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Rearranging the chromatin for pluripotency

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Pluripotent cells are characterized by infinite self-renewal and unrestricted differentiation potential with far-reaching implications in developmental biology and regenerative medicine. Somatic cells can be reprogrammed to a pluripotent state by ectopic expression of defined transcription factors (TFs) such as Oct4, Klf4, Sox2, and c-Myc, generating induced pluripotent stem cells (iPSCs). This process shows the power of TFs to overcome the epigenetic barriers that normally guard somatic cell identity and their ability to reestablish molecular and functional characteristics of pluripotency.[1]

Characterization of gene expression, DNA methylation, and histone modification patterns as well as the binding of TFs and other chromatin-associated proteins at different stages during reprogramming has been helpful for understanding the molecular mechanisms underlying induced pluripotency. In contrast, the role of 3-dimensional (3D) chromatin architecture in pluripotency and reprogramming has remained more elusive. Recent work from our and other laboratories therefore aimed at: (1) characterizing the dynamic change of 3D chromatin architecture during iPSC formation; (2) identifying molecules that are involved in long-range interactions; and (3) determining their relevance to pluripotency.

We addressed these questions by characterizing the genome-wide interaction network centered on the *Nanog* promoter in differentiated, pluripotent cells and during reprogramming.[2] Several experimental protocols have been developed in recent years to study chromatin folding.[3] We used a modified 4C (circular chromosome conformation capture) protocol coupled with deep sequencing (m4C-seq) to identify genome-wide chromatin interactions of the *Nanog* locus. This approach revealed a complex pluripotency-specific *Nanog* interactome, which was rearranged after differentiation and largely restored during reprogramming (**Fig. 1**). Meanwhile, others found that the 3D chromatin architecture around another key pluripotency gene (Oct4) also changed during mouse[4] and human reprogramming[5] by using regular 4C-seq and 3C technologies, thus reinforcing the notion that long-range chromatin interactions might be critical for the acquisition of pluripotency.

Nanog interactions were dependent on Mediator and cohesin complexes, which mediate promoter-enhancer chromatin loops.[6] Mediator and cohesin components physically associated with a large fraction of *Nanog*'s interactions in pluripotent cells, and their knockdown resulted in dramatic rearrangements of chromatin architecture from a pluripotency-specific to a differentiation-specific pattern. Notably, the conformational changes preceded morphological and transcriptional changes that normally occur upon cellular differentiation. Moreover, depletion of these proteins in somatic cells impaired iPSC formation, thus confirming their functional relevance in reprogramming. The key role of Mediator and/or cohesin in the pluripotency-associated interactome was independently confirmed by other recent studies.[4,5,7] Specific subunits of these complexes were also found to interact directly with the reprogramming factors,[2,4] suggesting that they collaborate to reorganize the 3D-chromatin architecture. Indeed,

another recent report proposed that pluripotency TFs (Nanog, Oct4, and Sox2) have a prominent role in organizing long-range interactions among their target loci in pluripotent cells,[8] supporting a similar "anchoring" function during the acquisition of pluripotency.

By analyzing the timing of long-range chromatin interactions and gene expression changes in intermediate stages of reprogramming,[2] we found that the formation of chromatin contacts around *Nanog* often preceded the transcriptional output of associated genes.[2] Consistent with this, *Oct4* long-range interactions were established specifically in the subset of cells poised to form iPSCs and before transcriptional activation of the respective genes.[4] The observation that chromatin and epigenetic modifications precede gene expression changes suggests a causative link, which warrants further investigation.

In comparing the aforementioned studies, differences in the experimental set-up, 3C/4C methodologies, and bioinformatic approaches can be sources of variability and affect the resolution of downstream analysis. For example, the m4C-seq technology, developed by our laboratory, and the related "enhanced-4C" method enable detection of weaker interchromosomal interactions but require a careful experimental design and bioinformatic analysis to control for variability and noise. In contrast, regular 4C-seq[4,8] approaches appear to favor the identification of stronger intra-chromosomal interactions with less sensitivity for trans interactions. 5C technology[7] provides an alternative, PCR-based technology with
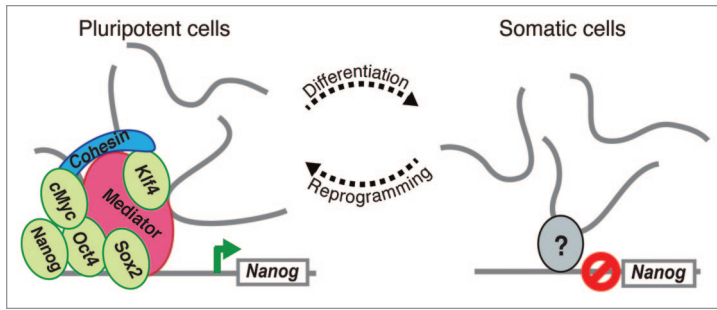
Figure 1. *Nanog* promoter engages in pluripotency-specific chromatin interactions. Mediator and cohesin complexes play key roles in establishing and maintaining the *Nanog* interactome. Pluripotency-associated TFs directly interact with Mediator and cohesin subunits. In differentiated cells, fewer interactions are found and they tend be more unstable. Moreover, the factors involved are not known.

great resolution of pair-wise interactions within spatially predefined regions, but lacks information on areas not covered by the primer sets and requires a greater amount of sequencing. A side-by-side comparison of all available methodologies seems critical to reveal relative caveats and advantages of each approach for specific applications.

Despite some methodological differences, a common theme emerging is that 3D chromatin architecture is crucial to maintenance and establishment of pluripotency. To clarify the potentially causal relationship between chromatin rearrangements and transcriptional activation, a mutational analysis of the TF

sites directly interacting with cohesin and Mediator might reveal functionally relevant domains that link the reprogramming machinery with chromatin structure. A characterization of reprogramming intermediates at different stages of iPSC formation should further elucidate the functional link between chromatin rearrangements and transcription, possibly identifying new regulators essential for coupling the 2 processes.

Many other questions remain, e.g., whether different reprogramming factor cocktails rearrange 3D chromatin structure with equal efficiency and fidelity, how the partitioning of the genome into relatively stable topological domains

impacts gene regulation, and what the distinguishing features of enhancer regions and mechanisms by which they mediate 3D interactions are. Technologies for studying 3D genome organization have been instrumental for discovering new links between epigenetic gene regulation and higher-order chromatin structure at the population level.[2,4-8] The continuing improvements in single-cell approaches are expected to provide an additional layer of resolution to this important connection.

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