al. (2017) study is that of a broader role for ZFP568. The work so far demonstrates a function only for one of these ZFP568 binding sites, at the Igf2-P0 locus. Its deregulation alone explained the early embryonic death of zfp568 knockout mice, since the latter could be prevented by additionally deleting Igf2. Previous work also involved ZFP568 in convergent extension in the mouse embryo (Garcia-Garcia et al., 2008). To determine more fully the roles of ZFP568, it will be important to examine its patterns of expression in newborn and adult mice (human KRAB-ZFP genes display broad yet highly specific modes of expression in precursor and differentiated cells alike), to define its genomic targets in these settings, and to study animals in which this factor is conditionally deleted in relevant tissues.

Proteins displaying strong homologies with murine ZFP568 are found in all examined eutherian mammals, suggesting conserved functions. One exception is humans, where the ZNF arrays of its putative orthologues, including the rapidly evolving znf568, depart sufficiently from their murine counterpart to suggest that these proteins target different sequences (Imbeault et al., 2017). Indeed, recent studies of some KRAB-ZFP paralogs differing by only few amino acids at critical positions within their poly-zinc finger sequences have revealed overlapping yet clearly distinguishable ranges of genomic targets (Ecco et al., 2016) (and our unpublished data). It will be interesting to determine over which range of species ZFP568 orthologues can substitute for each other. Moreover, consistent with the absence in humans of a true homologue of murine ZFP568, the human Igf2 gene not only does not contain a sequence corresponding to the ZFP568-binding site, but also lacks a placenta-specific promoter. Therefore, how expression of this essential placental growth factor is regulated in our species remains to be defined. Nevertheless, in both mouse and human the Igf2 locus is subjected to imprinting, explaining why it is expressed only from the paternal allele. Imprinting is established in germ cells, and its maintenance during the phase of early embryonic genome remodelling that takes place in both species is ensured by ZFP57, a mammalian-restricted KRAB-ZFP (Li et al., 2008; Quenneville et al., 2011). Interestingly, the murine and human orthologues of ZFP57 recognize the same highly conserved hexanucleotide within imprinting control regions (ICRs), and are functionally interchangeable. Thus, Igf2
can be regulated by not just one but two KRAB-ZFPs, one conserved between human and mouse, the other absent in our species.

In sum, the Yang et al. work (2017) not only sheds light on an essential aspect of mammalian embryogenesis, but also points to the complexity of the evolutionary interplay linking KRAB-ZFPs and the regulatory networks of higher vertebrates. No doubt that this topic will be the focus of intense exploration in the years to come.

REFERENCES


