STEM CELL DIFFERENTIATION

Dppa2 and Dppa4 safeguard bivalent chromatin in order to establish a pluripotent epigenome

Bivalent chromatin domains contain opposing histone modifications that assist cell lineage specification. Two studies report a role for Dppa2 and Dppa4 in the establishment of bivalency and the prevention of de novo DNA methylation at development-related genes in mouse embryonic stem cells.

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During embryonic development, the right genes must be expressed at the right time. As our understanding of cellular decision making during this process has matured, so has our appreciation of the epigenomic landscape and its role in 'priming' cells for lineage specification[1].

Part of this 'priming' relates to bivalent chromatin domains, an unusual epigenetic feature present in embryonic stem cells (ESCs), although not exclusively[2]. Genome-wide epigenetic profiling in ESCs revealed the twin occurrence of trimethylated histone H3 lysine 4 (H3K4me3) — an activating epigenetic mark — and trimethylated histone H3 lysine 27 (H3K27me3) — a mark found on repressive chromatin — on a significant number of developmentally regulated genes[3]. This is in contrast to almost all other mammalian cell types, where H3K4me3 and H3K27me3 seldom co-occur and are each present on a distinct set of promoters[4]. The functional relevance of bivalency in lineage specification has been further elucidated during mammalian and zebrafish development. Bivalency represents an elegant model for how epigenetic regulation could precede and direct transcriptional program switching in the developing organism[4]. Nonetheless, exactly how bivalent domains are initially formed in the early embryo remains an open question and a topic of interest in developmental biology.

Two new studies now implicate Dppa2 and Dppa4 (hereafter Dppa2/4) in establishing bivalency in ESCs[5,6]. Dppa2/4 are nuclear proteins capable of heterodimerization and they have been used as markers of pluripotency in mouse and human cells[7,8]. Curiously, pluripotent cells that lack Dppa2/4 do not lose stem-cell properties nor do these cells show altered levels of other pluripotency genes[5,9]. However, mice lacking Dppa2/4 succumb to defects later in development, long after the proteins themselves cease to be expressed, hinting at their possible role in epigenetic priming[9,10].

Building on previous work identifying Dppa2/4 as regulators of the zygotic transcriptional program (the 2-cell (2C)-like state in ESCs)[11], Eckersley-Maslin et al. now address the question of how bivalency is established in ESCs at the promoters of lineage-specifying genes[5]. Analyzing existing Dppa2/4 ChIP–seq (chromatin immunoprecipitation followed by sequencing) datasets in ESCs[12], the authors noticed that the majority (>60%) of bivalent promoters are bound by Dppa2/4. To find potential interactors of Dppa2/4 on chromatin, the authors employed a powerful proteomic technique called qPLEX-RIME (quantitative multiplexed rapid immunoprecipitation mass spectrometry of endogenous proteins). This recently developed method combines immunoprecipitation of crosslinked proteins with advanced mass spectrometry analysis, allowing for both the identification and quantification of chromatin-associating protein complexes[13]. Using qPLEX-RIME, the authors found that chromatin-bound Dppa2/4 interacted with members of the COMPASS and Polycomb complexes, which deposit H3K4me3 and H3K27me3 marks, respectively. Having identified a link between Dppa2/4 and the 'writers' of the bivalent marks, Eckersley-Maslin et al. analyzed the levels of H3K4me3 and H3K27me3 in Dppa2/4 double-knockout cell lines. Dppa2/4 depletion caused a considerable reduction in H3K4me3 and, to a lesser extent, H3K27me3 levels on Dppa2/4-bound genes, although the two bivalent marks were not altered globally. On a subset of these bivalent genes, both histone marks were reduced. These genes were thus dubbed 'Dppa2/4 dependent'. Notably, around a fifth of the 309 Dppa2/4-dependent genes are essential for normal development[9,10].

Beyond selective regulation of bivalent marks, what effect does Dppa2/4 have on gene expression? In ESCs, the authors found that the Dppa2/4-dependent genes were characterized by low levels of expression, low levels of H3K4me3 and the presence of the initiating but not the elongating form of RNA polymerase II. Interestingly, during differentiation, Dppa2/4-dependent but not independent genes remained silenced in Dppa2/4 double knockout cells. This silencing was accompanied by a significant gain in DNA methylation. However, knocking down Dppa2/4 in ESCs that lack all DNA methyltransferases still leads to downregulation of Dppa2/4-dependent genes, suggesting that DNA methylation itself is not required for silencing and that DNA methylation is not the cause but rather the consequence of the loss of bivalent marks at these genes.

A study by Gretarsson and Hackett[6], while starting from a very different experimental setup, corroborates these findings. By adopting and improving the reporter for genomic DNA methylation (RGM) system[14], the authors were able to monitor the levels of methylation in single cells. In this method, a fluorescent reporter protein is attached to a promoter capable of mimicking the methylation status of nearby regions. The authors modeled global resetting of DNA methylation by culturing cells in titrated 2i/LIF (t2i/L) medium before switching to 2i/LIF medium; this shifts the methylome of ESCs from a hyper- to a hypomethylated state[15]. By introducing a clustered regularly interspaced short palindromic repeats (CRISPR) guide RNA (gRNA) library and screening for cells that did not express the fluorescent reporter (that is, no RGM activation), the authors were able to identify putative regulators of global demethylation and factors that protect the genome from active DNA methylation. In this manner, Gretarsson and Hackett identified 56 genes and selected 24 of these for further validations. They generated CRISPR-knockout cell lines for each and

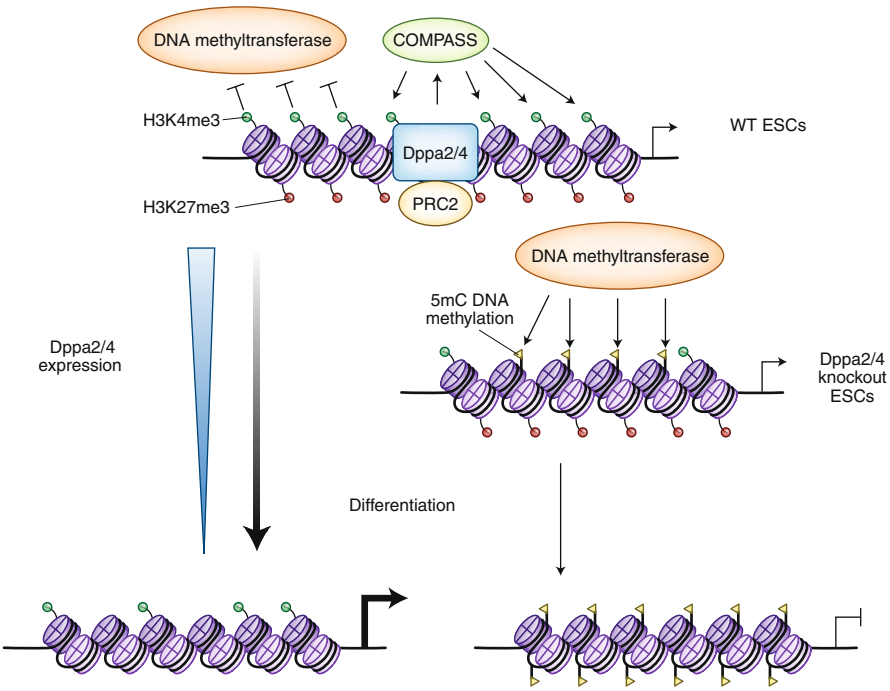

Fig. 1 | Bivalent domains are established at Dppa2/4-bound promoters in ESCs. At a subset of developmental promoters, bivalency is established in part by the association of Dppa2/4 with members of both the COMPASS and PRC2 complexes, which deposit H3K4me3 and H3K27me3 histone modifications, respectively. These promoters regulate genes that are generally expressed at low levels or are silent due to their 'poised' or metastable state. In wild-type ESCs, Dppa2/4 stabilizes H3K4me3 levels, preventing de novo DNA methylation by inhibiting both binding of Dnmt3L and DNA methyltransferase activity[16]. Upon differentiation, retargeting of PRC2 complexes alters the chromatin environment of bivalent domains. Although Dppa2/4 expression decreases following exit from the pluripotent state, retention of H3K4me3 maintains a permissive environment for elevated transcription at later developmental stages[17]. In the absence of Dppa2/4, these bivalent domains lose H3K4me3 and the balance is tilted in favor of repressive H3K27me3. Without the positive reinforcement from H3K4me3-induced repression of DNA methyltransferases, these promoters gain de novo DNA methylation and lose bivalency. No longer poised for activation, genes at these loci fail to be upregulated following differentiation, and this may contribute to defects occurring later in development.

analyzed the overall methylation content in 2i/LIF conditions. While, as expected, the loss of most of these genes led to a global increase in methylation, several of them, including Dppa2/4, displayed a focal affect. In Dppa2/4-knockout ESCs, DNA hypermethylation was observed at a subset of developmentally-associated promoters, many of which typically remain unmethylated throughout normal development, which is in agreement with the findings of Eckersley-Maslin et al. Curiously, Gretarsson and Hackett noticed that the absence of Dppa2/4 also results in the accumulation of focal de novo DNA methylation at LINE1 transposable elements. The authors speculate that these ancient viral remnants may have acquired Dppa2/4 binding sites as a way to commandeer Dppa2/4 activity and protect themselves from epigenetic silencing.

A question remains concerning the sequence of epigenetic events. There is prior evidence that H3K4me3 itself is able to repel the action of de novo DNA methyltransferases[16]. But does this occur at Dppa2/4-bound bivalent promoters? Eckersley-Maslin et al. used DNA-methylation-deficient ESCs and found that this did not significantly rescue expression changes following Dppa2/4 knockdown. By contrast, Gretarsson and Hackett observed that, in epiblast-like cells, genes that were repressed following Dppa2 knockout and that contained CpG islands at their promoters were partially rescued by the deletion of the maintenance DNA methyltransferase gene, *Dnmt1*, suggesting that DNMT1 is involved in repressing them. This implies that, although DNA methylation may not necessarily initiate silencing, it nonetheless contributes to its enforcement.

Collectively, the results from both groups convincingly demonstrate a role for Dppa2/4 in safeguarding H3K4me3 in a targeted manner, preventing DNA-methylation-induced silencing (Fig. 1). As this occurs both at a subset of bivalent promoters and at LINE1 elements, it raises an interesting dilemma of genetic conflict: how are the deleterious effects of these 'selfish' viral elements subdued during epigenetic reprogramming in early development? Importantly, these studies also highlight the extent to which epigenetic memory is intertwined with development — particularly as Dppa2/4 are downregulated following differentiation[17]. To expand on these findings, future efforts should aim to explore the functional relevance of losing bivalency at promoters of Dppa2/4-sensitive genes. For instance, to what extent does hypermethylation or repression of these genes contribute to the birth defects seen in Dppa2/4-mutant mice? Indeed, a deeper characterization of this group of bivalent promoters may uncover new patterns of cell-fate specification in development.

Offered here are useful examples of how epigenetic mechanisms can set the stage for multilineage commitment in the developing embryo. Strengthened by their unique approaches, Eckersley-Maslin et al. and Gretarsson and Hackett provide strong evidence of a predetermined epigenetic sequence of events orchestrated, at least in part, by Dppa2/4. ❐

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Competing interests

The authors declare no competing interests.