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# A fork in the road to differentiation

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**The symmetric inheritance of histone modifications by the nascent chromatin fibers during DNA replication is essential for proper developmental progression. Two new studies using mouse embryonic stem cells further illuminate the role of histone inheritance in early cell fate decisions.**

The preservation of cell identity relies on the maintenance of gene expression patterns over long periods of time. This is particularly challenging in proliferating cells, where the mechanisms required for cell division entail major changes in the chromatin, the molecular support of gene regulatory processes controlling transcription. Indeed, both DNA replication and mitosis lead to the eviction of many transcription regulators and the partial erasure of the chromatin modifications associated with gene regulatory states, such as post-translational histone modifications<sup>1,2</sup>. While the last couple of decades have been particularly fruitful regarding the molecular mechanisms associated with the maintenance of chromatin states during DNA replication<sup>3</sup>, a major question had not yet been addressed: to what extent is the inheritance of histone modifications during DNA replication important to control cell identity? In two papers<sup>4,5</sup> published in this issue of *Nature Genetics*, the Groth, Brickman, Andersson and Gan laboratories experimentally assess this key question, which stands at the heart of the field of epigenetics. The two studies use mouse embryonic stem (ES) cells as a model system and reach similar conclusions: interfering with the fidelity of histone modification inheritance during DNA replication leads to differentiation deficits *ex vivo*<sup>4,5</sup>, abolishes the defining capacity of ES cells to colonize the embryo upon injection into blastocysts<sup>4</sup>, and is embryonic lethal with developmental alterations appearing as soon as the 4-cells stage<sup>5</sup>. This is a key demonstration that in mammals the correct inheritance of histone modifications during DNA replication represents an essential feature of appropriate developmental progression.

The two studies take advantage of recent progress in our understanding of DNA replication, either using specific mutations of *Mcm2* (*Mcm2-2A*), a subunit of the DNA helicase whose activities are intrinsically associated with the replisome, or exploiting knock-out of *Pole3*, a subunit of the DNA polymerase complex<sup>6-9</sup>. In both cases, the resulting ES cells do not exhibit cell cycle problems and self-renew efficiently. However, they cannot symmetrically inherit parental histones in the nascent chromatin fibers upon the passage of the replication fork. Both studies show that while histone modifications are differentially enriched between the two nascent fibers in *Mcm2* or *Pole3* mutant cells, they are progressively equalized, possibly

by the interplay between the enzymes that read modifications on parental histones and write them on those that are newly incorporated and the action of transcription and its regulators<sup>3</sup>. However, they both find that repressive marks, in particular histone H3 lysine 27 trimethylation (H3K27me3), is particularly slow in being equalized between the two nascent fibers, to the point that the differences are inherited by daughter cells, producing epigenomic noise and heterogeneity.

In contrast to the naïve expectation of a progressive loss of H3K27me3 as *Mcm2* or *Pole3* mutant ES cells proliferate, both studies show a further complex scenario where the H3K27me3 landscape is reconfigured. This is particularly prominent at so called bivalent gene promoters, which are marked both by active (H3K4me3) and repressive (H3K27me3) modifications and widely characterize developmental regulators in ES cells<sup>10</sup>. H3K27me3 and components of the H3K27 trimethyltransferase complex (PRC2) are redistributed from promoters displaying high levels in wild-type cells to those characterized by lower levels. Whether this over-enrichment of H3K27me3 at selected promoters is due to histone-independent recruitment of PRC2 upon the passage of the replication fork<sup>4</sup>, or to replication-independent nucleosome assembly pathways involving the histone variant H3.3 and the HIRA chaperone<sup>5</sup>, remains somewhat unclear. However, regardless of the exact mechanisms, these observations point to a role of H3K27me3 inheritance as a determinant to specifically focus the enzymes responsible for its deposition, ensure a balanced chromatin restoration and promote epigenomic stability.

The fact that *Mcm2* and *Pole3* mutant ES cells self-renew efficiently and do not present major issues before triggering differentiation is a significant observation not thoroughly discussed in the two studies. Indeed, epigenetic mechanisms are typically seen as a way to maintain gene regulatory states and preserve cell identity. Instead, it is shown that self-renewing ES cells are largely independent of the symmetric inheritance of histone modifications, with defects clearly manifesting only upon inducing a change in cell fate. Paradoxically, this implies that preserving a balanced epigenetic inheritance, thus epigenomic stability, is primarily required to enable phenotypical changes, inverting the logic of the famous quote from *The Leopard* – “If we want things to stay as they are, things will have to change”<sup>11</sup> – in ES cells: if things have to change upon differentiation, the chromatin has to remain the same after each round of replication. Does this mean that epigenetic inheritance is more important for the acquisition of new cell fates than for their stabilization? While this is an attractive hypothesis that takes the concept of epigenetics closer to its original developmental definition<sup>12</sup>, it should be emphasized that self-renewing ES cells are perhaps not the best model to study the epigenetic maintenance of cell identity. First, because defining the identity of ES cells beyond their potential to adopt more defined identities (i.e. pluripotency), is not trivial. In this regard, it can be rightly considered that the loss of pluripotency observed in *Mcm2/Pole3* mutant ES cells represents a loss of identity. Second, because these cells are largely insensitive to the loss of histone and DNA modifying enzymes and represent a clear example of regulatory dominance by transcription factors<sup>13</sup>. Indeed, the fast restoration of symmetry between nascent fibers observed for most marks could in fact be due to the powerful activity of the transcription factors controlling self-renewal and pluripotency, since at least some are able to bind DNA almost immediately after or during the passage of the replication fork<sup>14</sup>. Nevertheless, it is noteworthy that the epigenetic inheritance of histone modifications, as clearly shown by these two studies, is

involved in the proper establishment of new cell identities, demonstrating that epigenetic control is not a mere mechanism stabilizing established cell states. Rather, it strongly contributes to the dynamic changes of cell fate required for development and, more specifically, to maintain pluripotency.

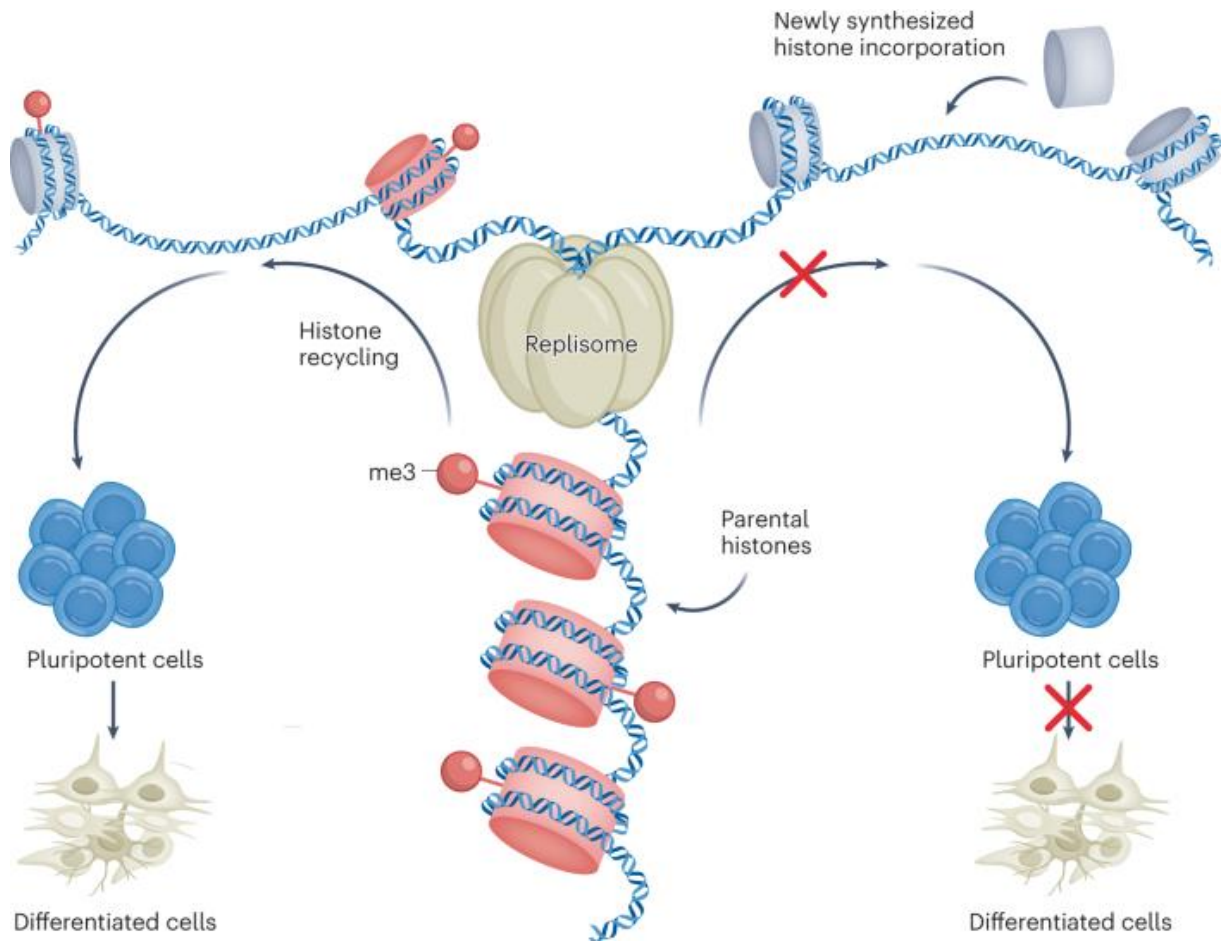


Figure 1 - **The replication balance of histone modification inheritance.** Perturbation of symmetric histone inheritance during DNA replication in embryonic stem cells negatively affects their differentiation capacity.

### Competing interests

The authors declare no competing interests.

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