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From Fibroblasts to iPS Cells: Induced Pluripotency by Defined Factors

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ABSTRACT

Patient-specific pluripotent cells may serve as a limitless source of transplantable tissue to treat a number of human blood and degenerative diseases without causing immune rejection. Recently, isolation of patient-specific induced pluripotent stem (iPS) cells was achieved by transducing fibroblasts with four transcription factors, Oct4, Sox2, Klf4, and c-Myc. However, the use of oncogenes and retrovirus in the current iPS cell establishment protocol raises safety concerns. To generate clinical quality iPS cells, the development of novel reprogramming methods that avoid permanent genetic modification is highly desired. The molecular mechanisms that mediate reprogramming are essentially unknown. We argue that establishment of a stable and self-sustainable ES-specific transcriptional regulatory network is essential for reprogramming. Such a system should include expression of Oct4, Sox2, Nanog and probably other pluripotenty-promoting factors from endogenous loci and establishment of a permissive epigenetic state to maintain such expression. In addition, though not yet proven experimentally, overcoming cellular senescence of fibroblasts by inactivating Rb and p53 pathways and up-regulating telomerase activity may also be required. J. Cell. Biochem. 105: 949–955, 2008.

KEY WORDS: PLURIPOTENCY; REPROGRAMMING; EMBRYONIC STEM CELLS; iPS CELLS

Embryonic stem (ES) cells are derived from pre-implantation embryos, can be propagated long-term in vitro, and can differentiate into all three embryonic germ layers. Pluripotent cells that are genetically matched to a specific patient may serve as a limitless source of transplantable tissues to treat a spectrum of blood and degenerative diseases without evoking immune rejection [for review, Lerou and Daley, 2005]. Although tremendous effort has been put into deriving such immune compatible cells, success was limited until very recently when induced pluripotent stem (iPS) cells were obtained by transducing embryonic and adult fibroblasts with defined transcription factors [Takahashi and Yamanaka, 2006]. In this review, we will summarize the landmark discoveries of iPS cell derivation, then discuss the potential problems and challenges faced by this field. Finally, we will provide an overview of the mechanisms that maintain pluripotency and discuss the potential mechanisms of reprogramming.

INDUCED PLURIPOTENCY: GANG OF FOUR

In mid 2006, Takahashi and Yamanaka reported that mouse embryonic fibroblasts (MEFs) and adult tail tip fibroblasts could be reprogrammed back to a pluripotent state by introducing four transcription factors Oct4, Sox2, Klf4 and c-Myc via retroviral delivery coupled to reactivation of a neomycin-resistant reporter gene knocked into the *Fbx15* locus [Takahashi and Yamanaka, 2006]. Colonies of iPS cells were isolated about 2 weeks after viral transduction. Although expressed in undifferentiated ES cells, the *Fbx15* gene is dispensable to maintain self-renewal and pluripotency [Tokuzawa et al., 2003]. iPS cells exhibit many features characteristic of ES cells: they are positive for alkaline phosphatase and the ES cell-specific surface marker SSEA-1, express Nanog from the endogenous locus, differentiate into all three germ layers in vitro, and form teratomas when injected into immunodeficient mice.

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However, iPS cells differ from ES cells in a number of ways. For example, when comparing genome-wide expression, a significant number of genes were found differentially expressed between ES cells and iPS cells. In addition, when iPS cells were injected into blastocysts, chimeric embryos arrested in mid-gestation stage, indicating that iPS cells possess limited developmental potential. These data suggest that iPS cells selected by *Fbx15* reactivation most likely represent an intermediate phenotype between somatic fibroblasts and true pluripotent ES cells.

A second major advance of the field came just months later—the isolation of germline-competent iPS cells [Maherali et al., 2007; Okita et al., 2007; Wernig et al., 2007]. In these studies, instead of driving selection via reactivation of the *Fbx15* locus, the authors chose either *Oct4* or *Nanog*. The rationale was that both Oct4 and Nanog are essential to the pluripotent state of ES cells, and reactivation of endogenous loci of genes that are essential to pluripotency may better correlate with a more complete degree of reprogramming than *Fbx15*. Blastocyst injection of iPS cells isolated via *Oct4* or *Nanog* reactivation produced highly chimerized live pups as well as germ cells [Maherali et al., 2007; Okita et al., 2007; Wernig et al., 2007]. As germ-line transmission is a feature of only the most pristine cells, it is considered one of the most stringent criteria to evaluate pluripotency.

Following on this work, at the end of 2007 three groups demonstrated the isolation of iPS cells from human embryonic, neonatal and adult fibroblasts [Takahashi et al., 2007; Yu et al., 2007b; Park et al., 2008]. In all three studies, human iPS cells were isolated based on the distinct colony morphology about 3 weeks after viral infection. Human iPS cells exhibited features of human ES cells: they are positive for alkaline phosphatase, express surface markers SSEA-3 and Tra-1-60, express Oct4 and Nanog from endogenous loci, differentiate into all three germ layers in vitro, and form mature teratomas when injected into immunodeficient mice. Genome-wide microarray analyses revealed that the global expression pattern of iPS cells is more similar to ES cells than fibroblasts, and that a majority of ES cell-specific genes are reactivated in iPS cells. It is interesting to note that two groups achieved isolation of human iPS cells using the same four factors that reprogram mouse fibroblasts [Takahashi et al., 2007; Park et al., 2008], while Yu et al. [2007b] identified a different combination of four factors Oct4, Sox2, Nanog and a RNA binding protein Lin28. Lin28 has recently been shown to regulate the biogenesis of the let7 family of microRNAs [Newman et al., 2008; Viswanathan et al., 2008], providing insights into the mechanisms of reprogramming (as discussed below). The identification of novel reprogramming factors suggests that there might be several gene combinations that can achieve reprogramming. However, whether the iPS cells isolated by the different combinations of factors are identical, and whether the various recipes are equally effective with mouse fibroblasts remains to be studied. Recently, a growing number of groups have succeeded in deriving human iPS cells, proving that the technique is robust [Lowry et al., 2008; Mali et al., 2008; Masaki et al., 2008].

In addition to reprogramming embryonic and neonatal fibroblasts, Park et al. successfully isolated iPS cells from primary skin fibroblasts obtained from a healthy adult donor via the aid of two additional factors, *hTERT* and SV40 large T antigen, albeit with very low efficiency [Park et al., 2008]. The role of *hTERT* and large T in reprogramming remains unclear, especially considering that neither of the factors was incorporated into the genome of iPS cells isolated. A recent study confirmed that large T antigen enhanced reprogramming efficiency by up to 70-fold, but also found integration of the large T viral sequence and chromosomal aneuploidy [Mali et al., 2008]. Nonetheless, the successful reprogramming of adult fibroblasts demonstrated that it is possible to generate iPS cells from a patient, which represents a step closer to the potential clinical use of these cells.

THE PROMISE, THE PROBLEM, AND THE CHALLENGE

The potential therapeutic value of iPS cells was well illustrated in a recent study [Hanna et al., 2007]. Here the authors successfully treated a mouse model of sickle cell anemia by combining cell and gene therapy. iPS cells were first derived from the tail tip fibroblasts of a mouse with sickle cell anemia. The mutant β-globin allele was repaired in the iPS cells by homologous recombination. Repaired iPS cells were differentiated into hematopoietic stem cells and transplanted back into the diseased mouse. Analysis of peripheral blood 12 weeks after transplantation revealed normal blood and amelioration of symptoms. Although a similar proof-of-principle experiment using genetically matched ES cells derived by somatic cell nuclear transfer (SCNT) was achieved previously [Rideout et al., 2002], derivation of human ES cells by SCNT has never been achieved. Thus, the success in isolating iPS cells from human primary fibroblasts makes this strategy extremely promising as a methodology for clinical use.

Compared to other existing strategies of deriving patient-specific pluripotent cells, such as SCNT, cell fusion, trans-differentiation of germ cells [for review, Hochedlinger and Jaenisch, 2006] and parthenogenesis [Kim et al., 2007a,b], direct reprogramming with defined factors is relatively easy and efficient. Contrast to SCNT and parthenogenesis, derivation of patient-specific iPS cells does not require a supply of human oocytes or pre-implantation embryos. In addition, iPS cells maintain a normal karyotype and imprinting status [Maherali et al., 2007], which differs from pluripotent cells generated by cell fusion, parthenogenesis and in vitro trans-differentiation of germ cells [Hochedlinger and Jaenisch, 2006; Kim et al., 2007a]. However, iPS cells generated via current technologies have their own problems that must be solved before any therapeutic use.

First, genes that are oncogenic must be omitted from the combination of reprogramming factors. Although the retroviral-delivered genes are silenced in most iPS cells, they could nevertheless be reactivated in more differentiated cells, leading to the risk for malignant progression. In keeping with this notion, about 20% of chimeric mice derived from iPS cells developed tumors within a 2- to 10-month period, most likely due to reactivation of the retroviral version of the *c-Myc* oncogene [Okita et al., 2007]. Recently, two groups independently reported that fully reprogrammed mouse and human iPS cells could be isolated at a much lower efficiency without using *c-Myc* [Nakagawa et al., 2008; Wernig et al., 2008]. Chimeric mice generated from the mouse iPS

cells were free of tumors within a 4-month period. In contrast, about 15% of mice derived from the iPS cells established by four factors developed tumors within this same time period [Nakagawa et al., 2008]. Alternatively, Yu et al. [2007b] isolated human iPS cells by substituting *Klf4* and *c-Myc* with *Nanog* and *Lin28*, which could be an alternative strategy to reduce the risk of tumors. While *c-Myc* is a well-known oncogene, *Klf4* has been shown to transform epithelial cells [Rowland and Peeper, 2006], and ectopic expression of *Oct4* has been demonstrated to induce dysplastic growth in epithelial tissues [Hochedlinger et al., 2005]. Elevated expression of *Oct4*, *Sox2*, *Klf4*, and *Nanog* has been reported in various tumors [Rowland and Peeper, 2006; Clark, 2007; Gu et al., 2007]. The risk of tumor formation by reactivating transgenes in tissues derived from reprogrammed cells, especially in the case of human iPS cells, has not yet been fully evaluated.

Beyond the direct concern stemming from the over-expression of oncogenes, the risk of indirect negative effects via insertions elsewhere in the genome presents additional caveats. It has been unfortunately demonstrated in a viral-based gene therapy trial viral integration may elevate transcription of a nearby oncogenic factors and could eventually lead to leukemia [Hacein-Bey-Abina et al., 2003]. As viral integration is permanent and irreversible, novel reprogramming methods that involve transient expression of factors or using chemical modulators may prove to be a superior way to generate clinically useful iPS cells.

Additionally, given the low efficiency of iPS cell derivation, it has been postulated that viral integration into particular genomic sites may enhance or be required for successful reprogramming. This question was addressed recently by mapping viral integration sites in several mouse iPS cell lines, where no common viral integration sites were found [Aoi et al., 2008]. Although the number of integration sites examined was too few to conclusively eliminate the possible effects of insertional mutagenesis, this study suggests that reprogramming capacity was intrinsic to the factors introduced, and more importantly that it might be possible to develop non-integrative methods to achieve reprogramming. In order to have the greatest chances of achieving such a feat, it will undoubtedly be of enormous utility to obtain a more complete understanding of what mediates the pluripotent state.

MOLECULAR MECHANISM UNDERLYING REPROGRAMMING

PLURIPOTENCY-PROMOTING TRANSCRIPTIONAL FACTORS

What is ''stemness'' is a question asked for many years, yet a complete answer remains elusive. There are many features that distinguish ES cells from differentiated cells, including gene expression, microRNA expression, epigenetic modifications, cell cycle regulation, and telomerase activity. Genome-wide expression profiling revealed a large set of ES cell-specific or enriched genes [Ramalho-Santos et al., 2002]. Among them are the aforementioned and extensively studied pluripotency-promoting transcription factors Oct4, Sox2, and Nanog [for review, Niwa, 2007]. Mouse embryos lacking either Oct4 or Sox2 do not form the epiblast, the population of pluripotent cells within mouse embryos. Over-expression of Nanog enhances ES cell self-renewal, however, over-expression of either Oct4 or Sox2 unexpectedly induces differentiation [Niwa, 2007]. It seems that the expression levels of Oct4 and Sox2 must be maintained within a very narrow range in order to support pluripotency. Genome-wide mapping of the binding sites of these three factors revealed that they bind alone or cooperatively to the promoters of several hundred target genes [Boyer et al., 2005; Loh et al., 2006]. On one hand, they serve as transcriptional activators that enhance the expression of genes that maintain pluripotency, which include themselves; while on the other hand, they also serve as transcription repressors to down-regulate lineage-specific genes and thereby prevent differentiation. Furthermore, a protein interaction network between Oct4, Nanog, and other pluripotency-promoting factors in mouse ES cells has also been described [Wang et al., 2006]. Within this network, there exist a large number of factors previously demonstrated as transcriptional targets of Oct4, Sox2, and Nanog [Boyer et al., 2005]. Many of these target genes are transcriptional factors and chromatin modifiers that have important roles in early mouse embryogenesis in vivo or in maintaining pluripotency in ES cells in vitro [Wang et al., 2006]. These data suggest that ES cells possess a unique transcriptional and protein interaction network, which when disrupted leads to differentiation.

The role of *Klf4* and its family members in the maintenance of pluripotency have not been realized until recently. Although *Klf4* is an abundant transcript in ES cells, knocking down *Klf4* does not exhibit an obvious phenotype [Nakatake et al., 2006], most likely due to functional redundancy among other *Klf* family members in ES cells. Simultaneous depletion of multiple Klf proteins leads to differentiation [Jiang et al., 2008]. Furthermore, expression of a small group of ES cell-specific genes requires *Klf* family proteins [Nakatake et al., 2006]. In addition, Klf family members appear to share a significant number of target genes with Nanog, suggesting Klf proteins are critical components of the transcriptional regulatory network in pluripotent cells [Jiang et al., 2008].

MICRORNAs

A group of microRNAs is specifically expressed in ES cells [Suh et al., 2004]. The exact role that microRNAs play in the maintenance of pluripotency remains elusive, as ES cells lacking either Dicer or DGCR8, enzymes required for microRNA processing, continue to express all the markers that are unique to the undifferentiated state [Murchison et al., 2005; Wang et al., 2007]. Instead, these cells have defects in differentiation, suggesting that microRNAs play an important role in lineage specification. Lin28, one of the factors used to reprogram human fibroblasts [Yu et al., 2007b], has recently been shown to block processing of the *let-7* family microRNA in ES cells [Newman et al., 2008; Viswanathan et al., 2008]. *Let-7* family members also have been implicated in the promotion of differentiation of cancer stem cells [Yu et al., 2007a; Kumar et al., 2008]. Thus, Lin28 may facilitate reprogramming by repressing *let-7*-induced differentiation in fibroblasts. These data suggest that pluripotency is maintained by at least two mechanisms, where genes that actively promote pluripotency are expressed alongside repression of microRNAs and other factors that regulate differentiation.

EPIGENETIC CHARACTERISTICS

ES cells possess a unique epigenetic state. Investigation of the methylation status of histone H3 reveals a unique bivalent modification pattern where both lysine 4 (K4) and lysine 27 (K27) are methylated at a number of loci that encode transcription factors important for lineage specification [Bernstein et al., 2006]. This is curious, considering that K4 methylation represents a permissive transcriptional state while K27 methylation is repressive. Genes whose histone H3s are bivalently modified usually express at low levels. As it is unusual that both permissive and repressive modifications coexist at the same loci, it has been hypothesized that bivalent modification is essential to balance the two central characteristics of ES cells—their capacity for self-renewal and differentiation [Bernstein et al., 2006].

Besides histone modifications, ES cells also bear a different DNA methylation pattern when compared to fibroblasts. The promoters of important pluripotency genes, such as *Oct4* and *Nanog*, are known to be free of methylation in ES cells but are heavily methylated in fibroblasts. During reprogramming, the methylation marks on these promoters are either removed or lost passively [Takahashi and Yamanaka, 2006; Maherali et al., 2007; Takahashi et al., 2007; Wernig et al., 2007; Yu et al., 2007b; Park et al., 2008]. Indeed, the extent of demethylation of these promoters may reflect the fidelity and completeness of cellular reprogramming. It has been found that the iPS cells selected by reactivation of the *Fbx-15* locus exhibit demethylation of the *Nanog* promoter, but not that of *Oct4* [Takahashi and Yamanaka, 2006]. Consistent with this, *Fbx-15* selected cells are only partially reprogrammed, as evidenced by their inability to from live chimeras.

CELL CYCLE

Cell cycle regulation is another unique feature that distinguishes ES cells from differentiated cells. ES cells transit through cell cycle much faster than differentiated cells mainly due to a shortened G1 phase. In mouse embryonic fibroblasts (MEFs), the G1 phase lasts 15–20 h and temporally accounts for more than 80% of the cell cycle. However, in both mouse and human ES cells, G1 lasts 2–4 h and temporally accounts for only 15–20% of the cell cycle. This unique cell cycle pattern is further characterized by hyperphosphorylated RB protein, constitutively high activity of cyclin E and A-associated kinases, and a lack of expression of major CDK inhibitors [Stead et al., 2002]. Upon differentiation, the ES cell-cycle pattern quickly switches to a MEF-like pattern [Savatier et al., 1996]. The role of a shortened G1 phase in maintaining pluripotency is not clear, though the exclusivity of this unique cell cycle among cells that are pluripotent suggests it is important. Another difference between ES cells and somatic cells is the activity of telomerase, where ES cells, as well as many adult stem cells, show a much higher telomerase activity. Similar to ES cells, iPS cells exhibit a cell cycle with a shortened G1 phase [Maherali et al., 2007] and elevated telomerase activity [Takahashi and Yamanaka, 2006; Takahashi et al., 2007; Yu et al., 2007b].

HOW DO THESE FEATURES YIELD PLURIPOTENCY?

Derivation of iPS cells is a gradual process that extends over weeks in tissue culture [Brambrink et al., 2008; Stadtfeld et al., 2008]. It starts with transducing fibroblasts with viruses carrying three or four factors and culminates in the isolation of iPS cells manifesting most of the features that ES cells have. The introduced reprogramming factors are sufficient to induce all the events required to establish pluripotency. Here, we argue that during reprogramming, fibroblasts gradually establish a stable, self-sustainable transcriptional regulatory network that includes expression of key pluripotent regulators, such as Oct4, Sox2, and Nanog from the endogenous loci in conjunction with the establishment of a permissive epigenetic state to maintain this expression. In addition, fibroblasts must also undergo a profound change in cell cycle regulation, which may be critical to overcome cellular senescence.

THE TIMING OF REPROGRAMMING

Two recent studies demonstrated that molecular markers are sequentially expressed during reprogramming of mouse fibroblasts [Brambrink et al., 2008; Stadtfeld et al., 2008]. In the first 3–5 days after viral transduction, the fibroblast-specific marker Thy-1 was down-regulated [Stadtfeld et al., 2008], and ES cell-specific marker alkaline phosphatase was up-regulated [Brambrink et al., 2008] in a large proportion of fibroblasts. In subsequent days, a population of SSEA-1 positive cells emerged within the previously Thy-1 negative or alkaline phosphatase positive cells. Around 10–14 days after the initial viral transduction, the endogenous *Oct4* or *Nanog* locus was reactivated in a small percentage of cells within the SSEA-1 positive population. Fully reprogrammed iPS clones that are independent from ectopically expressed factors can only be isolated at this stage.

Data from the two studies suggested that reprogramming should involve repression of the differentiated phenotype by turning off factors that induce lineage specification, while establishing the ES cell phenotype by turning on genes that promote pluripotency. The virally delivered reprogramming factors may serve as a trigger to initiate a process that activates expression of other pluripotency-promoting genes [Boyer et al., 2005; Loh et al., 2006]. Together with the products of the newly activated genes, the virally expressed factors might reconstitute the core elements of the ES cell-specific protein interaction network [Wang et al., 2006] and recruit other transcription factors and chromatin modifiers to induce more stable and global changes. In addition to direct activating transcription of pluripotency genes, these protein complexes should also be able to guide proper DNA methylation, demethylation, and histone modification at various loci, where two important outcome will be the erasure of fibroblast-specific epigenetic marks and the establishment of ES cell-specific epigenetic modifications. Epigenetic changes may ensure endogenous expression of *Oct4*, *Sox2*, *Nanog*, and other genes critical for pluripotency, which are the foundation for the long-term stability and self-sustainability of the transcription regulatory network.

DNA METHYLATION AND HISTONE MODIFICATION

Although Oct4, Sox2, and Nanog are known to bind to their own promoters and up-regulate gene expression [Boyer et al., 2005; Loh et al., 2006], activation of the endogenous loci of these genes only

comes at later stages of reprogramming [Brambrink et al., 2008; Stadtfeld et al., 2008]. Promoter demethylation is a prerequisite for activation [Maherali et al., 2007; Takahashi et al., 2007; Wernig et al., 2007; Yu et al., 2007b; Park et al., 2008]. DNA demethylation can be achieved in two ways. In active demethylation, an unidentified enzyme removes methyl groups from cytosine residues. This is a rapid process and has been demonstrated to occur in the paternal pronucleus during fertilization [Mayer et al., 2000] and probably in primordial germ cells during migration and entry into the genital ridge [Hajkova et al., 2002]. In contrast, passive demethylation requires cell proliferation, as methyl groups are lost due to the lack of methylation maintenance on newly synthesized DNA strands. Due to the slow kinetics of reprogramming, it is likely that demethylation of the *Oct4* and *Nanog* promoters occurs passively. As reactivation of the endogenous *Oct4* and *Nanog* loci may reflect the extent of reprogramming in fibroblasts, understanding when and how demethylation occurs, especially in the context of defined sequential molecular markers, will be critical [Brambrink et al., 2008; Stadtfeld et al., 2008].

To repress the fibroblast phenotype and keep iPS cells from differentiation, down-regulation of genes encoding lineage specification factors is required, where de novo methylation at lineage-specific loci may represent an additional mechanism. The role of methylation in reprogramming can be evaluated in fibroblasts that are deficient for Dnmt3a or 3b, the enzymes catalyzing de novo methylation. Establishment of the ES cell-specific bivalent H3 modification in iPS cells [Maherali et al., 2007] may serve as an alternative method to form repressive chromatin structures, as this type of modification usually correlates with a low level expression of genes encoding lineage specification factors [Boyer et al., 2005]. The mechanisms by which the bivalent H3 modification is established and maintained, and how this modification contributes to the establishment and maintenance of pluripotency are questions that have yet to be answered.

The unique histone modification pattern of ES cells suggests that activity of histone modification enzymes might be critical for reprogramming. It has been demonstrated that Oct4 regulates expression of two histone H3 demethylases [Loh et al., 2007]. These enzymes enhance expression of a group of pluripotency genes by removing transcriptionally repressive methylation from K9 of H3. Depletion of either of these enzyme leads to ES cell differentiation. As both enzymes directly link epigenetic modification to pluripotency, they may be key players in reprogramming.

OVERCOME CELLULAR SENESCENCE

During reprogramming, fibroblasts not only become pluripotent, they also become immortal. Fibroblasts proliferate a finite period of time before entering into senescence. In contrast, ES cells and iPS cells do not experience such a limitation. Immortalization requires that at least two barriers be overcome: cellular senescence and telomere shortening [for reviews, Drayton and Peters, 2002; Herbig and Sedivy, 2006]. Rb and p53 are the key senescence-inducing factors. Interestingly, in ES cells, the Rb pathway is constitutively inactivated due to hyperphosphorylation [Savatier et al., 1994], while certain aspects of p53 function are compromised [Qin et al., 2007]. Although activity of the Rb and p53 pathways have not been examined in iPS cells, a similar cell cycle profile to ES cells suggests that both of pathways are also down-regulated. However, escaping from cellular senescence does not automatically ensure immortality. Cells can enter a so-called replication crisis state where they undergo apoptosis if their telomere erodes below a critical length [Wright and Shay, 1992; Ducray et al., 1999]. To avoid telomere shortening, the activity of telomerase must be up-regulated. Myc directly up-regulates the transcription of *TERT*, the gene encoding the enzymatic subunit of the telomerase [Wu et al., 1999]. However, it is unclear if elevated telomerase activity in iPS cells is due to ectopic expression of *c-Myc* and how much the resulting change of telomerase activity contributes to reprogramming. It is also unclear how the four factors find ways to inactivate Rb and p53 and to what extent this contributes to reprogramming, where fibroblasts that are defective in Rb or p53 pathways may be informative resource to test this hypothesis. It is also worth stressing that immortalization by reprogramming is different from transformation. iPS cells are immortal in their undifferentiated state. Upon differentiation, both the Rb and p53 pathways become fully functional again through yet unknown mechanisms.

The successful isolation of iPS cells from fibroblasts has brought a new era of stem cell biology, one providing the opportunity for researchers to understand the nature of pluripotency. It will be necessary to develop novel methods to create iPS cells without modification. The next a few years will see new reprogramming factors and pathways identified, not to mention more studies designated to reveal the molecular mechanisms of reprogramming. Understanding these mechanism may in turn guide us to the development of novel methods to generate the next generation of iPS cells, ones with direct clinical utility.

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