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Epigenetics of Cellular Reprogramming

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Abstract

Cells are constantly changing their state of equilibrium in response to internal and external stimuli. These changes in cell identity are driven by highly coordinated modulation of gene expression. This coordinated regulation is achieved in large part due to changes in the structure and composition of the chromatin, driven by epigenetic modulators. Recent discoveries in cellular and genomic reprogramming have highlighted the importance of chromatin modifications to reach and uphold the fidelity of target cell states. In this review, we focus on the latest work addressing the mechanisms surrounding the epigenetic regulation of various types of reprogramming, including somatic cell nuclear transfer (SCNT), cell fusion and transcription factor- and microRNA-induced pluripotency. The studies covered herein showcase the interplay between these epigenetic pathways, and highlight the importance of furthering our understanding of these connections to form a clearer picture of the mechanisms underlying stable cell fate transitions.

INTRODUCTION

Cells have a specific molecular and physiological identity that dictates their function. However, many cell types are highly plastic and can transition efficiently from one type to another. This process requires loss of the molecular characteristics of the original cell, and acquisition of an entirely new but heritable molecular signature, in the context of an unchanging genomic sequence. This process, known as epigenetic reprogramming, often involves changes in transcription and chromatin structure as a result of changing covalent modifications on chromatin. Epigenetic reprogramming is highly temporally and spatially regulated, and a plethora of players cooperate to carefully orchestrate this process. There has recently been a large push towards understanding how to manipulate epigenetic changes to help convert one cell type into another in vitro.

Historically, the study of embryonic development, including fertilization of an oocyte and specification of primordial germ cells, has informed our view of epigenetic reprogramming. Recently, with the discovery of somatic cell reprogramming, studies have expanded to

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analyzing epigenetic reprogramming of diverse cell types in vitro. In vitro studies have made understanding of the molecular mechanisms of epigenetic reprogramming more attainable. This review focuses on recent progress made in understanding the dynamic epigenetic changes that are required to accurately and efficiently reprogram the epigenome of one cell type into another. We compare different methods of reprogramming cells from one type to another, and identify key epigenetic players that regulate these transitions. There are certain broad changes during reprogramming that have been identified in recent years, including genomic demethylation (both histone and DNA), histone acetylation and loss of heterochromatin (Fig. 1 and summarized in two excellent recent reviews [1,2]). The exact mechanisms by which these changes are achieved and the detailed interplay between the players responsible however remain relatively unclear. And while the pathways utilized during different forms of experimental reprogramming are not necessarily the same, there are emerging patterns common to most if not all cell state transitions.

SOMATIC CELL NUCLEAR TRANSFER

Somatic cell nuclear transfer (SCNT), involving the transfer of a somatic nucleus into an enucleated oocyte to produce cloned animals, is the earliest example of experimentally induced programming [3]. Interestingly, cloned animals have reduced survival relative to naturally fertilized embryos, and it is widely hypothesized that this lethality is due to improper epigenetic reprogramming in both the embryonic and extra-embryonic lineages [4]. Aberrant DNA methylation has been observed in swine, bovine and murine cloned embryos compared to their fertilized counterparts [5–7]. Repeat regions are especially susceptible, both in cow and mouse embryos. In both cases, satellite repeats (Satellite I repeat in cows, and LINEs and LTRs in mice) remain hypermethylated in SCNT embryos relative to wild-type counterparts. The transfer of globally hypomethylated somatic nuclei cells improves the efficiency of reprogramming of those nuclei by SCNT [8]. Additionally, fertilization-specific demethylation at specific promoters fails to occur during SCNT in mouse, suggesting the absence of signals directing specific demethylation events [5,6]. Together, these data support the notion that defects in DNA demethylation in part underlie the reduced survival of cloned embryos.

In recent years, genome-wide methylation has been shown to be more dynamic than previously thought, providing insights on how regulation of methylation contributes to epigenetic plasticity. During DNA demethylation, 5-methyl cytosines (5mc) can be converted to 5-hydroxymethyl cytosines (5hmc) by the Tet family of proteins [9]. Tet3, which is expressed in the oocyte, localizes to the somatic pseudo-pronucleus upon SCNT and Tet3 knockout oocytes fail to de-repress somatic Oct4 following SCNT [10]. 5hmc may be more than just a demethylation intermediate as it has been shown to have specific binding partners [11,12]. Furthermore, changing DNA methylation has to be accompanied by modulation of other epigenetic modifications in order to achieve a reprogrammed epigenome, and it is likely that these events are co-regulated (Fig. 2).

One way to achieve coordinated regulation of different epigenetic factors is to have common signaling molecules. It has been postulated that Vitamin C, a known co-factor for histone demethylases, might also work through the Tet family of DNA demethylating enzymes

[13,14]. Vitamin C can enhance blastocyst formation following SCNT and is associated with enhanced histone acetylation and increased expression of pluripotency markers such as Sox2 and Klf4 [15] (Fig. 2). Indeed, histone deacetylase (HDAC) inhibitors improve SCNT efficiency [16,17]. Vitamin C is unlikely to directly influence histone acetylation as it has not been shown to be a cofactor of acetyltransferases. Rather it may influence acetylation indirectly by acting through demethylase, tying together several epigenetic pathways (Fig. 2)

Tet-dependent hydroxymethylation of the somatic genome during reprogramming may result in recruitment of additional epigenetic modifiers. It is known that embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have higher levels of hydroxymethylation than somatic cells, further supporting a functional role for that modification and suggesting that the presence of 5hmc might help drive the unique epigenetic environment required to achieve pluripotency [18]. So the defects in the Tet3 mutant could be either due to persistence of 5mc or absence of 5hmc (or perhaps a combination of both). Efficiency of SCNT also seems to be associated with the histone modification status of the donor genome. Using nuclei from fibroblasts pre-treated with ooplasm greatly improves SCNT efficiency, specifically reducing histone H3K9 methylation and increasing acetylation [19]. Taken together, these papers showcase the importance of multiple pathways cooperating to coordinate reprogramming of various epigenetic modifications during SCNT (Fig 2.).

Modulation of histone and DNA marks goes hand-in-hand with changes in chromatin structure, which impacts the expression of underlying genes, and therefore, the state of the cell. Epigenetic reprogramming to pluripotency requires the dramatic loss of heterochromatin, which includes the re-activation of the inactive X chromosome that is characteristic of somatic cells. Intriguingly, oocytes are relatively ineffective at re-activating the X-chromosome following SCNT (as measured by aberrant expression of *Xist* RNA, the non-coding RNA responsible for coating and inactivating one X), suggesting that other areas of heterochromatin may also be poorly resolved [20]. The knockdown of the histone variant macroH2A facilitates X-reactivation through loss of heterochromatin, and improves pluripotency gene expression, in the context of SCNT in Xenopus oocytes [21]. However, the role of canonical X-inactivation factors does not appear to be restricted to reactivation of the X chromosome during epigenetic reprogramming. SCNT-derived embryos have ectopic expression of Xist associated with global mis-regulation of many genes until the morula stage in SCNT-derived embryos. RNAi-mediated knockdown of Xist improves the survival of mice cloned by SCNT[20]. This result suggests that preventing Xist expression during pre-implantation development helps SCNT-derived embryos obtain an epigenetic state beneficial for survival, and provides a yet unknown and uncharacterized role for Xist that extends beyond X-inactivation. Further investigation into the connection between chromatin modifications and general chromatin structure and architecture will shed light on how global changes in chromatin are coupled with highly specific changes in gene expression (Fig. 2).

REPROGRAMMING BY CELL FUSION

Oocytes are not the only cell type capable of reprogramming other cells. As early as half a century ago, scientists discovered that fusion of two somatic cell types resulted in epigenetic

reprogramming, highlighting the epigenetic plasticity of terminally differentiated cells [22]. About fifteen years ago, Surani and colleagues discovered that fusion of embryonic germ cells (EGCs – pluripotent cells derived from primordial germ cells) with thymic lymphocytes leads to the demethylation of imprinted loci as would normally occur during PGC reprogramming [23]. This experiment showed that these pluripotent cells possess the ability to override a somatic cell's inherent program providing an exciting platform to identify the factors that contribute to reprogramming. For example, the ability of ESCs to reprogram fibroblasts is dependent on the cell cycle stage of the ESCs in question. Specifically, cells in the S/G2 phases are more likely to successfully reprogram somatic cells than cells in G1, suggesting that the ongoing DNA synthesis is required to erase the memory of the fibroblast genome [24].

In fact, ESCs reprogram somatic cells in a bi-phasic manner, the second of which appears to require DNA synthesis. The somatic nucleus first undergoes a 'trans-reprogramming' that occurs with rapid kinetics, and has been hypothesized to require the rapid action of trans-activating transcription factors present in the ESCs. Following this, the somatic genome experiences 'cis-reprogramming', which requires a round of replication and has much slower kinetics, suggesting that some parts of the somatic chromatin are resistant to trans-reprogramming until they have been 'rebooted' by DNA replication [25]. However, it has recently been shown that pluripotency in heterokaryons can be achieved rapidly without cell division or DNA replication showing that cis-reprogramming is not always required [26].

Key trans-factors for reprogramming are now beginning to be identified, including a number of epigenetic factors. For example, two members of the Tet family of DNA demethylases, Tet1 and Tet2, have critical yet distinct roles in reprogramming during cell fusion. While Tet2 has a global demethylation role during cell fusion, and depletion of Tet2 in EGCs greatly reduces the efficiency with which they reprogram human B cells, Tet1's role appears to be focused on the demethylation of imprinted control regions [27]. This difference highlights once again how coordinated and regulated the process of reprogramming is, to the extent that two highly related proteins perform different non-redundant functions, both of which contribute to the ultimate success of reprogramming (Fig. 2).

While reprogramming to pluripotency is often associated with an increase in active chromatin marks, ESCs and EGCs lacking functional polycomb complex 2 (PRC2, responsible for depositing the repressive mark H3K27me3) are unable to reprogram B-cells upon fusion [28]. This suggests that the active repression of lineage-specific genes is also important. However, knockout of PRC2 components (and therefore loss of H3K27me3) does not cause the loss of pluripotency in ESCs [29,30]. PRC is therefore critical for the transition of a somatic genome to a pluripotent one, but not for its maintenance, suggesting that resulting de-repression of lineage-specific genes is not sufficient to exit pluripotency.

TRANSCRIPTION FACTOR- AND MIRNA-INDUCED PLURIPOTENCY

Cell fusion experiments highlight the importance of ESC trans-factors for reprogramming the somatic genome. This concept led Yamanaka and colleagues to identify key pluripotency-related transcription factors that when overexpressed can reprogram somatic

cells into induced pluripotent stem cells (iPSCs)[31]. This exciting discovery not only led to an explosion in the field of reprogramming, but also considerably advanced our understanding of stem cell biology. In the last few years, many studies have focused on elucidating the mechanisms by which exogenously introduced transcription factors can reprogram somatic cells to a pluripotent state. A recent review by Papp and Plath summarizes in great depth the relationship between chromatin state and reprogramming to pluripotency, as well as the role of various chromatin-modifying factors on the efficiency of reprogramming [1]. Here, we will focus on the interplay between transcription factors, microRNAs and epigenetic modifiers during this cell state transition.

There appear to be multiple stages in preparing the chromatin for pluripotency, each of which requires different players. Recent work has shown the broad requirement for a number of epigenetic modifiers for preparing cells for pluripotency [32], including identifying individual roles for specific proteins. For example, the histone H3K27 demethylase Utx is not necessary for maintaining pluripotency, but is required for somatic cells to transition to iPSCs [33]. Similarly, the interplay between the methyltransferases and demethylases of histone H3K9 establish a pre-pluripotency state that is responsive to BMP signaling [34]. The down-regulation of BMP signaling causes a switch of the H3K9 methylation status of pluripotency genes, resulting in their expression. The authors suggest that demethylation of repressive histone marks can establish a ‘pluripotency primed’ state during reprogramming. This state is not fully pluripotent, but in response to vitamin C (which is likely regulating the H3K9 demtheylases), the cells overcome the barrier separating the “primed” and fully pluripotent states (Fig. 2). It is conceivable that before iPSCs can acquire the appropriate transcriptome, their epigenome must be prepared in advance to initiate and sustain the expression of key genes [35].

Establishing the correct DNA methylation state of specific loci is vital to set up this pluripotency-permissive epigenetic state. Tet2 is required to increase 5-hydroxymethyl (5hmc) levels at critical loci. 5-methylcytosine (5mc) on the other hand is also regulated independently by additional factors such as the chromatin-binding protein PARP-1 [36]. This uncoupling of 5hmc and 5mc lends support to the growing theory that 5hmc is its own epigenetic modification rather than just a demethylation intermediate. It suggests that the factors recruited by 5hmc during reprogramming might be required in establishing an epigenetic state that allows complete reprogramming to pluripotency. Furthermore, recent work has shown that Oct4 can be replaced by Tet1 during reprogramming of mouse fibroblasts. Both methylation and hydroxymethylation are increased during the intermediate stages of reprogramming [37]. Interestingly, while Tet1 can replace Oct4, physical interaction with Nanog appears to be important for Tet1 function during reprogramming. In a recent study, Nanog is proposed to recruit Tet1 to pluripotency loci for hydroxymethylation and activation of gene expression [38]. These papers suggest that tight regulation of the balance between methylation and hydroxymethylation is critical for successful transition of the epigenetic landscape towards pluripotency.

Epigenetic modifiers are sensitive to environmental stimuli, raising the question of how external factors can affect somatic cell reprogramming. Recent work reveals that reprogramming is greatly improved by addition of vitamin C, as is the case with SCNT

[13,14,34,39,40]. This improved efficiency could be through the action of histone demethylases, as suggested by the requirement of H3K9 and H3K36 demethylation for iPSC formation [34,41]. Intriguingly, the H3K36 demethylase Kdm2a is vitamin C-dependent and promotes expression of cell cycle genes by demethylating and repressing Ink4a/Arf (Fig. 2). This highlights the importance of repressing of the somatic program in addition to activating the pluripotency program to achieve iPSC status. Indeed, during de-differentiation, a vast majority of cells are refractory to reprogramming, suggesting inadequate silencing of areas of chromatin regulating the somatic transcriptional program [42,43].

Kdm2a also cooperates with Oct4 to activate the miR302 family of microRNAs, which might further promote the transition to pluripotency. The miR302 family is part of a broader family of miRNAs, the ESCC miRNAs, known to regulate the unique cell cycle properties of ESCs [41,44]. Recent work has shown that miR302 and other ESCC family members, are capable of reprogramming mouse and human somatic cells to iPSCs, either alone or in concert with other factors [45–48]. In fact, among the targets of miR302 in human ESCs are a number of chromatin regulators such as MECP2, MBD2, LSD1 and SMARCC2, further highlighting the link between different methods of epigenetic modulation (for example, miRNAs and methylation) [48,49].

Covalent modification of chromatin is only one step in establishing the appropriate epigenetic environment. Modified chromatin recruits a multitude of other factors that regulate the structure of chromatin as well as the transcription of underlying genes. One example of a newly discovered chromatin-binding factor that affects reprogramming is the telomere-associated Zscan4 [50]. Although it is known that pluripotent cells maintain longer telomeres, and that Zscan4 has been shown to extend telomere length, it only enhances somatic cell reprogramming in the first 3 days of the process, suggesting roles that potentially extend beyond the maintenance of telomere length, as is the case in ESCs [50,51]. Indeed, Zscan4 has been shown to increase genomic stability during iPSC formation, and although it is known that telomeres rejuvenate during reprogramming, Zscan4 may also be acting via additional mechanisms [52]. For example, greatly altering the epigenetic state of a cell may cause undesirable transcriptional instability that can be buffered by factors such as Zscan4 [53].

FUTURE PERSPECTIVES

The highly complex regulatory mechanisms that define a cell state are carefully orchestrated. In attempting to replicate a specific cell state it is critical to mimic these mechanisms as closely as possible. Central to achieving that goal is a more thorough understanding of the epigenetic state of fully reprogrammed cells. For example, in order to achieve stable and high-fidelity iPSCs, it is essential to be able to reproduce the epigenetic environment of a pluripotent cell as accurately as possible, thus arming the iPSCs with the potential to produce normal downstream lineages.

While making ESC-like cells from somatic cells could transform the landscape of regenerative medicine, an alternative strategy is to directly convert one somatic cell type to another, termed transdifferentiation. Epigenetic reprogramming almost certainly plays an

equally important role in transdifferentation as it does in dedifferentiation to iPSCs, although very little is known in this context. One recent example is the transdifferentiation of pre-B cells to macrophages, during which key genes are hydroxymethylated. The transition fails in the absence of Tet2, suggesting that demethylation is critical for transdifferentiation, as is the case with reprogramming to pluripotency [54]. It will be important to determine whether there are intermediate or transitory epigenetic states during transdifferentiation, and how fully reprogrammed the final products are (Fig. 3). For example, are there certain molecular benchmarks that need to be reached, as is the case with reprogramming to pluripotency? What are the roles of stochasticity and hierarchy? How much memory of the originating fate remains following transdifferentiation? Similar to the production of iPSCs, answering these questions will allow for optimization of current methods of transdifferentiation.

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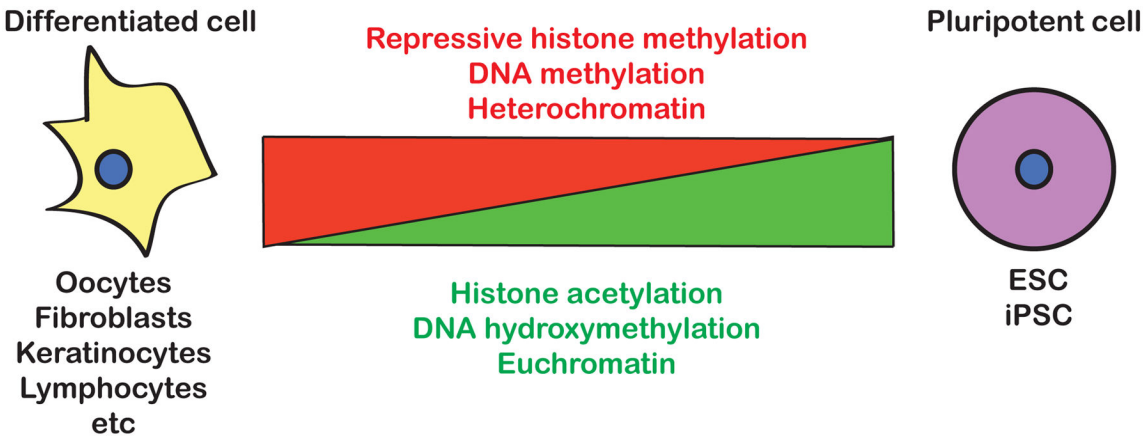

Fig. 1.

Key epigenetic changes during the transition between differentiated and pluripotent cells.

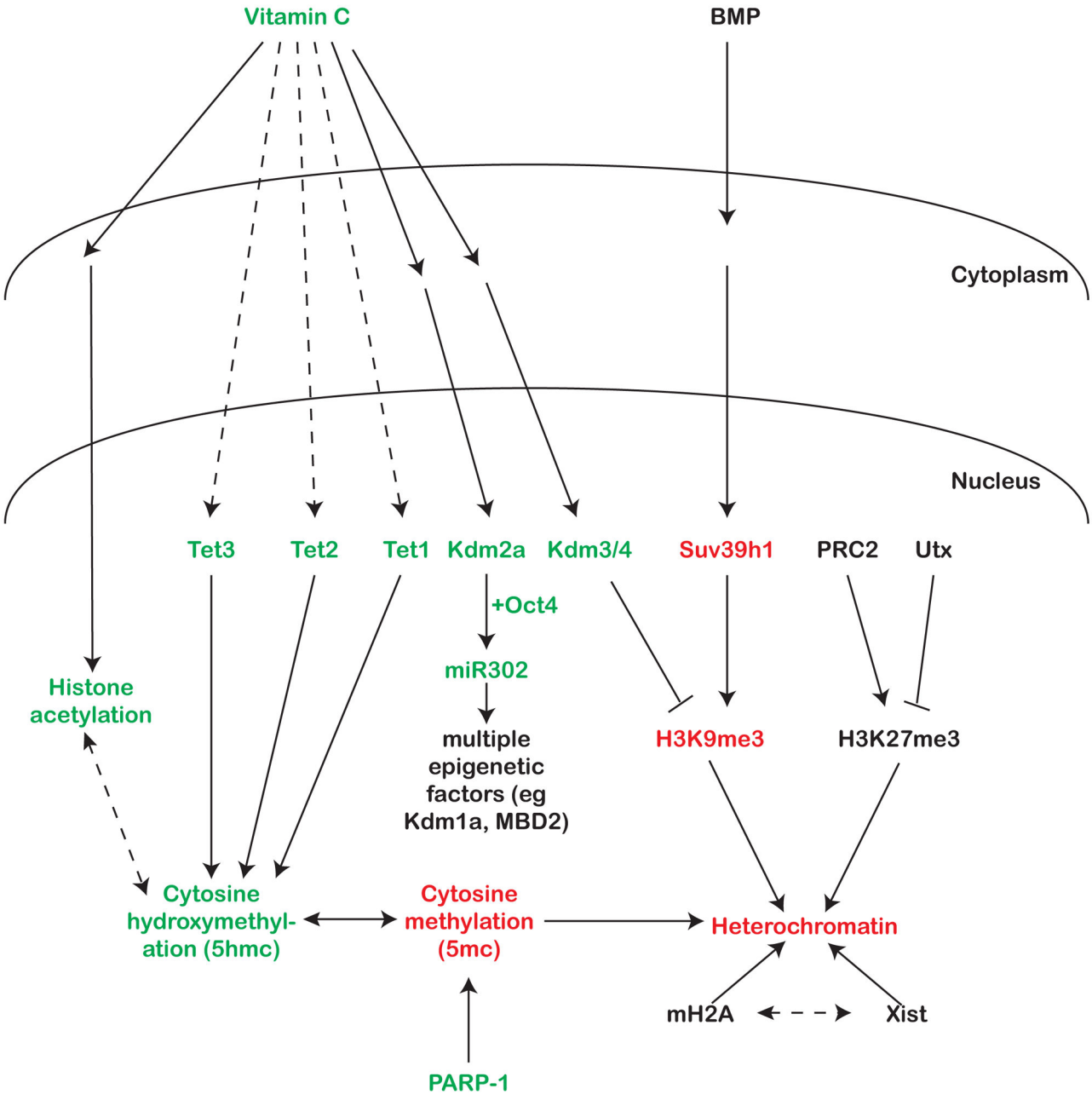

Fig. 2.

Pathways influencing the epigenetics of reprogramming. Green text -known enhancers of reprogramming. Red text - known inhibitors of reprogramming. Black text - unknown, unclear, or many different effects on reprogramming. Solid lines represent known connections and dashed lines represent speculative connections between factors.

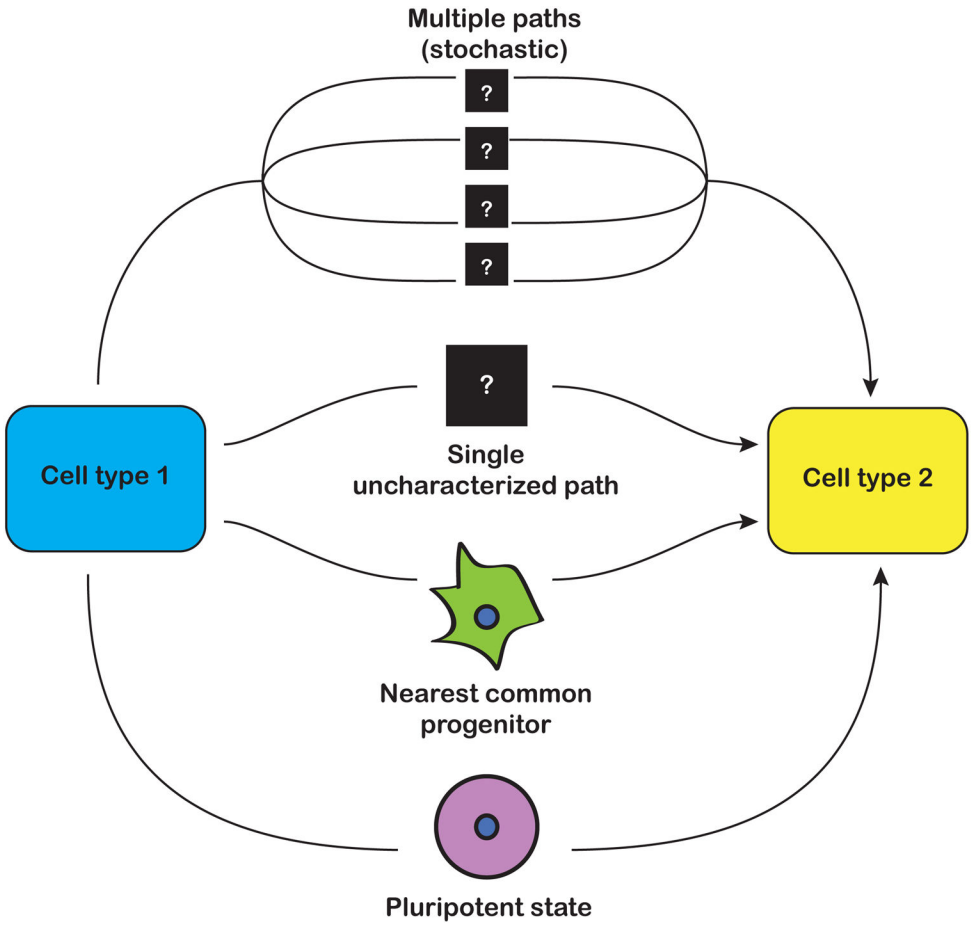

Fig. 3.

Unanswered questions in the field of transdifferentiation. Transitioning from one cell type to another could involve going through a pluripotent state, the nearest common progenitor state, or one or more unknown states.