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REVIEW ARTICLE

From clocks to dominoes: lessons on cell cycle remodelling from embryonic stem cells

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Cell division is a fundamental cellular process and the evolutionarily conserved networks that control cell division cycles adapt during development, tissue regeneration, cell de-differentiation and reprogramming, and a variety of pathological conditions. Embryonic development is a prime example of such versatility: fast, clock-like divisions hallmarking embryonic cells at early developmental stages become slower and controlled during cellular differentiation and lineage specification. In this review, we compare and contrast the unique cell cycle of mouse and human embryonic stem cells with that of early embryonic cells and of differentiated cells. We propose that embryonic stem cells provide an extraordinarily useful model system to understand cell cycle remodelling during embryonic-to-somatic transitions. We discuss how cell cycle networks help sustain embryonic stem cell pluripotency and self-renewal and how they safeguard cell identity and proper cell number in differentiated cells. Finally, we highlight the incredible diversity in cell cycle regulation within mammals and discuss the implications of studying cell cycle remodelling for understanding healthy and disease states.

Keywords: cell cycle remodelling; embryonic stem cells; embryonic-to-somatic transition

On clocks: the early embryonic cell cycle

Cell division is a fundamental cellular process, and the correct partitioning of chromosomes between two daughter cells requires tight regulation of the cell cycle machinery. In essence, cell division is dependent on the activation and de-activation of cyclin-dependent kinases (CDKs) and the oscillatory expression of cyclin proteins, which regulate CDK activities in all cells.

Biochemical oscillations of Cdk–cyclin activity are at the heart of early embryonic division cycles and allow the embryonic cell cycle to function as an oscillator that toggles rapidly between DNA duplication and chromosome segregation.

Work in classical embryonic cell cycle models, such as the frog *Xenopus laevis* [1,2], zebrafish *Danio rerio* [3,4] and the fruit fly *Drosophila melanogaster* [5], has shown that early embryonic divisions are fast, due to the absence of gap phases, and often show a degree of synchrony. These synchronous cell division cycles, due to their regulation by the abovementioned biochemical

Abbreviations
APC/C, Anaphase-promoting complex; ATM, Ataxia telangiectasia-mutated; ATR, Ataxia telangiectasia and Rad3-related protein; CDK, Cyclin-dependent kinase; CdkI, Cdk inhibitor; CHK2, Checkpoint kinase 2; CIP/KIP, CDK-interacting protein/kinase inhibitory protein; EpiSCs, Mouse postimplantation epiblast cells; ES, Embryonic stem; G1, Gap phase 1; G2, Gap phase 2; H2AX, H2A histone family X; hES, Human embryonic stem; ICM, Inner cell mass; M, Mitosis; MAPK, Mitogen-activated protein kinase; MCM, Minichromosome maintenance; mES, Mouse embryonic stem; miRNAs, MicroRNAs; ORC, Origin recognition complex; PCNA, Proliferating cell nuclear antigen; Plk1, Polo-like kinase 1; pre-RC, Prereplication complex; RB, Retinoblastoma; SAC, Spindle assembly checkpoint; SCF, Skp2-Cullin-Fbox; Skp2, S-phase kinase-associated protein 2; UNG, Uracil-DNA glycosylase; UVC, Ultraviolet radiation C.

oscillator, were termed 'clock-like' by Murray and Kirschner [1].

The cell cycle has evolved from this simple oscillatory system to include more complex regulatory features that are necessary to maintain genomic stability through generations in specialised, somatic cells.

Speed and regulation by the cyclin–CDK biochemical oscillator are features that seem to be conserved in mammals. Indeed, cell division in early-stage mammalian embryos closely resembles these classical models. Embryonic mammalian cells progress rapidly through cell division with truncated or absent gap (G1 and G2) phases [6,7] (Fig. 1). Mouse blastomeres are believed to undergo cell division every 4.4 to 10 h from embryonic days E4.5 to E7, expanding from 20 cells to 4000 [7,8]. Cell cycle duration in early human embryos, as measured by time between mitoses, is 11 h (between the first mitosis and the second mitosis) and 1 h (between the second mitosis and the third mitosis) [8].

Molecularly, knockout studies have demonstrated that while Cdk1 and cyclins A and B are essential for early mouse development, the majority of Cdk–cyclin proteins are fairly redundant [9–11]. The Cdk–cyclin oscillator is cell autonomous and therefore insensitive to serum deprivation; as such, it does not require mitogenic or developmental signals in order to progress through the G1 phase.

Furthermore, like frog or zebrafish embryos, early divisions in the pre-implantation mammalian embryo are independent of restriction point – the point at which cells become 'committed' to the cell cycle and no longer require extracellular proliferative cues [12] – and are typically not (or only very loosely) subjected to checkpoint control [13–17].

Key to this seems to be the fact that early embryonic divisions are independent of the retinoblastoma (RB) protein, which, in adult, somatic cells, plays a crucial role in controlling the G0/G1-phase and the G1/S-phase transitions in somatic cells. This gatekeeping role of RB results from negatively regulating the activity of E2F transcription factors, which are master regulators of G1-S transcription and, thereby, of the transition from G1 into S phase [18].

While RB null mice die at day E15, this is at least in part due to placental insufficiency [19]. In fact, loss of RB has little effects on cell division and differentiation prior to implantation [20], supporting the idea that early embryonic divisions might be independent of RB.

Does this mean that early embryonic divisions are totally devoid of gatekeepers? The answer seems to be no: although an apoptotic response does not take place in early divisions in the mouse embryo and it has been reported that both the cell cycle arrest and the apoptotic functions of p53 are not active, irradiation of 1-cell mouse embryos in G2-phase delays mitotic entry, suggesting the existence of some sort of checkpoint control mechanisms, at least in G2 [21–23].

Lastly, early embryonic divisions can occur before activation of zygotic transcription, by relying on maternal mRNA [2,4]. Early embryonic cells (blastomeres) have a typical morphology with a high nuclear-to-cytoplasmic ratio and, for the most part, do not undergo any cytoplasmic growth. In these early cells, embryonic divisions seem to serve the purpose of increasing cell number in order to prepare for lineage specification. Each blastomere is totipotent and can, in principle, give rise to a new whole organism.

From clocks to dominoes: cell cycle adaptations of embryonic stem cells

The first specification of the totipotent embryo gives rise to two distinct cellular populations during early mammalian development: the trophectoderm and the inner cell mass (ICM), which will in turn give rise to the extra-embryonic lineages and the embryo, respectively [24].

Embryonic stem (ES) cells originate from the ICM and have the following unique characteristics: they derive from the pre-implantation embryo; have

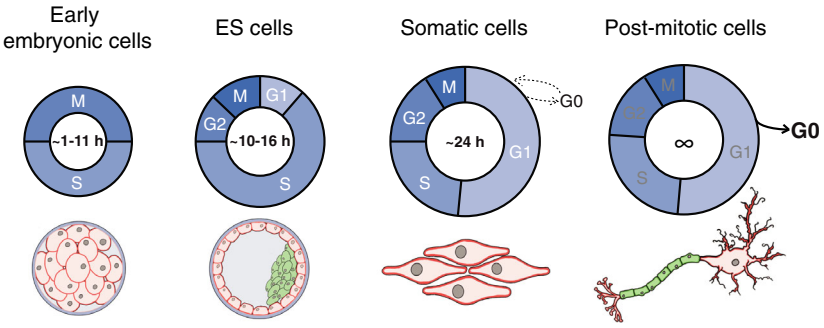


Fig. 1. Cell cycle remodelling during cellular differentiation. Schematic showing differences in cell cycle structure of early embryonic divisions, embryonic stem (ES), differentiated (somatic) and postmitotic cells. Average cell cycle duration is shown in hours. Cells are not to scale.

Table 1. Molecular differences between cell cycle regulators in somatic and embryonic stem cell division cycles. (M) and (H) indicate mouse and human embryonic stem cells, respectively

Cell cycle regulator	ES cells	Somatic cells	References
Cyclins			
Cyclin A	High and non-(M) or oscillatory (H) expression	Lower expression; cell cycle regulated	Savatier 1996, Stead 2002, Faast 2004, Fujii-Yamamoto 2005, Ghule 2007, Neganova 2009, Pauklin and Vallier 2013, Wang 2017
Cyclin B	High expression (M)(H)	Lower expression; cell cycle regulated	
Cyclin D	Low (M) or intermediate (H) expression	High expression; cell cycle regulated	
Cyclin E	High, non-(M) or oscillatory (H) expression	Lower expression; cell cycle regulated	
CDKs			
Cdk1	High expression, oscillatory activity (M)(H)	Cell cycle regulated activity	Stead 2002, Faast 2004, White 2005, Fujii-Yamamoto 2005, Ghule 2007, Neganova 2009, Bar-On 2010
Cdk2	High expression, constant (M) or oscillatory activity (H)	Cell cycle regulated activity	
Cdk4	Low (M) or intermediate (H) activity	Cell cycle regulated activity	
Cdk6	Low, oscillatory activity (M)	Cell cycle regulated activity	
CDK inhibitors			
p21/p27/57	Low or absent expression (M)(H)	Higher, cell cycle regulated expression	Stead 2002, Fujii-Yamamoto 2005, Egozi 2007, Neganova 2009, Li 2012,
p16/18/19	Low or absent expression (M)(H)	Higher, cell cycle regulated expression	
G1-phase			
RB	Hyperphosphorylated (M)(H), Hypophosphorylated (H)	Hypo/hyperphosphorylated	Savatier 1994, Stead 2002, Fujii-Yamamoto 2005, White 2005 Conkin and Sage 2009, Bar-On 2010, Ballabeni 2011, Yang 2012, Matson 2017
E2F	De-repressed form, cell cycle independent transcription (M)	Cell cycle dependent transcription	
APC/C Cdh1	Low activity (M)(H)	Cell cycle regulated activity	
p53	Does not induce G1-arrest in response to damage (M)(H)	p53-depedent arrest in response to damage	
Cdt1	High expression (M)(H)	Cell cycle regulated expression	
Emi1	High expression (M)(H)	Cell cycle regulated expression	
S-phase			
Cdc25A	High expression (H)	Cell cycle regulated expression	Fujii-Yamamoto H 2005, Zhang 2009, Ballabeni 2011, Matson 2017
MCM complex	Fast loading (M)(H)	Slower, cell cycle regulated loading	
Cdc6	High expression (M)(H)	Lower expression; cell cycle regulated	
Geminin	High expression (M)(H)	Lower expression; cell cycle regulated	
G2/M-phase			
Plk1	High activity (H)	Cell cycle regulated activity	Egozi 2007, Bar-on 2010, Ballabeni 2011, Yang 2011, Yang 2012, Zhang 2012, Gonzales 2015
Skp2	High levels throughout the cell cycle (H)	Lower expression, no longer present in G1	
ATM/ATR-CHEK2	Expressed and active G2-checkpoint (M)(H)	Expressed and active G2-checkpoint	
APC/C-Cdc20	Lower actity (M)	Higher activity, cell cycle regulated	

prolonged undifferentiated proliferation without undergoing senescence or quiescence; and have stable developmental potential to form derivatives of all three embryonic germ layers. Under specific conditions, much like early embryonic cells, these cells can remain proliferative, and maintain self-renewal capacity and pluripotency, thanks to a unique transcriptional profile [25–27]. The ability to grow them *in vitro* makes them an incredible trackable system to study cell division.

Pluripotency is sustained by rapid divisions cycles [28–34], with mouse ES (mES) cells having an average cell cycle duration of approximately 10 h and human ES (hES) cells of 15–16 h (Fig. 1). ES cells from other primates also divide on average every 15 h [35]. Shorter cell cycle durations in ES cells are coupled with a cell cycle structure that is not too dissimilar to that of early embryonic divisions: the pluripotent cell cycle is characterised by abbreviated G1 and G2 phases and on average over 50% of ES cells are found in S phase [35,36] (Fig. 1).

Embryonic stem cells display other characteristics of early embryonic divisions most notably the ability to self-renew without undergoing quiescence or senescence. ES cells are refractory to contact inhibition and are independent (or less dependent) of extracellular signals, such as mitogen signals, to divide. For example, inhibiting mitogen-activated protein kinase (MAPK) activation (a potent mitogenic cue) has no effect on embryonic cell cycle progression in ES cells [35,37].

Unique Cdk–cyclin activation patterns in embryonic stem cells

Four cell cycle regulatory mechanisms are at the heart of pluripotent cell division cycles (summarised in Table 1): (a) non-oscillatory activation dynamics of cyclin-dependent kinases, Cdks (Fig. 2), (b) overexpression of cyclin proteins (Fig. 2), (c) reduced activity of cell cycle inhibitors (Fig. 2) and (d) atypical (or impaired) checkpoint control (Figs 3 and 4).

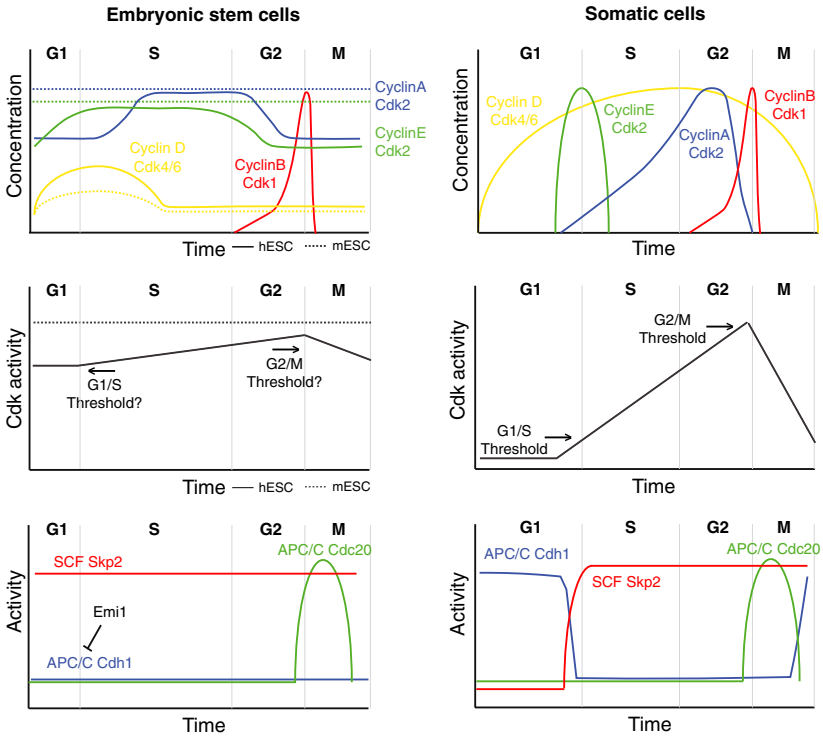


Fig. 2. Comparison of expression levels of cell cycle regulators in somatic and embryonic division cycles. Top: Expression levels of Cdk–cyclin complexes in embryonic (left) and somatic stem cells (right). Solid lines indicate human embryonic stem (hES) cells, and dashed lines indicate mouse embryonic stem (mES) cells. Middle: oscillations of CDK activity in embryonic (left) and somatic stem cells (right). Threshold levels for G1/S and G2/M transition are shown or only hypothesised (question marks) for somatic cells and for embryonic stem cells, respectively. Bottom: Expression levels of APC-Cdh1, APC-Cdc20 and SCF Skp2 in embryonic (left) and somatic stem cells (right).

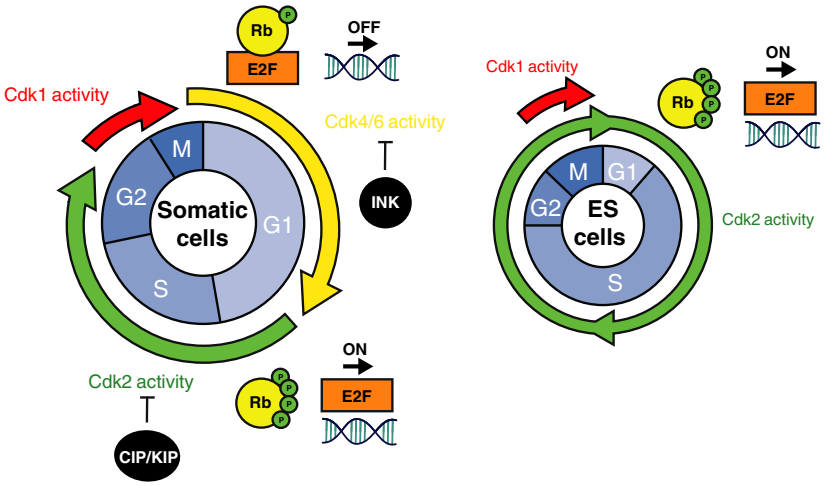


Fig. 3. G1/S checkpoint is compromised in embryonic stem cells. Schematic highlighting differences between G1/S checkpoint control in somatic and embryonic stem cells. E2F transcription and RB phosphorylation are cell cycle-regulated in somatic divisions and constitutive in embryonic stem cell divisions. Levels of CDK activities are shown for somatic and embryonic stem cell division cycles.

At the core of pluripotent cycles is a unique Cdk–cyclin pattern [38–41]. Cdk1, the kinase responsible for the onset of and progression through mitosis, is the only Cdk to show oscillatory activity in both mES and hES cells, despite the high expression of its regulatory partner cyclin B [42,43]. While Cdk4/6 shows minimal activity due to low expression of cyclins D1, D2 and D3, the G1/S master regulator, Cdk2, is constitutively active throughout the embryonic stem cell cycle due to the constitutive expression of its binding partner cyclins E and A [35,42,44–46].

These unusual activation patterns of cyclin-dependent kinases have been proposed to be important to maintain pluripotency in embryonic stem cells [38,41,47]. Mitotic cyclins, cyclins A and B, are essential in cell cycle progression in ES cells, whereas G1 cyclins have been shown to be only important for the maintenance of pluripotency [47]. Indeed, cyclins D and E, have been shown to phosphorylate and thereby prevent degradation of pluripotency factors Nanog, Oct4 and Sox2 [47].

Due to the aberrant activation pattern of Cdk2, the G1/S gatekeeper RB remains hyperphosphorylated

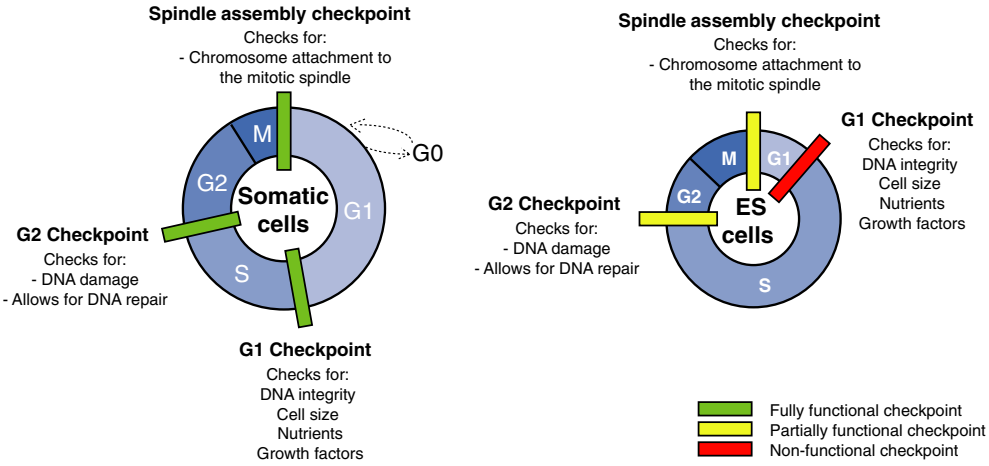


Fig. 4. Checkpoint control in somatic and embryonic stem cell divisions. Schematic showing the checkpoints at play in somatic and embryonic stem cells. Green, yellow and red bars indicate a fully functional, a partially functional and a nonfunctional checkpoint regulation, respectively.

and inactive throughout the cell cycle [48] (Fig. 3). As a result, embryonic stem cells rapidly enter S phase, following a fairly short G1 phase of approximately 1 h in mES cells and 2–3 h in hES cells [36], showing that truncated gap phases and absence of a restriction point are likely to be conserved features of mammalian pluripotent [42]. However, while elevated levels of Cdk2 activity are also seen in hES cells, most cyclins display relative cell cycle-dependent expression in these cells [33,36] (Fig. 2). In addition, Cdk4/6 shows cell cycle-regulated dynamics in hES cells with maximum activity, albeit timid, during G1 phase. As a consequence of Cdk4/6 and Cdk2 activity, slightly reduced levels RB hyperphosphorylation have been reported in G1 for hES cells [49,50].

The differences between mES and hES cells suggest that a more functional restriction point might be potentially present in hES cells. This idea is supported by the fact that knockout of all three RB (Rb^{p105}, $RbL1^{P107}$ and $RbL2^{p130}$) protein family, also known as pocket proteins, having an effect only on hES cell proliferation rate but showing no effect on mES cells [50–53]. A clear mechanistic insight into how G1/S regulators orchestrate this transition in hES cells remain, nevertheless, to be established. These differences in pluripotent divisions between mES and hES cells are likely to be a consequence of the different developmental stages of the two cell types: mES cells are thought to be in an earlier, 'naïve' pluripotent state, as opposed to hES cells, which are thought to be 'primed' pluripotent cells. In other words, hES cells might represent a slightly later developmental stage, equivalent to mouse postimplantation epiblast cells (EpiSCs) [54].

At the heart of the nonoscillatory cyclin expression and the unusually high CDK activation are the low expression levels of CDK inhibitors in ES cells (Fig. 2, Table 1). The CDK-interacting protein/kinase inhibitory protein CIP/KIP and INK Cdk inhibitor (CdkI) proteins, for example, play important roles in cell cycle arrest by binding to and inhibiting Cdks. These include $p21^{CIP}$ and $p27^{Kip1}$, which bind to and antagonise the activity of Cdk1 and Cdk2 and p15, p16 and p18, which inhibit Cdk4 and Cdk6 and all show low or no expression in pluripotent embryonic cell cycles [55,56,57].

In addition to CdkIs, regulation of cell division in ES cells depends on other important cell cycle inhibitors. In particular, the anaphase-promoting complex (APC/C), for example, targets a plethora of cell cycle regulators for degradation, including cyclins. In ES cells, the activity of the APC/C is predominantly low due to elevated levels of the APC/C inhibitor Emi1 [58,59]. The attenuation of APC by Emi1 in ES cells is thought to help increase the expression of cyclins and thereby elevate Cdk activity in ES cells. In addition to APC/C, other proteins that regulate protein degradation during the cell cycle contribute to the atypical expression patterns of cell cycle regulators in ES cells. One example is S-phase kinase-associated protein 2 (Skp2) which targets p27 for degradation. In ES cells, Skp2 is expressed at high levels throughout the cell cycle, as opposed to its restricted expression to S/G2/M in somatic divisions [57].

Together, these special features of high, nonoscillatory Cdk activity, high expression of cyclins and reduced CDK inhibition in ES cell cycles give rise to an atypical Cdk activity and promote a speedy entry into S phase. Interestingly, in this scenario, how Cdk activity is counteracted by phosphatases and how cell cycle events are ordered in ES cells is largely unanswered. For example, whether a quantitative model of Cdk activity is still relevant in embryonic division cycles when nonoscillatory levels of Cdk activity prevails remains to be understood [60,61] (Fig. 2).

Checkpoint control and maintenance of genomic integrity in embryonic stem cells

Maintenance of genome integrity is a demanding task for rapidly proliferating cells. Since ES cells retain the potential to produce any cell type in the adult body, mutations in ES cells can potentially compromise multiple cell lineages and subsequent generations. The question is, thus, how is the fast cycling through cell division in ES cells compatible with preservation of genomic integrity? One adaptation from early embryonic to pluripotent divisions is the acquisition of checkpoint control mechanisms that help with surveillance of genomic integrity (Fig. 4).

ES cells have different mechanisms to protect integrity of their genomes: (a) they overexpress genes important for repair pathways, (b) they show hypersensitivity to DNA damage and, importantly, (c) they show a lower threshold towards programmed cell death, apoptosis.

The modified G1/S checkpoint in ES cell cycles

The G1/S checkpoint serves to protect genomic integrity and to prevent cells with damaged DNA from entering S phase. However, as a consequence of hyperactivation of Cdk2, low level of CdkI expression and misregulation of RB, ES cells lack a fully functional G1/S checkpoint [16,35,62]. Accordingly, monkey ES cells do not arrest in G1 after gamma radiation, reflecting the absence of a classical G1 checkpoint [35].

Although the lack of a functional G1/S checkpoint facilitates speedy entry into S phase and shortened G1 phase, ES cells are particularly vulnerable to DNA damage and show constitutive replication stress [63,64]. Due to this vulnerability, ES cells have adapted to accommodate their dynamic cycling. In fact, ES cells have the capacity to repair a variety of DNA lesions by various agents more efficiently than somatic cells [65]. In addition, ES cells employ an effective replication-coupled repair mechanism to protect genome integrity, characterised by slow replication fork speed and frequent fork reversal [64].

While ES cells are able to upregulate p53 mRNA in response to DNA damage, p53 fails to translocate to the nucleus and activate cell cycle arrest [64]. In addition, after DNA damage, while p21, an important p53 target gene during cell cycle arrest, is elevated at the mRNA level, it is not detectable at a protein level [66]. In line with this, the mir-302 family of microRNAS (miRNAs), which negatively regulates p21 protein expression, is upregulated in ES cells in response to double-strand breaks [66].

Although the DNA damage response does not rely on cell cycle arrest in G1 by the p53-p21 axis in ES cells, an increased apoptotic response to damaged cells has been reported [16]. In line with this, the pro-apoptotic BAX protein has been shown to be constitutively active in ES cells [67]. Elimination of damaged cells may represent for these cells a safer alternative to cell cycle arrest.

Recently, it has been suggested that hES cells might be capable of partially executing the G1/S checkpoint activation after ultraviolet radiation C (UVC) light treatment [68]. After exposure, hES cells show lower levels of Cdk2 activity and undergo G1 arrest. This lowering of Cdk2 activity is not achieved via the classical ATM-CHK2-p53-p21 inactivation but instead by downregulation of the Cdk2-activating phosphatase Cdc25A. In contrast, downregulation of Cdk2 in hES cells triggers the G1 checkpoint through the activation of the ATM-CHK2-p53-p21 pathway, while also promoting differentiation and loss of pluripotency, highlighting the causality between Cdk2 hyperactivation and lack of a fully functional G1/S checkpoint [69].

Checkpoint control at G2/M in ES cell cycles

The absence of a fully operational G1/S checkpoint means that ES cells are prone to replication stress and DNA damage [64]. Therefore, alternative DNA repair mechanisms are necessary to be in place to maintain genomic integrity. This control is achieved during G2 phase.

DNA repair during G2 phase is surprisingly efficient in hES cells. This is partly due to increased expression levels of genes involved in base excision repair and involved in double-strand break repair such as uracil-DNA glycosylase (UNG), BRCA1 and ATM/ATR-dependent phosphorylation of H2A histone family X (H2AX) [65,70]. Moreover, in order to repair double-strand breaks, ES cells favour homologous recombination [16,71]. Rad51, which is critical for homologous recombination, is constitutively expressed throughout

the cell cycle in mES cells [72]. Rad51 suppression causes cells to accumulate in G2, suggesting a role for Rad51 in G2/M progression, while also contributing to maintenance of genome integrity.

Together, these mechanisms help protect genomic integrity at the earliest stages of mammalian development.

The spindle assembly checkpoint in ES cell cycles

The spindle assembly checkpoint (SAC) helps maintain chromosomal integrity during mitosis. While the SAC is active in ES cell cycle activation of SAC and apoptosis are not coupled, the SAC does not activate apoptosis as it does in differentiated cells after mitotic abnormalities [16,73]. This potentially can lead to an increased tolerance to changes in ploidy and karyotype abnormalities in embryonic stem cells [73]. In fact, following treatment with a microtubule poison, mES cells transiently activate the SAC but nevertheless exit mitosis and enter G1 with a 4C DNA content, without undergoing apoptosis [73], a process known as mitotic slippage. Upon differentiation, coupling between apoptosis and spindle assembly checkpoint control is activated and any deviations in ploidy are eliminated [73].

S-phase control in ES cells

To sustain their high proliferative requirements, ES cells must be primed to initiate DNA replication in an efficient manner.

Before DNA replication can start, replication origins need to be licensed so that helicases can unwind DNA and synthesis begin. Licensing factors include the six subunit origin recognition complex (ORC), which surrounds the replication origin and further recruits Cdt1 and Cdc6 in G1 phase. ORC, Cdt1 and Cdc6 then load the minichromosome maintenance (MCM) helicase complex to complete the fully licensed replication origin (i.e. prereplication complex, pre-RC). This DNA/protein complex is now primed for activation and thousands of these origins initiate DNA synthesis throughout S phase [74].

As licensing occurs exclusively in G1 (to prevent re-replication), ES cells have elevated levels of Cdt1 and Cdc6 [43,59,75] to compensate for the abbreviated G1 phase. This has been shown to mediate rapid MCM loading in pluripotent cells in comparison with their differentiated counterpart [75]. Slowing down MCM loading resulted in elongation of G1 and promoted differentiation [75], highlighting that efficient replication origin licensing is an intrinsic feature of embryonic cell cycles and is vital for pluripotency.

Histone biosynthesis during the G1/S transition is an essential process to package newly synthesised DNA in S phase [76]. Histone biosynthesis is in part regulated by Cdk2-dependent phosphorylation of p220 (NPAT), which localises to Cajal bodies and promotes histone H4 gene expression during S phase [77]. The hyperactivation of Cdk2 results in phosphorylation of p220(NPAT) and its premature accumulation in foci at Cajal bodies during G1, preceding the onset of DNA synthesis [78].

Regulation of transcription in ES cells

Cell cycle-regulated transcription is a vital mechanism to bring about changes in gene expression and coordinate progression through the cell cycle. As these dynamic changes in gene expression are bought about by changes in Cdk activities, a consequence of modified Cdk activities in ES cells means that the vast majority of cell cycle regulators have an elevated transcriptional activity. Interestingly, this is often coupled to histone acetylation of their promoter region [44].

In particular, since RB is predominantly inactive in ES cells, E2F transcription is cell cycle-independent. In other words, there is no cyclic expression of E2F targets and E2F targets are instead actively transcribed throughout the cell cycle. As a result, ES cells are primed to transcribe S-phase-specific genes immediately after mitosis and traverse G1 phase. How the E2F transcriptional program functions in hES cells is not fully established.

Control of G2/M phases in ES cells

The regulatory network that drives G2/M transition is conserved in early embryonic and in ES cells and relies on the activation of Cdk1–cyclin B complexes. The transcription factor B-MYB, a target of E2F, is thought to regulate Cdk activity by upregulating many Cdk1 regulatory proteins such as cyclin B1 and Plk1 [79,80].

Since Cdk1–cyclin B1 expression levels are high in ES cells, in order to prevent premature mitotic entry, oscillatory activity of Cdk1 is thought to be maintained by interaction with Oct4, one of the core pluripotent transcription factors, which forms a complex with and inhibits Cdk1 activation in ESC prior to mitosis [81]. Consistent with this, overexpression of Oct4 leads to delayed mitotic entry, whereas downregulations lead to a decrease in G2 duration [81].

Cdk1 is thought to be at the heart of a multitude of events in ES cells including self-renewal, pluripotency, genome maintenance, apoptosis and G2/M checkpoint

activation [32,34,41]. Knockdown of Cdk1 in hES cells leads to loss of pluripotency, spontaneous differentiation, gross genome instability and an inability to execute apoptosis or G2/M checkpoint [34].

In cooperation with Cdk1, mitotic kinases also serve to regulate multiple M phase events such as spindle assembly, APC activation and cytokinesis [82]. Aurora A is one such kinase and localises to centrosomes and spindle microtubules during mitosis and is vital for cell cycle progression, spindle pole orientation and microtubule stability in proliferation cells [83]. In mESC, Aurora A also displays roles outside of mitosis and is important in self-renewal [84]. Interestingly, a recent study has proposed that in hES cells, S/G2 regulators such as cyclin B1 and ATM/ATR-CHEK2 are important for maintenance of pluripotency or what they term 'pluripotency dissolution' outside of G1 phase [31], linking S/G2 cell cycle regulators to self-renewing and maintenance of pluripotency.

The interplay between cycle regulation and pluripotency is an emerging question in stem cell biology.

The interplay between cell cycle control and pluripotency

It has been hypothesised that short division cycles and, in particular, a rapid progression through G1 phase are important to sustain pluripotency, as an abbreviated G1 phase potentially minimises exposure to differentiation signals [28,49,85,86]. Mounting evidence has underscored the importance of cell cycle regulators to maintain pluripotency in ES cells.

Cdk4/6 bound to G1 cyclins partners are known to directly phosphorylate and stabilise the pluripotency factors Nanog, Oct4 and Sox2 [47]. In addition, studies showed that downregulation of key cell cycle regulators such as Cdk1, Cdk2, cyclin E and cyclin B resulted in loss of pluripotency and triggered spontaneous differentiation [29-34,87]. On the other hand, ectopic expression of cyclin E and cyclin B promotes self-renewal in ES cells [31]. In line with this, studies in hES cells have shown that artificially increasing Cdk4/6 activity during G1 or lowering RB hyperphosphorylation can lead to an increase in G1 duration and an enhanced differentiation potential [49,88]. It has been further proposed that Cdk4/6–cyclin D regulates the activation of the transcription factor Smad2/3, an important transducer of pluripotency [86].

Despite these observations, there is conflicting evidence on the importance of G1 length in maintaining pluripotency in ES cells. For example, elongating G1 in mES cells by overexpressing p21/p27 or knocking down CDK2/cyclin A is still compatible with the pluripotent state and does not induce differentiation [89]. In addition, mES cells grown in naïve pluripotency conditions (2i/LIF) have an extended G1 but are nevertheless more pluripotent than primed mES cells [45]. In hES cells, while p27 overexpression leads to the upregulation of Brachyury and Twist, two important factors in differentiation, it has no effect on core pluripotency factors [90]. While there is compelling evidence that the activity of key cell cycle regulators influences the pluripotent state in ES cells, the requirement for an abbreviated G1 phase may be less crucial.

Finally, an important shared regulator of both pluripotency and cell cycle control in ES cells is the transcription factor MYC [91]. MYC is essential for the maintenance of pluripotency [92] but also upregulates the transcription of Cdks and cyclins, including cyclins D, E and A, while simultaneously negatively regulates CdkIs, p21 and p27 [93]. Furthermore, Myc also targets the miRNA cluster mir-17-92, which negatively regulates translation of p21, E2F1, RB and cyclin D [94,95]. Altogether, these observations put this transcription factor at the heart of the interplay between pluripotency and cell cycle regulation.

On dominoes: the cell cycle of somatic cells

Somatic cells have requirements that are very different from those of embryonic cells. With cellular specification and the acquisition of restricted patterns of gene expression during embryogenesis arises, the need to maintain cellular identity and cell number within tissues. In addition, somatic cells lose self-renewing capacity and acquire the ability to reversibly exit the cell cycle, a process known as quiescence. The cell cycle has a crucial role in these properties of differentiated cells, and these somatic cells have adapted cell cycle control to fulfil these important requirements.

Differentiation of embryonic stem cells is accompanied by extensive gene expression changes, upregulation of lineage-specific programs and epigenetic modifications that maintain those programs. Cell morphology changes dramatically during differentiation: cells become larger and elongated and the ratio of nuclear-to-cytoplasmic volume inverts, due to extensive cytoplasmic growth. The cell cycle is also actively remodelled through differentiation. Somatic divisions are long (typically 24 h), asynchronous and have long gap phases, most notably G1 phase, and can have adopted mechanisms to maintain genome integrity and cellular identity [28,30,35,37,38] (Fig. 1).

Concomitantly with the lengthening of G1, differentiated (somatic) cells become dependent on external cues such as mitogenic factors to undergo cell division and develop sensitivity to serum deprivation. In other words, a restriction point is established. Mitogenic-dependent progression through G1 phase is mediated by induction of the cyclin D family of proteins, which, in somatic cells, is typically rapidly degraded but becomes stabilised in the presence of mitogenic signals. In fact, protein stabilisation and degradation play an important role in cell cycle control in somatic cells. The expression levels of other key cell cycle regulators such as p53, p21, p27, cyclin E and E2F are all regulated by degradation.

In somatic cells, G1 phase can be seen as a critical point in the cell cycle where decisions on whether to proliferate, enter a quiescent state or exit the cell cycle completely take place (Fig. 1). If a cell passes the so-called 'restriction point' at the end of G1 phase, it is irreversibly committed to progress through the cell cycle, undergo DNA replication and segregate chromosomes during mitosis.

Introduction of cell cycle-dependent activities of cell cycle regulators in somatic divisions

Degradation gives rise to one of the most fundamental control features of the transition from a pluripotent to somatic division cycle: the appearance of oscillatory activation and expression of cell cycle regulators (Fig. 2). Somatic cells downregulate cyclins and upregulate Cdk inhibitor proteins, including increasing the activation patterns of APC/C (Fig. 2). Important somatic cycle regulators are the CIP/KIP family (p21, p27, p57) inhibitors which control cell cycle progression by inhibiting Cdk1 and Cdk2 complexes [96], and the INK4 family (p15, p16, p18 and p19) proteins, which display specificity for regulating Cdk4 and Cdk6 [96]. CdkIs provide an effective way of slowing down the cell cycle by arresting cell division – CdkIs bind to and inhibit Cdk–cyclin complexes by steric inhibition, thereby blocking access to substrates or ATP and hence reducing specificity to cyclin proteins and destabilising Cdk–cyclin interactions.

In somatic cells, Cdk expression levels are downregulated relative to embryonic cells and remain constant throughout the cell cycle, while cyclins levels start to oscillate in a cell cycle-dependent way. Cyclin proteins are unstable in nature and their levels are regulated both at a transcriptional level and at the level of protein stability in a cell cycle-dependent manner in somatic cell divisions. The oscillatory expression of cyclin proteins during somatic divisions is critical for normal somatic cell cycle progression. Failure in regulating cyclin levels can trap cells in a particular cell cycle phase. Progression through each cell cycle phase requires the activation of at least one phase-specific Cdk–cyclin complex. Each of these promotes activation of the next complex ensuring that the cell cycle progresses in an ordered fashion.

The combination of the new waves of cyclin oscillations with the appearance of CdkIs results in an overall decrease in Cdk activity. Importantly, the activation dynamics of each Cdk–cyclin complex becomes cell cycle-dependent (Fig. 2, Tables 1 and 2).

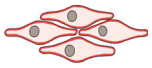
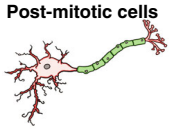
Introduction of a restriction point and checkpoint control mechanisms in somatic divisions

Sophisticated control mechanisms involved in the regulation of cell division in somatic cells couple extracellular signals to the cell cycle machinery. These not only provide directionality in cell cycle progression but also have strategically placed restriction points that provide safeguards for genomic integrity.

Somatic cells have intact checkpoint mechanisms in place to detect a plethora of DNA damage events in order to activate cell cycle arrest and repair the damage both during G1 and G2 phases [97]. Mutation frequency in somatic cells is two orders of magnitude that of embryonic stem cells ($\approx 10^{-4}$) [98]. Failure to repair DNA damage typically results in apoptosis. Checkpoint control mechanisms are fundamental to keep the four cell cycle phases of each round of cell division in the correct temporal order, and to ensure that chromosomal DNA is faithfully replicated and then distributed equally to each daughter cell. This ensures the maintenance of genomic integrity through generations of daughter cells (Fig. 4). Checkpoint control allows the cell cycle of a somatic cell to behave like the pieces in a falling line of dominoes: one phase only starts after full completion of the previous phase [1].

Checkpoints can be divided into two groups: (1) cell-intrinsic checkpoints that depend on intrinsic signals, for example, indicating the integrity of the genome; and (2) cell-extrinsic checkpoints that depend on mitogenic signals such as growth factors. One such extrinsic checkpoint controls the passage from early to late G1, or the restriction point. The appearance of the restriction point is one of the key features of somatic cell division cycles. The restriction point marks the commitment point after which a cell no longer needs external signals to undergo a new cell division cycle. Control of the restriction point relies

Table 2. Differences in cell cycle control amongst early embryonic, embryonic stem cell, somatic and terminally differentiated cells

Early embryonic cells	ES cells	Somatic cells	Post-mitotic cells
			
- Autonomous oscillations of active CDK cyclin complexes - Truncated or non-existent GAP phases - Absent or reduced checkpoints - Do not rely on external stimulus to progress through G1 phase - Independent of restriction point - Insensitive to serum deprivation - Divisions can occur without zygotic transcription	- Non cyclical, high expression of cyclins - Non cyclical, constitutively active CDKs - Low levels of CDK inhibitors - Lack of G1/S checkpoint control - Do not rely on external stimulus to progress through G1 phase - Independent of restriction point - Insensitive to serum deprivation - Non cyclical expression of E2F targets - Non cyclical Rb hyperphosphorylation - Acetylation of histones at promoter regions of cell cycle genes	- Cyclical expression of cyclins - Cell cycle regulated CDK activity - Cyclic levels of CDK inhibitors - Functional G1/S, G2 and SAC checkpoints - Rely on mitogenic and differentiation cues to progress through G1 phase - Dependent of restriction point - Sensitive to serum deprivation - Cyclic expression of E2F targets - Cyclic hyperphosphorylation of Rb - Deacetylation of histones at promoter regions of cell cycle genes	- Down-regulation of G1 cyclins - Down-regulation of CDK Activity - High levels of CDK inhibitors - Permanent Rb/E2F repression of genes - Silencing of cell cycle genes by methylation of histones at promoter regions

heavily on control of Cdk4/6-cyclin D complexes and RB (Fig. 3).

The acquired oscillatory activity of Cdk4/6 and Cdk2 is key in regulating the restriction point via the phosphorylation of RB. Under normal conditions in early G1, RB is in a hypophosphorylated, active state and binds to and suppresses the E2F transcription factor, thereby inhibiting transcription of S phase-specific genes, such as cyclin E or Cdc25A, and those involved in DNA replication such as thymidine kinase or DNA polymerase-α and proliferating cell nuclear antigen (PCNA). After mitogenic signalling, RB is hyperphosphorylated by Cdk4/6 and releases E2F inhibition, thereby promoting E2F-dependent transcription (Fig. 3). One such target of E2F is cyclin E, which forms a complex with and activates Cdk2 and goes on to promote further phosphorylation and inhibition of RB and progression through G1/S. This positive feedback loop marks a point of no return in cell cycle entry [99].

E2F regulation in somatic cell cycles leads to a transient repression of E2F-regulated transcription during G1, and so expression of E2F target genes becomes cell cycle-dependent in somatic cells. An overall decrease in the expression of cell cycle-related genes is further achieved by deacetylation of histones at promoter regions of cell cycle regulators [100].

The establishment of this G1/S checkpoint is one of the hallmarks of the somatic cell cycle. It restricts premature entry into S phase and allows for tuning of the speed of proliferation (Fig. 4). The main targets of the G1/S checkpoint are Cdk–cyclin complexes that drive the transition from G1 to S phase [18]. Following the activation of the checkpoint sensors, ATM and Cdh2, Cdc25A phosphatase becomes inactivated by phosphorylation, rendering Cdk2 inactive. This results in the rapid initiation of G1 arrest until Cdk2 activity is allowed to rise. Cdk2 inactivation and G1 arrest are actively maintained by p21 upregulation.

Later in the cycle, the G2/M checkpoint prevents cells containing damaged DNA from entering mitosis (M). During G2 phase, cyclin B is expressed and activates Cdk1 so that the complex can drive nuclear envelope breakdown and entry into M phase. Cdk1–cyclin B1 is activated until G2/M, when Cdk1 inhibitor Wee1 causes inactivation by phosphorylation of threonine 14 and tyrosine 15 residues. These inhibitory phosphorylations are counteracted by the phosphatase Cdc25C, a target for the checkpoint protein Chk2. Together with Chk2, the p53 targets p21 and GADD45 aid with maintenance of a G2 arrest and prevent somatic cells from entering into mitosis.

The last piece of the falling domino: the cell cycle of terminally differentiated cells

Terminal differentiation is characterised by permanent exit from the cell cycle and is the most common cellular state of fully differentiated adult cells. Its most prominent feature is a prolonged cell cycle arrest with a G1 (i.e. 1C) DNA content, that is G0 (Fig. 1).

Indeed, one of the hallmarks of postmitotic cells is that they become refractory to mitogen or other proliferative signals that promote cell cycle reactivation. A permanent withdrawal from the cell cycle causes irreversible loss of proliferative potential (Table 2).

Molecularly, cell cycle arrest is brought about by downregulation of G1 cyclins [47], high expression of

Cdk inhibitors and the presence of inactive, hypophosphorylated RB. Together, these give rise to downregulation of Cdk activity and a permanent inhibition of E2F-dependent transcription [100] (Table 2).

Cell cycle-driven gene expression is further silenced by methylation of histones at the promoters of genes encoding on cell cycle regulators. RB plays an important role in this, both by inhibiting E2F and by recruiting histone deacetylases and methyltransferases to help repress E2F target genes. This suggests that terminal differentiation might not be an easily reversible cellular state [101], but the very last piece of a falling domino.

In line with this, protein degradation machinery also plays a key role in mitotic exit [102]. While both APC/C and Skp2-Cullin-Fbox (SCF) ubiquitin ligases seem to promote quiescence, RB and APC/C have been shown to physically interact and induce degradation of Skp2, which normally targets the Cdk inhibitors p21 and p27 for degradation. The inhibition of Skp2 results in accumulation of the inhibitors and permanent cell cycle arrest.

Concluding remarks

Our current knowledge of the differences between embryonic and somatic division cycles mainly stems from research on established model systems (xenopus, zebrafish, drosophila model organisms, and yeast and mammalian cells). While these studies have paved the way to our understanding of cell division, they have perhaps fallen short of showing how the biochemical machinery regulating cell division cycles changes during developmental and cellular transitions (Figs 2–4 and Table 1). Embryonic stem cells can be instrumental in filling this gap and provide clues as to how the cell cycle changes while organisms develop and grow.

The pluripotent cell cycle has characteristics of both embryonic and somatic divisions, and the differences in cell cycle regulation between embryonic stem cells and their differentiated counterparts (summarised in Table 2) likely result from their specific functions. ES cells self-renew and retain the potential to produce all cell types in an adult organism and thereby can transmit any errors resulting from cell division to progeny during cell specification. On the contrary, genome integrity in somatic cells is important to maintain cellular identity and to protect these cells from malignant transformation. Cell cycle regulation in cells with different functions has therefore adapted to fulfil these specific requirements. Remarkably, this highlights how the diversity of cell cycle regulation within mammalian species can differ more than those of distantly related species.

Embryonic stem cells can be utilised to understand how one genome can produce such diverse phenotypic outcomes by monitoring developmental cell cycle changes in real time. Understanding the molecular mechanisms underlying these adaptations will not only shed light into embryonic-to-somatic transition during development but, importantly, it will also shed light on how rapid cell divisions boosting cell proliferation adapt to become slow and controlled division cycles. This has great implications for targeting cancer and other pathological conditions hallmarked by a failure to arrest proliferation. The fact that embryonic cell cycles rely on unusual activation dynamics of oncogenes, but yet do not undergo oncogenic transformation, opens new avenues of research with translational potential.

Finally, understanding how proper cell cycle progression is achieved when oscillations of key cell cycle regulators are lost in embryonic stem cells and how fidelity of genome replication and segregation are ensured in the absence of a functional G1-checkpoint control are open, fascinating and important questions.

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