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Epigenetic Obstacles Encountered By Transcription Factors: Reprogramming Against All Odds

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Abstract

Reprogramming of a somatic nucleus to an induced pluripotent state can be achieved *in vitro* through ectopic expression of Oct4 (Pou5f1), Sox2, Klf4 and c-Myc. While the ability of these factors to regulate transcription in a pluripotent context has been studied extensively, their ability to interact with and remodel a somatic genome remains underexplored. Several recent studies have begun to provide mechanistic insights that will eventually lead to a more rational design and improved understanding of nuclear reprogramming.

Introduction

With the report that ectopic expression of MyoD could induce a fibroblast to acquire myotube-like characteristics, many began to explore the effects of ectopic expression of lineage or cell type specific transcription factors (TFs) and their ability to induce cell state transitions [1–4]. TF mediated reprogramming experiments were complimented by studies using cell fusion and somatic cell nuclear transfer (SCNT) which demonstrated the ability to revert somatic nuclei to pluri- and totipotency, respectively [5–9]. However, the reprogramming field grew exponentially after 2006, when a seminal report by Takahashi and Yamanaka identified a combination of TFs whose ectopic expression was capable of reprogramming a somatic nucleus to a pluripotent state, now referred to as induced pluripotency or induced pluripotent stem cells (iPSCs)[10*]. Ectopic expression of Oct4 (Pou5f1), Sox2, Klf4 and c-Myc (OSKM) was shown to reprogram embryonic and adult fibroblast nuclei to a state resembling mouse embryonic stem cells (mESCs). Minor technical improvements to the original approach reported shortly thereafter enabled the establishment of iPSCs that were both molecularly and developmentally comparable to mESCs [11–14]. Successfully reprogrammed iPSCs exhibit the potential to contribute to each germ layer upon *in vitro* or *in vivo* differentiation [15–17], although at different efficiencies [18]. Despite notable variation that has been observed among iPSC cell lines [19*], their remarkable potential explains the continued excitement and therapeutic promise [20]. A recent perspective from Yamanaka suggested that the number of lines investigated in each study contributed notably to the respective conclusion whether or not differences exist between the tested ESC and iPSC lines [21].

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In this review, we will discuss selected advances in the reprogramming field with a particular focus on the interaction of TFs and the (epi)genome. This includes a closer inspection of characteristics that may influence the ability of various TFs to induce cell state conversions *in vitro*.

Transcription Factors and the Genome: An Epigenetic Barrier?

Epigenetic regulation of transcription through mechanisms such as posttranslational histone modifications and DNA methylation is essential for maintaining cellular identity both *in vivo* [22, 23] and *in vitro* [24, 25]. During differentiation and reprogramming, remodeling of the epigenetic landscape is dictated by TFs [26, 27] and also non-coding RNAs [28, 29]. Any newly acquired state will exhibit distinct DNA methylation and histone modification patterns as compared to the starting population [26, 30]. As a result, it is not too surprising that reprogramming can be assisted by the manipulation of epigenetic modifiers. For example, it was found that chemical depletion of DNA methyltransferases [26], inhibition of histone deacetylases [31], or knockdown of several chromatin modifying enzymes [32] improved the overall reprogramming efficiency.

Closer inspection of all TFs used in the various studies provides relevant insights for dissecting their ability to facilitate reprogramming (Box 1). In contrast to the more limited capacity of OSKM, some TFs used during direct cell state conversions exhibit a relatively unique ability to access and remodel target chromatin in a monomeric, ATP-independent fashion [33–35]. These factors have been termed “pioneering transcription factors,” a group defined by their ability to access and remodel heterochromatic regions towards open chromatin [36, 37]. It is noteworthy that many lineage-specific TF reprogramming combinations include pioneer TFs [38–42] (Table 1). The basic helix-loop-helix domain (bHLH) containing factor MyoD, for example, can bind to DNA in a heterochromatic chromatin state and initiate chromatin remodeling [43, 44]. More recently, members of the Fox family have been used to induce the transition from a fibroblast to a hepatocyte-like cell [40, 41] and ectopic expression of various Gata family TFs has been used to induce a cardiomyocyte-like state from fibroblasts [39]. As noted above, OSKM have not been shown to exhibit direct chromatin remodeling capabilities analogous to MyoD and the Fox/Gata families, but appear to cooperate with available chromatin regulators [45**].

Myc, a non-essential reprogramming factor, is categorized as a transcriptional pause release factor and is unlikely to cause chromatin decondensation given that it associates with the Pol II pre-initiation complex, which cannot assemble without preexisting H3K4 methylation [46–49]. Furthermore, a recent report suggested that Oct4 could promote the establishment of nucleosome-depleted regions (NDRs), another requirement for transcriptional activation, only in the absence of DNA methylation [50*]. This highlights Oct4’s limited ability to bind in a preexisting heterochromatic environment and explains why only selected Oct4 target genes show immediate changes in H3K4 methylation [51**]. In contrast to the restricted binding of c-Myc and Oct4, chromatin immunoprecipitation (ChIP) results detected binding of the pioneer factor FoxA1 at a distal regulatory element that contains DNA methylation [52] (Figure 1). During subsequent differentiation, DNA methylation was eliminated proximal to FoxA1 binding and this region exhibited *de novo* gain of H3K4me1, a modification associated with active and poised distal regulatory elements. Further supporting the Fox family’s abilities to remodel heterochromatic regions, this factor was also used in combination with other TFs to induce the conversion of fibroblasts to hepatocyte-like cells [41]. More recent technical advances combining ChIP with bisulfite sequencing (ChIP-BS-Seq) will enable a more detailed characterization of the direct relationships between TFs and DNA methylation in this context [53, 54].

Many pioneering factors such as FoxA1 and Gata4, perform their functions as monomers [33, 55–57] (Table 2), unlike Oct4, which requires dimerization with Sox2 in the pluripotent context [58, 59]. Further insight into the role of dimerization and cofactors during reprogramming was provided by a recent study that showed Sox17 could be transformed into a reprogramming factor by changing specific residues that promoted interaction with Oct4 [60**]. While the DNA binding profile of the new dimer was not directly interrogated, the ability of multiple *Sox*, but not *Oct*, family members to serve as iPSC reprogramming factors may suggest distinct roles for these families. Oct4 appears critical for DNA binding specificity while Sox2 or other Sox factors likely recruit histone acetyltransferases, such as p300 to these targets [61, 62]. More generally, these findings suggest that one could construct TFs with novel chromatin remodeling functions and DNA binding preferences. Systematic dissection of the functions associated with specific domains that have been retained by evolutionarily conserved TFs will provide the necessary insights. Interestingly, Hirayai *et al.* recently affixed the transactivation domain (TAD) of MyoD to Oct4, and examined this engineered factor's ability to promote the reprogramming of MEFs [63**]. Indeed they found that substituting the wildtype form of Oct4 with the TAD fusion construct accelerated the appearance of Oct4 positive colonies when ectopically expressed. This suggests that wildtype Oct4, in combination with the other reprogramming factors (SKM), has a limited ability to access and remodel heterochromatic regions, which is consistent with published observations [50*, 51**, 64]. However, it highlights that this constraint can be readily overcome by engineering improved reprogramming factors as shown for Oct4-TAD.

Although it appears that some of the direct conversion experiments do not require cell division for reprogramming, it is worth emphasizing that forkhead domain-containing factors remain bound to mitotic chromosomes [65], while Pou family members do not [66, 67]. The nuclear exclusion of Oct4 and Sox2 during mitosis, in combination with distinct Oct4-Sox2 stoichiometric requirements [68], which must be reestablished after each cell division, may therefore contribute to the low reprogramming efficiency.

Transcription Factor Induced Chromatin Dynamics and Remodeling

By isolating populations based on the number cell divisions post-OSKM induction, our lab previously reported that initial gene expression dynamics were predominantly observed at loci containing a preexisting euchromatic state as defined by H3K4 methylation and/or H3K27 methylation [51**]. However, a *de novo* or enhanced gain of H3K4me2 was observed at promoters of many developmental and pluripotency-associated genes prior to detectable transcription originating from these loci, suggesting that reprogramming cells transition through an early state of orchestrated epigenetic priming. The observed dynamics are in agreement with an independent study that found Oct4 to interact with Wdr5, a component of the MLL complex conferring H3K4 methylation [45**].

It appears that only parts of the somatic genome are amenable to immediate epigenetic remodeling as a result of ectopic OSKM expression. This is further supported by the observed correlation between the epigenetic state of the starting cell type and the reactivation of genes that are expressed in pluripotent cells [26]. Taberlay and colleagues found that putative enhancer regions enriched for the repressive mark H3K27me3 that also contain a NDR nearby, permit Oct4 binding [69**]. Ectopic expression of OCT4 in human fibroblasts confirmed its binding to the NDR within the H3K27me3-enriched enhancer of *MYOD*. This resulted in subsequent gain of H3K4me3 at the promoter while maintaining enrichment of H3K27me3 and transcriptional silencing [69**]. Examining the nucleosome deposition and DNA methylation dynamics during *in vitro* differentiation in more detail shows that genomic regions silenced via histone modifications remain generally more amenable to TF binding and subsequent activation, while regions that have already gained

DNA methylation are typically not reactivated within the same time frame [50*]. Interestingly, multiple-epithelial related genes such as *Cdh1* and *Ocln* that are activated during the early to intermediate stages of reprogramming [70, 71] contain CpG island (CGI)-promoters, and remain free of DNA methylation in MEFs and are instead silenced via H3K27 methylation. This permissive chromatin state explains why certain loci show more dynamic chromatin changes upon induction of OSKM than others [50*, 51**, 69**].

Facilitating the Elimination of Heterochromatin

In order to successfully convert one cell type into another, the original gene expression program needs to be silenced and the new transcriptional program must be established in such a way that it can be stably propagated. To systematically screen for novel factors that may facilitate reprogramming, Singhal *et al.* conducted a genome-wide shRNA screen and found that ectopic expression of Brg1 and Baf155 had a notable effect [72**]. The results suggest that nucleosome remodeling is a key factor in the cell state transition and that core components of the BAF complex can facilitate the establishment of euchromatin. Ectopic expression of these factors enhanced binding of Oct4 at multiple promoters [72**], which is consistent with the prior observations that its binding is generally limited by heterochromatic regions and DNA methylation [69**] (Figure 1). Recent unrelated DNA methylation studies in mESCs further support the antagonistic relationship between TF binding and DNA methylation on a global scale [73**, 74**]. The notion that TF binding protects regions from DNA methylation has been established by classic studies [75, 76] and is further supported by more recent evidence including FoxD3 binding at the *Alb1* enhancer in mESCs which is responsible for maintaining an unmethylated, primed state at this locus [77]. It seems that the creation of NDRs during reprogramming could facilitate loss of DNA methylation at discrete sites by allowing TFs to bind the DNA, consequently protecting regions from further inheritance of DNA methylation during ensuing cell divisions and thereby facilitate eventual activation.

Concluding Remarks

More than three decades of TF mediated reprogramming studies, including hundreds of recent papers focused on OSKM reprogramming, have begun to provide mechanistic insights that make the outcome of ectopic TF expression more predictable. Here, we have summarized selected reports regarding the role of some TFs and the extent to which they can function within a nuclear reprogramming context as illustrated in Figure 1. The distinct abilities of various TFs to induce epigenetic changes including chromatin remodeling have highlighted the need for methodical examination of domain-specific functions, their global- and context-specific abilities to access binding sites, the influence of context-specific co-factors and the presence of epigenetic remodeling complexes. The growing number of detailed epigenetic maps for a broad range of cell types will enable further integration and eventually lead to more targeted manipulations. In time, these analyses will provide the insight necessary to promote rationally designed reprogramming experiments, develop more powerful reprogramming factors and create more advanced methods for the production of desired cell types *in vitro*.

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Box 1. TFs utilized to induce cellular transitions

Many combinations of TFs have been utilized to induce cellular transitions (Table 1). In contrast to the factors that reprogram somatic cells to iPSCs, the combinations of factors used for these experiments generally include a factor with known chromatin remodeling capabilities, such as Fox and Gata TFs (Table 1). Additionally, these experiments commonly use factors with the ability to bind DNA as monomers, which are not reported features of OSKM in the specific context of reprogramming to iPSCs (Table 2). TF binding abilities are presumably context dependent, therefore the information included in Table 2, which was often derived *in vitro* or using limited cell types, should be considered with these caveats in mind.

Figure 1. Chromatin Remodeling induced by ectopic TF expression

Schematic of two types of TFs (Oct and Fox) and their ability to bind DNA methylated regions when overexpressed in fibroblasts. Oct4 cannot bind until DNA methylation is removed. Once it binds it can recruit factors like Wdr5 to induce gain of H3K4 methylation. This is insufficient to cause transcriptional activation and likely requires additional factors/complexes (indicated by the question mark) to create nucleosome depleted regions (NDRs) and expression. In contrast a pioneering TF, such as FoxA1 has the ability to bind multiple chromatin states including DNA methylated regions directly (as a monomer) and will subsequently induce remodeling.

Table 1

Starting cell type	Induced state	TFs used
MEFs	neural precursor	Brn2, Sox2, FoxG1 [78]
MEFs	motor neuron	Asc11, Brn2, Myt11, Lhx3, Hb9, Isl1, Ngn2 [42]
MEFs	cardiomyocyte	Gata4, Mef2c, Tbx5 [39]
MEFs	hepatocyte-like	Hnf4 α plus Foxa1, Foxa2 or Foxa3 [41]
MEFs	hepatocyte-like	Gata4, Hnf1 α , Foxa3 [40]
B cells	macrophage	CEBP α [3]
pancreatic exocrine cells	β -cells	Ngn3, Pdx1 and Mafa [79]
MEFs	macrophage-like	PU.1, CEBP α , CEBP β [38]
somatic cells	iPSC	Oct4, Sox2, Klf4, c-Myc [10]

Table 2

TF	Conserved domains/families	Binding preference
Asl1	HLH	homodimer/heterodimer [80]
Bm2	homeodomain, POU	Homodimer [81]
c-Myc	HLH, MYC-N, MYC-LZ	Heterodimer [82]
CEBPa	bZIP	Homodimer [76]
CEBPb	bZIP	Heterodimer/Homodimer [83]
FoxA1	FH, HNF_C	Monomer [33]
FoxA2	FH, HNF_C	Monomer [33]
FoxA3	FH, HNF_C	Monomer [84]
Gata4	ZnF_Gata, GATA_N	Monomers [33]
Hb9/mnx1	homeodomain	unknown
Hnf4a	NR_DBD	Homodimer [85]
Isil	homeodomain, LIM1, LIM2	unknown
Klf4	zf-H2C2_2, zf-C2H2	unknown
Lhx3	homeodomain, LIM1, LIM2	Monomer [86]
Mafa	bZIP, Maf_N	monomer, homodimer [87]
Mef2c	MADS	homodimer, heterodimer [88]
MyoD	HLH, Myf5	monomer, heterodimer [89]
Myt1l	MYT1, C2H2, TMF, SMC	unknown
Ngn2	HLH	heterodimer [90]
Ngn3	HLH	Heterodimer [91]
Pdx1	homedomain	monomer, heterodimer, heterotrimer [92]
POU5f1	homeodomain	homodimer, heterodimer [59]
PU.1	ETS	unknown
Sox2	HMG-box, SOXP	monomer, heterodimer [59]
Tbx5	T-box	Monomer, heterodimer [93]

Abbreviations: HLH (helix-loop-helix), FH (forkhead), HNF_C (Hepatocyte Nuclear Factor C terminal domain), GATA_N (Gata-type transcriptional activator, N terminus), NR_DBD (nuclear receptor, DNA binding domain), zf-H2C2_2 (Zinc-finger double domain), bZIP (basic region, leucine zipper), MYT1 (myelin TF 1), ZF-C2H2 (zinc-finger double domain), TMF (TATA element modulatory factor 1), NDR (Nucleosome Depleted Region)