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Crosstalk Between Stem Cell And Cell Cycle Machineries

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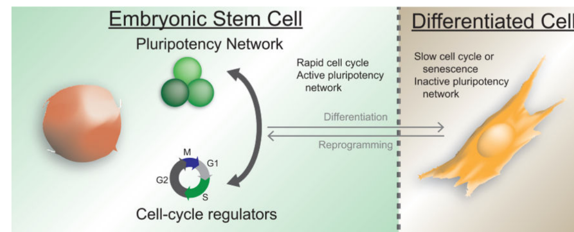
Abstract

Pluripotent stem cells, defined by an unlimited self-renewal capacity and an undifferentiated state, are best typified by embryonic stem cells. These cells have a unique cell cycle compared to somatic cells as defined by a rapid progression through the cell cycle and a minimal time spent in G1. Recent reports indicate that pluripotency and cell cycle regulation are mechanistically linked. In this review, we discuss the reciprocal co-regulation of these processes, how this co-regulation may prevent differentiation, and how cellular reprogramming can re-establish the unique cell cycle regulation in induced pluripotent stem cells.

Graphical abstract



Introduction

The proper development of a metazoan organism consisting of a variety of specialized cell types requires the strict co-regulation of the differentiation and cell cycle machineries. As a cell acquires its fully differentiated state, concomitant exit from the cell cycle ensures the integrity of the genome and prevents tumorigenesis. At the opposite end of this spectrum, pluripotent stem cells persist in a state of rapid proliferation. These cells have a unique cell

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cycle consisting of a short G1 phase, which in part serves to impede differentiation [1–3]. Once the purview of developmental biologists, the fundamental question of how the cell cycle and differentiation are linked has become critical to a broad swath of disciplines including regenerative medicine, cancer biology, and aging. This review will examine recent findings on the dynamic regulation between the pluripotency and cell cycle networks.

Reciprocal regulation of cell cycle and pluripotency networks: Pluripotency regulation of the cell cycle

The pluripotent network consists of a core set of transcription factors, including Oct4 (Pou5f1), Sox2, and Nanog, which serve to establish the undifferentiated state and the self-renewing capacity of embryonic stem (ES) cells [reviewed in 4,5]. While it is clear that a major role of these core transcription factors is the activation of the greater pluripotency network [6], an emerging emphasis on crosstalk with the cell cycle machinery has recently been identified (Figure 1, Table 1). Early studies of the core pluripotency network identified *Myc* as a target of Oct4 and Nanog in ES cells that is central to the maintenance of pluripotency [7–9]. Myc then binds to and regulates many cell cycle genes in ES cells [10,11]. It does so in part by overcoming paused Pol II at target genes allowing for successful transcriptional elongation [12,13]. The dependency of Myc, and PI3K signaling, which also promotes pluripotency [14], can be relieved by growth in media containing GSK3β and MEK1/2 inhibitors (2i conditions) [15].

Pluripotency and cell cycle control also converge on the Rb/E2F pathway (Table 1), one of the major regulators of the cell cycle, which is indeed critically involved in the regulation of the cell cycle in ES cells [16,17]. Rb, and its family members p107 and p130, comprise the family of “pocket proteins” which canonically repress E2F activity by an E2F-binding pocket domain. Through this pathway mitogen signaling can affect the activity of Cyclin/CDK complexes which, through phosphorylation of the pocket proteins, can relieve inhibition of the E2F family of transcription factors to initiate DNA replication [reviewed in 18,19]. ES cells are characterized by high CDK activity, subsequent phosphorylation of all three pocket proteins, and high E2F activity. Indeed Myc can directly regulate E2F activity [11]. Oct4 can also directly regulate the expression of E2F3a, which is partly responsible for the high proliferative rates in ES cells [20]. In addition, Nanog can upregulate CDKs and the CDK activator, Cdc25a [21]. To further enhance high CDK activity, several CDK inhibitors (including $p16^{Ink4a}$, $p15^{Ink4b}$, $p19^{Arf}$, $p21^{Cip1}$, and $p27^{Kip1}$) are repressed in part by core pluripotency members [19,22,23]. The core pluripotency network also upregulates miRNAs, particularly of the *miR290–295* cluster, *miR302*, and *miR590* (Table 1), which in turn repress CDK inhibitors, pocket proteins, pro-differentiation miRNAs, and apoptosis [24–28]. Beyond transcriptional regulation and post-transcriptional regulation by miRNAs, post-translational modifications of key pathway members are also utilized by the cell to enforce high proliferation in ES cells. For example, the F-box protein Fbw7 (Fbxw7), a component of the SCF-type ubiquitin ligase complex, targets c-Myc for degradation and is therefore downregulated in ES cells to maintain high c-Myc protein stability [29,30]. In addition, the O-GlcNAcylation of a RINGB, a member of the polycomb repressive complex 1 (PRC1),

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removes PRC1 from regulatory DNA elements of cell cycle genes to promote differentiation [31].

One complication of fast cell proliferation is the potentially increased accumulation of genetic mutations due to error-prone DNA synthesis. Oct4 has been shown to directly bind to and inhibit Cdk1 resulting in a lengthening of G2 phase which allows more time for the DNA repair machinery to correct *de novo* mutations [32]. Similarly, a *miR-590/Acvr2a/Rad51b* axis also serves to balance the needs of the cell to maintain fast proliferation and resolve DNA damage. This occurs through the expression of *miR-590*, which becomes upregulated in ES cells during damage and mediates the upregulation of Rb and p21 to slow the cell cycle, while *Activin* signaling induces expression of the DNA-damage repair gene *Rad51* [28].

Reciprocal regulation of cell cycle and pluripotency networks: Cell cycle regulation of pluripotency

As the core pluripotency network can control the cell cycle, there are multiple means by which cell cycle regulators control pluripotency (Figure 2). Indeed there are several examples of how the high CDK activity in ES cells may influence the pluripotency network. Loss of CDK1 in human ES cells results in a reduction of pluripotency gene expression, including the core factors OCT4, KLF4, and LIN28, and subsequently increases differentiation [33]. Additionally, these cells show increased DNA damage and ensuing apoptosis [33,34]. Similar results were found performing chemical CDK2-inhibition in human ES cells [35]. Sox2 can be phosphorylated by Cdk2, although this is dispensable for the maintenance of pluripotency [36]. Mediator, which is controlled by Cdk8, plays an important role in the activation of genes containing Oct4, Sox2, and Nanog bound at their enhancers by looping them to promoter regions using cohesion [4]. Rb and associated proteins can silence members of the core pluripotency network in differentiated tissues, therefore this high Cdk activity serves to block this repression on pluripotency [37–39]. Similarly, Cdk inhibitors such as $p27^{Kip1}$ also silence some pluripotency factors and are themselves repressed in ES cells [23,40]. This repression of Cdk inhibitors is E2F4-dependent, a reflection of the complex transcriptional role of the E2Fs, where E2F4–6 are transcriptional inhibitors rather than transcriptional activators and S-phase inducers [18,40]. Not surprisingly, activating E2Fs are implicated in the regulation of many pathways in ES cells, including the core pluripotency network genes *Oct4*, *Sox2*, and *Nanog*, as well as the Wnt and FGF pathways [39,41]. On the other hand, knockdown of E2F2 alone, reduced the proliferation of ES cells, but pluripotency was not effected [42]. This could reflect a specialization amongst the activating E2Fs to separate cell cycle and pluripotency functions. In addition, the Yes-associated protein (YAP), a member of the Hippo pathway which regulates organ size and cell cycling in somatic tissues, is indeed active in ES cells and can bind to many of the targets of the core pluripotency network [43].

A rapid cell cycle to inhibit differentiation

Not only does the cell cycle play an integral role in the maintenance of pluripotency, but rapid proliferation may also serve to inhibit differentiation, thereby maintaining the

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undifferentiated state [1]. By the action of the core pluripotency network, multiple lineage-specific transcription factors are repressed [4,44]. Similarly, recent advances have indicated that the rapid cycling of ES cells maintains pluripotency by resisting differentiation [3,45] and that slowing of the cell cycle aids differentiation [1,45]. The high CDK activity in ES cells results in inactivation of the pocket proteins, which have been implicated in differentiation as well [reviewed in 18]. Myc, while keeping ES cells in a proliferative state, also directly represses the FGF/ERK pathway to inhibit differentiation (Figure 1) [7,46]. *In silico* techniques have shed light on the interactions between cell cycle networks and differentiation, which could be the source of future mechanistic studies [47].

Cell cycle control in the reprogramming of induced pluripotent stem (iPS) cells

When Yamanaka and colleagues first successfully reprogrammed somatic cells to a pluripotent state, they observed that iPS cells grew at a rate similar to ES cells [48]. An integrative genomic analysis of human iPS cell reprogramming indicates that cell cycle genes are upregulated as early as day 5 of reprogramming [49]. Indeed, fully reprogrammed iPS cells acquire the minimal G1 phase typical of ES cells, and accelerated proliferation in the starting cell population aids in the efficiency of reprogramming [2,45]. Using nuclear transfer as a rapid means of reprogramming, Gurdon and colleagues observed a phenomena they termed “mitotic advantage”. They detected that mitotic nuclei are reprogrammed at rates significantly higher than interphase nuclei and that this is due in part to the de-ubiquitination of histones during mitosis resulting in gene derepression [50]. Similar studies have shown that enriching for proliferating cells enhances reprogramming [51]. This has been further supported by single cell studies that show that rapidly dividing cells are in a privileged state, whereby they account for the majority of successfully reprogrammed cells [52].

The mechanisms that underlie this increase in proliferation as cells reprogram have been the focus of multiple studies (Figure 3). Cells with Cdk inhibition are refractive to reprogramming [53–55], and the Cdk2-mediated phosphorylation of Sox2, while not required for pluripotency does indeed aid reprogramming [36]. The ability of the p53 pathway (Table 1) to restrict reprogramming is in part through increasing the proliferation of p53-null cells, although proliferation-independent roles for p53 activity cannot be strictly excluded [56,57]. The process of reprogramming stresses the cells, e. g. through DNA damage, induction of apoptotic programs, and oncogene expression [55], and reducing the checkpoint activity of p53 and Cdk inhibitors promotes successful reprogramming [58]. Loss of members of the Rb pathway also increases reprogramming [37,40]. However, the effects of Rb loss on reprogramming involves a direct repression of the core pluripotency network, rather than perturbations of the cell cycle, indicating that at least this critical tumor suppressor may possess separate functions to both regulate proliferation and pluripotency [37]. Along with CDK regulation, modulating the proper levels of CDK partner Cyclins is fundamental to proper reprogramming [59,60]. As in ES cells, Myc activity increases the rate of proliferation and reprogramming by helping to establish a pluripotent cell cycle in reprogrammed cells [10–12,61].

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Concluding comments

There are multiple molecular connections between the cell cycle and pluripotency. It appears plausible that these links are critical to establishing and maintaining the undifferentiated state, while setting the stage for later differentiation. The two processes of cell cycle regulation and pluripotency appear to exist in a circular relationship in ES cells where disruption of one will affect the other, resulting in generally two outcomes: cell death and/or differentiation. Indeed there are many proteins that coordinately link both processes, such as Rb and p53 [37,38,57]. Many signaling pathways can also simultaneously induce rapid proliferation and the core pluripotency gene network [15,62]. In ES cells it does appear, though, that there may be conditions in which cell cycle regulation and pluripotency may be uncoupled [37,63]. For example, Rb regulates the induction of pluripotency in a manner that is distinct from its cell cycle functions [37]. Perhaps the apparent connection of these two processes in ES cells reflects a fine balance that is achieved in the culture conditions that effectively pause these cells in their developmental state. This however, may not be true during normal development where a hierarchy between the cell cycle and pluripotency is still unclear. To address this question more studies should be performed in rodent and non-human primate models of early development, rather than ES cells that exist in a developmentally paused state, which may acquire the balanced co-regulation of proliferation and pluripotency to maintain *ex vivo* self-renewal. Furthermore, reprogramming techniques, such as nuclear transfer, cell fusion, or in vitro reprogramming with defined factors can further illuminate the mechanistic interrelatedness and potential hierarchy of cell cycle regulation and pluripotency.

It is perhaps not surprising that the genes and signaling pathways that simultaneously regulate proliferation and pluripotency in ES cells are often misregulated in cancer. After all, one of the main hallmarks of cancer is uncontrolled cell division. In addition, cancer has many characteristics reminiscent of ES and iPS cells including self-renewal, the ability to bypass senescence, oncogene activation, tumor suppressor repression, and tumor formation [64,65]. Of note, many cancer types are refractive to reprogramming to iPS cells, and examples of cancer cell-derived iPS cells are few [66–69]. This observation is counterintuitive because inhibition of tumor suppressors and activation of oncogenes facilitates reprogramming [66–69]. It is possible that these cancer cells are unable to acquire the unique ES cell cycle with a short G1 phase. Therefore, a greater elucidation of the crosstalk between cell cycle and pluripotency pathways can not only advance the fields of developmental biology and regenerative medicine, but may also provide novel insights into the biology of cancerous transformation.

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Highlights

- The pluripotent state has a unique cell cycle relative to somatic cells
- Extensive reciprocal crosstalk exists to maintain both pluripotency and a rapid cell cycle
- Unique cell cycle maintains pluripotency by inhibiting differentiation
- iPS cell reprogramming re-establishes theunique cell cycle of ES cells

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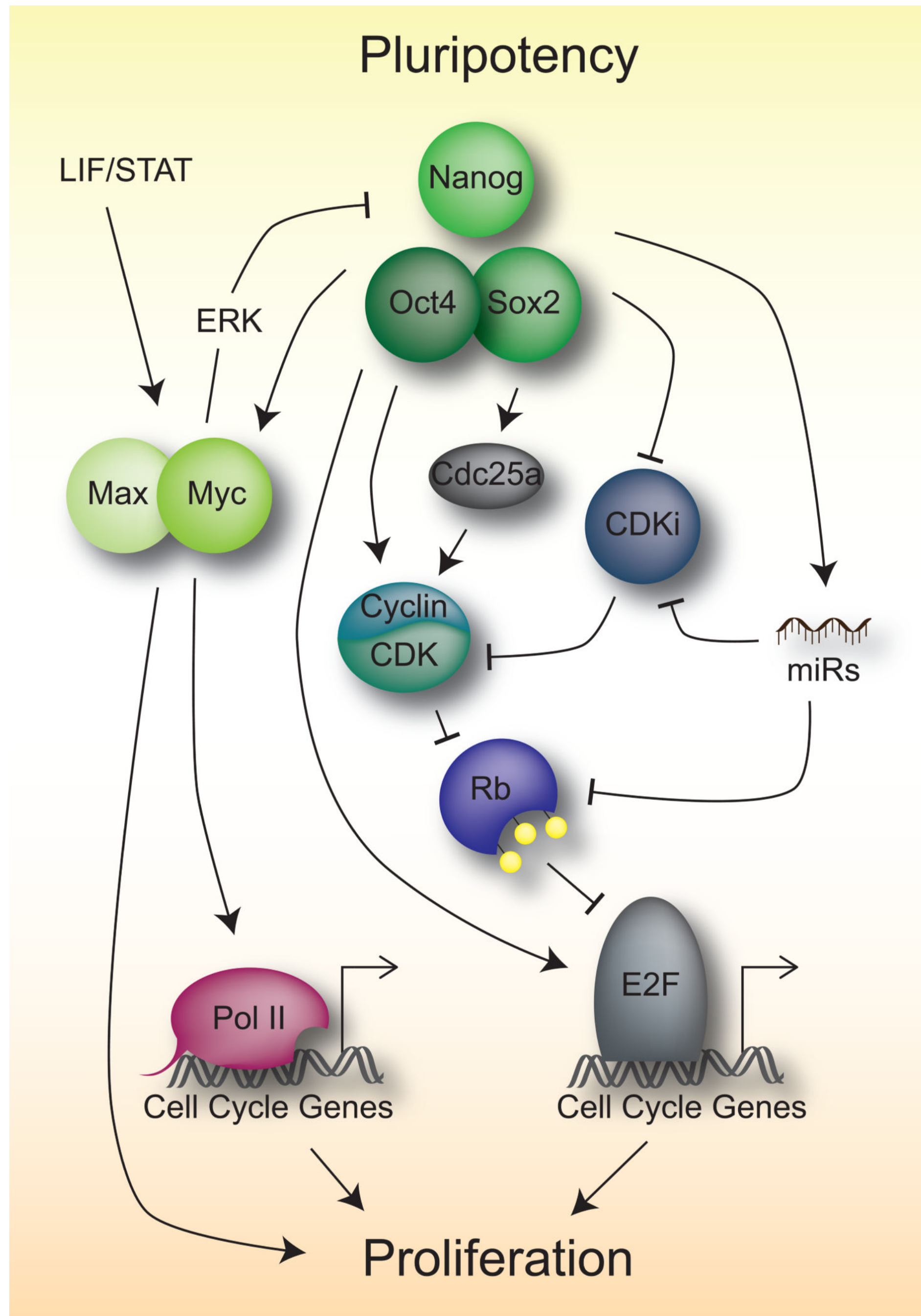

Figure 1.
Means of pluripotency control of the cell cycle

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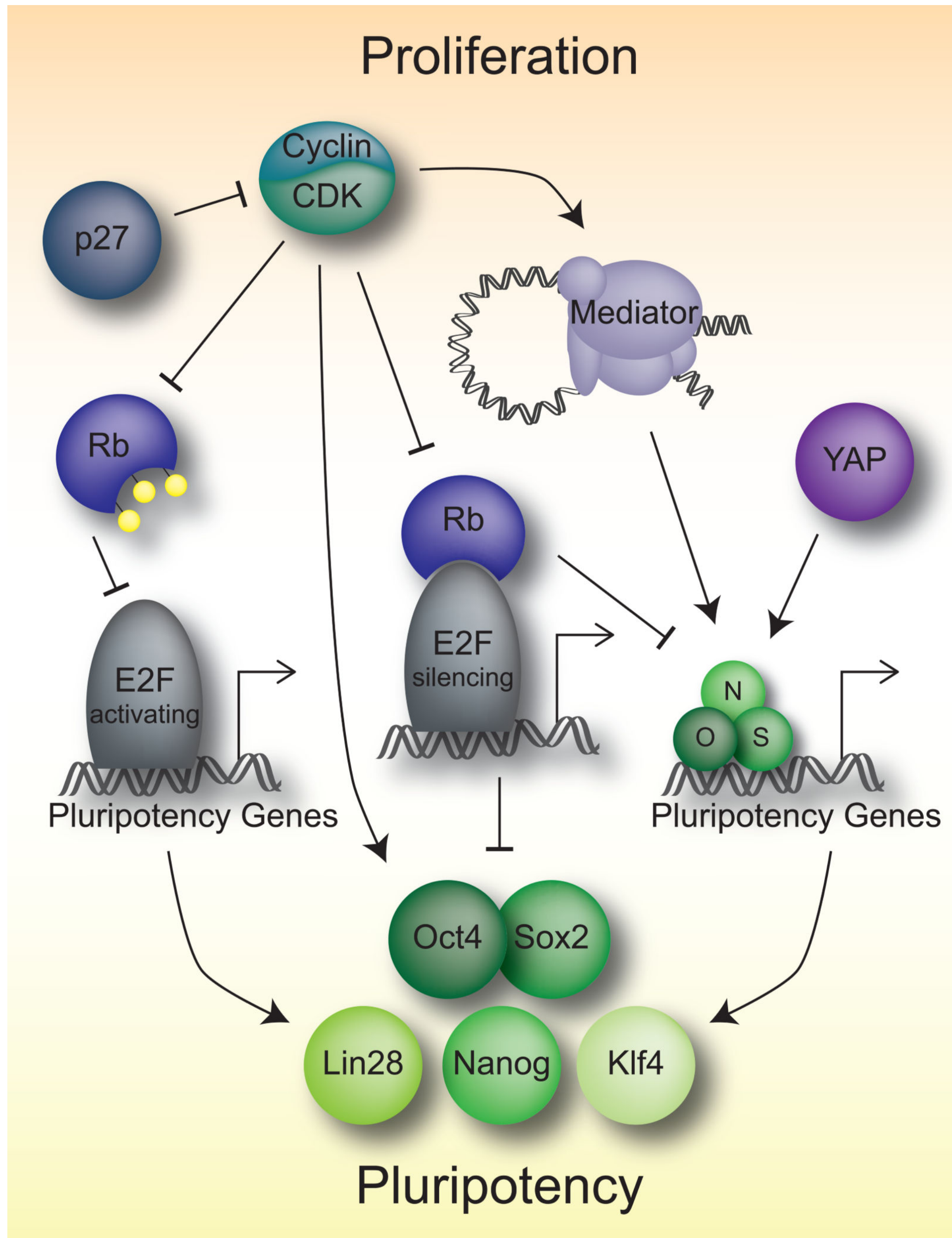

Figure 2.
Means of cell cycle control of pluripotency

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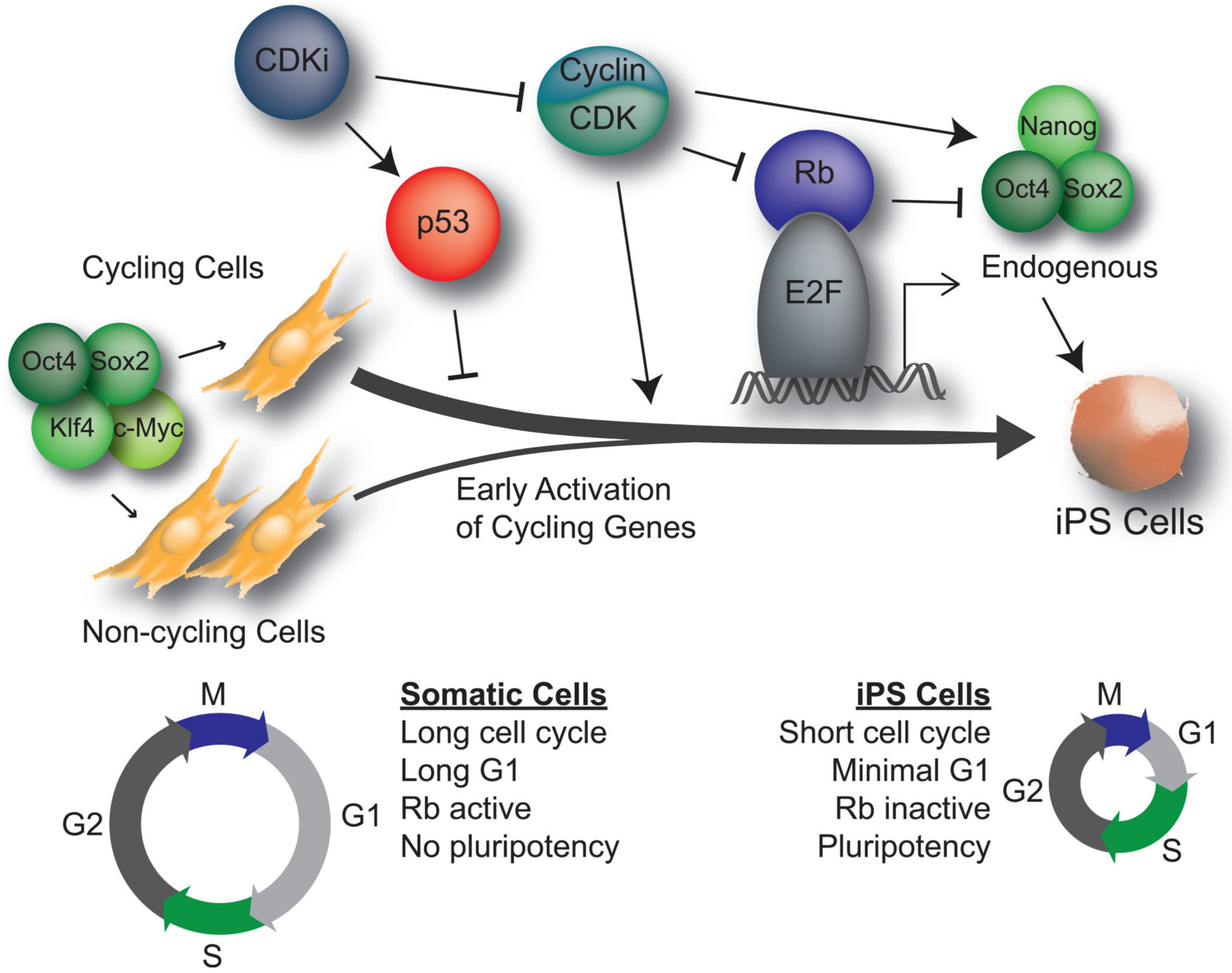

Figure 3.
Cell cycle control in reprogramming to pluripotency

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Table 1

Molecular Pathways which regulate pluripotency and the cell cycle in ES cells

Pathway	Role	Members	Ref
Core Pluripotency Network	Maintain the undifferentiated state and self-renewal of pluripotent cells	Oct4 (Pou5f1), Sox2, Nanog	[4,5]
Myc	Upregulate expression of genes to promote rapid proliferation. Often oncogenic driver of cancer	c-Myc	[11]
Rb/E2F	Establish G1/S restriction point to regulate proliferation with growth signals	Pocket Proteins (Rb, p107, p130) E2F transcription factors (E2F1-5), DP1, CyclinD/E, Cdk2/4, Cdk-inhibitors ($p16^{Ink4a}$, $p15^{Ink4b}$ $p19^{Arf}$, $p21^{Cip1}$, and $p27^{Kip1}$)	[37]
p53	Genomic stability, stress response, growth arrest, apoptosis	p53 (Trp53, TP53), Mdm2, $p21^{Cip1}$, ATM, Chk2	[53–55,70,71]
LIF/Stat3	ES cell self-renewal, Myc activation	LIF receptor (LIFR), gp130, Stat3, Jak	[7]
PI3K/Akt	Growth factor signaling, apoptosis, and cell survival	Receptor tyrosine kinases, PI3K, Akt	[15,62]
MAPK/ERK	Extracellular signaling to nuclear transcription factors	FGF, Ras, Raf, Mek, Erk	[46]
Hippo	Organ size control, cell proliferation	Mst1/2, Lats1/2, Yap/Taz	[43]
ESC cell cycle regulating (ESCC) miRs	Pluripotent gene activation, rapid cell cycle in ES cells	*miR290–295* cluster, *miR302*	[25,27]