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The Chromatin Accessibility Landscape in Cell Plasticity and Reprogramming: Understanding and Overcoming the Barriers

Diyi Yang[1,2] | Xingting Guo[1] | Rongwen Xi[1,3]

[1]National Institute of Biological Sciences, Zhongguancun Life Science Park, Beijing, China | [2]Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China | [3]Tsinghua Institute of Multidisciplinary Biomedical Research, Tsinghua University, Beijing, China

Correspondence: Xingting Guo (guoxingting@nibs.ac.cn) | Rongwen Xi (xirongwen@nibs.ac.cn)

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ABSTRACT

Cell plasticity enables the dynamic changes in cell identities necessary for normal development and tissue repair. Induced cell reprogramming, which leverages this plasticity, holds great promise for regenerative medicine and personalized therapies. However, the success of cell reprogramming is often impeded by various molecular barriers, such as epigenetic marks, cell senescence, and the activation of alternative or refractory routes. In this review, we examine the cell reprogramming events that occur within or between germ layers and adult stem cell lineages and propose that the overall similarity in the pre-existing chromatin accessibility landscape is a major determinant of reprogramming efficiency from one cell type to another. A better understanding of the regulation and control of chromatin accessibility should facilitate the development of new methods and strategies to improve cell reprogramming efficiency and advance translational research.

1 | Introduction

In 1962, John B. Gurdon [1] demonstrated a groundbreaking concept by showing that differentiated intestinal nuclei from frogs could, when inserted into nuclei-deprived frog egg cells, give rise to normal frogs. This pivotal discovery revealed that the DNA in differentiated cells retains the complete genetic information required to develop into various tissue types, underscoring the concept that cell fate is not irrevocably fixed. More than four decades after Gurdon's pioneering work, Shinya Yamanaka and his team introduced four specific genes—*Oct4*, *Sox2*, *Klf4*, and *c-Myc* (collectively known as OSKM or "Yamanaka factors")—into adult mouse cells (embryonic fibroblasts and adult tail fibroblasts) using retroviral vectors. This intervention successfully reprogrammed these cells into a pluripotent state similar to that of mouse embryonic stem cells, thus creating induced pluripotent stem cells (iPSCs) [2]. Together, these discoveries highlight the fundamental concept of cell plasticity, the ability of cells to change their properties and differentiation states.

Cell plasticity is essential for normal development, tissue regeneration, and homeostasis. The multipotent tissue stem cells play important roles in maintaining tissue homeostasis and regeneration [3–5]. The intrinsically high plasticity in certain specialized cells also allows them to contribute to the maintenance of tissue integrity, especially following damage. For example, after liver injury or partial hepatectomy, bile duct epithelial cells can proliferate and reprogram to replace lost hepatic cells, facilitating

liver regeneration and restoring liver function [6, 7]. Similarly, when intestinal stem cells (ISCs) are compromised by pathogen infection, differentiated intestinal cells such as enterocytes (ECs) or enteroendocrine cells (EECs) can dedifferentiate to replenish the ISC pool [8–10].

To date, a wide range of successful cell fate manipulations, including the generation of iPSCs from various starting cells and direct transdifferentiation between distinct specialized cell types [11], have been achieved through the use of endogenous factors [1, 2] or exogenous chemical compounds [12, 13]. Cell reprogramming holds significant promise for clinical applications, particularly by utilizing patient-derived cells for therapeutic purposes, which may reduce organ transplantation-related challenges such as immune rejection or donor scarcity [14]. Despite the promise of directed cell reprogramming, the field faces unresolved challenges, including issues with delivery efficiency, incomplete reprogramming, and the risk of tumor formation due to sustained ectopic expression [2]. Addressing these challenges is essential for advancing the clinical translation of reprogramming technologies.

Over the past few decades, extensive research has identified various barriers to cell reprogramming, including cellular and molecular constraints [15–17], structural limitations (e.g., extracellular matrix composition) [18, 19], cell–cell communication dynamics [20], and signaling pathways [21]. Based on a recent finding from our group, along with the literature overview, here we propose that the similarity in chromatin accessibility landscapes, which is usually predictable based on the phylogenetic relationship between the original cell type and the target cell type, may serve as a major determinant of reprogramming efficiency.

2 | Similarity in Chromatin Accessibility Landscape: A Major Determinant in Reprogramming Efficiency

2.1 | *Drosophila* Study Reveals Robust Cell Identity Plasticity Between Specialized Cells Within a Common Stem Cell Lineage

Due to the advantage in genetic manipulation and conservation of regulatory mechanisms, the *Drosophila* midgut epithelium has become an attractive experimental system to study stem cell self-renewal and cell lineage differentiation [22, 23]. The renewal of the midgut epithelium is maintained by ISCs, which periodically generate committed progenitor cells, which then further differentiate into either ECs or EECs [24, 25]. We have recently shown that these intestinal epithelial cells—including ISCs, ECs, and EECs—exhibit a high degree of cell plasticity. Depletion of a single transcription factor (TF) named *Tramtrack* induces direct reprogramming of ECs into EEC identity, while depletion of the EEC fate regulator *Prospero* induces dedifferentiation of EECs into ISC identity, with a reprogramming efficiency of approximately 20% and 30%, respectively [26]. Further chromatin accessibility analysis by ATAC-seq suggests that this high interswitchable ability in cell identity may be due to a very similar chromatin accessibility landscape among cells within this stem cell lineage, including a very similar chromatin accessibility on gene loci of TFs that determine cell identity of these cells. The highly similar pre-existing chromatin accessibility landscape may provide an amenable chromatin environment allowing rapid transcriptome switch from one cell type to another cell type (Figure 1A).

In the mammalian intestinal epithelium, progenitor cells of absorptive and secretory cell types also exhibit a broadly permissive and highly similar chromatin landscape [27, 28], enabling them to be inter-switchable. In the absence of the secretory cell fate-specific regulator *Atoh1*, secretory cells transit into an absorptive cell fate [27], while depleting HNF4α and HNF4γsimultaneously in ECs induces them to adopt a secretory cell program [29]. The similarity in chromatin accessibility landscape may also explain why, following damage to ISCs, both absorptive and secretory progenitor cells can undergo dedifferentiation to replenish new ISCs [30].

It is found that various primitive endodermal cells—including pancreatic exocrine cells, hepatocytes, and gastrointestinal cells—can be transdifferentiated into β cells by overexpressing the TFs *Pdx1*, *Ngn3*, and *MafA* [31–33]. In comparison, pancreatic α cells have a higher transdifferentiation efficiency, as these cells have a close lineage relationship with β cells and exhibit higher similarity in chromatin accessibility. Not surprisingly, α-to-β cell transdifferentiation can be efficiently achieved by downregulating *Arx* or upregulating *Pax4* (which inhibits *Arx* expression) [34–36].

These observations support the hypothesis that different types of cells in closely related cell lineages might exhibit a higher propensity for spontaneous or manipulated transdifferentiation events, compared to cells from distantly related lineages, possibly due to the similarity in chromatin accessibility that allows efficient transcription program switching without the need of extensive interventions (Figure 1A). In contrast, transdifferentiation between cells from distantly related lineages may require external factors to overcome the inherent chromatin and epigenetic barriers that distinguish these lineages, thus may compromise the reprogramming efficiency (Figure 1B).

2.2 | Chromatin Accessibility Changes During Development Correlate With Lineage Restriction

Thus, it appears that cell identity plasticity is closely related to the cell lineage relationships during development. In multi-cellular organisms, fertilization signifies the beginning of life, with the subsequent cleavage stages marking the onset of development. Cell identity, which emerges during subsequent differentiation processes, is controlled by TFs. These TFs work in concert with coregulators, chromatin modifiers, and signaling pathways to precisely guide cell fate decisions and regulate gene expression for cell identity establishment [37, 38]. A continuous coordination occurs between chromatin structure and gene expression patterns as cells develop into specialized cell identities. As a result, chromatin accessibility becomes more restricted as lineage specification continues [39].

Cell reprogramming, however, involves rewiring the chromatin landscape. This involves the silencing of chromatin regions associated with the original cell identity and the opening of chromatin regions associated with the target cell identity. This coordinated

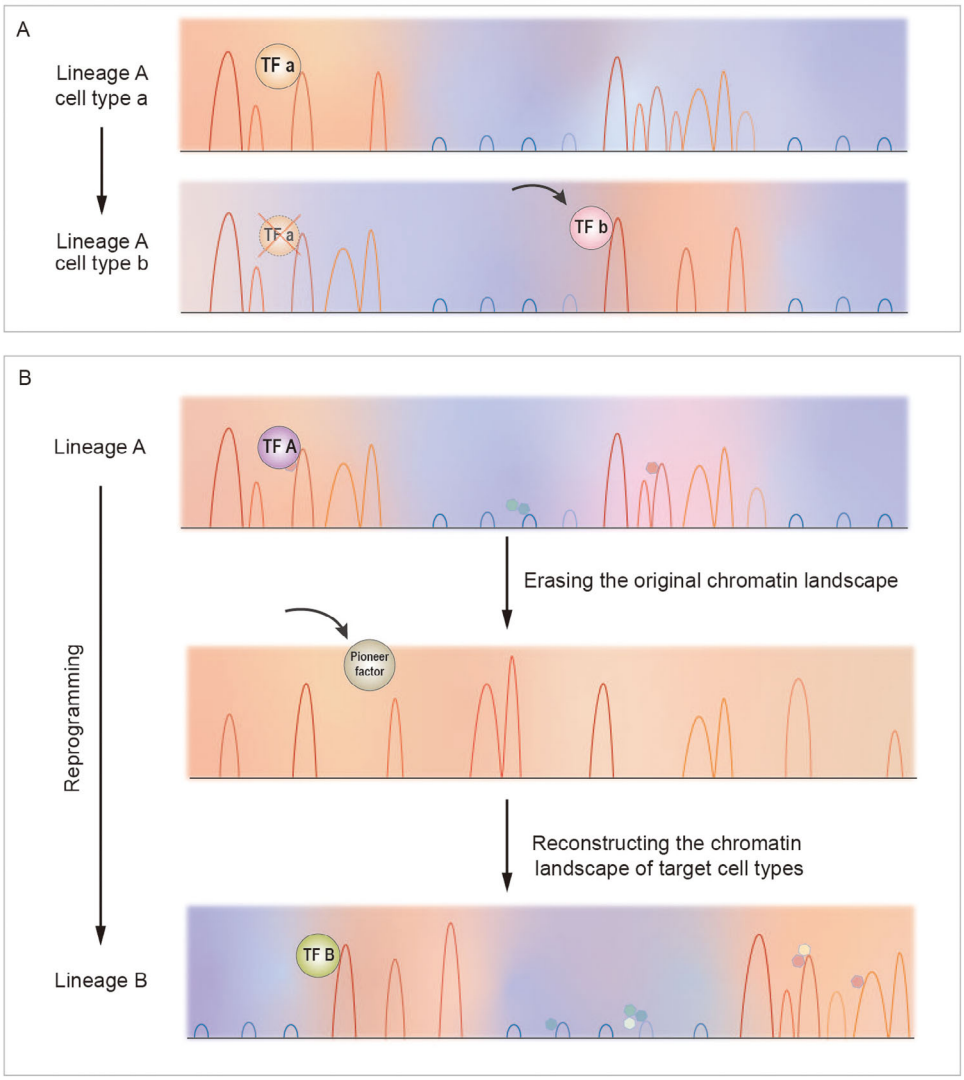

FIGURE 1 | The impact of pre-existing chromatin accessibility landscape on cell reprogramming efficiency. Cell reprogramming within a common stem cell lineage. Cells sharing a similar pre-existing chromatin landscape enable efficient lineage conversion through the induction of master transcription factors (TFs). Chromatin openness is depicted by color and wave amplitude: red with tall waves indicates high accessibility, while blue with short waves indicates low accessibility. (B) Cell reprogramming across distant cell lineages. When reprogramming involves cells from distantly related lineages, the pre-existing chromatin landscape is often highly condensed due to accumulated epigenetic modifications (e.g., histone deacetylation or DNA methylation). These modifications impose a major barrier to reprogramming. To overcome this, the epigenetic marks must first be erased to reset the chromatin to an initial, more open state. Subsequently, pioneer factors (e.g., Oct4, Sox2) and lineage-specific TFs (e.g., TF A) work together to remodel the chromatin landscape, enabling the activation of target genes and successful lineage conversion. Hexagons represent the epigenetic modifications.

shift in chromatin states underlies the overall transcriptional transition between the two cell types during reprogramming [40]. Thus, the feasibility of rewiring the chromatin landscape between the original and target cells may represent a major barrier to cell reprogramming efficiency.

2.3 | Comparative Analysis of Intra- Versus Intergerm Layer Reprogramming Efficiency

To further evaluate the hypothesis raised above—that similarity in chromatin accessibility landscape determines the reprogramming efficiency, here we summarize some representative dedifferentiation and transdifferentiation events reported in the past (Table 1). It can be observed that cells within the same germ layer, particularly within the same adult stem cell lineage, tend to exhibit a higher propensity for transdifferentiation (higher than 10%) compared to cells across germ layers (mostly lower than 10%). For instance, the efficiency of converting fibroblasts (derived from mesoderm) into neurons [41] (derived from ectoderm) or hepatocytes [42] (derived from endoderm) is typically under 10% [2]. In contrast, transdifferentiation within the same germ layer—such as reprogramming pancreatic exocrine cells to β cells—can reach efficiencies of up to 25% [31]. Similarly, using cardiac reprogramming factors *Gata4*, *Mef2c*, and *Tbx5* (GMT), the in vivo reprogramming efficiency of cardiac fibroblasts into cardiac cells is approximately 10%–15% [43].

These observations collectively highlight the importance of assessing the similarity of the pre-existing chromatin accessibility

TABLE 1 | Comparison of cell reprogramming efficiency in iPSC reprogramming and direct cell reprogramming events.

Induced pluripotent stem cell reprogramming

Organism	Starting cell type	Target cell type	Reprogramming factor	Delivery method	Reprogramming efficiency	Reference
Murine	Fibroblasts	iPSC	OSKM	Retrovirus	0.001%–0.5%	[2, 44, 45]
Murine	Hematopoietic and nonhematopoietic cells	iPSC	OSKM	Retrovirus	6.5%	[46]
Murine	Mature B lymphocytes	iPSC	OSKM	Retrovirus	3.3%	[47]
Murine	Hepatocytes and gastric epithelial cells	iPSC	OSKM	Retrovirus	0.1%–0.5%	[48]
Murine	Pancreatic β cells	iPSC	OSKM	Lentivirus	0.1%–0.2%	[49]
Murine	Fibroblasts	iPSC	OSKM OSKM and p16 deletion	AAV	0.05% 0.14%	[50]
Murine	Fibroblasts	iPSC	OSKM and Dppa2/4^{OE}	AAV	~32%	[51]
Murine	Fibroblasts	iPSC	OSKM and Ink4a/Arf deletion	Retrovirus	~8%	[52]
Murine	Fibroblasts	iPSC	OSK and p53 deletion	Retrovirus	3%–20%	[53, 54]

Direct cell reprogramming (transdifferentiation)—across germ layers

Organism	Starting cell type	Target cell type	Reprogramming factor	Delivery method	Reprogramming efficiency	Reference
Murine	Fibroblasts (mesoderm)	Induced neuronal (iN) cells (ectoderm)	Ascl1, Brn2, and Myt1l	Lentivirus	1.8%–19.5%	[41]
Human	Fibroblasts (mesoderm)	iN cells (ectoderm)	Ascl1, Brn2, and Myt1l	AAV	0.4%–5.9%	[55]
Murine	Hepatocytes (endoderm)	iN cells (ectoderm)	Ascl1, Brn2, and Myt1l	Lentivirus	~6%	[56]
Murine	Fibroblasts (mesoderm)	GABAergic INs (ectoderm)	Foxg1, Sox2, Ascl1, Dlx5, and Lhx6	Lentivirus	~12%	[57]
Murine	Fibroblasts (mesoderm)	Hepatocytes (endoderm)	Hnf4α plus Foxa1, Foxa2 or Foxa3	Retrovirus	30%–40%	[42]
Murine	Fibroblasts (mesoderm)	Endothelial cells (endoderm)	Foxo1, Er71, Klf2, Tal1, and Lmo2	Lentivirus	4%	[58]
Murine	Fibroblasts (mesoderm)	Hepatic stem cells (endoderm)	Hnf1β and Foxa3	Lentivirus	0.4% ± 0.082%	[59]
Murine	Fibroblasts (mesoderm)	Hepatocyte-like cells (endoderm)	Gata4, Hnf1α and Foxa3, and p19^{Arf} deletion	Lentivirus	23%	[60]
Human	Fibroblasts (mesoderm)	Hepatocyte-like cells (endoderm)	ATF5, PROX1, FOXA2, FOXA3, and HNF4A	Lentivirus	22%–27%	[61]

Direct cell reprogramming (transdifferentiation)—within germ layer

Organism	Starting cell type	Target cell type	Reprogramming factor	Delivery method	Reprogramming efficiency	Reference
Murine	Astrocytes	Neuronal cells	Ascl1, Bcl2	Retrovirus	~40%	[62]
Murine	Astrocytes	Neuronal cells	Ascl1	AAV	30%–40% (P12–P15); 10%–20% (P60)	[63]
Murine	Astrocytes	Proliferative neuroblasts	Sox2	Retrovirus	23.2% ± 5.3%	[64, 65]
Murine	Cardiac fibroblasts	Cardiomyocytes	GMT	Retrovirus	10%–15%	[43]
Murine	Cardiac fibroblasts	Cardiomyocytes	GMT	Retrovirus	25%	[66]
Murine	Cardiac fibroblasts	Cardiomyocytes	GMT and Ybx1 depletion	Lentivirus	40%	[67]

(Continues)

TABLE 1 | (Continued)

Direct cell reprogramming (transdifferentiation)—within germ layer						
Organism	**Starting cell type**	**Target cell type**	**Reprogramming factor**	**Delivery method**	**Reprogramming efficiency**	**Reference**
Human	Cardiac fibroblasts	Cardiomyocytes	GMT + miR-133	Retrovirus	40%–60%	[68]
Murine	Cardiac fibroblasts	Cardiomyocytes	miRNAs: 1, 133, 208, and 499	AAV	12%	[69]
Murine	Pancreatic exocrine cells	Pancreatic β cells	Ngn3, Pdx1, and Mafa	AAV	25%	[31]
Murine	Gastrointestinal	Pancreatic β cells	Ngn3, Pdx1, and Mafa	AAV	14.6%–41.5%	[70]
Murine	Cardiac fibroblasts	Endothelial cells	Sox7 and Erg	Retrovirus	22.5%	[71]
Drosophila	Enterocyte	Enteroendocrine cell	Depletion of Ttk69 and Dpn	RNAi	65.3% ± 7.1%	[26]

landscape to determine the starting cells for the induction of the required functional cells. With the development of methods for chromatin accessibility analysis and comparative analysis tools for different cell types, here we propose that cells with a similar chromatin accessibility landscape may become strong candidates to be selected as the starting cells for in vitro or in vivo transdifferentiation to achieve high efficiency.

3 | Modulating Chromatin Accessibility to Improve Cell Reprogramming Efficiency

Cell reprogramming efficiency is affected not only by the phylogenetic relationship between the starting and target cell types but also by the cellular state of individual cells. Variations in reprogramming efficiency arise from factors, such as the specific factors or cocktails used, the age, and cell cycle state of the cell.

3.1 | Transcription Factors Orchestrate Chromatin Accessibility to Facilitate Transcriptional Remodeling

During cell reprogramming, chromatin undergoes dynamic temporal changes, transitioning from a closed to an open state to enable transcriptional activation. These epigenetic changes are tightly regulated and exhibit distinct phases, with cells that fail to reprogram often retaining their original chromatin state [72]. At the core of this process are pioneer factors, which play a pivotal role in initiating chromatin remodeling by binding to closed chromatin regions and triggering local chromatin opening. For instance, *Oct4* and *Sox2* are among the first factors to bind and destabilize nucleosomes, creating accessible regions for subsequent transcriptional machinery [72–74]. This initial remodeling is further enhanced by the recruitment of additional factors like *Klf4* and *Myc*, which stabilize the open chromatin state and activate target genes essential for reprogramming [75]. Importantly, the binding of these factors is guided by the topological features of chromatin, with nucleosome positioning and histone modifications serving as critical "signposts" that direct the coordinated recruitment of TFs [76]. Together, these mechanisms highlight the intricate, phase-specific regulation of chromatin accessibility during reprogramming.

Pioneer factors are uniquely equipped to drive cell fate transitions due to their ability to recognize and bind condensed chromatin, even in the absence of prior epigenetic marks. This capability allows them to initiate widespread chromatin remodeling, which is essential for enabling the binding of additional regulatory factors and activating lineage-specific gene expression [77–81]. For example, during OSKM-induced reprogramming, *Oct4*, *Sox2*, and *Klf4* collaborate to activate distal enhancers critical for pluripotency, while *c-Myc* primarily amplifies transcriptional activity rather than initiating chromatin opening [82, 83]. Beyond pluripotency, pioneer factors, such as *Pax7* in muscle stem cells [84] and *FoxA* in fibroblast-to-hepatocyte transdifferentiation, have been shown to have a conserved role in promoting chromatin decondensation and facilitating fate transitions across diverse biological contexts [60, 61, 85]. These examples underscore the universal importance of pioneer factors in overcoming epigenetic barriers to cell fate changes.

Functionally, TFs can either act as barriers or facilitators of reprogramming. For example, a set of TFs, including ATF7IP, JUNB, SP7, and ZNF207 (AJSZ) has been identified to maintain the differentiated cell state by modulating the chromatin landscape, and depleting their expression can increase chromatin accessibility, thereby promoting reprogramming [86]. Similarly, our recent study showed that knocking down the master TF governing the origin cell identity promotes EC-to-EEC transdifferentiation in the *Drosophila* intestine [26]. In the context of GMT-mediated direct cardiac reprogramming, *Ybx1* maintains a repressive chromatin state that prevents the fibroblasts from fully acquiring a cardiomyocyte fate [67]. Knocking down *Ybx1* alleviates this repressive state, allowing the chromatin to become permissive for the reprogramming process [67]. Thus, understanding the regulatory mechanisms controlling lineage specification and cell identity maintenance could greatly aid the identification of key pioneer factors that facilitate chromatin remodeling and promote an efficient reprogramming process.

3.2 | Persistent Epigenetic Modifications as Reprogramming Barriers

Changes in the chromatin accessibility landscape are regulated by a variety of epigenetic modification processes, including

chromatin remodeling, histone modifications, and DNA methylation. Persistent epigenetic modifications accumulated after mature cell specification restrict chromatin accessibility of other cell identity gene loci while depleting these suppressive epigenetic landmarks may facilitate reopening chromatin of target cell identity genes and enhance reprogramming efficiency. Indeed, in our study of the EC-to-EEC transdifferentiation in *Drosophila*, we performed a genetic screen to identify gene barriers to the transdifferentiation process and found that simultaneous knockdown of genes encoding the nucleosome remodeling and deacetylase (NuRD) complex components, MEP-1 and Mi-2, enhances the efficiency of the EC to EEC cell fate transition [26]. Similarly, DNA methylation often correlates with reduced chromatin accessibility and the maintenance of cell identity, resulting in a loss of cell plasticity [87]. This suggests that epigenetic modification status may pose a barrier to reprogramming in individual cells.

Studies have shown that cell reprogramming efficiency can be improved by erasing the repressive chromatin modifications. For example, a study using single-cell transcriptomic analysis reveals that epigenetic regulation, including the modulation of chromatin structure, DNA methylation patterns, and the use of epigenetic modifiers, can improve the efficiency of human cardiac reprogramming [68]. The expression of JMJD3 and UTX, the H3K27me3 demethylases, is higher in ESCs than in MEFs and increases during cell reprogramming [88]. Overexpression of histone demethylases can improve reprogramming efficiency [89–91]. Additionally, in quiescent liver cells, the silencing of proliferation-associated genes by H3K27me3 is reversed during liver regeneration, promoting rapid gene activation and enhancing regenerative capacity [92]. Moreover, loss of *Arid1a* disrupts the SWI/SNF chromatin remodeling complex, resulting in significant changes in chromatin accessibility, thereby inhibiting the binding of cell fate-specific TFs and promoting cell fate transitions [93].

These findings collectively suggest that chromatin modifications that maintain cell identity—such as repressive histone marks and DNA methylation—pose significant obstacles to reprogramming. Effective cell reprogramming requires the removal of these modifications, enabling the chromatin to adopt a more permissive state and facilitating the establishment of new cell fates. In addition to the nontargeted epigenetic modification by modulating the expression of pioneer factors or epigenetic regulators, the recently developed CRISPR-based epigenetic editing technology offers a more precise approach. By utilizing a "dead" Cas9 protein (dCas9) fused with an epigenetic effector domain, this method enables precise tuning of the epigenetic states at specific target regions. This strategy allows for greater control over chromatin landscape remodeling and may significantly enhance the efficiency and directionality of cell reprogramming [94–96].

3.3 | Cell Senescence as a Reprogramming Barrier

Cell senescence, a state of permanent cell cycle arrest induced by stress or damage, poses significant challenges to epigenetic reprogramming. Unlike organismal aging, which refers to the gradual decline of physiological function over time, cell senescence is a cellular response that can occur at any stage of life. Persistent epigenetic modifications can be inherited through cell division, enabling progeny cells to maintain stable expression profiles in response to external stimuli—a phenomenon known as epigenetic memory [97–99]. As cells mature, undergo endoreplication, or enter senescence, this epigenetic memory consolidates. Importantly, the accumulation of senescent cells increases with organismal aging, creating a link between cell senescence and the aging process [100]. Consequently, young or newly differentiated cells, which have not yet undergone significant senescence, exhibit higher plasticity and are more amenable to being reprogrammed. Consistent with this notion, our study in the *Drosophila* midgut observed that cell plasticity is relatively high in young or newly differentiated ECs but declines rapidly with age [26]. Knocking down *E2F1*, a TF essential for driving EC endoreplication, significantly enhances the transdifferentiation efficiency from EC-to-EEC [26]. A similar trend has been observed in astrocyte-to-neuron transdifferentiation, where young mice show higher reprogramming efficiency than older ones [63], underscoring the critical role of aging or cell senescence in determining cell plasticity and reprogramming potential.

Senescent cells undergo significant changes in chromatin structure, including the formation of heterochromatin, histone modifications, and activation of the DNA damage response [101]. These epigenetic alterations are distinct from the gradual epigenetic drift observed during organismal aging, which involves more widespread and stochastic changes in the epigenome. Targeting senescence-associated chromatin changes has emerged as a promising therapeutic strategy, particularly in the context of aging-related diseases. Recent advancements in senolytics and senomorphics have opened new avenues for mitigating the detrimental effects of cell senescence [102, 103]. Both approaches may exert their effects by remodeling the epigenetic landscape of senescent chromatin, thereby reducing the expression of proinflammatory genes, restoring a healthier chromatin state, and facilitating the elimination of proinflammatory or growth-inhibitory factors.

3.4 | Alternative or Refractory Route as a Reprogramming Barrier

Cell reprogramming is a heterogeneous and unsynchronized process that is not strictly unidirectional. While pioneer factors open chromatin and enable TFs of the target cell identity to bind to their target loci, other TFs—such as gatekeeper TFs of the original cell identity or mis-expressed master TFs of other lineages—can also bind to the poised chromatin. This competition often leads to refractory routes (reversion to the original state) or alternative fates, significantly compromising reprogramming efficiency. We also observed such fate conflict in our *Drosophila* model, depletion of *Ttk69* not only activated the EEC master TF *Prospero* but also turned on *Dpn* and other neuronal identity factors, and the failed EC-to-EEC transformation attributes to activation of these factors and redirecting into reprogramming of neuronal lineages [26, 104]. Similarly, in *Ascl1*-overexpression induced fibroblast-to-neuron reprogramming [105], a significant portion of reprogrammed cells adopted a myocyte-like signature. In fibroblast to cardiac reprogramming mediated by GMT, cells often deviated into vasculature-like lineages or reverted to fibroblasts [68, 106]. Collectively, these observations highlight the prevalence of alternative and refractory routes during reprogramming.

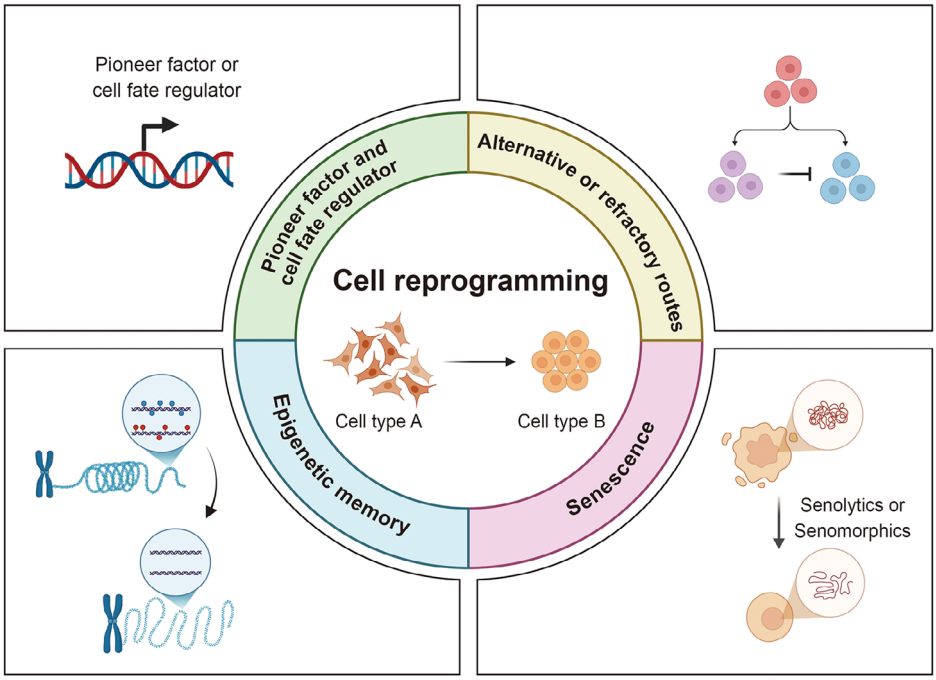

FIGURE 2 | Molecular barriers and regulators of cell reprogramming. This diagram summarizes the four key factors that influence the efficiency of cell reprogramming described in this review: *Pioneer factors or cell fate regulators*: Key transcription factors (TFs) that initiate chromatin remodeling and activate lineage-specific gene expression. *Epigenetic memory*: Persistent epigenetic modifications (e.g., DNA methylation, histone modifications) that maintain the original cell identity and impede reprogramming. *Alternative or refractory routes*: Mis-expression of lineage-specific TFs or activation of conflicting pathways that divert cells toward undesired fates. *Senescence*: Age-related changes in chromatin structure and gene expression that reduce cellular plasticity and reprogramming efficiency. Strategies to address these barriers include: *Enhancing pioneer factor activity*: Optimizing the expression or function of pioneer factors to initiate chromatin opening. *Erasing epigenetic memory*: Using small molecules or CRISPR-based tools to remove repressive epigenetic marks. *Suppressing alternative routes*: Depleting mis-expressed TFs or inhibiting conflicting pathways to redirect cells toward the desired fate. *Reversing senescence*: Applying senolytics or senomorphics to restore chromatin accessibility and cellular plasticity. This figure was created with BioRender.com.

Conversely, suppressing these alternative or refractory routes can significantly enhance reprogramming efficiency. As we observed in the *Drosophila* model, depleting the mis-expressed TF *Dpn* upon *Ttk69* loss arrested the conflicting neuronal route and significantly increased the efficiency of reprogramming toward the intended EEC lineage [26]. These findings underscore the importance of identifying key modulators that drive alternative or refractory routes and developing strategies to suppress them. By revealing the different trajectories during reprogramming and targeting these conflicting pathways, we can achieve more precise and efficient cell fate conversion.

4 | Conclusion and Future Perspective

Cell plasticity is essential for development, regeneration, and cellular adaptation, yet reprogramming efficiency remains limited by epigenetic barriers, cell senescence, and the activation of alternative or refractory routes (Figure 2). Our review emphasizes the importance of pre-existing chromatin accessibility landscapes in determining reprogramming outcomes and proposes the need to manipulate these landscapes to overcome molecular challenges. Key strategies include identifying universal regulators such as pioneer factors and master TFs that drive chromatin remodeling across diverse cell types, as well as erasing epigenetic memory (e.g., via H3K9me3 erasers in primate cloning [107–109]) to reset chromatin states for precise fate conversion (Figure 1B).

The integration of cutting-edge technologies, such as single-cell multi-omics and spatial omics, has begun to revolutionize our ability to dissect cellular heterogeneity and resolve conflicting trajectories. For instance, single-cell RNA-seq (scRNA-seq) combined with ATAC-seq (scATAC-seq) enables comprehensive mapping of gene expression and chromatin dynamics during reprogramming [110], while spatial omics delineates microenvironmental cues and cell-cell communication networks critical for in vivo modeling [111, 112]. Trajectory analysis has further identified refractory routes marked by AKR1C1 and FAP expression, which can be suppressed by targeted inhibitors to enhance lineage conversion [68]. Similarly, modulating Dnmt3a level in acinar to β-cell reprogramming, co-expression of *Brn2* and *Myt1l* with *Ascl1* in fibroblast-to-neuron reprogramming, and overexpressing inflammatory cytokines in fibroblast-to-dendritic cell (DC) reprogramming all orchestrate trajectories toward successful reprogramming [105, 113, 114]. These tools, coupled with computational modeling and machine learning, will predict regulatory nodes and optimize reprogramming paths, paving the way for efficient strategies.

Despite significant progress, many challenges remain to be addressed. The molecular mechanisms by which age or cell

senescence impacts chromatin accessibility are still poorly understood and warrant further investigation. Additionally, the dynamic interplay between epigenetic regulation, transcriptional networks, and cell metabolism during reprogramming needs to be elucidated to develop more efficient strategies. Ultimately, advancing our understanding of the molecular mechanisms underlying cell plasticity and reprogramming will not only enhance basic research but also accelerate the translation of these technologies into clinical applications. By addressing current limitations and harnessing the power of emerging technologies, we can unlock the full potential of cell reprogramming for regenerative medicine and disease modeling.

Author Contributions

D.Y., X.G., and R.X. conceptualized the idea, drafted, and revised the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article, as no new data were created or analyzed in this study.

References

1. J. B. Gurdon, "The Developmental Capacity of Nuclei Taken From Intestinal Epithelium Cells of Feeding Tadpoles," *Journal of Embryology and Experimental Morphology* 10 (1962): 622–640.

2. K. Takahashi and S. Yamanaka, "Induction of Pluripotent Stem Cells From Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors," *Cell* 126, no. 4 (2006): 663–676, https://doi.org/10.1016/j.cell.2006.07.024.

3. C. Blanpain and E. Fuchs, "Plasticity of Epithelial Stem Cells in Tissue Regeneration," *Science* 344, no. 6189 (2014): 1242281.

4. A. Wabik and P. H. Jones, "Switching Roles: The Functional Plasticity of Adult Tissue Stem Cells," *The EMBO Journal* 34, no. 9 (2015): 1164–1179.

5. G. Careccia, L. Mangiavini, and F. Cirillo, "Regulation of Satellite Cells Functions During Skeletal Muscle Regeneration: A Critical Step in Physiological and Pathological Conditions," *International Journal of Molecular Sciences* 25, no. 1 (2023): 512.

6. X. Deng, X. Zhang, W. Li, et al., "Chronic Liver Injury Induces Conversion of Biliary Epithelial Cells Into Hepatocytes," *Cell Stem Cell* 23, no. 1 (2018): 113.

7. W. Li, L. Li, and L. Hui, "Cell Plasticity in Liver Regeneration," *Trends in Cell Biology* 30, no. 4 (2020): 329–338, https://doi.org/10.1016/j.tcb.2020.01.007.

8. K. S. Yan, O. Gevaert, G. X. Y. Zheng, et al., "Intestinal Enteroendocrine Lineage Cells Possess Homeostatic and Injury-Inducible Stem Cell Activity," *Cell Stem Cell* 21, no. 1 (2017): 76, https://doi.org/10.1016/j.stem.2017.06.014.

9. F. de Sousa e Melo and F. J. de Sauvage, "Cellular Plasticity in Intestinal Homeostasis and Disease," *Cell Stem Cell* 24, no. 1 (2019): 54–64, https://doi.org/10.1016/j.stem.2018.11.019.

10. Y. Liu and Y.-G. Chen, "Intestinal Epithelial Plasticity and Regeneration via Cell Dedifferentiation," *Cell Regeneration* 9, no. 1 (2020): 14, https://doi.org/10.1186/s13619-020-00053-5.

11. C. Jopling, S. Boue, and J. C. I. Belmonte, "Dedifferentiation, Transdifferentiation and Reprogramming: Three Routes to Regeneration," *Nature Reviews Molecular Cell Biology* 12, no. 2 (2011): 79–89.

12. P. Hou, Y. Li, X. Zhang, et al., "Pluripotent Stem Cells Induced From Mouse Somatic Cells by Small-Molecule Compounds," *Science* 341, no. 6146 (2013): 651–654.

13. J. Guan, G. Wang, J. Wang, et al., "Chemical Reprogramming of Human Somatic Cells to Pluripotent Stem Cells," *Nature* no. 7909 (2022): 325–331.

14. Y. Wang, Q. Zheng, Z. Sun, et al., "Reversal of Liver Failure Using a Bioartificial Liver Device Implanted With Clinical-Grade Human-Induced Hepatocytes," *Cell Stem Cell* 30, no. 5 (2023): 617–631, https://doi.org/10.1016/j.stem.2023.03.013.

15. H. Wang, Y. Yang, J. Liu, and L. Qian, "Direct Cell Reprogramming: Approaches, Mechanisms and Progress," *Nature Reviews Molecular Cell Biology* 22, no. 6 (2021): 410–424, https://doi.org/10.1038/s41580-021-00335-z.

16. K. K. Haridhasapavalan, K. Raina, C. Dey, P. Adhikari, and R. P. Thummer, "An Insight Into Reprogramming Barriers to iPSC Generation," *Stem Cell Reviews and Reports* 16, no. 1 (2020): 56–81, https://doi.org/10.1007/s12015-019-09931-1.

17. D. H. Arabacı, G. Terzioğlu, B. Bayırbaşı, and T. T. Önder, "Going Up the Hill: Chromatin-Based Barriers to Epigenetic Reprogramming," *FEBS Journal* 288, no. 16 (2021): 4798–4811, https://doi.org/10.1111/febs.15628.

18. D. J. Huels and J. P. Medema, "Think About the Environment: Cellular Reprogramming by the Extracellular Matrix," *Cell Stem Cell* 22, no. 1 (2018): 7–9, https://doi.org/10.1016/j.stem.2017.12.006.

19. M. d'Angelo, E. Benedetti, M. G. Tupone, et al., "The Role of Stiffness in Cell Reprogramming: A Potential Role for Biomaterials in Inducing Tissue Regeneration," *Cells* 8, no. 9 (2019): 1036.

20. E. Apostolou and K. Hochedlinger, "Chromatin Dynamics During Cellular Reprogramming," *Nature* 502, no. 7472 (2013): 462–471, https://doi.org/10.1038/nature12749.

21. K. Hawkins, S. Joy, and T. McKay, "Cell Signalling Pathways Underlying Induced Pluripotent Stem Cell Reprogramming," *World J Stem Cells* 6, no. 5 (2014): 620–628, https://doi.org/10.4252/wjsc.v6.i5.620.

22. H. Jiang and B. A. Edgar, "Intestinal Stem Cells in the Adult *Drosophila* Midgut," *Experimental Cell Research* 317, no. 19 (2011): 2780–2788, https://doi.org/10.1016/j.yexcr.2011.07.020.

23. I. Miguel-Aliaga, H. Jasper, and B. Lemaitre, "Anatomy and Physiology of the Digestive Tract of *Drosophila melanogaster*," *Genetics* 210, no. 2 (2018): 357–396, https://doi.org/10.1534/genetics.118.300224.

24. C. A. Micchelli and N. Perrimon, "Evidence That Stem Cells Reside in the Adult *Drosophila* Midgut Epithelium," *Nature* 439, no. 7075 (2006): 475–479, https://doi.org/10.1038/nature04371.

25. B. Ohlstein and A. Spradling, "The Adult *Drosophila* Posterior Midgut Is Maintained by Pluripotent Stem Cells," *Nature* 439, no. 7075 (2006): 470–474, https://doi.org/10.1038/nature04333.

26. X. Guo, C. Wang, Y. Zhang, R. Wei, and R. Xi, "Cell-Fate Conversion of Intestinal Cells in Adult *Drosophila* Midgut by Depleting a Single Transcription Factor," *Nature Communications* 15, no. 1 (2024): 2656, https://doi.org/10.1038/s41467-024-46956-8.

27. T.-H. Kim, F. Li, I. Ferreiro-Neira, et al., "Broadly Permissive Intestinal Chromatin Underlies Lateral Inhibition and Cell Plasticity," *Nature* 506, no. 7489 (2014): 511–515, https://doi.org/10.1038/nature12903.

28. U. Jadhav, M. Saxena, N. K. O'Neill, et al., "Dynamic Reorganization of Chromatin Accessibility Signatures During Dedifferentiation of Secretory Precursors Into Lgr5+ Intestinal Stem Cells," *Cell Stem Cell* 21, no. 1 (2017): 65, https://doi.org/10.1016/j.stem.2017.05.001.

29. L. Chen, N. H. Toke, S. Luo, et al., "A Reinforcing HNF4–SMAD4 Feed-Forward Module Stabilizes Enterocyte Identity," *Nature Genetics* 51, no. 5 (2019): 777–785, https://doi.org/10.1038/s41588-019-0384-0.

30. K. Murata, U. Jadhav, S. Madha, et al., "Ascl2-Dependent Cell Dedifferentiation Drives Regeneration of Ablated Intestinal Stem Cells," *Cell Stem Cell* 26, no. 3 (2020): 377–390, https://doi.org/10.1016/j.stem.2019.12.011.

31. Q. Zhou, J. Brown, A. Kanarek, J. Rajagopal, and D. A. Melton, "In Vivo Reprogramming of Adult Pancreatic Exocrine Cells to β-Cells," *Nature* 455, no. 7213 (2008): 627–632, https://doi.org/10.1038/nature07314.

32. Y. J. Chen, S. R. Finkbeiner, D. Weinblatt, et al., "De Novo Formation of Insulin-Producing "Neo-β Cell Islets" From Intestinal Crypts," *Cell Reports* 6, no. 6 (2014): 1046–1058, https://doi.org/10.1016/j.celrep.2014.02.013.

33. H. Nagasaki, T. Katsumata, H. Oishi, et al., "Generation of INSULIN-PRODUCING Cells From the Mouse Liver Using β Cell-Related Gene Transfer Including Mafa and Mafb," *PLoS ONE* 9, no. 11 (2014): 113022, https://doi.org/10.1371/journal.pone.0113022.

34. A. M. Mawla, T. van der Meulen, and M. O. Huising, "Chromatin Accessibility Differences Between Alpha, Beta, and Delta Cells Identifies Common and Cell Type-Specific Enhancers," *BMC Genomics* 24, no. 1 (2023): 202, https://doi.org/10.1186/s12864-023-09293-6.

35. P. Collombat, X. Xu, P. Ravassard, et al., "The Ectopic Expression of Pax4 in the Mouse Pancreas Converts Progenitor Cells Into α and Subsequently β Cells," *Cell* 138, no. 3 (2009): 449–462, https://doi.org/10.1016/j.cell.2009.05.035.

36. M. Courtney, E. Gjernes, N. Druelle, et al., "The Inactivation of Arx in Pancreatic α-Cells Triggers Their Neogenesis and Conversion Into Functional β-Like Cells," *PLoS Genetics* 9, no. 10 (2013): 1003934, https://doi.org/10.1371/journal.pgen.1003934.

37. B. E. J. Maven, C. A. Gifford, M. Weilert, et al., "The Multi-Lineage Transcription Factor ISL1 Controls Cardiomyocyte Cell Fate Through Interaction With NKX2.5," *Stem Cell Reports* 18, no. 11 (2023): 2138–2153, https://doi.org/10.1016/j.stemcr.2023.09.014.

38. X. Guo, Y. Zhang, H. Huang, and R. Xi, "A Hierarchical Transcription Factor Cascade Regulates Enteroendocrine Cell Diversity and Plasticity in *Drosophila*," *Nature Communications* 13, no. 1 (2022): 6525, https://doi.org/10.1038/s41467-022-34270-0.

39. Y. Liu, X. Wan, H. Li, et al., "CTCF Coordinates Cell Fate Specification via Orchestrating Regulatory Hubs With Pioneer Transcription Factors," *Cell Reports* 42, no. 10 (2023): 113259, https://doi.org/10.1016/j.celrep.2023.113259.

40. J. A. Martinez-Sarmiento, M. P. COSMA, and M. Lakadamyali, "Dissecting Gene Activation and Chromatin Remodeling Dynamics in Single Human Cells Undergoing Reprogramming," *Cell Reports* 43, no. 5 (2024): 114170, https://doi.org/10.1016/j.celrep.2024.114170.

41. T. Vierbuchen, A. Ostermeier, Z. P. Pang, Y. Kokubu, T. C. Südhof, and M. Wernig, "Direct Conversion of Fibroblasts to Functional Neurons by Defined Factors," *Nature* 463, no. 7284 (2010): 1035–1041, https://doi.org/10.1038/nature08797.

42. S. Sekiya and A. Suzuki, "Direct Conversion of Mouse Fibroblasts to Hepatocyte-Like Cells by Defined Factors," *Nature* 475, no. 7356 (2011): 390–393, https://doi.org/10.1038/nature10263.

43. L. Qian, Y. Huang, C. I. Spencer, et al., "In Vivo Reprogramming of Murine Cardiac Fibroblasts Into Induced Cardiomyocytes," *Nature* 485, no. 7400 (2012): 593–598, https://doi.org/10.1038/nature11044.

44. M. Wernig, A. Meissner, R. Foreman, et al., "In Vitro Reprogramming of Fibroblasts Into a Pluripotent ES-Cell-Like State," *Nature* 448, no. 7151 (2007): 318–324, https://doi.org/10.1038/nature05944.

45. A. Meissner, M. Wernig, and R. Jaenisch, "Direct Reprogramming of Genetically Unmodified Fibroblasts Into Pluripotent Stem Cells," *Nature Biotechnology* 25, no. 10 (2007): 1177–1181.

46. M. Abad, L. Mosteiro, C. Pantoja, et al., "Reprogramming In Vivo Produces Teratomas and iPS Cells With Totipotency Features," *Nature* 502, no. 7471 (2013): 340–345, https://doi.org/10.1038/nature12586.

47. J. Hanna, S. Markoulaki, P. Schorderet, et al., "Direct Reprogramming of Terminally Differentiated Mature B Lymphocytes to Pluripotency," *Cell* 133, no. 2 (2008): 250–264, https://doi.org/10.1016/j.cell.2008.03.028.

48. T. Aoi, K. Yae, M. Nakagawa, et al., "Generation of Pluripotent Stem Cells From Adult Mouse Liver and Stomach Cells," *Science* 321, no. 5889 (2008): 699–702, https://doi.org/10.1126/science.1154884.

49. M. Stadtfeld, K. Brennand, and K. Hochedlinger, "Reprogramming of Pancreatic β Cells Into Induced Pluripotent Stem Cells," *Current Biology* 18, no. 12 (2008): 890–894, https://doi.org/10.1016/j.cub.2008.05.010.

50. B. B. Grigorash, D. van Essen, G. Liang, et al., "p16High Senescence Restricts Cellular Plasticity During Somatic Cell Reprogramming," *Nature Cell Biology* 25, no. 9 (2023): 1265–1278, https://doi.org/10.1038/s41556-023-01214-9.

51. C. Hernandez, Z. Wang, B. Ramazanov, et al., "Dppa2/4 Facilitate Epigenetic Remodeling During Reprogramming to Pluripotency," *Cell Stem Cell* 23, no. 3 (2018): 398.

52. H. Li, M. Collado, A. Villasante, et al., "The Ink4/Arf Locus Is a Barrier for iPS Cell Reprogramming," *Nature* 460, no. 7259 (2009): 1136–1139, https://doi.org/10.1038/nature08290.

53. R. M. Marión, K. Strati, H. Li, et al., "A p53-Mediated DNA Damage Response Limits Reprogramming to Ensure iPS Cell Genomic Integrity," *Nature* 460, no. 7259 (2009): 1149–1153, https://doi.org/10.1038/nature08287.

54. H. Hong, K. Takahashi, T. Ichisaka, et al., "Suppression of Induced Pluripotent Stem Cell Generation by the p53–p21 Pathway," *Nature* 460, no. 7259 (2009): 1132–1135, https://doi.org/10.1038/nature08235.

55. O. Torper, U. Pfisterer, D. A. Wolf, et al., "Generation of Induced Neurons via Direct Conversion In Vivo," *Proceedings of the National Academy of Sciences USA* 110, no. 17 (2013): 7038–7043, https://doi.org/10.1073/pnas.1303829110.

56. S. Marro, Z. P. Pang, N. Yang, et al., "Direct Lineage Conversion of Terminally Differentiated Hepatocytes to Functional Neurons," *Cell Stem Cell* 9, no. 4 (2011): 374–382, https://doi.org/10.1016/j.stem.2011.09.002.

57. G. Colasante, G. Lignani, A. Rubio, et al., "Rapid Conversion of Fibroblasts Into Functional Forebrain GABAergic Interneurons by Direct Genetic Reprogramming," *Cell Stem Cell* 17, no. 6 (2015): 719–734, https://doi.org/10.1016/j.stem.2015.09.002.

58. J. K. Han, S. H. Chang, H. J. Cho, et al., "Direct Conversion of Adult Skin Fibroblasts to Endothelial Cells by Defined Factors," *Circulation* 130, no. 14 (2014): 1168–1178, https://doi.org/10.1161/circulationaha.113.007727.

59. B. Yu, Z.-Y. He, P. You, et al., "Reprogramming Fibroblasts Into Bipotential Hepatic Stem Cells by Defined Factors," *Cell Stem Cell* 13, no. 3 (2013): 328–340, https://doi.org/10.1016/j.stem.2013.06.017.

60. P. Huang, Z. He, S. Ji, et al., "Induction of Functional Hepatocyte-Like Cells From Mouse Fibroblasts by Defined Factors," *Nature* 475, no. 7356 (2011): 386–389, https://doi.org/10.1038/nature10116.

61. D. Nakamori, H. Akamine, K. Takayama, F. Sakurai, and H. Mizuguchi, "Direct Conversion of Human Fibroblasts Into Hepatocyte-Like Cells by ATF5, PROX1, FOXA2, FOXA3, and HNF4A Transduction," *Scientific Reports* 7, no. 1 (2017): 16675, https://doi.org/10.1038/s41598-017-16856-7.

62. S. Gascón, E. Murenu, G. Masserdotti, et al., "Identification and Successful Negotiation of a Metabolic Checkpoint in Direct Neuronal Reprogramming," *Cell Stem Cell* 18, no. 3 (2016): 396–409, https://doi.org/10.1016/j.stem.2015.12.003.

63. Y. Liu, Q. Miao, J. Yuan, et al., "Ascl1 Converts Dorsal Midbrain Astrocytes Into Functional Neurons In Vivo," *Journal of Neuroscience* 35, no. 25 (2015): 9336–9355, https://doi.org/10.1523/jneurosci.3975-14.2015.

64. W. Niu, T. Zang, D. K. Smith, et al., "SOX2 Reprograms Resident Astrocytes Into Neural Progenitors in the Adult Brain," *Stem Cell Reports* 4, no. 5 (2015): 780–794, https://doi.org/10.1016/j.stemcr.2015.03.006.

65. W. Niu, T. Zang, Y. Zou, et al., "In Vivo Reprogramming of Astrocytes to Neuroblasts in the Adult Brain," *Nature Cell Biology* 15, no. 10 (2013): 1164–1175, https://doi.org/10.1038/ncb2843.

66. M. Ieda, J. D. Fu, P. Delgado-Olguin, et al., "Direct Reprogramming of Fibroblasts Into Functional Cardiomyocytes by Defined Factors," *Cell* 142, no. 3 (2010): 375–386, https://doi.org/10.1016/j.cell.2010.07.002.

67. Y. Xie, Q. Wang, Y. Yang, et al., "Translational Landscape of Direct Cardiac Reprogramming Reveals a Role of Ybx1 in Repressing Cardiac Fate Acquisition," *Nature Cardiovascular Research* 2, no. 11 (2023): 1060–1077, https://doi.org/10.1038/s44161-023-00344-5.

68. Y. Zhou, Z. Liu, J. D. Welch, et al., "Single-Cell Transcriptomic Analyses of Cell Fate Transitions During Human Cardiac Reprogramming," *Cell Stem Cell* 25, no. 1 (2019): 149–164, https://doi.org/10.1016/j.stem.2019.05.020.

69. T. M. Jayawardena, E. A. Finch, L. Zhang, et al., "MicroRNA Induced Cardiac Reprogramming In Vivo," *Circulation Research* 116, no. 3 (2015): 418–424, https://doi.org/10.1161/CIRCRESAHA.116.304510.

70. C. Ariyachet, A. Tovaglieri, G. Xiang, et al., "Reprogrammed Stomach Tissue as a Renewable Source of Functional β Cells for Blood Glucose Regulation," *Cell Stem Cell* 18, no. 3 (2016): 410–421, https://doi.org/10.1016/j.stem.2016.01.003.

71. G. Farber, Y. Dong, Q. Wang, et al., "Direct Conversion of Cardiac Fibroblasts Into Endothelial-Like Cells Using Sox17 and Erg," *Nature Communications* 15, no. 1 (2024): 4170, https://doi.org/10.1038/s41467-024-48354-6.

72. A. S. Knaupp, S. Buckberry, J. Pflueger, et al., "Transient and Permanent Reconfiguration of Chromatin and Transcription Factor Occupancy Drive Reprogramming," *Cell Stem Cell* 21, no. 6 (2017): 836.

73. G. A. Roberts, B. Ozkan, I. Gachulincová, et al., "Dissecting OCT4 Defines the Role of Nucleosome Binding in Pluripotency," *Nature Cell Biology* 23, no. 8 (2021): 834–845.

74. R. Guan, T. Lian, B.-R. Zhou, D. Wheeler, and Y. Bai, "Structural Mechanism of LIN28B Nucleosome Targeting by OCT4," *Molecular Cell* 83, no. 12 (2023): 1970–1982.

75. A. Zviran, N. Mor, Y. Rais, et al., "Deterministic Somatic Cell Reprogramming Involves Continuous Transcriptional Changes Governed by Myc and Epigenetic-Driven Modules," *Cell Stem Cell* 24, no. 2 (2019): 328–341.e9.

76. M. R. O'Dwyer, M. Azagury, K. Furlong, et al., "Nucleosome Fibre Topology Guides Transcription Factor Binding to Enhancers," *Nature* 638 (2024): 251–260, https://doi.org/10.1038/s41586-024-08333-9.

77. M. A. Frederick, K. E. Williamson, M. Fernandez Garcia, et al., "A Pioneer Factor Locally Opens Compacted Chromatin to Enable Targeted ATP-Dependent Nucleosome Remodeling," *Nature Structural & Molecular Biology* 30, no. 1 (2023): 31–37, https://doi.org/10.1038/s41594-022-00886-5.

78. L. Miao, Y. Tang, A. R. Bonneau, et al., "The Landscape of Pioneer Factor Activity Reveals the Mechanisms of Chromatin Reprogramming and Genome Activation," *Molecular Cell* 82, no. 5 (2022): 986–1002, https://doi.org/10.1016/j.molcel.2022.01.024.

79. J. Lerner, A. Katznelson, J. Zhang, and K. S. Zaret, "Different Chromatin-Scanning Modes Lead to Targeting of Compacted Chromatin by Pioneer Factors FOXA1 and SOX2," *Cell Reports* 42, no. 7 (2023): 112748, https://doi.org/10.1016/j.celrep.2023.112748.

80. S. L. Klemm, Z. Shipony, and W. J. Greenleaf, "Chromatin Accessibility and the Regulatory Epigenome," *Nature Reviews Genetics* 20, no. 4 (2019): 207–220, https://doi.org/10.1038/s41576-018-0089-8.

81. R. J. Geusz, A. Wang, D. K. LAM, et al., "Sequence Logic at Enhancers Governs a Dual Mechanism of Endodermal Organ Fate Induction by FOXA Pioneer Factors," *Nature Communications* 12, no. 1 (2021): 6636, https://doi.org/10.1038/s41467-021-26950-0.

82. A. Soufi, G. Donahue, and K. S. Zaret, "Facilitators and Impediments of the Pluripotency Reprogramming Factors' Initial Engagement With the Genome," *Cell* 151, no. 5 (2012): 994–1004, https://doi.org/10.1016/j.cell.2012.09.045.

83. A. Soufi, M. F. Garcia, A. Jaroszewicz, N. Osman, M. Pellegrini, and K. S. Zaret, "Pioneer Transcription Factors Target Partial DNA Motifs on Nucleosomes to Initiate Reprogramming," *Cell* 161, no. 3 (2015): 555–568, https://doi.org/10.1016/j.cell.2015.03.017.

84. A. Gouhier, J. Dumoulin-Gagnon, V. Lapointe-Roberge, J. Harris, A. Balsalobre, and J. Drouin, "Pioneer Factor Pax7 Initiates Two-Step Cell-Cycle-Dependent Chromatin Opening," *Nature Structural & Molecular Biology* 31, no. 1 (2024): 92–101, https://doi.org/10.1038/s41594-023-01152-y.

85. K. Horisawa, M. Udono, K. Ueno, et al., "The Dynamics of Transcriptional Activation by Hepatic Reprogramming Factors," *Molecular Cell* 79, no. 4 (2020): 660–676, https://doi.org/10.1016/j.molcel.2020.07.012.

86. M. A. Missinato, S. Murphy, M. Lynott, et al., "Conserved Transcription Factors Promote Cell Fate Stability and Restrict Reprogramming Potential in Differentiated Cells," *Nature Communications* 14, no. 1 (2023): 1709, https://doi.org/10.1038/s41467-023-37256-8.

87. T. Fujimori, C. Rios-Martinez, A. R. Thurm, et al., "Single-Cell Chromatin state Transitions During Epigenetic Memory Formation," *bioRxiv* 560616 (2023), https://doi.org/10.1101/2023.10.03.560616.

88. Y. Huang, H. Zhang, L. Wang, et al., "JMJD3 Acts in Tandem With KLF4 to Facilitate Reprogramming to Pluripotency," *Nature Communications* 11, no. 1 (2020): 5061, https://doi.org/10.1038/s41467-020-18900-z.

89. J. Wei, J. Antony, F. Meng, et al., "KDM4B-Mediated Reduction of H3K9me3 and H3K36me3 Levels Improves Somatic Cell Reprogramming Into Pluripotency," *Scientific Reports* 7, no. 1 (2017): 7514.

90. W. Liu, X. Liu, C. Wang, et al., "Identification of Key Factors Conquering Developmental Arrest of Somatic Cell Cloned Embryos by Combining Embryo Biopsy and Single-Cell Sequencing," *Cell Discovery* 2, no. 1 (2016): 1–15.

91. R. Xu, Q. Zhu, Y. Zhao, et al., "Unreprogrammed H3K9me3 Prevents minor Zygotic Genome Activation and Lineage Commitment in SCNT Embryos," *Nature Communications* 14, no. 1 (2023): 4807, https://doi.org/10.1038/s41467-023-40496-3.

92. C. Zhang, F. Macchi, E. Magnani, and K. C. Sadler, "Chromatin States Shaped by an Epigenetic Code Confer Regenerative Potential to the Mouse Liver," *Nature Communications* 12, no. 1 (2021): 4110, https://doi.org/10.1038/s41467-021-24466-1.

93. X. Sun, J. C. Chuang, M. Kanchwala, et al., "Suppression of the SWI/SNF Component Arid1a Promotes Mammalian Regeneration," *Cell Stem Cell* 18, no. 4 (2016): 456–466, https://doi.org/10.1016/j.stem.2016.03.001.

94. M. Nakamura, Y. Gao, A. A. Dominguez, and L. S. Qi, "CRISPR Technologies for Precise Epigenome Editing," *Nature Cell Biology* 23, no. 1 (2021): 11–22, https://doi.org/10.1038/s41556-020-00620-7.

95. P. Liu, M. Chen, Y. Liu, L. S. Qi, and S. Ding, "CRISPR-Based Chromatin Remodeling of the Endogenous Oct4 or Sox2 Locus Enables Reprogramming to Pluripotency," *Cell Stem Cell* 22, no. 2 (2018): 254, https://doi.org/10.1016/j.stem.2017.12.001.

96. J. K. Nuñez, J. Chen, G. C. Pommier, et al., "Genome-Wide Programmable Transcriptional Memory by CRISPR-Based Epigenome Editing," *Cell* 184, no. 9 (2021): 2503–2519, https://doi.org/10.1016/j.cell.2021.03.025.

97. Z. Chen and R. Natarajan, "Epigenetic Modifications in Metabolic Memory: What Are the Memories, and Can We Erase Them?," *American Journal of Physiology – Cell Physiology* 323, no. 2 (2022): C570–C582.

98. S. Sun and L. B. Barreiro, "The Epigenetically-Encoded Memory of the Innate Immune System," *Current Opinion in Immunology* 65 (2020): 7–13.

99. T. Paniza, M. Deshpande, N. Wang, et al., "Pluripotent Stem Cells With Low Differentiation Potential Contain Incompletely Reprogrammed DNA Replication," *Journal of Cell Biology* 219, no. 9 (2020): e201909163, https://doi.org/10.1083/jcb.201909163.

100. Y. Ovadya, T. Landsberger, H. Leins, et al., "Impaired Immune Surveillance Accelerates Accumulation of Senescent Cells and Aging," *Nature Communications* 9, no. 1 (2018): 5435, https://doi.org/10.1038/s41467-018-07825-3.

101. S. W. Criscione, Y. V. Teo, and N. Neretti, "The Chromatin Landscape of Cellular Senescence," *Trends in Genetics* 32, no. 11 (2016): 751–761, https://doi.org/10.1016/j.tig.2016.09.005.

102. S. Chaib, T. Tchkonia, and J. L. Kirkland, "Cellular Senescence and Senolytics: The Path to the Clinic," *Nature Medicine* 28, no. 8 (2022): 1556–1568, https://doi.org/10.1038/s41591-022-01923-y.

103. X. Hao, C. Wang, and R. Zhang, "Chromatin Basis of the Senescence-Associated Secretory Phenotype," *Trends in Cell Biology* 32, no. 6 (2022): 513–526, https://doi.org/10.1016/j.tcb.2021.12.003.

104. Z. Li, X. Guo, H. Huang, et al., "A Switch in Tissue Stem Cell Identity Causes Neuroendocrine Tumors in *Drosophila* Gut," *Cell Reports* 30, no. 6 (2020): 1724, https://doi.org/10.1016/j.celrep.2020.01.041.

105. B. Treutlein, Q. Y. Lee, J. G. Camp, et al., "Dissecting Direct Reprogramming From Fibroblast to Neuron Using Single-Cell RNA-seq," *Nature* 534, no. 7607 (2016): 391–395, https://doi.org/10.1038/nature18323.

106. Z. Liu, L. Wang, J. D. Welch, et al., "Single-Cell Transcriptomics Reconstructs Fate Conversion From Fibroblast to Cardiomyocyte," *Nature* 551, no. 7678 (2017): 100–104, https://doi.org/10.1038/nature24454.

107. Z. Liu, Y. Cai, Y. Wang, et al., "Cloning of Macaque Monkeys by Somatic Cell Nuclear Transfer," *Cell* 172, no. 4 (2018): 881–887.e7, https://doi.org/10.1016/j.cell.2018.01.020.

108. S. Matoba, Y. Liu, F. Lu, et al., "Embryonic Development Following Somatic Cell Nuclear Transfer Impeded by Persisting Histone Methylation," *Cell* 159, no. 4 (2014): 884–895, https://doi.org/10.1016/j.cell.2014.09.055.

109. W. Liu, X. Liu, C. Wang, et al., "Identification of Key Factors Conquering Developmental Arrest of Somatic Cell Cloned Embryos by Combining Embryo Biopsy and Single-Cell Sequencing," *Cell Discovery* 2, no. 1 (2016): 16010, https://doi.org/10.1038/celldisc.2016.10.

110. C. Zhu, M. Yu, H. Huang, et al., "An Ultra High-throughput Method for Single-Cell Joint Analysis of Open Chromatin and Transcriptome," *Nature Structural & Molecular Biology* 26, no. 11 (2019): 1063–1070, https://doi.org/10.1038/s41594-019-0323-x.

111. K. Vandereyken, A. Sifrim, B. Thienpont, and T. Voet, "Methods and Applications for Single-Cell and Spatial Multi-Omics," *Nature Reviews Genetics* 24, no. 8 (2023): 494–515, https://doi.org/10.1038/s41576-023-00580-2.

112. L. Liu, A. Chen, Y. Li, J. Mulder, H. Heyn, and X. Xu, "Spatiotemporal Omics for Biology and Medicine," *Cell* 187, no. 17 (2024): 4488–4519, https://doi.org/10.1016/j.cell.2024.07.040.

113. G. Liu, Y. Li, M. Li, et al., "Charting a High-Resolution Roadmap for Regeneration of Pancreatic β Cells by In Vivo Transdifferentiation From Adult Acinar Cells," *Science Advances* 9, no. 21 (2023): adg2183, https://doi.org/10.1126/sciadv.adg2183.

114. F. F. Rosa, C. F. Pires, I. Kurochkin, et al., "Single-Cell Transcriptional Profiling Informs Efficient Reprogramming of Human Somatic Cells to Cross-Presenting Dendritic Cells," *Science Immunology* 7, no. 69 (2022): abg5539, https://doi.org/10.1126/sciimmunol.abg5539.