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Mitotic Bookmarking: Maintaining the Stem Cell Identity during Mitosis

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In *Cell Reports*, Liu et al. (2017) investigate mechanisms for how pluripotent stem cells maintain their identity during cell division. They show that the histone mark H3K27ac and pluripotency transcription factors remain associated with mitotic chromatin in ESCs and during iPSC reprogramming, demonstrating the importance of mitotic bookmarking in pluripotency.

During mitosis, it is challenging for all dividing cells to maintain their identity, including embryonic stem cells (ESCs). ESCs have a rapid cell cycle and a short G1 phase. Each G1 phase is a critical period to initiate new transcriptional programs (Dalton, 2015), since most transcription factors (TFs) dissociate from condensed chromatin during the last M phase (Mitosis). Therefore, an important unresolved question is how to maintain the pluripotent identity of ESCs after each cell cycle. It is reported that some pioneer TFs remain bound to specific regions in mitotic chromatin, such as FOXA1 in adult liver cells (Caravaca et al., 2013) and GATA1 in hematopoietic cells (Kadauke et al., 2012). These so-called mitotic bookmarking TFs are critical for maintaining the cell-type-specific transcription programs through the cell cycle. In a recent issue of *Cell Reports*, Apostolou and colleagues (Liu et al., 2017) discover that enhancers of pluripotency-related genes in ESCs are bookmarked by OCT4, SOX2, and KLF4 (OSK) and histone 3 lysine 27 acetylation (H3K27ac), while promoters of housekeeping genes are bookmarked by H3K27ac throughout the cell cycle. They further employ an elegant cell-cycle-dependent temporal OCT4 degradation model demonstrating that activity of OCT4 in M-to-G1 transition is important for both the maintenance and establishment of pluripotency.

The ESC pluripotent state is maintained by well-known master TFs, which act collaboratively in driving pluripotency transcriptional programs, and several are important mitotic bookmarking factors for ESC self-renewal (Deluz et al., 2016; Festuccia et al., 2016; Teves et al., 2016). Using live-cell imaging and in vitro biochemical assays, these studies demonstrate that pluripotency TFs OCT4, SOX2 (Deluz et al., 2016; Teves et al., 2016), and ESRRB (Festuccia et al., 2016) associate with the mitotic chromatin and function as mitotic bookmarking factors. However, it is still unknown how these bookmarking TFs coordinate with epigenetic features such as histone marks in mitosis. In the current study, Liu et al. (2017) first survey the abundance of specific histone modifications on mitotic chromatin. They find that the histone methylation marks H3K27me3 and H3K9me3 are highly retained during mitosis, and bookmarking for the repressive regions by these marks is important because the associated repressors dissociate from condensed chromosomes in mitosis (Egli et al., 2008). By contrast, H3K27ac is the only histone acetylation mark enriched in mitotic chromatin. Thus, Liu et al. (2017) profile the genome-wide mitotic retention of H3K27ac in asynchronous and mitotic ESCs using the chromatin immunoprecipitation and sequencing (ChIP-seq) technique. Compared to the H3K27ac profile in asynchronous cells, they discover that H3K27ac is enriched at enhancers of pluripotency-related genes and at promoters of housekeeping genes in mitotic ESCs. This observation is significant, because most studies on mitotic bookmarking merely focus on cell-specific TFs. By comparing the H3K27ac peaks of ESCs to those of an erythroblast, the authors further reveal that commonly bookmarked H3K27ac peaks are related to promoters of genes with roles in fundamental cellular processes among different cell types, suggesting that promoter enrichment of H3K27ac is cell-type independent.

Following the same strategy, Liu et al. (2017) investigate the protein retention at mitotic chromatin, revealing that pluripotency TFs are significantly enriched, which is in agreement with previous studies (Deluz et al., 2016; Festuccia et al., 2016; Teves et al., 2016). Further studies of the OSK ChIP-seq profiles between mitotic and interphase ESCs confirm that a large proportion (~25%–60%) of the OSK peaks are maintained during mitosis. Interestingly, about ~40%–50% of typical enhancers and 60% of super enhancers identified in ESCs remain bookmarked by these TFs, suggesting that ESC identity is maintained by the enhancer landscape that is bookmarked by TFs and H3K27ac. However, there are also some discrepancies between the current study and previous work. SOX2, for example, is reported to be only retained on a small number (<1%) of ChIP-seq peaks during mitosis (Deluz et al., 2016). This discrepancy may be caused by a fixation artifact, which is a phenomenon whereby a TF appears excluded from chromosomes after chemical fixation but is highly enriched on mitotic chromosomes as determined by fixation-free techniques such as fluorescence imaging (Teves et al., 2016). So far, formaldehyde-based crosslinking is still a standard step in ChIP experiments; therefore, all published ChIP-seq data in mitotic cells, including that of the

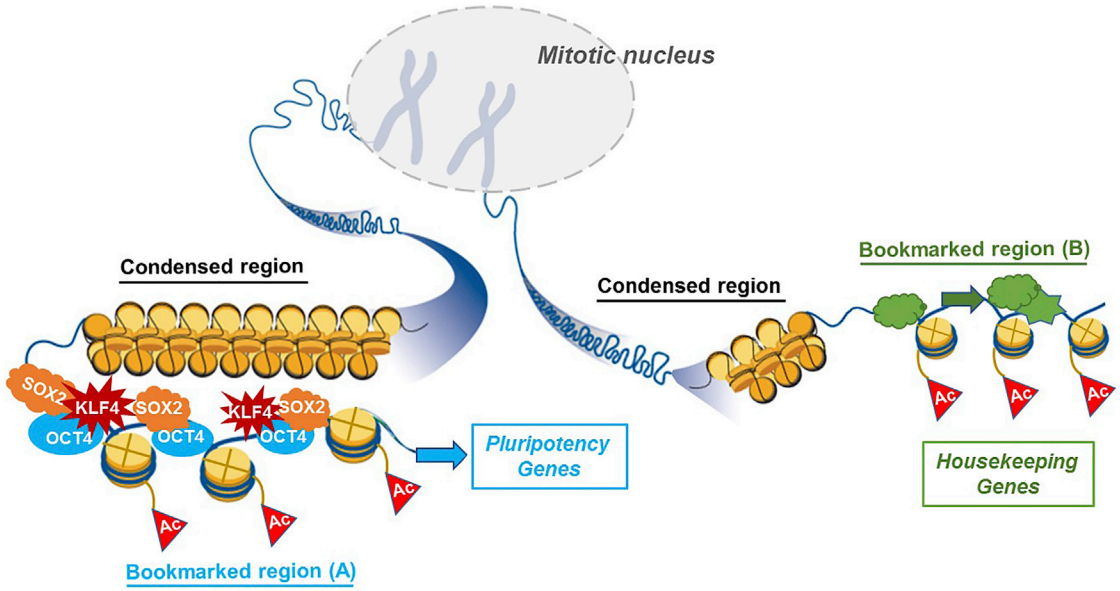

Figure 1. Mitotic Bookmarking for Maintaining Stem Cell Identity

During mitosis of embryonic stem cells (ESCs), most regions are occupied by condensed chromatin without transcription factor (TF) binding. However, some regions are bookmarked by histone mark H3K27ac and pluripotency TFs, OCT4, SOX2, and KLF4 (OSK), to maintain ESC identity. Liu et al. (2017) demonstrate that OSK together with H3K27ac are important for bookmarking enhancers of pluripotency genes (as illustrated in bookmarked region A), while H3K27ac (with other bookmarking TFs) is important for bookmarking promoters of housekeeping genes (as illustrated in bookmarked region B).

current study, may underestimate the binding intensity of any given TF. Indeed, by using a fixation-free ATAC-seq method, Teves et al. (2016) find that global SOX2 occupancy is quantitatively similar between asynchronous and mitotic cells. Therefore, an advanced technique is still necessary to overcome the limitations of ChIP for an unbiased study of bookmarked TFs at target DNA regions.

By introducing an elegant system that specifically degrades OCT4 in the M-to-G1 transition, Liu et al. (2017) demonstrate that OCT4 activity in M-to-G1 transition is required for reprogramming the somatic cell fate to a pluripotent state. However, using the same cell-cycle-dependent degradation system, Deluz et al. report that SOX2, another bookmaking TF that is a close partner of OCT4, is dispensable for reprogramming (Deluz et al., 2016). The disparate reported roles of OCT4 and SOX2 in reprogramming may be due to technical differences from the two independent studies or may suggest that OCT4 is a dominant gatekeeper compared to SOX2 in successful reprogramming (Sterneckert et al., 2012). Interestingly, the bookmarking OSK TFs in this study "coincidentally" match the three pluripotency-related Yamanaka factors, while the fourth TF, c-MYC, mainly facilitates cell division. During the G1 entry of a cell cycle, bookmarking OSK TFs gradually initiate the pluripotency gene transcription program after each cell division in the progression of reprogramming (Koche et al., 2011). Therefore, the work by Liu et al. (2017) strongly suggests that bookmarking TFs and mitosis are two requisites for cell-fate change and reprogramming by Yamanaka factors.

How cells faithfully maintain their identity during mitosis is a central question in developmental biology. The study by Liu et al. (2017) offers a synthetic view on the role of both histone marks and TFs in bookmarking pluripotent stem cell identity (as illustrated in Figure 1). This work thus advances our knowledge on how cell fate choices are implemented in ESCs and during early development, which is possibly applicable to adult and cancer stem cells under developmental and oncogenic processes.

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