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Mechanisms for enhancing cellular reprogramming

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During development, the genome adopts specific chromatin states to establish and maintain functionally distinct cell types in a well-controlled environment. A select group of transcription factors have the ability to drive the transition of the genome from a pluripotent to a more specialized chromatin state. The same set of factors can be used as reprogramming factors to reset the already established chromatin state back to pluripotency or directly to an alternative cell type. However, under the suboptimal reprogramming conditions, these factors fall short in guiding the majority of cells to their new fate. In this review, we visit the recent findings addressing the manipulation of chromatin structure to enhance the performance of transcription factors in reprogramming. The main emphasis is on the mechanisms underlying the conversion of somatic cells to pluripotency using OSKM. This review is intended to highlight the windows of opportunities for developing mechanistically based approaches to replace the phenotypically guided methods currently employed in reprogramming, in an attempt to move the field of cell conversion towards using next generation technologies.

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Introduction

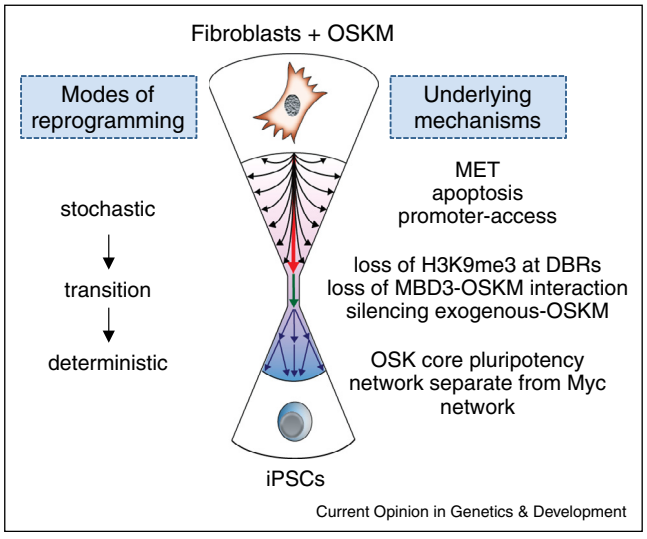
Within adult tissues and organs, fully differentiated cells rarely, if ever, change from one type to another. Somatic cells can be forcibly reprogrammed to pluripotency by nuclear transfer experiments, in which the somatic genome is exposed to a large number of factors found in the egg cytoplasm [1]. Thus it seems remarkable to discover that it can take so few transcription factors to convert cells from one somatic type to another. For instance, MyoD alone can reprogram fibroblasts to myoblasts [2], but more strikingly, the four transcription factors: Oct4, Sox2, Klf4, and c-Myc (OSKM) are able to convert fibroblasts to induced pluripotent stem cells (iPSCs) [3]. However, somatic cells still show a high resistance to such transcription factor-based reprogramming, raising a major obstacle in understanding the molecular mechanism underlying cellular conversion to pluripotency. With this in mind, many studies have recently reported various ways to enhance reprogramming, usually involving a change in the chromatin state of somatic genome to enable a change in cell fate.

Our understanding so far is that the conversion of somatic cells to pluripotency follows a step-wise process (recently reviewed in [4–6]). However, it has been argued that reprogramming can operate in two modes; a stochastic mode, by which iPS colonies appear with variable latencies, and a deterministic mode in which differentiated cells follow a hierarchal process to pluripotency (Figure 1) [7,8••]. Here we will review recent findings of how the different methods to enhance reprogramming fit this model, with a specific emphasis on the chromatin-basis behind both the pliancy and the rigidity of the somatic genome.

The pioneer concept in reprogramming

A select group of transcription factors, but not others, have the mechanistic ability to reprogram cells. It is intriguing to note that transcription factors involved in the early stages of embryonic development have provided an attractive route for cell fate conversion [9]. Pioneer factors are expressed early in development, and represent a special class of factors that can bind target DNA on nucleosomes [10–13]. This allows pioneer factors to engage silent chromatin and endow the competence for subsequent gene activation [14]. Early in reprogramming, the OSK factors, but not c-Myc, are able to access closed chromatin at distal element and before activation of silent genes, including those necessary for pluripotency such as *ESRRB* and *SALL4* [15••]. The pioneer activity of Oct4 has been confirmed in the maternal-to-zygotic transition at which Oct4 occupies SOX-POU binding sites before the onset of zygotic transcription [16•,17•]. The pioneer activity of Oct4 has been carefully assessed in binding to the enhancer elements of *NANOG*, *POU5F1*, and *MYOD1* genes [18,19]. The concept of pioneer factors expands beyond reprogramming to pluripotency as shown for the case of Ascl1, which can convert fibroblasts to become induced neuronal (iN) cells [20••]. Altogether, pioneer factors seem to possess an inherent ability to prime the genome to become susceptible for adapting chromatin states, which are more suited for alternative cell types.

Figure 1



Reprogramming somatic cells to pluripotency is initiated by a stochastic phase followed by a deterministic phase. The ectopic expression of OSKM in fibroblasts drives cells to go through many pathways stochastically (represented by black arrows). Some of these routes represent dead ends and others will lead to successful reprogramming (red arrow). The transition phase (green arrow) is a hallmark of initiating a cascade of deterministic events (blue arrows) resulting in fully reprogrammed iPSCs. The main pathways and processes that define each phase are displayed on the left.

Dissecting OSKM function in reprogramming by a transcription factor substitution approach

Soon after the discovery of OSKM, another set of factors including Oct4, Sox2, Nanog, and Lin28 have been shown to convert somatic cells to pluripotency [21]. Despite these factors being picked as candidates for reprogramming on the basis of their role in pluripotency in ES cells [3,21,22], subsequent studies have attempted to dissect the role of OSKM in reprogramming by using substitutes, and surprisingly showing that they can be replaced with functionally divergent factors. For example the nuclear receptor Nr5a2 and its close family member Nr5a1 are capable of both enhancing reprogramming and replacing Oct4 [23]. The pioneer factors Gata3, Gata4, and Gata6 can replace Oct4, while Sox1 and Sox3 can replace Sox2 if combined with Klf4 and c-Myc [24••,25••]. The reprogramming activity of Oct4 is conserved among species, as axolotl Oct4, the xenopus Oct91, as well as medaka Pou2 are each able to act together with mouse SKM to generate iPSCs from mouse fibroblasts [26•,27]. Oct1 and Oct6 are thought to lack reprogramming capability due to their unstructured linker region between Pou-specific and Pou-homeodomain; the two DNA-binding domains found in the POU (Pit1, Oct1/Oct2, UNC-86) family members [28•]. Other Sox family members including Sox7 and Sox17 can be converted into reprogramming factors by engineering a single mutation within the High-Mobility-Group (HMG) DNA-binding domain that promotes co-binding with Oct4 to the canonical Oct-Sox motif [29,30]. Interestingly, Oct4 can switch partners from Sox2 to Sox17 for endoderm specification and binds a compressed version of the Oct-Sox motif at enhancers [31]. Similarly, Sox2 switches partners with another Pou-family member BRN2 (Pou3f2) co-occupying enhancers for neuron progenitors [32]. Sox2 targets yet another distinct network for specifying trophoblast stem cells and marks adult cells in several epithelial tissues [33,34]. Esrrb, an orphan nuclear receptor that is expressed highly in ES cells, has been reported to replace Klf4 [35]. c-Myc was the first factor found to be dispensable for reprogramming and can be replaced by other family members such as n-Myc and l-Myc, as well as by the maternal factor Glis-1 [36–38]. The ability of maternal factors to enhance reprogramming may reflect the potent ability of oocytes to reprogram somatic cells. It is important to note that pioneer factors also appear to interfere with OSKM and counteract their induction to pluripotency, once co-expressed with OSKM [39,40]. Remarkably, these interfering factors extensively co-bind with OSKM [39]. For example, Gata4 can replace Oct4 in reprogramming, yet Gata4 blocks reprogramming if co-expressed with OSKM [24••,40]. This indicates that OSKM substitutes engage the somatic genome by binding many overlapping targets, creating alternative, yet conflicting, routes to pluripotency.

Dissecting OSKM function in reprogramming by small molecule substitution

Small molecules can also enhance reprogramming and replace the four OSKM factors. Vitamin C enhances the generation of iPS cells, at least partly, due its Tet-dependent induction of DNA demethylation [41–43]. Valporic acid (VPA), suberoylanilide hydroxamic acid (SAHA), trichostatin A (TSA), and butyrate are histone deacetylases (HDAC) inhibitors that can improve reprogramming and replace c-Myc [44,45]. Tranylcypromine, an H3K4 demethylation inhibitor, also promotes iPSC generation in the absence of c-Myc [46]. 5-Azacytidine (AZA), a DNA methyltransferase inhibitor, also enhances reprogramming [47,48]. Kenpaullone, like CHIR99021, both inhibit GSK-3β and increase OSKM-based reprogramming and Kenpaullone can replace Klf4 [49]. Tgf-β inhibitor (named 616452), on the other hand, can replace Sox2 and induce reprogramming [50,51]. Forskolin (FSK), a cAMP agonist, can act as a chemical substitutes for Oct4 [52•]. The small-molecule combination of VPA, CHIR99021, 616452, and Tranylcypromine enables reprogramming of mouse fibroblasts with only a single gene, Oct4 [46]. And more dramatically, the combination of CHIR99021, 616452, Forskolin and DZNep, an *S*-adenosylhomocysteine (SAH) hydrolase inhibitor, can replace all four reprogramming factors [52•]. Albeit, this cocktail of chemicals can induce pluripotency in mouse