The need for Eed

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Eed, a member of the Polycomb group family of chromatin regulators, acts early in mouse development to maintain imprinted X-chromosome inactivation in females. But does this protein also contribute to imprinting on mouse autosomes?

Genomic imprinting, the process that causes genes to be expressed in a parental origin–specific manner, is a useful model for studying the epigenetic control of genome function in mammals. Parallels between the mechanisms of X inactivation and autosomal imprinting have been proposed[1]. On page 502 of this issue, Jesse Mager and colleagues[2] address whether Eed, required for X inactivation, is also required for imprinting on autosomes. Their results offer further insight into the similarities and differences between X inactivation and autosomal imprinting. The finding that a few silent imprinted alleles are activated in mice with mutations in *Eed* allows us to assess regulatory mechanisms at those loci from a different perspective.

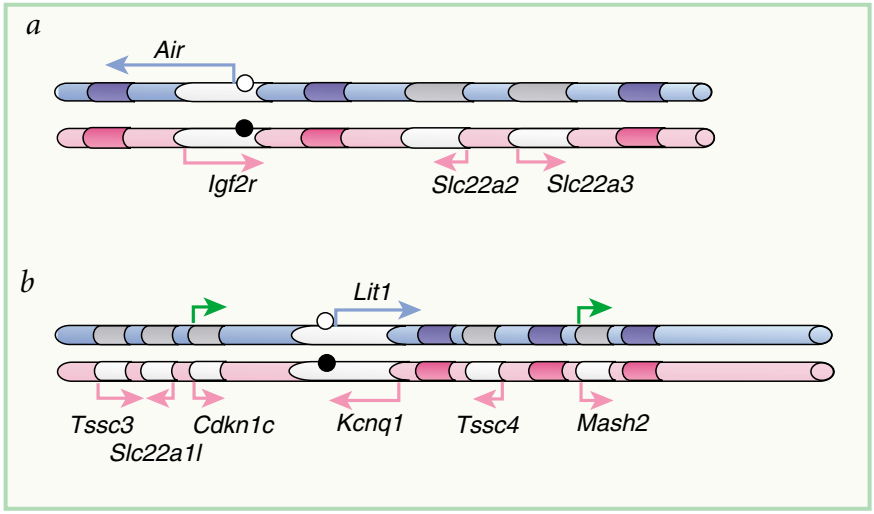
Polycomb in development

The Polycomb group (PcG) family of proteins function in multimeric complexes and are believed to maintain long-term gene silencing during development, acting at the level of the chromatin and involving post-translational modification of core histones[3–5]. In *Drosophila melanogaster* and mammals, two members of the PcG family, encoded by Enhancer of zeste (E(Z) in *D. melanogaster*; *Ezh2* in mouse) and extra sex combs (*esc* in *D. melanogaster*; *Eed* in mouse) function in the same complex. ESC–E(Z) complexes have histone methyltransferase activity that maps to the SET domain of E(Z), and the complex has been shown to include histone deacetylases, consistent with a role in epigenetic modification of chromatin[4,5]. An important early function for *Eed* was indicated when it was first identified as the mutated gene responsible for a lethal gastrulation defect with anterior–posterior patterning defects and abnormalities in mesoderm production and localization[6]. *Ezh2*$^{-/-}$ mouse mutants also die early and have gastrulation defects[7].

Mice with mutations in *Eed* do not maintain imprinted X inactivation (of an X-linked transgene) specifically in extra-embryonic trophoblast cells[8]. But new data from Silva *et al.*[9] suggest that in mouse pre-implantation development, Eed–Ezh2 complexes are not lineage-specific and their recruitment to the inactive X chromosome is temporally regulated, required early in development around the onset of differentiation. The work shows that *Eed* has a role not only in imprinted (extra-embryonic) X inactivation but also in X inactivation in embryonic lineages. The *Xist* RNA, required in *cis* for X inactivation, is expressed normally in *Eed* mutants[8,9]. It has been proposed that *Xist* RNA may recruit Eed–Ezh2 complexes to the inactive X chromosome inducing chromatin modifications, providing a template for more permanent silencing components[9]. Thereafter, both Eed–Ezh2 and *Xist* RNA are no longer required for silencing. Might a similar mechanism act at autosomal imprinted domains?

A role in autosomal imprinting

Mager *et al.*[2] analyzed the allelic expression of 14 informative imprinted genes from 6 unlinked domains in normal and *Eed*$^{-/-}$ mice at embryonic day 7.5. Of the imprinted genes analyzed, eight are



Polycomb group proteins and parental-origin effects. Two imprinted domains behave differently in *Eed*$^{-/-}$ embryos. ***a***, The *Igf2r* domain is unaffected in *Eed*$^{-/-}$ mutants. The three paternally silent imprinted genes in this domain are regulated by a non-coding antisense transcript (*Air*) expressed from the paternal chromosome. Although parallels have been drawn between X inactivation and the imprinting mechanism at this locus, no loss of imprinting is observed at this locus in *Eed* mutants. ***b***, The *Kcnq1* domain contains six genes that are expressed from the maternally inherited chromosome. In mice lacking the DMR, loss of imprinting of all six genes occurs on the paternal chromosome. In *Eed*$^{-/-}$ mutants, two of the genes are expressed from the paternal chromosome with no change to the methylation status or expression of *Kcnq1*/*Lit1*. The paternal chromosome is blue and the maternal chromosome is red. Active alleles of imprinted genes are white with a red arrow to denote expression from the maternal allele and a blue arrow to denote expression from the paternal allele. Arrows indicate the orientation of transcription. Silent alleles are gray and non-imprinted genes in the locus are darker red or blue. Circles show the location of germline-inherited DMRs; filled circles represent the methylated allele and open circles, the unmethylated allele. The green arrows indicate normally silent alleles that are expressed in *Eed*$^{-/-}$ mutants.

expressed from the maternally inherited chromosome and six from the paternally inherited chromosome. Notably, their results did not show loss of imprinting of all silent alleles in mutants. Rather, expression from four normally silent alleles was observed. This indicates that whereas X-chromosome silencing may involve a widespread function for Eed, autosomal silencing involving Eed may be considerably more restricted. This raises questions of how the Eed complex is targeted and how the regional extent of its function is controlled.

So do the four genes that are inappropriately expressed in the *Eed*$^{-/-}$ mouse share anything in common? Most obvious is the fact that all four genes are normally repressed on the paternally inherited allele. All six maternally inactive alleles tested were unaffected. If a paternal chromosome–specific function for Eed were more than a coincidence, this would suggest that paternally and maternally inherited chromosomes can use different chromatin-silencing mechanisms. This is consistent with the epigenetic differences known to occur between maternal and paternal genomes in the zygote. Furthermore, the paternal origin–specific loss of imprinting may imply that paternal and maternal chromosomes harbor different germline-specific epigenetic signals that may subsequently be differentially recognized by chromatin-modification complexes.

The imprinted genes not affected in *Eed*$^{-/-}$ mice are also of interest. For example, silencing of the maternally expressed *Igf2r* and linked *Slc22a2* and *Slc22a3* genes on the paternal chromosome is regulated by a paternally expressed antisense RNA (*Air*) that acts bidirectionally and in *cis* (see figure; ref. 10). Parallels have therefore been drawn between the function of *Air* RNA in imprinting the *Igf2r* domain and the *Xist* RNA in X inactivation[10]. In contrast with X inactivation, however, normal imprinting of *Igf2r* was observed in the *Eed*$^{-/-}$ mutants, indicating that there are mechanistic differences in the regulation of the two domains. In addition, an imprinting control element has recently been shown to be required for the inactivity of at least six paternally inherited alleles at the *Kcnq1* imprinted cluster (see figure; ref. 11). This controlling element is the promoter for a paternally expressed antisense transcript (*Lit1*), although a direct role for *Lit1* RNA in regional imprinting has not yet been shown. Two of the six genes regulated by the antisense-controlling element are *Mash2* and *Cdkn1c*. Their loss of imprinting (green arrows in figure) in *Eed*$^{-/-}$ mutants occurs in the absence of any effect on imprinting of *Kcnq1*, *Lit1* or other genes in the cluster. This indicates that Eed acts downstream from the controlling element and is only involved in the regulation of a subset of genes in this cluster (see figure). It also proves that the controlling element is necessary but not sufficient for *Mash2* and *Cdkn1c* silencing.

A choosy regulator

In situations in which DNA methylation cannot be maintained, imprinting is perturbed[12]. The relationship between DNA methylation and chromatin modification has been studied in several different organisms. Does DNA methylation recruit histone-modification complexes, or is DNA methylation secondary to modifications to chromatin? Examples of both have been reported[13,14], but few studies have addressed this question at mammalian imprinted domains. At least one recent study has shown that histone methylation can confer imprinting in the absence of DNA methylation and seems required to maintain DNA methylation[15].

To determine whether the Eed effect on imprinting was associated with changes in DNA methylation, Mager *et al.*[2] studied associated regions that are differentially methylated on the two parental chromosomes (DMRs). The DMRs in affected and unaffected imprinted genes were analyzed in mutants. Notably, some specific differences were observed at the different DMRs but no striking correlation between these and the behavior of the genes was evident. Changes in methylation could be caused directly by altered chromatin modifications in the mutants or could be secondary to changes in local expression or regional conformation. Regardless, the findings indicate that Eed largely acts downstream of DNA methylation.

This study[2] suggests that Eed is not a global imprinting regulator but rather is involved in maintaining the silencing of some alleles on paternal chromosomes while other imprinted alleles in the same epigenetically regulated domain remain unaffected. This seems to be different from its role in X inactivation. But in *Eed*$^{-/-}$ mutants, expression of genes on the inappropriately activated X are limited to the analysis of a transgene and two endogenous genes[8,9]. Although chromosome-wide changes in histone modification are observed on the X chromosome in *Eed* mutants, more X-linked genes should be tested to rigorously prove that Eed is required to maintain all inactivated genes on the X chromosome. Nonetheless, these studies provide new insights into the regulation of large imprinted domains and the relationship between DNA methylation and histone modifications in mammalian epigenetic silencing. □

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