

# A Determined “Hesitation” on H3K27me3 Empowers Stem Cells to Differentiate

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To uncover the precise mechanisms coordinating proliferation and fate choice of stem cells, in this issue of *Molecular Cell* and in an accompanying paper in *Cell Reports*, Mazo and colleagues (Petruk et al. 2017a, 2017b) reveal that delayed accumulation of H3K27me3 on nascent DNA is essential to recruit pioneer transcription factors in stem cell differentiation.

All stem cells, regardless of their sources, have two general features: they are capable of dividing and renewing themselves for long periods, and they can give rise to other cell types. For self-renewal, DNA sequence, along with its organization into chromatin, must be precisely inherited and maintained in cell division. In contrast, while differentiating, they change to a more specialized cell fate in which the epigenetic landscape including DNA methylation and histone modification patterns is switched to the destined cell type. Stem cell differentiation usually goes through several stages, becoming more specialized at each step. Inheritance of DNA methylation occurs immediately after DNA replication. An important component of the replication fork, Proliferating Cell Nuclear Antigen (PCNA), serves as a platform to recruit factors involved in DNA methylation including DNMT1 and UHRF1 (Sharif et al., 2007). However, compared to the faithful inheritance of DNA methylation, transmission of information during replication at the nucleosomal level may or may not be stably maintained in the face of dramatic changes of epigenetic landscape triggered by stem cell differentiation (Corpet and Almouzni, 2009). Now in *Molecular Cell* (Petruk et al., 2017a) and in *Cell Reports* (Petruk et al., 2017b), Alexander Mazo and colleagues demonstrate that upon differentiation signals, accumulation of H3K27me3 on nascent chromatin is delayed following DNA replication, providing a critical “window of opportunity” for access of pioneer transcriptional factors (TFs) associated with different cell fates

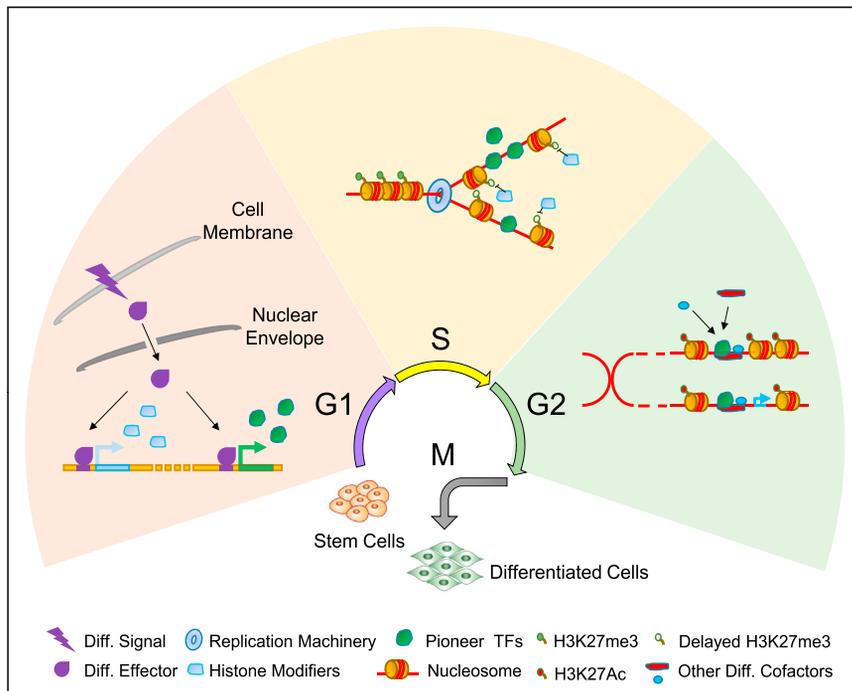
to initiate new gene transcription program and change cell fate.

Understanding the mechanisms underlying chromatin dynamics during DNA replication in the maintenance and differentiation of stem cells is of fundamental importance. A cell cycle can be divided into four distinct phases: G1 phase, S phase (DNA replication), G2 phase, and M phase (mitosis). Previous studies on cell-cycle regulation and differentiation of embryonic stem cells (ESCs) revealed that ESCs sense differentiation signals specifically in G1 phase. Using a fluorescent ubiquitination-based cell-cycle indicator (Fucci) system, several studies have shown that transcripts important for neuroectoderm (Pauklin and Vallier, 2013) and mesoderm/definitive endoderm (Singh et al., 2013) development are all selectively upregulated in G1 phase. However, the importance of S and G2 phase in ESC differentiation is less explored. Recently Huck-Hui Ng and colleagues (Gonzales et al., 2015) identified factors that associate with pluripotent state dissolution (PSD) using an siRNA screen in human ESCs and revealed that inhibition of cycle-cycle genes specifically in S and G2 phases significantly causes delay of PSD. Further investigation indicated that PSD is attenuated by DNA-damage accumulation and subsequent activation of the S phase checkpoint and upregulation of Cyclin B1 at G2 phase (Gonzales et al., 2015). Although the molecular link between ESC differentiation and DNA replication checkpoint is intriguing, the relationship between normal cell-cycle progression,

especially in the DNA post-replication (S/G2 phases) period, and ESC fate-decision remains to be fully determined.

Mazo and colleagues developed a chromatin assembly assay (CAA) to study the structure of nascent chromatin (Petruk et al., 2012). CAA is based on labeling of nascent DNA with EdU that is subsequently chemically conjugated with biotin, then the proximity of tested protein to the nascent DNA is examined by a proximity ligation assay (PLA) using antibodies to biotin and the protein of interest. Applying this unique system in *Drosophila* embryos, they have demonstrated that histone H3K4me3 and H3K27me3 are largely absent from cells in S phase and readily re-established after S phase (Petruk et al., 2012). Their further studies on the order of nucleosome assembly after DNA replication indicated that various histone modifiers are associated with PCNA during replication. By contrast, major nucleosome-remodeling complexes are subsequently recruited to nascent DNA following replication (Petruk et al., 2013). This model suggests that nascent histones are temporarily regulated by the context of PCNA with specific histone modifiers, therefore providing an opportunity for changing the fate on daughter cells after replication.

H3K27me3 is a repressive histone mark for condensed chromatin and low accessibility of TFs. In the current study, Mazo and colleagues discovered that H3K27me3 deposition on newly replicated DNA in human and mouse ESCs is delayed during early stages of the ESC differentiation (Petruk et al., 2017a).



**Figure 1. Cell Cycle-Dependent Stepwise Action of Stem Cell Differentiation**

In G1 phase, the extracellular differentiation (diff.) signal activates its downstream effectors, which upregulates differentiation-promoting pioneer transcription factors (TFs) and specific histone modifiers. During DNA replication in S phase, the histone modifiers (e.g., KDM6A as in Petruk et al., 2017a, 2017b) delay the re-assembly of H3K27me3 on nascent chromatin, providing a “window of opportunity” for pioneer TFs to access repressive genomic regions. In G2 phase, the pioneer TFs may recruit other differentiation cofactors and histone modifiers to further turn on these repressive regions (e.g., by removing H3K27me3 or changing H3K27me3 to H3K27Ac). After the cell division (M phase), cell fate will switch from a stem cell to a more differentiated state.

Interestingly, although the time window (~30 min to 1 hr) of delayed H3K27me3 re-assembly differs in human/mouse ESCs and by different differentiation signals, it is critical for the timely access of lineage-specific pioneer TFs on daughter DNA strand. Importantly, this observation is in agreement with the notion that chromatin with H3K27me3 and H3K4me3 (also called bivalent region) is considered to poise expression of developmental genes, allowing timely activation while maintaining repression in the absence of differentiation signals. By using a small-molecular inhibitor specific for H3K27me3 lysine demethylases, including KDM6A (also known as UTX), the authors further confirmed that delayed H3K27me3 re-assembly is caused by high activity of lysine demethylase (KDM), but not by inhibition of histone methyltransferase (HMT) such as EZH2.

Taken together, these results and previous studies on the role of G1 phase in ESC differentiation imply a model that lineage-

specific pioneer TFs are activated during the G1 phase while their binding to chromatin occurs at S/G2 phases (Figure 1). The model could explain how self-renewal and cell-fate commitment are balanced during ESC maintenance and upon differentiation. The next question is whether this model is generally applicable to differentiation of other multipotent stem cells and/or progenitors. In another study (Petruk et al., 2017b), the authors investigated the behavior of H3K27me3 re-assembly in hematopoietic progenitor cells (HPCs) and their differentiation, revealing that essentially the same mechanism is at play. Nevertheless, before the model can be applied to any stem cell, a few caveats must be kept in mind. First, lengthening of the G1 phase has been noted during differentiation of ESCs (Singh et al., 2013) and neural stem cells (NSCs) (Salomoni and Calegari, 2010). It seems likely that the connection between the G1 length and cell commitment ca-

capacity may also apply to other multipotent stem cells, but more work is required to address such a generality. Second, change of cell fate requires de novo assembly of histones with distinct modifications. However, it is not clear how replication-dependent de novo histone deposition and recycling of parent histones are coordinated in stem cell differentiation. Third, both histone KDMs (e.g., KDM6A and KDM6B) and HMTs (e.g., EZH2) are important for reshaping the H3K27me3 landscape. It is unclear whether KDM6A is the only histone modifier that is generally responsible for regulating H3K27me3 deposition during replication.

Overall, the two current studies by the Mazo group (Petruk et al. 2017a, 2017b) represent an important step forward in our understanding of the interplay between proliferation and fate choice of stem cells. Such knowledge not only provides a molecular explanation of the biological plasticity of stem cells but also is essential for developing novel strategies in manipulating stem cell fate for both therapeutic and regenerative medicine.

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