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## Defining the chromatin signature of pluripotency

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► **To cite this version:**

Nathalie Beaujean. Defining the chromatin signature of pluripotency. *Nature Cell Biology*, 2024, 26 (1), pp.24-25. 10.1038/s41556-023-01268-9 . hal-04399339

**HAL Id: hal-04399339**

**<https://hal.science/hal-04399339>**

Submitted on 17 Jan 2024

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1 **Strapline: Historical News & Views**

2 **Defining the chromatin signature of pluripotency**

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7 COI statement: N. Beaujean has no conflicts of interest to declare.

8 **Abstract**

9 The interplay between DNA and its associated proteins plays a crucial role in regulating gene  
10 expression and determining cellular identity. Here we revisit an earlier *Nature Cell Biology*  
11 study that established the chromatin signature associated with pluripotency.

12 **Main text**

13 Pluripotent stem cells are a valuable tool in regenerative medicine and disease modeling due to  
14 their ability to differentiate into various cell types. These cells can exist in different states,  
15 including the so-called 'naive' stem cells, which possess the remarkable ability to give rise to  
16 any cell type within an organism. In 2006, Amanda Fisher and colleagues published a cutting-  
17 edge study in *Nature Cell Biology* that shed light on the chromatin signatures associated with  
18 pluripotency<sup>1</sup>. To investigate the replication timing of candidate genes, the researchers  
19 employed a technique based on BrdU (5-bromo-2'-deoxyuridine) pulse -labeling of the cells.  
20 They discovered that the replication timing of embryonic stem (ES) cells was distinct from that  
21 of embryonic carcinoma cells, hematopoietic stem cells, and their differentiated progeny.  
22 Specifically, lineage-specific genes replicated earlier during the S phase of the cell cycle in ES  
23 cells. Although silent, these lineage-specific genes also had high levels of acetylated H3K9 and  
24 methylated H3K4, two markers of open chromatin structures usually associated with active  
25 gene expression. Indeed, epigenetic modifications that occur on histone proteins are intimately  
26 linked with gene expression as they directly affect chromatin compaction and the accessibility  
27 of chromatin to the transcriptional machinery. On the other hand, several non-expressed genes  
28 in ES cells also had trimethylation of H3K27, which is a repressive modification. Furthermore,  
29 the authors found that loss of H3K27 methylation in ES cells resulted in the premature  
30 expression of several neural-specific genes, suggesting that lineage-specific genes in ES cells  
31 are accessible but repressed by 'opposing' chromatin modifications (Fig. 1). This result raised  
32 questions about the coexistence of active and repressive histone modifications within the same  
33 nucleosome.

34 The concept of 'bivalent' chromatin, characterized by such juxtaposition of both activating  
35 (H3K4me3) and repressive (H3K27me3) histone marks is now well accepted as a major player  
36 involved in regulating differentiation processes and mediating phenotypical plasticity during  
37 cell fate determination<sup>2</sup>. They exhibit spatiotemporal specificity during different developmental  
38 stages and when the balance between activating and repressive histone marks is disrupted upon  
39 receiving developmental signals, irreversible and spontaneous differentiation occurs (Fig. 1).  
40 Since the publication of this landmark paper, several studies confirmed that replication-timing

41 profiles can serve as a distinguishing feature for different stem cell types. A study conducted  
42 by Ryba et al. in various human and mouse stem cell types, for example, showed that  
43 differentiation-induced changes in replication timing occur in coordinated units and are  
44 correlated with transcription changes<sup>3</sup>. Additionally, it was confirmed by high-resolution  
45 analysis that ES cells and their neural progenitors have distinct replication timing profiles<sup>4</sup>.

46 The paper by Amanda Fisher and colleagues also emphasized the need for further research to  
47 understand the role of epigenetic modifications in regulating gene expression and pluripotency.  
48 In this context, recent findings have shed light on the role of DNA methylation, specifically the  
49 methylation of cytosine residues in CpG islands, in regulating transcription in stem cells<sup>5</sup>. In  
50 ES cells, it can act as a repressive or activation mark for gene expression depending on the sites  
51 of DNA methylation. Upon differentiation, DNA methylation changes gradually restrict cell  
52 potency and drive the acquisition of a more restricted, tissue-specific phenotype. Additionally,  
53 the use of mouse ES cells has provided insights into the relationships between H3K27, H3K36  
54 and DNA methylation, highlighting the importance of H3K36me2 as a major determinant of  
55 the developmental epigenome<sup>6</sup>. Remarkably, DNA methylation and histone modifications are  
56 associated with the expression state of pluripotency-associated genes, including OCT4,  
57 NANOG, and SOX2. Of note, these modifications are not limited to gene promoter regions but  
58 can occur at other locations in the genome. For example, different pluripotent states, such as  
59 naive and primed pluripotency, exhibit distinct epigenetic states at pericentromeric  
60 heterochromatin satellite sequences<sup>7</sup>. Recent research also highlighted the role of RNA  
61 methylation (m6A) in regulating stem cell maintenance and cell-fate decisions<sup>8</sup>. All these  
62 findings emphasize the interconnected nature of epigenetic modifications and how their  
63 interaction with transcription factors plays a defining role in determining cell fate and sustaining  
64 pluripotency within stem cells. This knowledge has been further used to understand how  
65 cellular identity is established and maintained during development. These modifications indeed  
66 play a crucial role in specifying the developmental stage of cells within a multicellular  
67 organism. Chromatin changes are notably observed during the reprogramming of primordial  
68 germ cells (PGCs), which is linked to DNA demethylation and the erasure of parental imprints<sup>5</sup>.

69 On the other hand, understanding the chromatin signatures associated with pluripotency can  
70 also aid in improving reprogramming methods for generating induced pluripotent stem cells  
71 (iPSCs). iPSC technology, a groundbreaking study published in 2006 by Takahashi and  
72 Yamanaka<sup>9</sup>, has revolutionized the field of regenerative medicine, offering the potential to  
73 generate patient-specific cells for transplantation and disease modeling. However, iPSCs inherit  
74 epigenetic memory from their parental cells<sup>10</sup>. This epigenetic memory, often characterized by  
75 a residual DNA methylation signature, favors differentiation along lineages related to the  
76 parental cell. Because the presence of this epigenetic memory has important implications for  
77 clinical applications, researchers are trying to enhance the efficiency and safety of iPSC  
78 generation. In particular, epigenetic modifications in iPSCs can be influenced by the culture  
79 conditions, such as three-dimensional (3D) culture, leading to changes in pluripotency-  
80 associated factors and gene expression patterns<sup>11</sup>. Researchers are also exploring the use of  
81 specific growth factors and small inhibitory molecules, such as chromatin-modifying drugs, to  
82 reset this epigenetic memory (Fig. 1). This 'reprogramming' process often involves inhibitors  
83 of signaling pathways and the resetting of the epigenetic profile to initiate the reactivation of  
84 endogenous pluripotency genes<sup>10</sup>. Thanks to these improved protocols, the epigenetic  
85 landscapes of fully reprogrammed iPSCs are found to be closer to embryonic stem cells than to  
86 the original somatic cells. As expected, this reprogramming is associated with widespread  
87 relocalization of pluripotency-associated factors such as OCT4 and rearrangement of enhancer  
88 chromatin landscapes.

89 Future research in the field holds great potential thanks to the development of new techniques  
90 to study chromatin architecture and epigenetic modifications. Several –omics methods, such as  
91 ATAC-seq to map chromatin accessibility or ChIP-Seq to map the location of post-translational  
92 modifications of histones, can now be applied at the single-cell level, providing insights into  
93 heterogeneity within pluripotent cell populations. At the cellular level, live imaging and super-  
94 resolution microscopy can be used to investigate the dynamic changes in epigenetic  
95 modifications and even perform single-molecule resolution analysis. The emergence of  
96 CRISPR-based technologies enables precise epigenome editing to evaluate the impact of the  
97 epigenome at specific genomic regions. All these methods will contribute to deepening our  
98 knowledge of gene expression regulation in stem cells and potentially lead to therapeutic  
99 applications.

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125 Figure legend

126 Fig.1: Epigenetic regulation of gene expression in cells.

127 Depending on the genomic region (pluripotency genes or lineage-specific genes), different  
128 epigenetic marks may be present, particularly DNA methylation and histone post-translational  
129 modifications, conferring a more or less “open” structure to the chromatin. These chromatin  
130 modifications are intimately linked with the state of gene expression. In the case of lineage-  
131 specific genes in ES cells, a peculiar combination of opposing modifications (so-called  
132 ‘bivalent’ chromatin) maintains silencing of these genes even if chromatin is already accessible.  
133 Upon differentiation, the cells will gradually shift towards a tissue-specific epigenomic and  
134 transcriptomic profile. Reversely, cellular 'reprogramming' involves resetting of the epigenetic  
135 profile and reactivation of pluripotency-associated genes.

136

**PLURIPOTENT CELLS**

**DIFFERENTIATED CELLS**

