DEVELOPMENTAL EPIGENOMICS

The Polycomb landscape in mouse development

Polycomb-group proteins assemble into two primary complexes—Polycomb repressive complex (PRC) 1 and 2—that safeguard cell fate by repressing gene transcription. Two new studies explore the PRC1 landscape during the transition from gametes to embryos in mice, thus providing insight into the intergenerational transmission of epigenetic information and gene regulation dynamics as embryos prepare for gastrulation.

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PRC1 and PRC2 are two evolutionarily conserved complexes that affect cell identity and fate via an epigenetic mode of gene repression. In classical Polycomb-mediated regulation, PRC2 catalyzes the formation of trimethylated histone H3 lysine 27 (H3K27me3), a mark recognized by PRC1, which in turn deposits the histone H2A lysine 119 monoubiquitin mark (H2AK119ub1). However, recent data have indicated that in animals the relationship is not quite so straightforward. Variant forms of PRC1 (vPRC1) can act upstream of PRC2 and initiate the canonical pathway in flies and mice[1–5]. Furthermore, genetic perturbation studies have indicated that PRC2-mutant mice die during gastrulation[6], whereas complete PRC1 mutants exhibit an even more severe arrest at the two-cell stage[7]. Thus, the relationship between the two complexes is not unidirectional. Although genome-wide patterns of H3K27me3 in mouse gametes and embryos have been described[8], H2AK119ub1 maps have not been so readily achieved. In this issue, Mei et al.[9] and Chen et al.[10] have used emerging technology—as well as complementary genetic approaches—to shed light on the interplay between PRC1 and PRC2 during the earliest windows of mammalian development.

H2AK119ub1 dynamics

To assess allele-specific H2AK119ub1 profiles during embryogenesis, both groups used cleavage under targets and release by using nuclease (CUT&RUN), a method for genome-wide semiquantitative profiling of chromatin-bound factors[11], in F_1 hybrid mice. The technique is particularly useful for samples that are difficult to harvest, because high signal-to-noise ratios decrease the number of cells required. These H2AK119ub1 maps add to a publicly available tapestry of histone-modification, RNA polymerase II, DNA methylation and transcriptional profiles in early mouse embryos[12]. Of note, neither group collected CUT&RUN samples derived from reciprocal crosses of F_1 hybrids, which can confound parent-specific effects with strain-specific effects[13].

Exploring these data, both groups showed widespread H2AK119ub1 establishment during oogenesis, thus resulting in large domains overlapping the promoters of genes (Fig. 1a). Integrated analysis with existing datasets[8] revealed a strong correlation between H2AK119ub1 and H3K27me3 in mature oocytes. Because the dynamics between PRC1 and PRC2 during oocyte progression was not sampled, ascertaining the stepwise establishment of the Polycomb landscape in the female germline was not possible.

Both groups generated maps of H2AK119ub1 at key stages of early embryogenesis. Strikingly, after fertilization, the symmetry between H2AK119ub1 and

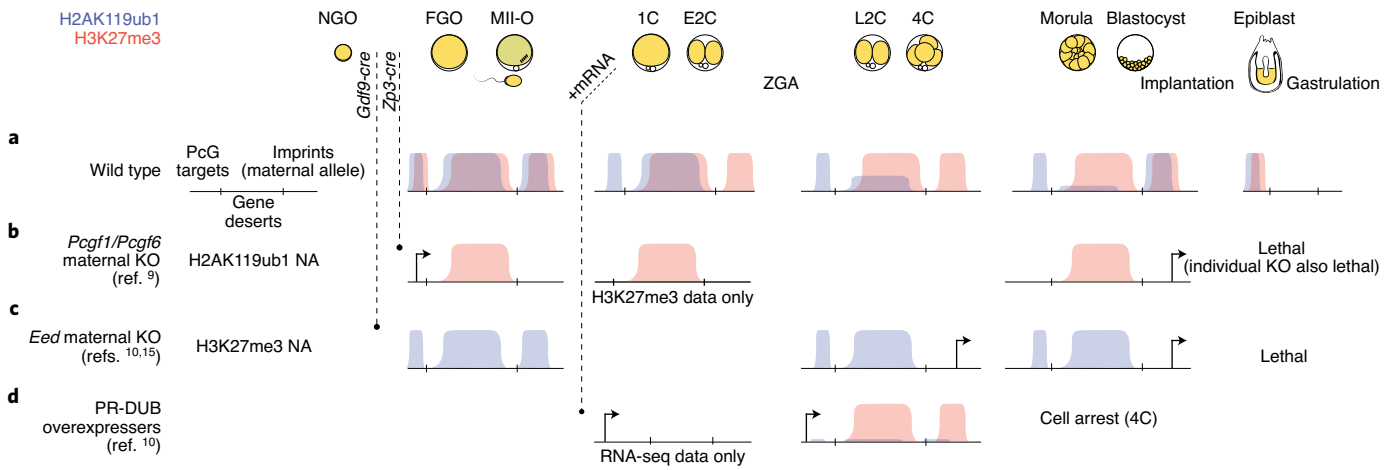

Fig. 1 | Polycomb and transcriptional landscapes in normal and modified mouse embryos. a–d, H2AK119ub1 and H3K27me3 dynamics over Polycomb group (PcG) target-gene promoters, gene deserts and the maternal allele of non-canonically imprinted genes in ooyctes and embryos in wild type (**a**), *Pcgf1/Pcgf6* maternal knockouts (**b**), *Eed* maternal knockouts (**c**) and PR-DUB overexpressers (**d**). KO, knockouts; NGO, non-growing oocyte; FGO, fully grown oocyte; MII-O, meiosis II oocyte; 1C, one cell; E2C, early two cell; ZGA, zygotic genome activation; L2C, late two cell; 4C, four cell; NA, not available. Arrows represent ectopic transcription.

H3K27me3 observed in oocytes was lost over gene deserts. While H2AK119ub1 was depleted, H3K27me3 was stably retained through the blastocyst stage. In contrast, a distinct set of gene-rich loci devoid of both marks in oocytes showed a sequential gain of H2AK119ub1 in early two-cell embryos followed by an H3K27me3 gain in blastocysts and epiblasts. The findings reported here provide noteworthy in vivo data consistent with findings from the studies performed largely in cell culture demonstrating that PRC1 can act upstream PRC2 (refs. [3,5]). In embryos, symmetry is restored after implantation, in which H2AK119ub1 and H3K27me3 domains are restricted to their canonical targets: the promoters of developmentally important genes.

PRC1 affects embryogenesis

To understand the biological importance of H3K27me3 and H2AK119ub1 dynamics, genetic interrogation of the relationship between PRC1 and PRC2 is paramount. To circumvent the severe PRC1-mutant phenotype, the two groups undertook complementary approaches: Mei et al. genetically ablated PRC1 subunits (*Pcgf1/Pcgf6*) in oocytes to assess the role of PRC1 in the female germline and embryo (Fig. 1b). Importantly, PCGF1 and PCGF6 are components of vPRC1. Chen et al. used a PRC2 mutant (*Eed*) to determine the role of H3K27me3 in H2AK119ub1 establishment in oocytes (Fig. 1c). Additionally, Chen et al. overexpressed Polycomb repressive deubiquitinase (PR-DUB) in zygotes, which efficiently removes H2AK119ub1 (Fig. 1d).

Pcgf1/Pcgf6-knockout oocytes exhibited substantial loss of H2AK119ub1 but still retained most of the H3K27me3 (~88%) (Fig. 1b). RNA-sequencing analysis of oocytes globally devoid of H2AK119ub1 showed specific upregulation of the subset of genes that had lost H3K27me3. Previous work has demonstrated that PRC2 controls a non-canonical form of transient maternal imprinting and imprinted X-inactivation[14,15]. Pertinently, embryos generated by fertilizing *Pcgf1/Pcgf6*-knockout oocytes with wild-type sperm exhibited upregulation of a subset of this class of imprinted genes, including *Xist*, encoding the key upstream regulator of X-inactivation. Analysis of extraembryonic tissues demonstrated a failure of imprinting associated with increased placental size. In summary, vPRC1 has a key role in establishing repressive modifications transmitted from oocytes to embryos.

Genetic deletion of *Eed* (which is essential for PRC2 activity) revealed that while H3K27me3 was globally lost, H2AK119ub1 was largely unaffected in oocytes and morula-stage embryos (Fig. 1c). Exceptionally, H2AK119ub1 was lost over non-canonically imprinted regions and *Xist*[14,15]. Hence, a notably strong link appears to exist between PRC1 and PRC2 in regulating this class of genes.

Chen et al. interrogated the role of H2AK119ub1 by directly injecting mRNA encoding PR-DUB into fertilized zygotes (Fig. 1d). Interestingly, whereas H2AK119ub1 was lost, H3K27me3 was unaffected, and RNA-sequencing analysis showed no change in imprinted-gene expression in four-cell embryos. Together, the results suggest that PRC1 regulation of imprints probably occurs in oocytes, possibly through PRC2 recruitment, but is dispensable in embryos. Crucially, however, depletion of H2AK119ub1 resulted in premature activation of canonical target Polycomb-regulated genes and cell arrest at the four-cell stage.

The PR-DUB overexpression experiment strongly indicates that H2AK119ub1 is immensely important in the earliest stages of embryo progression. Both groups made the striking finding that PRC1 regulation at this class of genes occurred seemingly independently of PRC2, thus perhaps explaining the milder PRC2 mutant phenotype. Although formal confirmation remains to be reported, these findings suggest that upstream vPRC1 activity represses Polycomb-regulated genes. However, this finding prompts the question of why PRC2 does not nucleate at these regions soon after fertilization. Are key factors that recruit PRC2 to vPRC1-marked regions absent, or are antagonistic factors perhaps present? Moreover, what is the biological rationale for limiting PRC2 activity at these stages? Finally, what is the mechanistic explanation for the sufficiency of vPRC1 for repression in this window? Future work will ideally address these exciting questions, thus further elucidating Polycomb-mediated gene regulation in the context of in vivo mammalian development. ❐

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