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The cell cycle in stem cell proliferation, pluripotency and differentiation

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Abstract

Cyclins, cyclin-dependent kinases and other components of the core cell cycle machinery drive cell division. Growing evidence indicates that this machinery operates in a distinct fashion in some mammalian stem cell types, such as pluripotent embryonic stem cells. In this review, we discuss our current knowledge of how cell cycle proteins mechanistically link cell proliferation, pluripotency and cell fate specification. We focus on embryonic stem cells, induced pluripotent stem cells, and embryonic neural stem/progenitor cells.

The core cell cycle machinery operating in the cell nucleus orchestrates cell division. The key components of this machinery are proteins called cyclins that bind, activate and provide substrate specificity to their associated catalytic partners, the cyclin-dependent kinases (CDKs)[1–4]. Cell cycle progression can be divided into four phases: gap 1 (G1), DNA synthesis (S), gap 2 (G2) and mitosis (M). Depending on the mitogenic environment, cells traversing G1 phase either activate a program that will result in cell division, or they enter a quiescent G0 state[1–4] (Fig. 1a). At the molecular level, stimulation of cells with growth-promoting factors results in upregulation of the D-type cyclins (D1, D2 and D3), which activate the cyclin-dependent kinase 4 (CDK4) and CDK6[1–5]. In a classical cell cycle model, cyclin D-CDK4/6 complexes, together with E-type cyclins (E1 and E2) and their associated kinases (primarily CDK2, but also CDK1 and CDK3) phosphorylate and functionally inactivate the retinoblastoma protein RB1, and pRB1-related RBL1 and RBL2 proteins[1–4]. This leads to the activation or de-repression of E2F transcription factors, which then transactivate genes required for the entry and progression of cells into S phase[1–4,6,7]. This model has been questioned by the demonstration that throughout most of G1 phase, RB1 exists in a mono-phosphosphorylated state, and becomes fully phosphorylated by cyclin E-CDK2 at the end of G1 phase[8]. In addition to RB1 phosphorylation, inactivation of Cdh1, a substrate recognition subunit of the anaphase promoting complex (APC/C), contributes to an

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irreversible commitment of cells to cell division[9]. Inhibition of Cdh1 by cyclin E-CDK2 at the end of G1 phase inactivates APC/C and allows accumulation of S-phase cyclins that are normally degraded by Cdh1-APC/C[9–11].

The entry of mammalian cells into S phase is driven by cyclin E-CDK2 acting in concert with the DBF4-associated kinase[12–14]. Later during S phase, cyclin A2 becomes upregulated, pairs with CDK2 and CDK1 and promotes S-phase progression[1–4]. Following a second gap phase (G2), cyclin B translocates to the nucleus, activates CDK1, and drives separation of genetic material to daughter cells[3,15].

In addition to these positive regulators of the cell cycle, mammalian cells also express two classes of cell cycle inhibitors. The INK proteins ($p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$, $p19^{INK4d}$) interact with CDK4 or CDK6 and block their association with D cyclins[1–4]. The KIP/CIP proteins ($p21^{Cip1}$, $p27^{Kip1}$, $p57^{Kip2}$) form ternary complexes with cyclin-CDK2 and cyclin-CDK1 molecules and inhibit their kinase activities[1–4].

Cell cycle machinery in embryonic stem cells

Work of several laboratories revealed that the core cell cycle machinery operates differently during early embryonic development[16]. In developing flies, fish and frogs, first cell cycles are very rapid, lack obvious gap phases and consist of alternating S and M phases[16]. Analyses of peri-implantation mouse embryos demonstrated that murine embryonic cells display remarkably short division times *in vivo* (~4.4 – 7.5h)[17–19] with a very small fraction of cells residing in G1 phase[20,21]. Intriguingly, the first two cell cycles are considerably longer, with significant shortening occurring during the third cell division[22]. Later in development, following gastrulation and formation of the endodermal, mesodermal and ectodermal lineages, the cell cycle length substantially increases, due to extension of the gap phases[19].

Murine embryonic stem ES cells (mESCs), derived from inner cell masses of blastocysts and cultured *in vitro* in conditions that favor retention of their pluripotent state, recapitulate this unique organization of the cell cycle[20]. They divide very rapidly, although not as fast as their *in vivo* counterparts (division time ~12h), and have a short G1 phase that lasts only 3h[16,23–25] (Table 1). These cells express high levels of cyclins E, A and B, and display elevated levels of Cdk1 and Cdk2 kinase, that greatly exceed those seen in somatic cells[20,26,27]. Strikingly, high levels of cyclins E and A are observed throughout the cell cycle, in contrast to their periodic expression in somatic cells[20,28] (Fig. 1a, b). Consequently, Cdk2, cyclin E- and cyclin A-associated kinases are constitutively active throughout the entire cell cycle, in contrast to somatic cells, where these kinases become transiently activated at specific cell cycle phases[16,20,28]. The only cell cycle kinase that retains periodicity in mESCs is cyclin B-Cdk1, but its levels are substantially higher than those in somatic cells[20,27]. As a consequence of hyperactivated Cdk1 and Cdk2 kinases, RB1 is constitutively phosphorylated/inactivated throughout the cell cycle, and E2F activity is constitutively de-repressed[16,20,23] (Fig. 1, Table 1).

Several mechanisms likely contribute to very high levels of Cdk1 and Cdk2 kinase activity in ESCs. In contrast to somatic cells, ESCs do not express KIP/CIP inhibitors, which normally mitigate the activity of Cdk1/2[16,20,26]. ESCs also express high levels of the APC/C inhibitor Emi1, leading to a decreased activity of APC/C[29,30]. This mechanism allows cell-cycle-wide accumulation of S-phase cyclins and of another APC/C target Skp2[11,29]. Skp2, in turn, triggers degradation of $p27^{Kip1}$, thereby further contributing to elevated Cdk1/2 kinase activity[16] (Fig. 1a).

Initial reports documented that mESCs express low levels of D cyclins and are refractory to cell cycle inhibition by $p16^{INK4a}$ (ref.[31]). Consistent with these results, another study[32] demonstrated that prior to gastrulation, mouse embryos do not express D cyclins. In contrast to these findings, others reported expression of cyclins D1 and D3 in the pluripotent cells of mouse embryonic epiblast[33]. Cyclin D3 was also shown to be expressed in ESCs where it complexes with Cdk6; however, these cyclin D3-Cdk6 complexes are refractory to inhibition by $p16^{INK4a}$ (ref.[33]). A similar cell cycle organization was reported in ESCs derived from rhesus monkeys[34]. It should be noted that these studies utilized ESCs grown in the presence of serum and leukemia inhibitory factor (LIF). Recent studies indicate that murine cells cultured in a defined medium with inhibitors of MEK and GSK3 kinases resemble true pluripotent stem cells from inner cell masses, and are hence termed to reside in a 'ground state'[35,36]. The cell cycle organization of these ground state cells may be different from that of cells cultured in serum/LIF, including a longer G1 phase and the presence of hypo-phosphorylated RB1[37]. This surprising finding calls for more side-by-side comparisons between mESCs cultured in different conditions versus pluripotent cells of early embryos *in vivo*.

The organization of the cell cycle in human ESCs (hESCs) is slightly different (Fig.1b, Table 1). This likely reflects the fact that hESCs are more similar to 'primed' pluripotent cells derived from the late epiblast layer of post-implantation embryos (epiblast-derived stem cells, EpiSCs)[19,38,39]. It is now appreciated that different forms of pluripotent stem cells exist during early development and that their cell cycle organization might show some differences[19]. Like mESCs, hESCs cells proliferate rapidly, display a short G1 phase and express high levels of CDK1 and CDK2 kinases[40,41]. However, these cells express appreciable levels of D cyclins and KIP/CIP inhibitors, show cell-cycle-dependent fluctuations of CDK2 kinase, and contain both hyper- and hypo-phosphorylated RB1[19,40–42] (Fig. 1b, Table 1).

Molecular links between cell cycle and pluripotency

One of the main questions in the field has been whether the unique cell cycle organization of pluripotent stem cells simply reflects the necessity to rapidly expand this cell population or it plays an active role in enforcing the pluripotent state. Several observations suggested a role for cell cycle proteins in enforcing pluripotency[24,27,40,43–46] (Fig. 2). The knock-down of CDK1, CDK2, cyclin E or B1, and treatment with CDK-inhibitors all resulted in the loss of the pluripotent state and triggered differentiation[24,27,40,43–46], whereas ectopic overexpression of cyclins E or B1 promoted ESC self-renewal[24,44]. However, several of these manipulations resulted in cell cycle arrest, apoptosis or perturbed cell cycle

progression, which confounded interpretation of the results[40,44,45,47]. At the mechanistic level, Wang et al.[27] proposed that CDK1 kinase maintains the pluripotent state by regulating the PI3K/Akt pathway. Conversely, Kim et al.[48] reported that CDK1 inhibits chromatin binding of Oct4 through an indirect mechanism involving Aurkb and PP1. The notion that the core cell cycle machinery actively regulates pluripotency was further supported by genetic studies using ESCs derived from cyclin D-null[49] or E-null[50] mice. Although ablation of all three D-type ($D1^{-/-}D2^{-/-}D3^{-/-}$) or both E-type ($E1^{-/-}E2^{-/-}$) cyclins had no effect on the pluripotent state, a combined acute shutdown of all five G1 cyclins resulted in the strong attenuation of pluripotency[25]. Importantly, cells lacking all G1 cyclins proliferated *in vitro*, albeit at a somewhat reduced rate, revealing that D and E cyclins are not essential for the proliferation of mESCs[25]. At the molecular level, G1 cyclin-CDK kinases were shown to directly phosphorylate core pluripotency factors Oct4, Sox2 and Nanog, resulting in their stabilization[25]. Ablation of all G1 cyclins strongly diminished phosphorylation of the critical residues of Oct4, Sox2 and Nanog, thereby triggering their proteasomal degradation and attenuation of the pluripotent state[25] (Fig. 2).

Although G1 cyclins are dispensable for the proliferation of mESCs, no ESC colonies were observed upon ablation of cyclin A, revealing an essential role for this cyclin in stem cells[51]. These observations are in stark contrast to the situation seen in fibroblasts, where G1 cyclins are essential, but cyclin A is not (as its function can be carried out by cyclin E)[51]. Collectively, these observations indicate that, in contrast to differentiated cells, ESCs rely on cyclins A and B for cell cycle progression, whereas G1 cyclins contribute to the maintenance of the pluripotent state.

In addition to cell cycle proteins regulating pluripotency factors, the reverse was also noted, namely the ability of Nanog and Oct4 proteins to affect proliferation[52–54]. Specifically in hESCs, Nanog was shown to bind the regulatory regions of the *CDK6* and *CDC25* genes and to upregulate their expression, thereby promoting cell proliferation[52]. Oct4 was postulated to stimulate cell growth by repressing the expression of $p21^{Cip1}$, a cell cycle inhibitor gene[53]. In contrast to these findings, another study reported that Oct4 inhibits cell proliferation by forming a complex with cyclin-Cdk1 and inhibiting Cdk1 activation[54]. Pluripotency factors can also affect proliferation through indirect mechanisms. For instance, Oct4 and Nanog upregulate c-Myc, which, in turn regulates expression of several cell cycle genes[55–57].

Dissolution of pluripotency and cell fate specification

The unusual cell cycle properties of ESCs change upon dissolution of pluripotency, when stem cells undergo cell fate specification and differentiate into different lineages[16,]. During this process, cell division length increases, mainly due to an expanded G1 phase[16,24,28,34,43,58]. D cyclins become upregulated, the activity of CDK1 and CDK2 decreases in part due to expression of KIP/CIP inhibitors, and the activation CDK2-, cyclin E- and cyclin A-associated kinases becomes restricted to specific cell cycle phases[16,26–29,31,34,45]. Downregulation of Emi1 causes an increased APC/C activity, resulting in enhanced degradation of APC/C targets, such as cyclins[29,30]. All these changes

lead to the appearance of hypophosphorylated RB1 during G1 phase, and subject E2F transcriptional activity to a tight cell-cycle-dependent control[16,28].

Several studies document that ESCs initiate differentiation in the G1 phase[59–62] (Fig. 3). This observation was best demonstrated using a FUCCI system which allows the sorting of cells in defined cell cycle phases[24,60,61]. One possible mechanistic explanation was provided by the observation that cells traversing the G1 phase express higher levels of developmentally-regulated transcription factors, suggesting that G1 phase ESCs may exist in a 'lineage-primed' state[61,62]. Several developmentally regulated genes in ESCs are marked by overlapping activatory (H3K4me3) and inhibitory (H3K27me3) histone marks[63,64]. In late G1, the activating H3K4me3 mark increases on many of these 'bivalent' developmental genes, resulting in increased levels of their transcripts, which might promote differentiation[62]. In addition, 5-hydroxymethylation of cytosine increases on some developmental genes during G1 phase, and this mechanism may contribute to their elevated expression[61] (Fig. 3).

Collectively, these observations indicate that G1 phase represents a permissive phase for the initiation of cell fate decisions[19]. In the light of these findings, it has been argued that a long G1 phase may enable the accumulation of factors needed for the dissolution of pluripotency and differentiation and, conversely, a short G1 phase minimizes the exposure to differentiation-promoting signals and helps to maintain the pluripotent state[65–67]. Work by Pauklin et al. has further extended this model[68]. The authors reported that during early G1 phase of hESCs, when D cyclins are not yet expressed, Smad2 and Smad3 transcription factors bind and activate endoderm genes, thereby specifying endodermal differentiation. Upregulation of cyclin D-CDK4/6 kinases during late G1 phase leads to phosphorylation of Smad2/3, thereby blocking Smad2/3 entry into the nucleus. This mechanism prevents endodermal cell fate and renders cells susceptible only to neuroectodermal differentiation[68]. In another study[69], these authors reported that in hESCs D cyclins regulate cell fate specification via a CDK4/6- and Smad-independent mechanism. According to this model, cyclin D1 recruits transcriptional coactivators to neuroectoderm genes, thereby promoting neuroectodermal differentiation[69]. D cyclins also bind endodermal gene promoters, but in this case they recruit transcriptional corepressors, which silence gene expression[69] (Fig. 3). Indeed, cyclin D1 was previously shown to interact with gene regulatory regions and regulate gene expression *in vivo* in a CDK4/6-independent fashion[70,71].

These two models[68,69] are at odds with several observations. For instance, knockout mice lacking individual D cyclins, all D cyclins ($D1^{-/-}D2^{-/-}D3^{-/-}$), or Cdk4 and 6 ($Cdk4^{-/-}Cdk6^{-/-}$) can develop at least until mid-gestation and undergo organogenesis, indicating that specification into different lineages is not significantly affected[49,72–75]. In addition, mESCs lacking all three D-type cyclins do not upregulate differentiation markers[25]. A lack of all five G1 cyclins in mESCs increases expression of several neuroectodermal genes and accelerates neural differentiation *in vivo* in chimeric embryos and teratoma assays[25], an opposite outcome to the one described for shRNA-mediated silencing of D cyclins in hESCs[68]. In agreement with the genetic data, several other reports document that cyclin D-CDK4/6 activity inhibits neuroectodermal differentiation *in vivo*, whereas its silencing promotes neuroectodermal fate[76–80]. One possible explanation for

these discrepancies would be that the molecular mechanism of lineage specification is fundamentally different between mouse and human cells.

Whereas cells in G1 are prone to differentiation, pathways operating in S and G2 phases may actively repress the dissolution of the pluripotent state[44]. At the molecular level, it was suggested that cyclin B1 might represent a key component of such pluripotency-promoting pathway during G2 phase[44] (Fig. 3). Van Oudenhove et al.[81] indicated that ESCs differentiating into mesodermal or endodermal lineages pause during G2 phase due to upregulation of Wee1, a kinase that inhibits CDK1[82], which may attenuate the pluripotency-promoting mechanism.

Cell cycle alterations during somatic reprogramming

Expression of a defined set of transcription factors, such as Oct4, Sox2, Klf4 and c-Myc, can convert differentiated somatic cells into induced pluripotent stem cells (iPSCs) in a process called somatic reprogramming[83–85] (Fig. 2). One of the early events accompanying this process is a strong acceleration of the cell cycle[86–89]. Guo et al.[88] demonstrated that during reprogramming of mouse fibroblasts, a small subpopulation of ‘ultra-fast’ dividing cells (cell division time of ~ 8h) accounts for >99% of the reprogramming. Others have shown that by sorting highly proliferative cells, one can substantially increase the efficiency of reprogramming[80]. These observations suggest that an acceleration of the cell cycle, which occurs only in a fraction of cells, may represent a rate-limiting factor in reprogramming[88]. Indeed, ectopic expression of several cyclins and CDKs increased the reprogramming efficiency whereas their depletion had the opposite effect[27,89,90]. Moreover, the reprogramming potential of primary murine fibroblasts declines after serial passaging, concomitantly with their reduced proliferation rates[91]. Conversely, genetic lesions in somatic cells that increase cell cycling, such as the inactivation of $p19^{ARF}$/$p16^{INK4a}$, p53, or $p21^{Cip1}$, increased the efficiency of reprogramming[91–93].

At the molecular level, Cdk2-mediated phosphorylation of Sox2 as shown to promote the ability of Sox2 to establish pluripotency during reprogramming, in combination with other factors[94], consistent with an observation that cyclin E-Cdk2 stabilizes Sox2 protein[25]. According to another study[27], cyclin B1 and CDK1 play a rate-limiting role in somatic cell reprogramming by upregulating and maintaining the expression of the reprogramming factor LIN28[95]. Inactivation of RB1 promotes reprogramming via a cell-cycle-independent mechanism, which is related to the ability of RB1 to directly bind pluripotency genes to repress their expression[96].

The organization of cell cycle in iPSCs, namely a rapid division time (16–18h) and a very short G1 phase (~2.5h), is similar to that in ESCs[89,97]. Similarly to ESCs, depletion of CDK1 in reprogrammed iPSCs also attenuates the pluripotent state[46]. Collectively, these observations suggest that the acquisition of a unique ‘ESC-like’ cell cycle organization in the process of reprogramming is functionally linked to the acquisition of pluripotency[97]. However, the exact molecular mechanisms by which cell cycle proteins help to acquire and enforce the pluripotent state in iPSCs remain largely unknown.

Cell cycle machinery in neural stem cells

Similarly to pluripotent ESCs, embryonic neural stem/progenitor cells display rapid cell division times (~8h)[98–100]. Interestingly, a subpopulation of slowly dividing neural progenitors was also identified, which could give rise to adult neural stem cells (NSCs)[101]. Expression of the cell cycle inhibitor $p57^{Kip2}$ is essential to maintain this slow dividing pool and is required for the emergence of adult NSCs[101]. As development proceeds, the cell cycle length of the embryonic neural progenitor cells increases (to up to ~18h), due to a four-fold increase in the length of G1[80,98,99,102].

Manipulations that prevent the physiological lengthening of G1 phase, such as ectopic overexpression of cyclin D1, E1, or CDK4, increase self-renewal and inhibit neurogenic differentiation[76–78]. Conversely, depletion of cyclin D1 and CDK4, or treatment with CDK4- or pan-CDK-inhibitors stimulates neurogenesis[76,80,103]. Likewise, NSCs of Cdk2/Cdk4 double-knockout mice display an increased propensity for neuronal differentiation, resulting in enhanced neurogenic divisions in the brains of these embryos[79]. Intriguingly, studies of the developing primate cortex revealed that local variations of cyclin E and $p27^{Kip1}$ levels in neuronal precursors residing in different areas correlate with their cell cycle length (and with G1 length), suggesting that area-specific levels of cyclin-E-associated kinase might locally influence neurogenesis[104].

Collectively these analyses strongly suggest that the activity of cyclin-CDK kinases inhibits neuronal differentiation. Lim et al.[105] proposed that in self-renewing NSCs, phosphorylation of Sox2 by high levels of cyclin-dependent kinases enhances the ability of Sox2 to inhibit neuronal differentiation[105]. When the activity of CDKs decreases, the shift towards non-phosphorylated Sox2 allows the proteolytically cleaved, truncated Sox2 species to bind and transactivate proneural genes, thereby promoting neurogenesis[105] (Fig. 4). Ali et al.[106] postulated that in rapidly proliferating neural stem/progenitor cells, cyclin A- and B-dependent kinases phosphorylate the proneural basic helix-loop-helix transcription factor neurogenin 2 (Ngn2), a master regulator of neuronal differentiation. This phosphorylation inhibits the ability of Ngn2 to bind neurogenic genes and promote neurogenesis[106]. According to this model, upon lengthening of G1 phase, decreased CDK activity relieves this repression and allows Ngn2 to turn on neurogenesis[106]. Interestingly, Ngn2 can repress expression of cyclins D1 and E2[107], suggesting the presence of a positive feedback loop that enforces the cell cycle exit during neural differentiation.

It should be noted that an opposite conclusion has also been reported[108], namely that in embryonic chick spinal cord, cyclin D1 serves to promote neurogenesis through a cell cycle-independent function mediated by Hes6. As mentioned before, Pauklin et al. noted that in hESCs, cyclin D1 and CDK4 promote neurogenic differentiation[68,69].

In addition to cyclins and CDKs, other cell cycle proteins were shown to play roles in neurogenesis. For instance, $p27^{Kip1}$ can promote neuronal differentiation by stabilizing Ngn2 protein, independently of its ability to inhibit CDKs[109]. In contrast, a related inhibitor, $p57^{Kip1}$, interacts with pro-neural basic helix-loop-helix factor Mash1 and represses its transcriptional activity, thereby inhibiting neurogenesis[110]. In addition, Rbl1 protein

regulates neural precursor cells in the mammalian brain by repressing the Notch1 pathway[111]. Lastly, CDK inhibitors $p27^{Kip1}$, $p57^{Kip2}$ and RB1 play cell-cycle-independent roles in regulating neuronal cell migration[109,112–117].

In contrast to highly proliferative ESCs, which are the focus of this Review, tissue-specific stem cells of adult organisms are largely quiescent[65,66]. It was proposed that their G0 arrest serves the same purpose as G1 phase truncation in ESCs, namely to limit the window of opportunity for cell differentiation[66]. Several cell cycle proteins, including CDK inhibitors, can enforce a quiescent state[118–124], as detailed in excellent Reviews on this subject published elsewhere[125,126].

Conclusions and outlook

One of the major questions in the field is whether the unusual cell cycle organization seen in ESCs and iPSCs plays a direct role in maintaining pluripotency. In addition, it remains to be resolved whether the reorganization of the cell cycle upon pluripotency dissolution represents the cause or consequence of cell differentiation. The combined evidence to date suggests that cell cycle proteins are causally involved in these processes, however, the mechanistic underpinnings are only beginning to emerge. The physiological role of very high Cdk1 and Cdk2 kinase activities in pluripotent ESCs remains a mystery. These kinases might likely phosphorylate additional substrates in stem cells that are different from those in differentiated cells, and this might contribute to pluripotency through some currently unknown mechanisms. Proteome-wide identification of cyclin-CDK substrates in pluripotent cells and during pluripotency dissolution will help to decipher the full repertoire of cyclin-CDK functions in these processes. The role of cell cycle proteins in cell fate specification and differentiation remains largely unclear, with different reports proposing different mechanisms and outcomes. Combinations of genome- and proteome-wide approaches might help to address this point. The contribution of 3D chromatin conformation, promoter-enhancer looping, insulated neighborhoods and topologically associating domains, as well as enhancer repertoire and decommissioning has recently been acknowledged in pluripotency and differentiation[127–132], however it remains unclear whether these processes are linked to cell cycle proteins. Another largely unaddressed issue is the heterogeneity within the stem cell compartments. Single-cell approaches such as live single-cell microscopy[9,133–135] or single-cell RNA sequencing[136–139] will help to ascribe specific molecular functions to cell cycle proteins at discreet stages of fate specification and cell differentiation.

An improved understanding of the cell cycle machinery in stem cell compartments will likely have important implications for regenerative medicine. For example, a transient expression of cyclin D1 and Cdk4 in NSCs *in vivo* resulted in increased neurogenesis and improved neuronal function[140]. Other work revealed the utility of inhibiting cell cycle proteins to promote hepatic and pancreatic differentiation[68,141]. Given the growing armamentarium of specific cell cycle inhibitors[82], these studies offer opportunities to specifically promote cell differentiation into a lineage of interest. Conversely, activation of the cell cycle machinery can be used as a mean to augment somatic reprogramming[27,89,90]. For these reasons, the elucidation of the full range of molecular functions of cell cycle proteins in stem cell self-renewal, differentiation and reprogramming should provide

important insights to help increase the efficiency of these processes in a therapeutically meaningful way.

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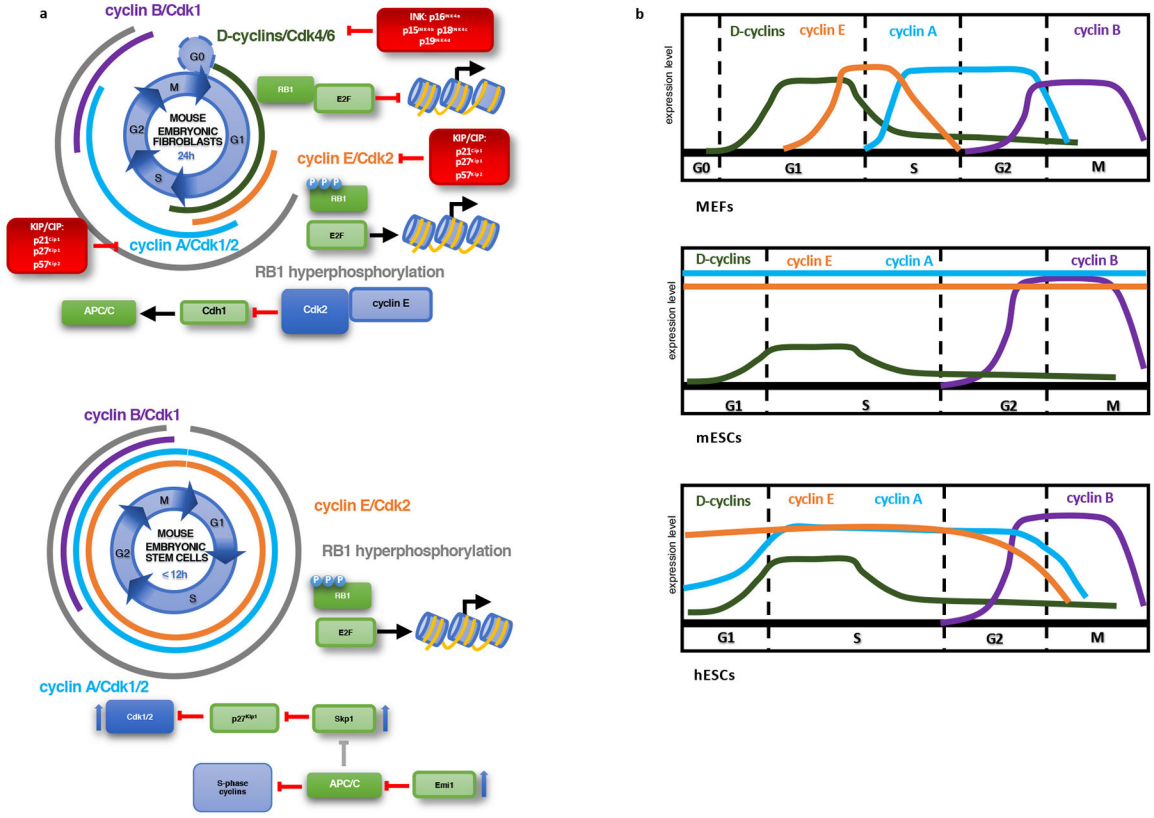

Figure 1 |. Organization of the cell cycle in somatic cells (MEFs) and in different types of ESCs.

a, Differences in activity and expression of cell cycle components between MEFs and mESCs. In contrast to MEFs, mESCs lack expression of D cyclins and continuously express cyclin A and cyclin E. This allows them to maintain RB1 hyperphosphorylation throughout the cell cycle and results in a very short G1 phase. Cyclin-dependent kinase inhibitors are absent from mESCs. Upward blue arrows indicate increased expression. b, Oscillations of cyclin levels in MEFs, mESCs and hESCs. Abbreviations: MEFs: mouse embryonic fibroblasts; mESCs: mouse embryonic stem cells; hESCs: human embryonic stem cells.

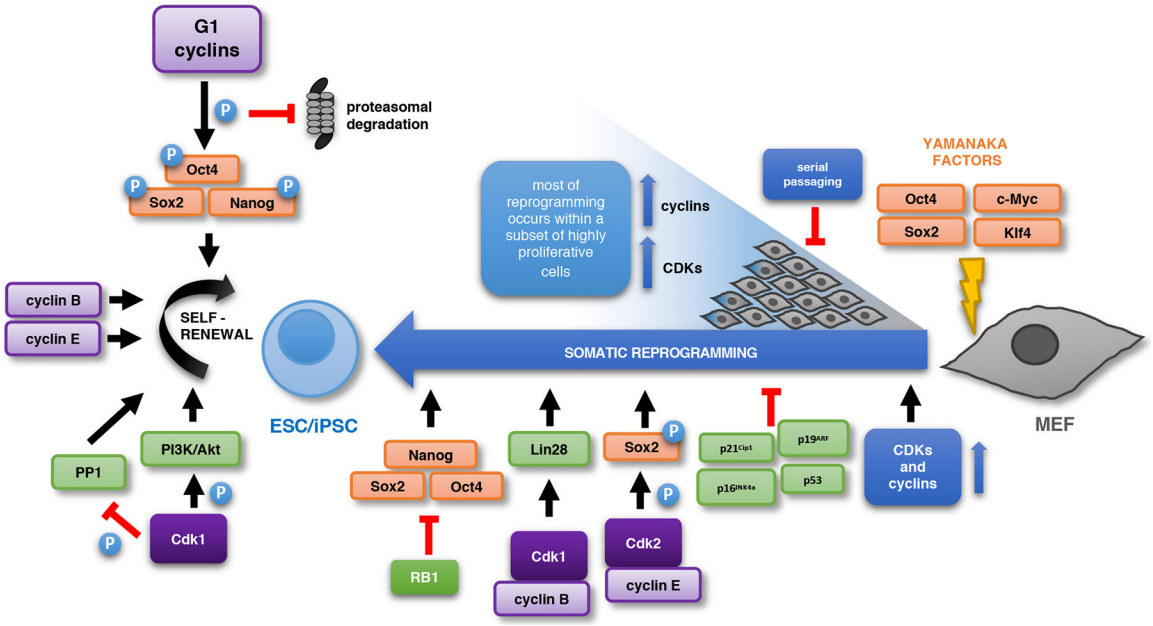

Figure 2 |. The cell cycle in somatic reprogramming and pluripotency maintenance.

During somatic reprogramming by expression of Oct4, Sox2, Klf4 and c-Myc, somatic cells rapidly accelerate the cell cycle. Ectopic overexpression of cell cycle proteins or inactivation of cell cycle inhibitors increases the efficiency of reprogramming. Conversely, serial passaging leads to a decreased reprogramming rate. CDK2-dependent phosphorylation of Sox2 or cyclin BCDK1-dependent upregulation of LIN28 were postulated to aid reprogramming. RB1 represses expression of core pluripotency factors. The cell cycle machinery is also important for the maintenance of ESC pluripotency. G1 cyclins stabilize core pluripotency factors through phosphorylation, thereby preventing their proteasomal degradation. High levels of cyclins and Cdk1-dependent phosphorylation of PI3K/Akt pathway components likely contribute to the maintenance of pluripotency. Cdk1 also inhibits Oct4 activity during the M phase, acting through PP1 and Aurkb. Upward blue arrows indicate increased expression. Abbreviations: ESC: embryonic stem cell; IPSC: induced pluripotent stem cell.

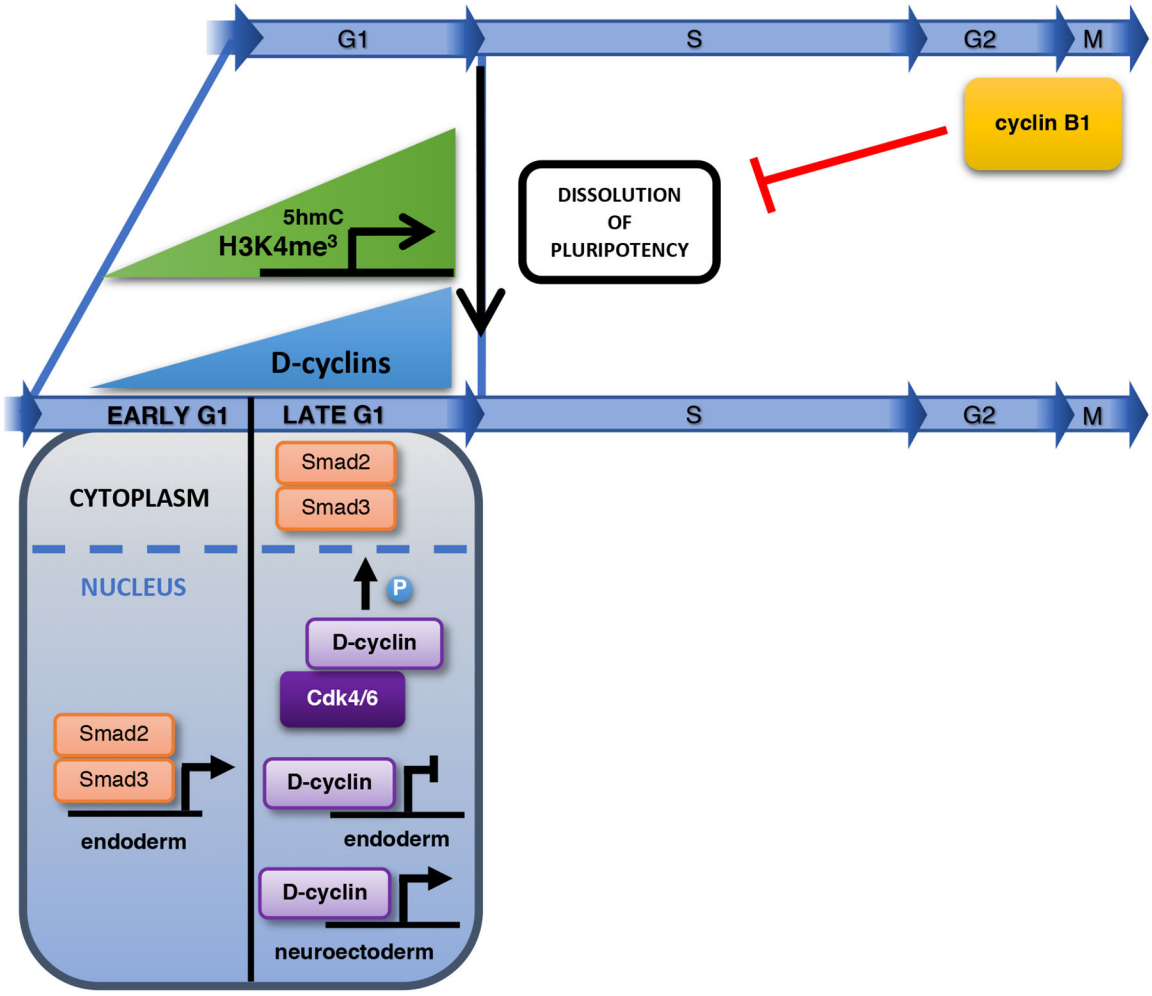

Fig. 3 |. The cell cycle during dissolution of pluripotency and cell differentiation.

G1 phase may provide a window of opportunity for the dissolution of pluripotency, as during this phase many developmental genes contain permissive epigenetic marks (H3K4me3 and 5-hydroxymethylcytosine). Cyclin B1 actively prevents pluripotency exit. Increased expression of D cyclins in late G1 triggers the phosphorylation and cytoplasmic retention of Smad2/3, thereby inhibiting endodermal differentiation. In addition, D cyclins directly repress the expression of endodermal genes and augment the expression of neuroectodermal genes. Abbreviations: 5hmC: 5-hydroxymethylcytosine.

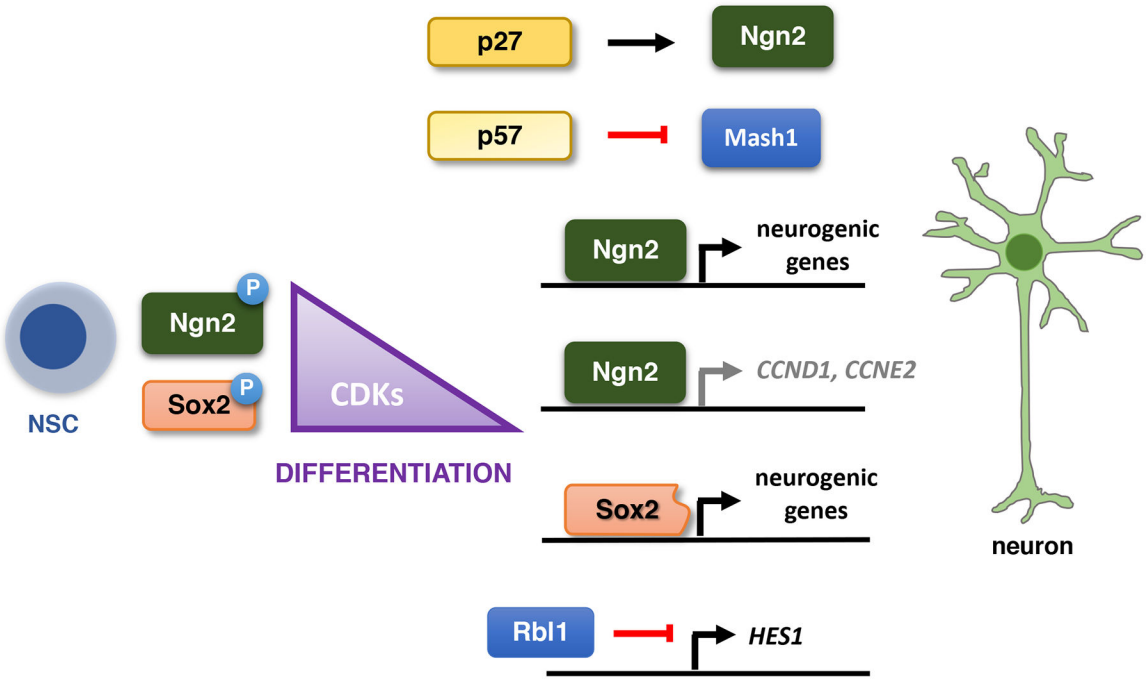

Fig. 4 |. The cell cycle in neurogenesis.

A decrease of CDK activity during neurogenic divisions enables the transactivation of proneural genes by a truncated form of Sox2 and Ngn2. Ngn2, in turn, inhibits the expression of G1 cyclins. A cell cycle inhibitor $p27^{Kip1}$ stabilizes Ngn2, whereas $p57^{Kip2}$ interacts with pro-neural factor Mash1 and represses its transcriptional activity. Rbl1 modulates the Notch pathway and affects the expression of its target genes. Abbreviations: NSC: neural stem cell.

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Table 1 |

Comparison of cell cycle features in MEFs, mESCs and hESCs.

	MEFs	mESCs	hESCs
Cell cycle length	24h	4.4 – 7.5h *in vivo*; up to 12h *in vitro*	16h
G1 phase length	11h	3h	3h
CDK1 and CDK2 activity	periodical	very high and constant	very high and periodical
D-cyclins expression	++	+/−	+
RB1 phosphorylation status	hypo- and hyperphosphorylated	hyperphosphorylated	hypo- and hyperphosphorylated
KIP/CIP inhibitors expression	present	absent	present

Abbreviations: MEFs: mouse embryonic fibroblasts; mESCs: mouse embryonic stem cells; hESCs: human embryonic stem cells.