Cycling through developmental decisions: how cell cycle dynamics control pluripotency, differentiation and reprogramming

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Abdenour Soufi[1*] and Stephen Dalton[2*]

[1]MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, UK

[2]Center for Molecular Medicine and Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia, USA

[*] corresponding authors: sdalton@uga.edu, Abdenour.Soufi@ed.ac.uk

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Summary

A strong connection exists between the cell cycle and mechanisms required for executing cell fate decisions in a wide-range of developmental contexts. Terminal differentiation is often associated with cell cycle exit, while cell fate switches are frequently linked to cell cycle transitions in dividing cells. These phenomena have been investigated in the context of reprogramming, differentiation and *trans*-differentiation but the underpinning molecular mechanisms remain unclear. Most progress to address the connection between cell fate and the cell cycle has been made in pluripotent stem cells where the transition through mitosis and G1-phase is critical for establishing a window of opportunity for pluripotency exit and the initiation of differentiation. This Review will summarize recent developments in this area and place them in a broader context that has implications for a wide-range of developmental scenarios.

Introduction

The identity of a cell can be defined by its specific, metastable program of transcription and by the activity of cell-type specific transcription factors. Cell-type specific patterns of chromatin organization and epigenetic modifications are crucial for the establishment and maintenance of these transcriptional programs. In order to transition from one state to another, cells must modify their transcriptome, epigenetic landscape and chromosome architecture in a highly coordinated way. Over the last quarter of a century, numerous observations have established a role for the cell cycle in broad aspects of cell fate decisions, and have shown that the expression of cell fate 'decision' genes is often coupled to cell cycle regulatory mechanisms (Fig. 1). These studies show that the cell cycle machinery impacts chromosome architecture, the epigenome and transcriptional programs required for cell identity in multiple contexts including differentiation, reprogramming and *trans*-differentiation.

Reprogramming differentiated cells was first demonstrated by exposing the nucleus of a somatic cell to the cytoplasm of an enucleated egg using somatic cell nuclear transfer (SCNT), generating animals ranging from frogs to primates (Briggs and King,

1952; Campbell et al., 1996; Gurdon, 1962; Tachibana et al., 2013; Wakayama et al., 1998). Similarly, it is possible to *trans*-differentiate cells from one differentiated type to another by exposing the nucleus of a donor cell to the cytoplasm of another cell. An example of this is the fusion between human fibroblasts and mouse muscle cells, which results in the induction of muscle-specific genes in the human genome (Blau et al., 1983). Pre-existing *trans*-acting factors in the recipient cytoplasm can therefore reset the transcriptional program of a donor somatic genome. Shortly after this discovery, ectopic expression of MYOD was shown to be sufficient for the conversion of fibroblasts to myoblasts, demonstrating that specific transcription factors can redirect cell identity (Davis et al., 1987). More recently, Takahashi and Yamanaka showed that OCT4, SOX2, KLF4, and MYC (together abbreviated as OSKM), are sufficient to convert somatic cells to the pluripotent state, thereby generating induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006). Together, these studies reveal that cell-type specific transcription factors are central to the cell fate decision making process (Graf and Enver, 2009; Xu et al., 2015). Numerous observations have identified important connections between these transcriptional master regulators, cell-state transitions and the cell cycle, although the molecular mechanisms that connect these processes are only starting to be elucidated. This Review will begin by broadly summarizing the role of the cell cycle in differentiation, reprogramming and *trans*-differentiation in developmental models ranging from yeast to humans. Then, attention will specifically focus on the role of cell cycle regulatory mechanisms in cell fate decisions made by pluripotent stem cells (PSCs). Finally, we put forth our views on how placing cell fate decisions within the context of cell cycle will have implications for a broad spectrum of developmental decisions and will likely change our current methods in manipulating cell identity for clinical purposes and for understanding human disease.

Progression through the cell cycle as a cell fate decision

Progression through the cell cycle involves a sequence of events in which chromosomes are replicated during S-phase and then segregated to daughter cells during M-phase (Morgan, 1995). These key events are separated by gap phases that serve as regulatory

windows to ensure that cell cycle events occur at the correct time and in the right order. All of these events are orchestrated by the activity of cyclin-dependent kinases (CDKs) that phosphorylate substrates required for the different cell cycle transitions. The gap phase separating M-phase from S-phase is known as G1-phase and marks the time when cells make the decision to exit the cell cycle or continue through further rounds of division. This decision is classically thought of as being controlled through the phosphorylation of retinoblastoma (RB) family proteins by CDKs, thereby establishing a binary switch mechanism known as the Restriction (R-) point that gates cell cycle progression in G1-phase (Blagosklonny and Pardee, 2002). Recent evidence suggests that the CDK-dependent G1 decision point in cycling cells may actually initiate upon mitotic exit and may precede the classically defined R-point (Cappell et al., 2016; Spencer et al., 2013). The R-point mechanism links the cell cycle machinery to mitogenic signals and under the appropriate signaling conditions, genes required for G1-S progression are activated. This mechanism has enormous implications for control of normal cell growth and de-regulated proliferation in cancer. In a stem cell context, R-point control is critical in determining the balance between self-renewal, quiescence and differentiation of stem cell populations (Li and Clevers, 2010; Tetteh et al., 2015). For example, proliferative control of hematopoietic stem cells is a critical determinant that distinguishes normal and cancer-related hematopoietic function (Pietras et al., 2011). The developmental state of pluripotent cells can also be regulated by exit from the cell cycle. For example, PSCs exit the cell cycle and enter a 'dormant' developmental state that mimics diapause following MYC depletion (Scognamiglio et al., 2016). This is likely to be related to MYCs ability to control CDK activity and is an interesting example of how proliferation and developmental status are coupled.

Another broad example where cell cycle decisions are coupled to cellular decisions is exemplified by size control mechanisms in a wide-range of organisms (Ginzberg et al., 2015). In principle, cells are required to achieve a critical volume in G1-phase before entering S-phase and committing to another round of cell division. If a cell is too small to sustain itself, it will delay progression into S-phase in an attempt to acquire sufficient volume by growth before it commits to another round of DNA replication and cell division. If size homeostasis is deregulated, a cell could potentially overgrow or reduce in size to

the point where its function would be severely compromised. This process has been studied extensively in the budding yeast where size-regulated commitment to the cell cycle occurs at a point in G1-phase known as 'Start' (Jorgensen and Tyers, 2004). This control point is generally considered to be the equivalent of the mammalian R-point and involves a regulatory mechanism where nutrient and biosynthesis-regulated signaling pathways converge with the cell cycle machinery to coordinate cell volume with progression into S-phase (Ferrezuelo et al., 2012). The cell cycle machinery is also linked to cellular decisions that occur following environmental stress where cells undergo a checkpoint arrest or, alternatively undergo apoptosis (Carvajal and Manfredi, 2013). These fundamental examples of coordination between cellular decisions and the cell cycle have broad relevance to the function of multipotent cells in development.

Cell identity and its coordination with the cell cycle

Early work in yeast provided a mechanistic link between cell cycle machinery and changes in cell identity through double-strand break-induced recombination (Haber, 1998). In this study, haploid budding yeast were found to switch between two different alleles of the mating-type (MAT) locus; **a** and α , leading to mixtures of **a** and α cells that can mate and form **a**/α diploids. In each cell cycle, haploid cells could potentially undergo a mating type switch driven by expression of the *HO* endonuclease that acts on the MAT locus in G1-phase. In *Dictyostelium* development, amoeba decide to become pre-spore cells if they sense starvation conditions in G1-phase but, choose a pre-stalk fate under the same conditions in S- and G2-phases (Gomer and Firtel, 1987). This example is interesting because it indicates that commitment to different cell fates is determined at different stages of the cell cycle in multipotent cells. A similar conclusion has emerged from studies of *C. elegans* vulval development (Ambros, 1999). Here, the point at which *lin-12* acts in the cell cycle impacts cell fate choice. Overall, these reports show that linking the cell cycle to cell fate decisions is a common theme in multipotent cells and is not restricted by species boundaries (Fig. 1). This principle also broadly applies to mammalian embryogenesis. During pancreatic development for example, endocrine progenitor cells adopt different fates depending on whether they are exposed to

differentiation signals in early or late G1-phase (Kim et al., 2015). If exposure to signals occurs in early G1-phase, cells differentiate and exit the cell cycle through an asymmetric cell division. In contrast, if pancreatic progenitors are programmed in late G1-phase they complete the cell cycle and generate two differentiated endocrine cells. The time at which pancreatic progenitors receive induction signals in G1-phase is therefore critical in determining how they respond. This concept is reiterated in studies of murine neocortical development (McConnell and Kaznowski, 1991). Here, multipotent cortical progenitors respond to local induction cues generating different cell fate outcomes depending on where they are in the cell cycle at the time of induction. In murine fetal erythropoiesis, entry and progression through S-phase is required for activation of the erythroid differentiation program through the erythroid master regulator, GATA1 (Pop et al., 2010). Down-regulation of the cyclin-dependent kinase inhibitor (CDKI) $KIP2^{p57}$ and the GATA1 antagonist PU1 are key requirements of this cell cycle-dependent regulatory mechanism. Linking S-phase progression to cell fate decisions in multipotent cells has also been reported in the *Drosophila* central nervous system (Weigmann and Lehner, 1995).

So far, examples of cell fate decisions being initiated during G1- and S-phase have been described, but G2-phase is also potentially important for cellular decisions. During bristle patterning in *Drosophila*, Notch signaling controls transition through late stages of the cell cycle such that cells with elevated Notch signaling divide first and those with lower signaling extend their G2-phase and delay division (Hunter et al., 2016). This G2-phase transition time is critical in determining the decision of sensory organ precursor cells to undergo a microchaete fate or a neural fate. The timing of mitotic entry and duration of G2-phase in which cells are exposed to differentiation cues therefore impacts Notch-mediated lateral inhibition and consequently, cell fate decisions. Studies in zebrafish (Bouldin and Kimelman, 2014), sea squirts (Ogura et al., 2011), frogs and flies (Davidson et al., 2009) reinforce this concept. Together, these studies show that although there are variations to the central theme, the mechanistic coupling of the cell cycle to cell fate decisions is a strong recurring biological theme in multipotent cells from diverse origins.

Terminal differentiation and cell cycle exit

Exit from the cell cycle in G1-phase is frequently required for terminal differentiation of cells during development. The mechanisms underlying this have been reviewed elsewhere (Buttitta and Edgar, 2007; Ruijtenberg and van den Heuvel, 2016) and so only the coordination between developmental programs and terminal differentiation will be highlighted here. In most cases, terminal differentiation is linked to the up-regulation of CDK inhibitor proteins (CDKIs), ensuring inhibition of CDK during G1 as well as hypo-phosphorylation of the RB tumor suppressor protein family, which serves to repress the E2F target genes required for further cell cycle activity. It has been difficult to unequivocally establish the precise mechanism linking terminal differentiation to cell cycle exit but the two processes seem to be linked at several levels and are often mutually antagonistic. During skeletal myogenesis, CDKs inhibit the activity of myogenic transcription factors such as MYOD, thereby maintaining cells in a proliferative, immature state (Guo and Walsh, 1997; Rao et al., 1994; Skapek et al., 1995). A similar scenario has been described during neurogenesis where CDKs inhibit pro-differentiation transcription factors such as NGN2 (Hardwick and Philpott, 2014). It is also true, however, that MYOD counteracts the impact of CDKs by activating the expression of genes for CDK inhibitors such as $CIP1^{p21}$ and $KIP2^{p57}$ (Busanello et al., 2012; Halevy et al., 1995; Parker et al., 1995). Inhibition of CDKs then leads to cell cycle arrest in conjunction with the activation of terminal myogenic events. This theme is also seen during terminal differentiation of *Drosophila* neuroblasts (Choksi et al., 2006; Li and Vaessin, 2000). Here, the homeo-domain transcription factor Prospero (Pro) activates genes required for differentiation but also inhibits transcription of key cell cycle regulatory genes such as *cyclin E* and *string* and promotes transcription of the CDKI gene, *dacapo* (Choksi et al., 2006; Li and Vaessin, 2000). These and other studies (Ruijtenberg and van den Heuvel, 2016) indicate an inverse mechanistic relationship between the cell cycle and terminal differentiation in a broad spectrum of cell types. These events depend on the activity of G1-specific CDKs and their regulation of transcription factors required for developmental decisions. Conversely, transcription factors required for cell fate decisions serve to modulate CDK activity and drive exit from the proliferative state. The balance between CDK activity and transcription factor activity therefore serves as a cell fate decision 'tipping point'.

Reprogramming, *trans*-differentiation and tissue regeneration

The examples considered so far cover the relationship between cell cycle stage and cellular decisions during the process of differentiation. It is important however, to emphasize the requirement for cell cycle controls in other decision-making contexts such as reprogramming, *trans*-differentiation and regeneration, and to establish the similarities and differences among these contexts. Interestingly, several reports indicate that cell proliferation *per se* is not a critical determinant for changes in cell identity. In addition to terminal differentiation (Falcone et al., 1984), *trans*-differentiation of fibroblasts to myoblasts (Chiu and Blau, 1984) and reprogramming by SCNT (Halley-Stott et al., 2010; Jullien et al., 2010; Jullien et al., 2011) have no requirement for active cell division. This observation may also apply to some examples of transcription factor-induced cell fate changes. This includes the switch in exocrine to endocrine pancreatic identity following ectopic expression of NGN3, PDX1 MAFA in mice (Zhou et al., 2008) and the generation of neurons from fibroblasts following ectopic expression of ASCL1, BRN2 and MYT1L (Vierbuchen et al., 2010). Interestingly, ASCL1 was found to drive somatic cells to exit the cell cycle in *trans*-differentiation (Treutlein et al., 2016), while ASCL1 seems to drive quiescent adult hippocampal stem cells to re-enter the cell cycle during differentiation (Urbán et al., 2016). This suggests that the mechanistic coupling between transcription factors and the cell-cycle machinery is context dependent and may be determined by the specific level of the protein as well as the pre-existing molecular landscape specific to the starting cell (masserdotti et al., 2016). C/EBPα-induced conversion of pre-B cells to macrophage-like cells however, can occur under proliferative and non-proliferative conditions (Di Tullio and Graf, 2012), but whether this involves two separate mechanisms is unclear. A caveat to some studies is that limited molecular characterization of *trans*-differentiated cells has been performed, making it unclear whether a complete cell fate switch was accomplished in the absence of cell division (Cahan et al., 2014; Morris et al., 2014). A growing amount of evidence however, does show a key role for active cell division in cell fate switching. During regeneration in *Medusae*, *trans*-differentiation of mono-nucleated muscle cells to other cell types including sensory cells requires active

cell division (Schmid et al., 1988). Cardiac regeneration in zebrafish is also intimately linked to cell cycle regulators such as *polo-like kinase 1* (*plk1*) in proliferating cardiomyocytes (Jopling et al., 2010; Poss et al., 2002). It is also clear that reduced proliferative capacity represents a major barrier for reprogramming to the pluripotent state (Hong et al., 2009; Kawamura et al., 2009; Ruiz et al., 2011; Utikal et al., 2009) and there are several cases where active cell division is an important requirement for directed *trans*-differentiation (Feng, 2016; Jiang et al., 2015) in response to reprogramming factors.

Although continued cell division is a requirement for cell fate changes in many instances, it is unclear why it may not be necessary in all situations. Subtle differences in the barriers that cells face as they undergo identity changes could explain variations in cell cycle-dependency. For example, reprogramming of fibroblasts to the pluripotent state is generally associated with extensive erasure and reestablishment of a DNA methylation signature (Lister et al., 2009; Lister et al., 2011) but in B-cells, no major changes in DNA methylation are required (Di Tullio and Graf, 2012). If the erasure of DNA methylation or other epigenetic signatures requires active cell division, this could explain some of the discrepancies described above. It is also possible that DNA replication rather than cell division is mechanistically important for reprogramming (Lavagnolli et al., 2015). Also, the dependency of a lineage-specifying transcription factor on CDK for activation could explain differences in various stem/progenitor cells.

Cell fate decisions are linked to G1-phase progression in PSCs

In the early mammalian embryo, cells transition through pluripotency during the pre-, peri, and post-implantation phases. Pluripotent cells that exist during peri-implantation development have short generation times but the cell cycle lengthens significantly as cells differentiate along the germ layer lineages (Lawson and Pedersen, 1992; Mac Auley et al., 1993; Snow and Bennett, 1978). Similar trends have been described for PSCs cultured *in vitro* (Boward et al., 2016). Rapid cell division is associated with a truncated G1-phase and only a short delay before cells enter S-phase after exiting M-phase. The absence of fully-formed gap phases establishes a situation wherein PSCs spend 50-65%

of their time in S-phase. As PSCs commit to one of the three embryonic germ layers their progeny acquire an extended G1-phase, resulting in increased cell division times. This can be accounted for by a fundamental change in the regulation of CDK activity (Faast et al., 2004; Stead et al., 2002; White et al., 2005). It has been assumed, mainly for anecdotal reasons, that the low G1-phase/high S-phase cell cycle structure of PSCs supports pluripotency by limiting the time cells are exposed to specification signals. As differentiation initiates, an elongated G1-phase would then make cells more susceptible to irreversible germ-layer commitment. Several reports have now established this concept experimentally. For example, if the length of G1-phase is increased through inhibition of CDK activity, PSCs spontaneously differentiate (Neganova et al., 2008; Ruiz et al., 2011). More recently, the strategic advantage of having a cell cycle with a short G1-phase has been demonstrated at the molecular level (Boward et al., 2016).

Although multiple laboratories showed that PSCs respond to induction signals in G1-phase over two decades ago (Mummery et al., 1987; Pierce et al., 1984; Wells, 1982) this general observation was not fully explored until recently, when the fluorescence ubiquitin cell cycle indicator (Fucci) reporter system was used to explore this phenomenon (Sakaue-Sawano et al., 2008). In a seminal report, Pauklin and Vallier (Pauklin and Vallier, 2013) confirmed that PSCs initiate cell fate decisions when in G1-phase, but they also identified an unanticipated mechanism where mesoderm and endoderm commitment occurs in early G1-phase and ectoderm commitment is restricted to late G1-phase (Fig. 2). This partitioning of G1-phase along germ layer boundaries is related the elevated activity of SMAD2,3 in early G1 and its inactivation in late G1. The former is dependent on ACTIVIN A signaling and the latter dependent on the removal of SMAD2,3 from target genes by Cyclin D activity. Because mesoderm and endoderm differentiation requires SMAD2,3 activity and because ectoderm requires pan-SMAD inhibition, this mechanism provides an insightful explanation for how cell fate commitment is partitioned into different stages of G1-phase. It also provides a mechanistic link between the cell cycle regulated activity of G1-phase cyclins and developmental genes required for cell fate decisions.

An additional report using the Fucci system to dissect cell cycle events has since been published, focusing on the links between epigenetic events and the cell cycle (Singh

et al., 2016). In this study, Singh and co-workers characterized the epigenetic changes that occur in self-renewing hPSCs and found that H3K4 trimethylation within bivalent domains of developmental genes increases in G1-phase while H3K27me3 repressive marks remain constant (Fig. 2). This establishes that bivalent domains of H3K4me3 and H3K27me3 are enriched in G1-phase and that throughout the remaining part of the cell cycle developmental genes are marked primarily by H3K27me3. This is an intriguing observation because it establishes a level of dynamic epigenetic regulation at developmental genes that was not previously appreciated. Consistent with changes in H3K4me3 in G1-phase, developmental genes become transcriptionally competent (Singh et al., 2013). The study suggests that developmental genes are primed for activation each time they go through G1-phase but are not activated unless the appropriate signaling networks are also active. In support of this, chromatin conformation-capture assays (4C) showed that G1-specific epigenetic changes at developmental genes coincide with the establishment of DNA loops that bridge distal enhancers with proximal promoters (Fig. 2). Chromosome architectural changes in G1-phase require increased H3K4me3 at the bivalent domain along with increased CDK2 activity, indicating a functional link between epigenetic remodeling, chromosome architectural changes and the cell cycle machinery. Although the mechanism by which CDKs control chromosome architecture at bivalent genes hasn't yet been established these studies indicate that in each G1-phase, the epigenetic landscape and chromosome architecture changes so that it puts developmental genes in a 'lineage-primed' state. The potential mechanisms by which CDKs remodel chromatin and activate developmental genes in G1-phase could be direct or indirect, but potentially could involve the direct phosphorylation of chromatin remodeling enzymes or sequence-specific transcription factors. In summary, a combination of epigenetics, chromosome architecture and transcription factor recruitment appear to be involved in priming developmental genes for G1-specific differentiation. CDK activity has been implicated in control of these regulatory steps (Pauklin and Vallier, 2013; Singh et al., 2015) (Fig. 2), establishing a link between cell fate decisions and the cell cycle machinery.

Entry to and exit from G1 phase

The idea that the transition from mitosis to G1-phase might establish conditions in which switching of lineage-specific transcriptional programs is possible is supported by studies showing that this window of the cell cycle represents a hyperactive, dynamic transcriptional state (Hsiung et al., 2016). This is consistent with earlier reports showing that developmental genes are primed for transcription in G1-phase (Singh et al., 2013; Singh et al., 2015). Increased transcription as cells transition into G1-phase is thought to localize at genomic regions pre-marked with H3K27ac. This hyper-transcriptional activity is not uniform and may also account for cellular heterogeneity (Hsiung et al., 2016). A recent study has revealed another interesting connection between exit from pluripotency and early cell fate decisions, this time involving S-phases and G2-phase. In this study, a high-throughput RNAi screen performed in hESCs identified cell-cycle genes involved in DNA replication and G2-phase progression that restrict exit from pluripotency or, 'pluripotent state dissolution' (Gonzales et al., 2015). This is another line of evidence that supports the idea that the cell cycle state of PSCs is related to maintenance of the pluripotency. By devoting most time to S-phase and minimizing the time spent in G1-phase, cells have little opportunity for dissolution of the pluripotent state. As G1-phase lengthens during differentiation, this would presumably cause an irreversible breakdown of the pluripotency network and enhance germ layer commitment. This is further evidence indicating that G1-phase represents a gateway for the initiation of cell fate decisions.

By integrating what we know so far, it is possible to envisage a model where the initiation of differentiation consists of at least two phases. The first phase would be pluripotent state dissolution in which the pluripotency network is inactivated, and second phase would be lineage commitment, where new transcriptional programs corresponding to germ layer formation are established. This latter step could involve a combination of bookmarking events during mitosis (see below and Box 1) and lineage priming in G1-phase. Pluripotent state dissolution is then attenuated in S-phase and G2-phase by activities in these respective cell cycle phases, maintaining high levels of pluripotency factors such as NANOG and OCT4. This increase of NANOG and OCT4 is tightly controlled and more homogenous at the transcriptional level after DNA replication

(Skinner et al., 2016). These findings support the model that G1-phase represents a window of time when cells are predisposed to initiate fate decisions. Thus, entry and exit from G1-phase is mechanistically linked to cell fate decisions in PSCs.

Cyclin D connects the cell cycle to activation of developmental genes

Paulkin and Vallier previously found that the CDK4,6-Cyclin D complexes impact ACTIVIN/NODAL signaling and promote human embryonic stem cell differentiation through phosphorylation of SMAD2,3 (Pauklin and Vallier, 2013). More recently however, the same laboratory reported that Cyclin D can influence cell fate decisions independently of SMAD2,3 inhibition and independently of its association with CDK4,6 (Pauklin et al., 2016). This second mechanism of action requires the ability of Cyclin D to recruit transcriptional co-activators and co-repressors to developmental target genes as it accumulates during G1-phase (Fig. 2). For example, when Cyclin D1 is absent in early G1, the co-activator p300 is recruited to endoderm genes. However, when Cyclin D1 accumulates in late G1, it recruits histone deacetylases (HDAC) in place of p300, which functions as a histone acetyltransferase (HAT) resulting in loss of the active histone acetylation and an increase in the repressive histone methylation H3K27me3 at these genes. Consistent with the G1-phase partitioning model, neuroectoderm genes are activated in late G1 by Cyclin D-dependent recruitment of p300 and increased H3K4me3 at these genes. Recruitment of Cyclin D to ectoderm genes is dependent on SP1 whereas Cyclin D recruitment to endoderm genes is E2F-dependent. This work reinforces a number of principles that help us to understand how pluripotent stem cells initiate cell fate decisions from G1-phase. Importantly, Cyclin Ds can interact with transcription factors bound to developmental genes in G1-phase and have vastly differing effects on their regulation via their ability to recruit co-repressors or co-activators. The principle that CDK activities coordinate cell fate decisions at developmental genes in G1-phase is underscored by these observations.

Mitosis and epigenetic memory

DNA replication and mitosis represent two events that could facilitate global restructuring of chromatin during the cell cycle. During S-phase, re-establishing the chromatin state on newly synthesized DNA is potentially important for maintaining or switching cell identity. It is therefore conceivable that this represents a time when new, cell-type specific epigenetic landscapes can be founded. The inheritance of this epigenetic state through the S-phase can use the mother chromatin state as a template or be directly coupled to the DNA replication machinery (for recent reviews see (Ma et al., 2015; Probst et al., 2009)). It is mitosis however, that has recently attracted most attention in this area, in part because of studies where asymmetric cell divisions have been implicated in cell fate changes (Arsenio et al., 2015; Congdon and Reya, 2008; Tran et al., 2012). The hallmarks of mitosis include chromosome condensation and nuclear envelope breakdown - both of which are under control of CDK activity. During M-phase, most transcription-associated factors including RNA polymerases dissociate from chromatin and the cell-type specific transcription programs temporarily halts (Egli et al., 2008; Gottesfeld and Forbes, 1997; Spencer et al., 2000). Furthermore, histone modifications such as global histone acetylation, which are associated with active gene expression, are generally diminished during mitosis (Kruhlak et al., 2001; McManus and Hendzel, 2006). Loss of histone tail acetylation such as H4K16 has been found to be directly linked to the mitotic-specific histone phosphorylation (H3S10P), promoting chromatin fiber condensation (Wilkins et al., 2014). However residual amounts of histone acetylation, which bookmarks a select group of gene promoters, has also been reported (Dey et al., 2009; Kouskouti and Talianidis, 2005; Valls et al., 2005; Zhao et al., 2011). Despite many repressive marks such as H3K9me3 and H3K27me3 being retained in mitotic chromatin (Follmer et al., 2012; Li et al., 2006; McManus et al., 2006; Peters et al., 2002; Singh et al., 2015), the functional proteins - known as 'writers' and 'readers' - that deposit and associate with these marks, such as SUV39H1, HP1 and BMI1, are generally excluded from mitotic chromatin (Egli et al., 2008; Kellum et al., 1995; Minc et al., 1999; Voncken et al., 1999). Histone modifications during mitosis have been reviewed in more detail elsewhere (Wang and Higgins, 2013). In addition, the precise nucleosome positioning and histone variant distribution that usually mark cell-type specific promoters and enhancers are also lost during mitosis (Deniz et al., 2016; Kelly et al., 2010; Komura and Ono, 2005). This

culminates in the erasure of cell type-specific, three-dimensional genomic organization (Dileep et al., 2015; Naumova et al., 2013) and the loss of genome-nuclear lamina interactions (Kind et al., 2013). Epigenetic erasure and chromosome remodeling could therefore explain the requirement for transition through mitosis in order to enhance cellular reprogramming, as discussed in more detail below.

Despite the global reorganization of the epigenome during mitosis, mitotic memory of active gene expression programs is retained. In effect, this may allow newly divided cells to pick up where they left off, either by preventing mitotic compaction of previously active DNA loci, or by facilitating reassembly of transcription complexes on the promoter, or both. This phenomenon is referred to as mitotic bookmarking (see Box 1), and has been shown to be important for the rapid reactivation of certain genes upon entry into G1-phase. Thus, it is possible that the selective retention of certain marks over others may represent a means to instructing cell fate change in the newly divided cell.

Mitosis as a window of opportunity for changes in cell fate

It is well-established that chromosome condensation and nuclear envelope breakdown during mitosis are important for successful SCNT reprogramming of mammalian cells (Campbell et al., 1996). This general model is also supported by studies of SCNT in amphibians where somatic cells are most responsive to reprogramming factors present in the cytoplasm of unfertilized, metaphase II (MII)-arrested oocytes (Halley-Stott et al., 2014). Histone H2A de-ubiquitination on mitotic chromatin but not interphase chromatin seems to contribute to this reprogramming responsiveness. Surprisingly, no other histone modification or epigenetic mechanism has been identified that, together with H2A de-ubiquitination, enhances SCNT reprogramming in M-phase. It has been postulated that histone de-ubiquitination and chromatin condensation may enhance SCNT reprogramming not through the eviction or exclusion of factors from mitotic chromatin but instead, by facilitating factor exchange (Halley-Stott et al., 2014). Hyper-dynamic protein exchange is a feature of pluripotency which decreases following exit from the pluripotent state (Meshorer et al., 2006). This is also consistent with the dynamic binding of pioneer

factors to mitotic chromatin (Caravaca et al., 2013). This pattern of dynamic protein exchange on chromatin may therefore be critical for establishing of the pluripotent state, but it could also establish conditions that predispose PSCs to differentiation or reprogramming following mitotic exit under the appropriate signaling conditions.

Mitosis may serve to provide a window of opportunity for reprogramming because reprogramming factors have preferential access to condensed chromatin and therefore face less competition from other factors that are excluded from target loci. It has been shown that OSK can access closed chromatin by acting as pioneer factors during iPSC reprogramming (Soufi et al., 2012; Soufi et al., 2015). However, whether these pioneer factors interact with mitotic chromatin is yet to be examined. Nevertheless, one can hypothesize that reprogramming factors have a unique ability to bookmark mitotic chromatin, enabling gene priming immediately following exit from mitosis and prior to gene reactivation (Fig. 3). Another mitotic advantage may be due to the exclusion of HP1 and SUV39H1 from heterochromatin, which blocks access of the reprogramming factors to key pluripotency genes during interphase (Soufi et al., 2012) (Fig. 3). The association and dynamic exchange of reprogramming factors with mitotic chromatin functions to bookmark the genome, both specifically and non-specifically (Fig. 3). This landscape is then reset upon mitotic exit and potentially establishes a new epigenomic state for cell fate conversion.

The mitotic advantage in reprogramming is not restricted to unfertilized oocytes but is applicable to other advanced embryonic stages as well. For example, mouse zygotes and electro-fused blastomeres arrested in mitosis can acquire a reprogramming capacity in SCNT (Egli et al., 2007; Riaz et al., 2011). However, a recent study has shown that even the cytoplasm of interphase two-cell mouse embryos can reprogram somatic cells in SCNT, if the donor nucleus and recipient cytoplasm are synchronized (Kang et al., 2014). This suggests that the mitotic advantage is due to effective cell-cycle coordination, and not necessarily the presence of special reprogramming proteins only present in the recipient mitotic cytoplasm or a special mitotic chromatin configuration of the donor nucleus. It has long been known that the cytoplasm of an MII-arrested oocyte retains high activity of CDK that can efficiently lead to nuclear envelope breakdown,

chromatin condensation and subsequent DNA replication of the donor nucleus from all cell cycle stages (Campbell et al., 1993). However, the cytoplasm of S-phase cells is less effective at synchronizing a G2-phase donor nucleus, unless the nuclear envelope is chemically permeabilized (Blow and Laskey, 1988). Thus, the nuclear envelope may act as a barrier for cell synchronization, which is eliminated during mitosis. Taken together, these seemingly conflicting reports seem to agree on the idea that resetting the somatic epigenome to pluripotency or totipotency can only be tolerated if supported by active cell division.

Conclusions

Throughout this Review, the link between cell fate decisions and the cell cycle has been emphasized in a developmental context using examples ranging from yeast to humans. The emerging general themes from this work indicate that cell fate decisions are context dependent with regard to their requirement for cell division. Both cell cycle-dependent and cell cycle-independent mechanisms have been highlighted. For example, terminal differentiation of muscle cells from immature precursors requires cell cycle exit whereas differentiation towards pancreatic and erythroid progenitors requires active division and phase-specific cell cycle activities. Similar variations have been reported in reprogramming to the pluripotent state and *trans*-differentiation across lineages. Irrespective of the requirement for cell division, the cell cycle machinery impacts these cellular decisions (see Table 1). For example, CDKI-regulated CDK inhibition is central to cell fate decisions made in a cell cycle-independent context, while elevated CDK activity is a requirement for most cell cycle-dependent cell fate decisions. Clearly then, it is critical to better understand the molecular basis underpinning cell fate decisions in each scenario in order to place the cell cycle in a broader developmental perspective.

We used PSCs as an example to highlight mechanisms that coordinate cell fate commitment with the cell cycle. In this scenario, cells transitioning through G1-phase are highly responsive to differentiation cues that target developmentally-regulated transcription factors and chromatin remodelers. In conjunction with developmental

signals, the G1-CDK machinery collaborates with signal-regulated transcription factors such as SMADs to recruit co-repressors and co-activators to developmental genes. All together, these events result in chromosome remodeling, enhancer recruitment and the coordinated activation of a transcriptional program required for cell fate decisions. We refer to this G1-specific mechanism as lineage-priming. Interestingly, SMADs are known to act on loci primed by master transcription factors (Mullen et al., 2011), indicating the existence of a more elaborate mechanism for cell fate specification. Along with lineage-priming, G1-phase represents a time when the pluripotency network is vulnerable to inactivation, also called dissolution, whereas in S-phase and G2-phase it is stabilized. Since pluripotent cells spend most time in the S-phase, this further suggests a connection between cell cycle stage and pluripotency wherein S-phase supports pluripotency while G1-phase represents a period of differentiation competency. Establishing a cell cycle where cells spend most time in S-phase and a brief time in G1-phase therefore minimizes the opportunity for pluripotent cells to switch state. Partitioning networks that sustain pluripotency or promote differentiation between different cell cycle phases seems a logical strategy to activate developmental genes and silence pluripotency genes in a coordinated manner. Another mechanism implicated in marking chromatin for future decisions in pluripotent cells is mitotic bookmarking. These observations indicate that mitosis is required to lay the epigenomic foundations for a cell fate switch in G1-phase. In total, these observations imply that the entire cell cycle is part of a coordinated network that orchestrates cell fate decisions.

It is important to note that the cell cycle machinery components present in the cytoplasm must co-ordinate with the chromatin configuration of the genome in the nucleus in order to effectively make a cell fate decision. This is highly relevant to reprogramming and *trans*-differentiation as the field is moving towards generating cell types for clinical applications and disease modeling - especially when the efficiency and the fidelity of the current protocols represent the biggest challenge in the field. In order to achieve these prerequisite requirements, future research must not only concentrate on how to manipulate chromatin and gene expression but also how these changes fit within the cell cycle. Defining in greater detail the mechanistic aspects that connect cell fate switches to the cell cycle machinery will be instrumental in developing novel and effective methods

to control cell types, as well as understanding whether this mechanism is a general feature of cell fate decisions or is restricted to specific cases.

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References

Ambros, V. (1999). Cell cycle-dependent sequencing of cell fate decisions in Caenorhabditis elegans vulva precursor cells. *Development* **126**, 1947–1956.

Arsenio, J., Metz, P. J. and Chang, J. T. (2015). Asymmetric Cell Division in T Lymphocyte Fate Diversification. *Trends Immunol.* **36**, 670–683.

Blagosklonny, M. V. and Pardee, A. B. (2002). The restriction point of the cell cycle. *Cell Cycle* **1**, 103–110.

Blau, H. M., Chiu, C. P. and Webster, C. (1983). Cytoplasmic activation of human nuclear genes in stable heterocaryons. *CELL* **32**, 1171–1180.

Blobel, G. A., Kadauke, S., Wang, E., Lau, A. W., Zuber, J., Chou, M. M. and Vakoc, C. R. (2009). A reconfigured pattern of MLL occupancy within mitotic chromatin promotes rapid transcriptional reactivation following mitotic exit. *Mol. Cell* **36**, 970–983.

Blow, J. J. and Laskey, R. A. (1988). A role for the nuclear envelope in controlling DNA replication within the cell cycle. *Nature* **332**, 546–548.

Bouldin, C. M. and Kimelman, D. (2014). Cdc25 and the importance of G2 control: insights from developmental biology. *Cell Cycle* **13**, 2165–2171.

Boward, B., Wu, T. and Dalton, S. (2016). Concise Review: Control of Cell Fate Through Cell Cycle and Pluripotency Networks. *STEM CELLS* **34**, 1427–1436.

Briggs, R. and King, T. J. (1952). Transplantation of Living Nuclei From Blastula Cells into Enucleated Frogs' Eggs. *Proceedings of the National Academy of Sciences* **38**, 455–463.

Busanello, A., Battistelli, C., Carbone, M., Mostocotto, C. and Maione, R. (2012). MyoD regulates p57kip2 expression by interacting with a distant cis-element and modifying a higher order chromatin structure. *Nucleic Acids Res.* **40**, 8266–8275.

Buttitta, L. A. and Edgar, B. A. (2007). Mechanisms controlling cell cycle exit upon terminal differentiation. *Current Opinion in Cell Biology* **19**, 697–704.

Cahan, P., Li, H., Morris, S. A., Lummertz da Rocha, E., Daley, G. Q. and Collins, J. J. (2014). CellNet: network biology applied to stem cell engineering. *CELL* **158**, 903–915.

Campbell, K. H., Loi, P., Otaegui, P. J. and Wilmut, I. (1996). Cell cycle co-ordination in embryo cloning by nuclear transfer. *Rev. Reprod.* **1**, 40–46.

Campbell, K. H., Ritchie, W. A. and Wilmut, I. (1993). Nuclear-cytoplasmic interactions during the first cell cycle of nuclear transfer reconstructed bovine embryos: implications for deoxyribonucleic acid replication and development. *Biol. Reprod.* **49**, 933–942.

Cappell, S. D., Chung, M., Jaimovich, A., Spencer, S. L. and Meyer, T. (2016). Irreversible APC(Cdh1) Inactivation Underlies the Point of No Return for Cell-Cycle Entry. *CELL* **166**, 167–180.

Caravaca, J. M., Donahue, G., Becker, J. S., He, X., Vinson, C. and Zaret, K. S. (2013). Bookmarking by specific and nonspecific binding of FoxA1 pioneer factor to mitotic chromosomes. *Genes & Development* **27**, 251–260.

Carvajal, L. A. and Manfredi, J. J. (2013). Another fork in the road--life or death decisions by the tumour suppressor p53. *EMBO Rep.* **14**, 414–421.

Chiu, C. P. and Blau, H. M. (1984). Reprogramming cell differentiation in the absence of DNA synthesis. *CELL* **37**, 879–887.

Choksi, S. P., Southall, T. D., Bossing, T., Edoff, K., de Wit, E., Fischer, B. E., van Steensel, B., Micklem, G. and Brand, A. H. (2006). Prospero acts as a binary switch between self-renewal and differentiation in Drosophila neural stem cells. *Developmental Cell* **11**, 775–789.

Congdon, K. L. and Reya, T. (2008). Divide and conquer: how asymmetric division shapes cell fate in the hematopoietic system. *Curr. Opin. Immunol.* **20**, 302–307.

Davidson, G., Shen, J., Huang, Y.-L., Su, Y., Karaulanov, E., Bartscherer, K., Hassler, C., Stannek, P., Boutros, M. and Niehrs, C. (2009). Cell cycle control of wnt receptor activation. *Developmental Cell* **17**, 788–799.

Davis, R. L., Weintraub, H. and Lassar, A. B. (1987). Expression of a single transfected cDNA converts fibroblasts to myoblasts. *CELL* **51**, 987–1000.

Deniz, Ö., Flores, O., Aldea, M., Soler-López, M. and Orozco, M. (2016). Nucleosome architecture throughout the cell cycle. *Sci Rep* **6**, 19729.

Dey, A., Nishiyama, A., Karpova, T., McNally, J. and Ozato, K. (2009). Brd4 marks select genes on mitotic chromatin and directs postmitotic transcription. *Mol. Biol. Cell* **20**, 4899–4909.

Di Tullio, A. and Graf, T. (2012). C/EBPα bypasses cell cycle-dependency during immune cell transdifferentiation. *Cell Cycle* **11**, 2739–2746.

Dileep, V., Ay, F., Sima, J., Vera, D. L., Noble, W. S. and Gilbert, D. M. (2015). Topologically

associating domains and their long-range contacts are established during early G1 coincident with the establishment of the replication-timing program. *Genome Res.* **25**, 1104–1113.

Egli, D., Birkhoff, G. and Eggan, K. (2008). Mediators of reprogramming: transcription factors and transitions through mitosis. *Nat Rev Mol Cell Biol* **9**, 505–516.

Egli, D., Rosains, J., Birkhoff, G. and Eggan, K. (2007). Developmental reprogramming after chromosome transfer into mitotic mouse zygotes. *Nature* **447**, 679–685.

Faast, R., White, J., Cartwright, P., Crocker, L., Sarcevic, B. and Dalton, S. (2004). Cdk6-cyclin D3 activity in murine ES cells is resistant to inhibition by p16(INK4a). *Oncogene* **23**, 491–502.

Falcone, G., Boettiger, D., Alemà, S. and Tatò, F. (1984). Role of cell division in differentiation of myoblasts infected with a temperature-sensitive mutant of Rous sarcoma virus. *The EMBO Journal* **3**, 1327–1331.

Feng, J. (2016). Kinetic barriers in transdifferentiation. *Cell Cycle* **15**, 1019–1020.

Ferrezuelo, F., Colomina, N., Palmisano, A., Garí, E., Gallego, C., Csikász-Nagy, A. and Aldea, M. (2012). The critical size is set at a single-cell level by growth rate to attain homeostasis and adaptation. *Nature Communications* **3**, 1012.

Follmer, N. E., Wani, A. H. and Francis, N. J. (2012). A polycomb group protein is retained at specific sites on chromatin in mitosis. *PLoS Genet.* **8**, e1003135.

Ginzberg, M. B., Kafri, R. and Kirschner, M. (2015). Cell biology. On being the right (cell) size. *Science* **348**, 1245075–1245075.

Gomer, R. H. and Firtel, R. A. (1987). Cell-autonomous determination of cell-type choice in Dictyostelium development by cell-cycle phase. *Science* **237**, 758–762.

Gonzales, K. A. U., Liang, H., Lim, Y.-S., Chan, Y.-S., Yeo, J.-C., Tan, C.-P., Bin Gao, Le, B., Tan, Z.-Y., Low, K.-Y., et al. (2015). Deterministic Restriction on Pluripotent State Dissolution by Cell-Cycle Pathways. *CELL* **162**, 564–579.

Gottesfeld, J. M. and Forbes, D. J. (1997). Mitotic repression of the transcriptional machinery. *Trends Biochem. Sci.* **22**, 197–202.

Graf, T. and Enver, T. (2009). Forcing cells to change lineages. *Nature* **462**, 587–594.

Guo, K. and Walsh, K. (1997). Inhibition of myogenesis by multiple cyclin-Cdk complexes. Coordinate regulation of myogenesis and cell cycle activity at the level of E2F. *Journal of Biological Chemistry* **272**, 791–797.

Gurdon, J. B. (1962). The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J Embryol Exp Morphol* **10**, 622–640.

Haber, J. E. (1998). Mating-type gene switching in Saccharomyces cerevisiae. *Annu. Rev. Genet.* **32**, 561–599.

Halevy, O., Novitch, B. G., Spicer, D. B., Skapek, S. X., Rhee, J., Hannon, G. J., Beach, D. and

Lassar, A. B. (1995). Correlation of terminal cell cycle arrest of skeletal muscle with induction of p21 by MyoD. *Science* **267**, 1018–1021.

Halley-Stott, R. P., Jullien, J., Pasque, V. and Gurdon, J. (2014). Mitosis gives a brief window of opportunity for a change in gene transcription. *PLoS Biol.* **12**, e1001914.

Halley-Stott, R. P., Pasque, V., Astrand, C., Miyamoto, K., Simeoni, I., Jullien, J. and Gurdon, J. B. (2010). Mammalian nuclear transplantation to Germinal Vesicle stage Xenopus oocytes - a method for quantitative transcriptional reprogramming. *Methods* **51**, 56–65.

Hardwick, L. J. A. and Philpott, A. (2014). Nervous decision-making: to divide or differentiate. *Trends Genet.* **30**, 254–261.

Hong, H., Takahashi, K., Ichisaka, T., Aoi, T., Kanagawa, O., Nakagawa, M., Okita, K. and Yamanaka, S. (2009). Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. *Nature* **460**, 1132–1135.

Hsiung, C. C.-S., Bartman, C. R., Huang, P., Ginart, P., Stonestrom, A. J., Keller, C. A., Face, C., Jahn, K. S., Evans, P., Sankaranarayanan, L., et al. (2016). A hyperactive transcriptional state marks genome reactivation at the mitosis-G1 transition. *Genes & Development* **30**, 1423–1439.

Hsiung, C. C.-S., Morrissey, C. S., Udugama, M., Frank, C. L., Keller, C. A., Baek, S., Giardine, B., Crawford, G. E., Sung, M.-H., Hardison, R. C., et al. (2015). Genome accessibility is widely preserved and locally modulated during mitosis. *Genome Res.* **25**, 213–225.

Hunter, G. L., Hadjivasiliou, Z., Bonin, H., He, L., Perrimon, N., Charras, G. and Baum, B. (2016). Coordinated control of Notch-Delta signalling and cell cycle progression drives lateral inhibition mediated tissue patterning. *Development* dev.134213.

Jiang, H., Xu, Z., Zhong, P., Ren, Y., Liang, G., Schilling, H. A., Hu, Z., Zhang, Y., Wang, X., Chen, S., et al. (2015). Cell cycle and p53 gate the direct conversion of human fibroblasts to dopaminergic neurons. *Nature Communications* **6**, 10100.

Jopling, C., Sleep, E., Raya, M., Martí, M., Raya, A. and Izpisua Belmonte, J. C. (2010). Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. *Nature* **464**, 606–609.

Jorgensen, P. and Tyers, M. (2004). How cells coordinate growth and division. *Current Biology* **14**, R1014–27.

Jullien, J., Astrand, C., Halley-Stott, R. P., Garrett, N. and Gurdon, J. B. (2010). Characterization of somatic cell nuclear reprogramming by oocytes in which a linker histone is required for pluripotency gene reactivation. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 5483–5488.

Jullien, J., Pasque, V., Halley-Stott, R. P., Miyamoto, K. and Gurdon, J. B. (2011). Mechanisms of nuclear reprogramming by eggs and oocytes: a deterministic process? *Nat Rev Mol Cell Biol* **12**, 453–459.

Kadauke, S., Udugama, M. I., Pawlicki, J. M., Achtman, J. C., Jain, D. P., Cheng, Y., Hardison, R. C. and Blobel, G. A. (2012). Tissue-specific mitotic bookmarking by hematopoietic transcription factor GATA1. *CELL* **150**, 725–737.

Kang, E., Wu, G., Ma, H., Li, Y., Tippner-Hedges, R., Tachibana, M., Sparman, M., Wolf, D. P., Schöler, H. R. and Mitalipov, S. (2014). Nuclear reprogramming by interphase cytoplasm of two-cell mouse embryos. *Nature* **509**, 101–104.

Kawamura, T., Suzuki, J., Wang, Y. V., Menendez, S., Morera, L. B., Raya, A., Wahl, G. M. and Izpisua Belmonte, J. C. (2009). Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature* **460**, 1140–1144.

Kellum, R., Raff, J. W. and Alberts, B. M. (1995). Heterochromatin protein 1 distribution during development and during the cell cycle in Drosophila embryos. *Journal of Cell Science* **108 (Pt 4)**, 1407–1418.

Kelly, T. K., Miranda, T. B., Liang, G., Berman, B. P., Lin, J. C., Tanay, A. and Jones, P. A. (2010). H2A.Z maintenance during mitosis reveals nucleosome shifting on mitotically silenced genes. *Mol. Cell* **39**, 901–911.

Kim, Y. H., Larsen, H. L., Rué, P., Lemaire, L. A., Ferrer, J. and Grapin-Botton, A. (2015). Cell cycle-dependent differentiation dynamics balances growth and endocrine differentiation in the pancreas. *PLoS Biol.* **13**, e1002111.

Kind, J., Pagie, L., Ortabozkoyun, H., Boyle, S., de Vries, S. S., Janssen, H., Amendola, M., Nolen, L. D., Bickmore, W. A. and van Steensel, B. (2013). Single-cell dynamics of genome-nuclear lamina interactions. *CELL* **153**, 178–192.

Komura, J.-I. and Ono, T. (2005). Disappearance of nucleosome positioning in mitotic chromatin in vivo. *Journal of Biological Chemistry* **280**, 14530–14535.

Kouskouti, A. and Talianidis, I. (2005). Histone modifications defining active genes persist after transcriptional and mitotic inactivation. *The EMBO Journal* **24**, 347–357.

Kruhlak, M. J., Hendzel, M. J., Fischle, W., Bertos, N. R., Hameed, S., Yang, X. J., Verdin, E. and Bazett-Jones, D. P. (2001). Regulation of global acetylation in mitosis through loss of histone acetyltransferases and deacetylases from chromatin. *Journal of Biological Chemistry* **276**, 38307–38319.

Lake, R. J., Tsai, P.-F., Choi, I., Won, K.-J. and Fan, H.-Y. (2014). RBPJ, the major transcriptional effector of Notch signaling, remains associated with chromatin throughout mitosis, suggesting a role in mitotic bookmarking. *PLoS Genet.* **10**, e1004204.

Lavagnolli, T., Gupta, P., Hörmanseder, E., Mira-Bontenbal, H., Dharmalingam, G., Carroll, T., Gurdon, J. B., Fisher, A. G. and Merkenschlager, M. (2015). Initiation and maintenance of pluripotency gene expression in the absence of cohesin. *Genes & Development* **29**, 23–38.

Lawson, K. A. and Pedersen, R. A. (1992). Clonal analysis of cell fate during gastrulation and early neurulation in the mouse. *Ciba Found. Symp.* **165**, 3–21– discussion 21–6.

Li, L. and Clevers, H. (2010). Coexistence of quiescent and active adult stem cells in mammals. *Science* **327**, 542–545.

Li, L. and Vaessin, H. (2000). Pan-neural Prospero terminates cell proliferation during Drosophila neurogenesis. *Genes & Development* **14**, 147–151.

Li, Y., Kao, G. D., Garcia, B. A., Shabanowitz, J., Hunt, D. F., Qin, J., Phelan, C. and Lazar, M. A. (2006). A novel histone deacetylase pathway regulates mitosis by modulating Aurora B kinase activity. *Genes & Development* **20**, 2566–2579.

Lister, R., Pelizzola, M., Dowen, R. H., Hawkins, R. D., Hon, G., Tonti-Filippini, J., Nery, J. R., Lee, L., Ye, Z., Ngo, Q.-M., et al. (2009). Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* **462**, 315–322.

Lister, R., Pelizzola, M., Kida, Y. S., Hawkins, R. D., Nery, J. R., Hon, G., Antosiewicz-Bourget, J., O'Malley, R., Castanon, R., Klugman, S., et al. (2011). Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature* **471**, 68–73.

Lodhi, N., Kossenkov, A. V. and Tulin, A. V. (2014). Bookmarking promoters in mitotic chromatin: poly(ADP-ribose)polymerase-1 as an epigenetic mark. *Nucleic Acids Res.* **42**, 7028–7038.

Ma, Y., Kanakousaki, K. and Buttitta, L. (2015). How the cell cycle impacts chromatin architecture and influences cell fate. *Front Genet* **6**, 19.

Mac Auley, A., Werb, Z. and Mirkes, P. E. (1993). Characterization of the unusually rapid cell cycles during rat gastrulation. *Development* **117**, 873–883.

Martínez-Balbás, M. A., Dey, A., Rabindran, S. K., Ozato, K. and Wu, C. (1995). Displacement of sequence-specific transcription factors from mitotic chromatin. *CELL* **83**, 29–38.

McConnell, S. K. and Kaznowski, C. E. (1991). Cell cycle dependence of laminar determination in developing neocortex. *Science* **254**, 282–285.

McManus, K. J. and Hendzel, M. J. (2006). The relationship between histone H3 phosphorylation and acetylation throughout the mammalian cell cycle. *Biochem. Cell Biol.* **84**, 640–657.

McManus, K. J., Biron, V. L., Heit, R., Underhill, D. A. and Hendzel, M. J. (2006). Dynamic changes in histone H3 lysine 9 methylations: identification of a mitosis-specific function for dynamic methylation in chromosome congression and segregation. *Journal of Biological Chemistry* **281**, 8888–8897.

Meshorer, E., Yellajoshula, D., George, E., Scambler, P. J., Brown, D. T. and Misteli, T. (2006). Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. *Developmental Cell* **10**, 105–116.

Minc, E., Allory, Y., Worman, H. J., Courvalin, J. C. and Buendia, B. (1999). Localization and phosphorylation of HP1 proteins during the cell cycle in mammalian cells. *Chromosoma* **108**, 220–234.

Morgan, D. O. (1995). Principles of CDK regulation. *Nature* **374**, 131–134.

Morris, S. A., Cahan, P., Li, H., Zhao, A. M., San Roman, A. K., Shivdasani, R. A., Collins, J. J. and Daley, G. Q. (2014). Dissecting engineered cell types and enhancing cell fate conversion via CellNet. *CELL* **158**, 889–902.

Mullen, A. C., Orlando, D. A., Newman, J. J., Lovén, J., Kumar, R. M., Bilodeau, S., Reddy, J., Guenther, M. G., DeKoter, R. P. and Young, R. A. (2011). Master transcription factors

determine cell-type-specific responses to TGF-β signaling. *CELL* **147**, 565–576.

Mummery, C. L., van Rooijen, M. A., van den Brink, S. E. and de Laat, S. W. (1987). Cell cycle analysis during retinoic acid induced differentiation of a human embryonal carcinoma-derived cell line. *Cell Differ.* **20**, 153–160.

Naumova, N., Imakaev, M., Fudenberg, G., Zhan, Y., Lajoie, B. R., Mirny, L. A. and Dekker, J. (2013). Organization of the mitotic chromosome. *Science* **342**, 948–953.

Neganova, I., Zhang, X., Atkinson, S. and Lako, M. (2008). Expression and functional analysis of G1 to S regulatory components reveals an important role for CDK2 in cell cycle regulation in human embryonic stem cells. *Oncogene* **28**, 20–30.

Ogura, Y., Sakaue-Sawano, A., Nakagawa, M., Satoh, N., Miyawaki, A. and Sasakura, Y. (2011). Coordination of mitosis and morphogenesis: role of a prolonged G2 phase during chordate neurulation. *Development* **138**, 577–587.

Parker, S. B., Eichele, G., Zhang, P., Rawls, A., Sands, A. T., Bradley, A., Olson, E. N., Harper, J. W. and Elledge, S. J. (1995). p53-independent expression of p21Cip1 in muscle and other terminally differentiating cells. *Science* **267**, 1024–1027.

Pauklin, S. and Vallier, L. (2013). The Cell-Cycle State of Stem Cells Determines Cell Fate Propensity. *CELL* **155**, 135–147.

Pauklin, S., Madrigal, P., Bertero, A. and Vallier, L. (2016). Initiation of stem cell differentiation involves cell cycle-dependent regulation of developmental genes by Cyclin D. *Genes & Development* **30**, 421–433.

Peters, A. H. F. M., Mermoud, J. E., O'Carroll, D., Pagani, M., Schweizer, D., Brockdorff, N. and Jenuwein, T. (2002). Histone H3 lysine 9 methylation is an epigenetic imprint of facultative heterochromatin. *Nat Genet* **30**, 77–80.

Pierce, G. B., Aguilar, D., Hood, G. and Wells, R. S. (1984). Trophectoderm in control of murine embryonal carcinoma. *Cancer Research* **44**, 3987–3996.

Pietras, E. M., Warr, M. R. and Passegué, E. (2011). Cell cycle regulation in hematopoietic stem cells. *The Journal of Cell Biology* **195**, 709–720.

Pop, R., Shearstone, J. R., Shen, Q., Liu, Y., Hallstrom, K., Koulnis, M., Gribnau, J. and Socolovsky, M. (2010). A key commitment step in erythropoiesis is synchronized with the cell cycle clock through mutual inhibition between PU.1 and S-phase progression. *PLoS Biol.* **8**, e1000484.

Poss, K. D., Wilson, L. G. and Keating, M. T. (2002). Heart regeneration in zebrafish. *Science* **298**, 2188–2190.

Probst, A. V., Dunleavy, E. and Almouzni, G. (2009). Epigenetic inheritance during the cell cycle. *Nat Rev Mol Cell Biol* **10**, 192–206.

Rao, S. S., Chu, C. and Kohtz, D. S. (1994). Ectopic expression of cyclin D1 prevents activation of gene transcription by myogenic basic helix-loop-helix regulators. *Molecular and Cellular Biology*

14, 5259–5267.

Riaz, A., Zhao, X., Dai, X., Li, W., Liu, L., Wan, H., Yu, Y., Wang, L. and Zhou, Q. (2011). Mouse cloning and somatic cell reprogramming using electrofused blastomeres. *Cell Res.* **21**, 770–778.

Ruijtenberg, S. and van den Heuvel, S. (2016). Coordinating cell proliferation and differentiation: Antagonism between cell cycle regulators and cell type-specific gene expression. *Cell Cycle* **15**, 196–212.

Ruiz, S., Panopoulos, A. D., Herrerías, A., Bissig, K.-D., Lutz, M., Berggren, W. T., Verma, I. M. and Belmonte, J. C. I. (2011). A High Proliferation Rate Is Required for Cell Reprogramming and Maintenance of Human Embryonic Stem Cell Identity. *Current Biology* **21**, 45–52.

Sakaue-Sawano, A., Kurokawa, H., Morimura, T., Hanyu, A., Hama, H., Osawa, H., Kashiwagi, S., Fukami, K., Miyata, T., Miyoshi, H., et al. (2008). Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. *CELL* **132**, 487–498.

Schmid, V., Alder, H., Plickert, G. and Weber, C. (1988). Transdifferentiation from striated muscle of medusae in vitro. *Cell Differ. Dev.* **25 Suppl**, 137–146.

Scognamiglio, R., Cabezas-Wallscheid, N., Thier, M. C., Altamura, S., Reyes, A., Prendergast, Á. M., Baumgärtner, D., Carnevalli, L. S., Atzberger, A., Haas, S., et al. (2016). Myc Depletion Induces a Pluripotent Dormant State Mimicking Diapause. *CELL* **164**, 668–680.

Singh, A. M., Chappell, J., Trost, R., Lin, L., Wang, T., Tang, J., Matlock, B. K., Weller, K. P., Wu, H., Zhao, S., et al. (2013). Cell-cycle control of developmentally regulated transcription factors accounts for heterogeneity in human pluripotent cells. *Stem Cell Reports* **1**, 532–544.

Singh, A. M., Sun, Y., Li, L., Zhang, W., Wu, T., Zhao, S., Qin, Z. and Dalton, S. (2015). Cell-Cycle Control of Bivalent Epigenetic Domains Regulates the Exit from Pluripotency. *Stem Cell Reports* **5**, 323–336.

Skapek, S. X., Rhee, J., Spicer, D. B. and Lassar, A. B. (1995). Inhibition of myogenic differentiation in proliferating myoblasts by cyclin D1-dependent kinase. *Science* **267**, 1022–1024.

Skinner, S. O., Xu, H., Nagarkar-Jaiswal, S., Freire, P. R., Zwaka, T. P. and Golding, I. (2016). Single-cell analysis of transcription kinetics across the cell cycle. *Elife* **5**, e59928.

Snow, M. H. and Bennett, D. (1978). Gastrulation in the mouse: assessment of cell populations in the epiblast of tw18/tw18 embryos. *J Embryol Exp Morphol* **47**, 39–52.

Soufi, A., Donahue, G. and Zaret, K. S. (2012). Facilitators and impediments of the pluripotency reprogramming factors' initial engagement with the genome. *CELL* **151**, 994–1004.

Soufi, A., Garcia, M. F., Jaroszewicz, A., Osman, N., Pellegrini, M. and Zaret, K. S. (2015). Pioneer transcription factors target partial DNA motifs on nucleosomes to initiate reprogramming. *CELL* **161**, 555–568.

Spencer, C. A., Kruhlak, M. J., Jenkins, H. L., Sun, X. and Bazett-Jones, D. P. (2000). Mitotic transcription repression in vivo in the absence of nucleosomal chromatin condensation. *The*

Journal of Cell Biology **150**, 13–26.

Spencer, S. L., Cappell, S. D., Tsai, F.-C., Overton, K. W., Wang, C. L. and Meyer, T. (2013). The proliferation-quiescence decision is controlled by a bifurcation in CDK2 activity at mitotic exit. *CELL* **155**, 369–383.

Stead, E., White, J., Faast, R., Conn, S., Goldstone, S., Rathjen, J., Dhingra, U., Rathjen, P., Walker, D. and Dalton, S. (2002). Pluripotent cell division cycles are driven by ectopic Cdk2, cyclin A/E and E2F activities. *Oncogene* **21**, 8320–8333.

Tachibana, M., Amato, P., Sparman, M., Gutierrez, N. M., Tippner-Hedges, R., Ma, H., Kang, E., Fulati, A., Lee, H.-S., Sritanaudomchai, H., et al. (2013). Human embryonic stem cells derived by somatic cell nuclear transfer. *CELL* **153**, 1228–1238.

Takahashi, K. and Yamanaka, S. (2006). Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *CELL* **126**, 663–676.

Tetteh, P. W., Farin, H. F. and Clevers, H. (2015). Plasticity within stem cell hierarchies in mammalian epithelia. *Trends Cell Biol.* **25**, 100–108.

Tran, V., Lim, C., Xie, J. and Chen, X. (2012). Asymmetric division of Drosophila male germline stem cell shows asymmetric histone distribution. *Science* **338**, 679–682.

Treutlein, B., Lee, Q. Y., Camp, J. G., Mall, M., Koh, W., Shariati, S. A. M., Sim, S., Neff, N. F., Skotheim, J. M., Wernig, M., et al. (2016). Dissecting direct reprogramming from fibroblast to neuron using single-cell RNA-seq. *Nature* **534**, 391–395.

Urbán, N., van den Berg, D. L. C., Forget, A., Andersen, J., Demmers, J. A. A., Hunt, C., Ayrault, O. and Guillemot, F. (2016). Return to quiescence of mouse neural stem cells by degradation of a proactivation protein. *Science* **353**, 292–295.

Utikal, J., Polo, J. M., Stadtfeld, M., Maherali, N., Kulalert, W., Walsh, R. M., Khalil, A., Rheinwald, J. G. and Hochedlinger, K. (2009). Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature* **460**, 1145–1148.

Valls, E., Sánchez-Molina, S. and Martínez-Balbás, M. A. (2005). Role of histone modifications in marking and activating genes through mitosis. *Journal of Biological Chemistry* **280**, 42592–42600.

Vierbuchen, T., Ostermeier, A., Pang, Z. P., Kokubu, Y., Südhof, T. C. and Wernig, M. (2010). Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* **463**, 1035–1041.

Voncken, J. W., Schweizer, D., Aagaard, L., Sattler, L., Jantsch, M. F. and van Lohuizen, M. (1999). Chromatin-association of the Polycomb group protein BMI1 is cell cycle-regulated and correlates with its phosphorylation status. *Journal of Cell Science* **112 (Pt 24)**, 4627–4639.

Wakayama, T., Perry, A. C., Zuccotti, M., Johnson, K. R. and Yanagimachi, R. (1998). Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* **394**, 369–374.

Wang, F. and Higgins, J. M. G. (2013). Histone modifications and mitosis: countermarks, landmarks, and bookmarks. *Trends Cell Biol.* **23**, 175–184.

Wapinski, O. L., Vierbuchen, T., Qu, K., Lee, Q. Y., Chanda, S., Fuentes, D. R., Giresi, P. G., Ng, Y. H., Marro, S., Neff, N. F., et al. (2013). Hierarchical mechanisms for direct reprogramming of fibroblasts to neurons. *CELL* **155**, 621–635.

Weigmann, K. and Lehner, C. F. (1995). Cell fate specification by even-skipped expression in the Drosophila nervous system is coupled to cell cycle progression. *Development* **121**, 3713–3721.

Wells, R. S. (1982). An in vitro assay for growth regulation of embryonal carcinoma by the blastocyst. *Cancer Research* **42**, 2736–2741.

White, J., Stead, E., Faast, R., Conn, S., Cartwright, P. and Dalton, S. (2005). Developmental activation of the Rb-E2F pathway and establishment of cell cycle-regulated cyclin-dependent kinase activity during embryonic stem cell differentiation. *Mol. Biol. Cell* **16**, 2018–2027.

Wilkins, B. J., Rall, N. A., Ostwal, Y., Kruitwagen, T., Hiragami-Hamada, K., Winkler, M., Barral, Y., Fischle, W. and Neumann, H. (2014). A cascade of histone modifications induces chromatin condensation in mitosis. *Science* **343**, 77–80.

Xu, J., Du, Y. and Deng, H. (2015). Direct lineage reprogramming: strategies, mechanisms, and applications. *Cell Stem Cell* **16**, 119–134.

Young, D. W., Hassan, M. Q., Yang, X.-Q., Galindo, M., Javed, A., Zaidi, S. K., Furcinitti, P., Lapointe, D., Montecino, M., Lian, J. B., et al. (2007). Mitotic retention of gene expression patterns by the cell fate-determining transcription factor Runx2. *Proceedings of the National Academy of Sciences* **104**, 3189–3194.

Zaret, K. S. (2014). Genome reactivation after the silence in mitosis: recapitulating mechanisms of development? *Developmental Cell* **29**, 132–134.

Zhao, R., Nakamura, T., Fu, Y., Lazar, Z. and Spector, D. L. (2011). Gene bookmarking accelerates the kinetics of post-mitotic transcriptional re-activation. *Nature Publishing Group* **13**, 1295–1304.

Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J. and Melton, D. A. (2008). In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature* **455**, 627–632.

Box 1: Mitotic bookmarking

Mitotic bookmarking refers to the retention of epigenetic marks during mitosis that enables rapid gene activation upon entry into G1-phase. The 'bookmarks' include DNase-hypersensitive sites that mark accessible and active promoters and enhancers (Hsiung et al., 2015; Martínez-Balbás et al., 1995), acetylated H4K5 marks and residual bromodomain protein 4 (BRD4) marks (Dey et al., 2009; Zhao et al., 2011). The mixed lineage leukemia (MLL), an epigenetic modifier that maintains gene activity through

catalyzing trimethylation of H3K4 at promoters (Blobel et al., 2009) and the poly(ADP-ribose) polymerase-1 (PARP-1) are also retained in mitotic chromatin (Lodhi et al., 2014). Interestingly, 'bookmarked' genes are rapidly reactivated upon entry into G1-phase (Fig. 3). In addition to chromatin modifiers, a select group of transcription factors such as FOXA1, GATA1, RUNX2, ESRRB and RBPJ also bind and 'bookmark' mitotic chromatin (Caravaca et al., 2013; Kadauke et al., 2012; Lake et al., 2014; Young et al., 2007; Festuccia et al., 2016). Interestingly however, these factors maintain mitotic chromatin binding at only a subset of the specific sites bound during interphase (Caravaca et al., 2013; Kadauke et al., 2012). This reduced number of specific binding sites is not due to lower transcription/chromatin factor levels but instead, the majority of bookmarking events occur at non-specific sites with a highly dynamic exchange rate (Caravaca et al., 2013). Transcription factors with bookmarking activity such as FOXA1 and GATA1 are thought to act as pioneer factors that reset the chromatin landscape and potentially, re-establish cell identity after mitosis (Zaret, 2014). Because OCT4, SOX2 and KLF4 (OSK) act as pioneer factors during reprogramming to pluripotency (Soufi et al., 2012) and because ASCL1 functions as a pioneer factor during trans-differentiation of fibroblasts to neurons (Wapinski et al., 2013), it will be important to investigate the association of these reprogramming factors with mitotic chromatin and the role of this in cell cycle regulated cell fate decisions.

Table. Cell cycle regulation of cell fate decisions and its regulation

Cell cycle target	Cell fate decision	Mechanism
HDAC, SP1	pluripotent cell fate determination	CYCLIN D (G1-phase)
SMAD2,3	pluripotent cell fate determination	CDK4/6-CYCLIN D (G1-phase)
MLL2	pluripotent stem cell differentiation	CDK2 (G1-phase)
HO	budding yeast	CDK (G1-phase)
unknown	pancreatic endocrine cell fate	G1-phase CDK regulated (?)
GATA1	erythroid differentiation	$KIP2^{p57}$ (S-phase)

NOTCH	*Drosophila* sensory organ formation	unknown (G2-phase)
NGN2	neural progenitor differentiation	inhibition by CDK (G1-phase)
unknown	cortical progenitor differentiation	unknown (G1-phase)
Prospero machinery	*Drosophila* neuroblast differentiation	pro-differentiation, inhibits cell cycle
H2A	differentiation, reprogramming	window for reprogramming factors (mitosis)
chromatin	reprogramming	OSK binding (mitosis)
unknown	*Dictyostylium* pre-spore/stalk decision	nutritional conditions (G1-, S-, G2-phases)

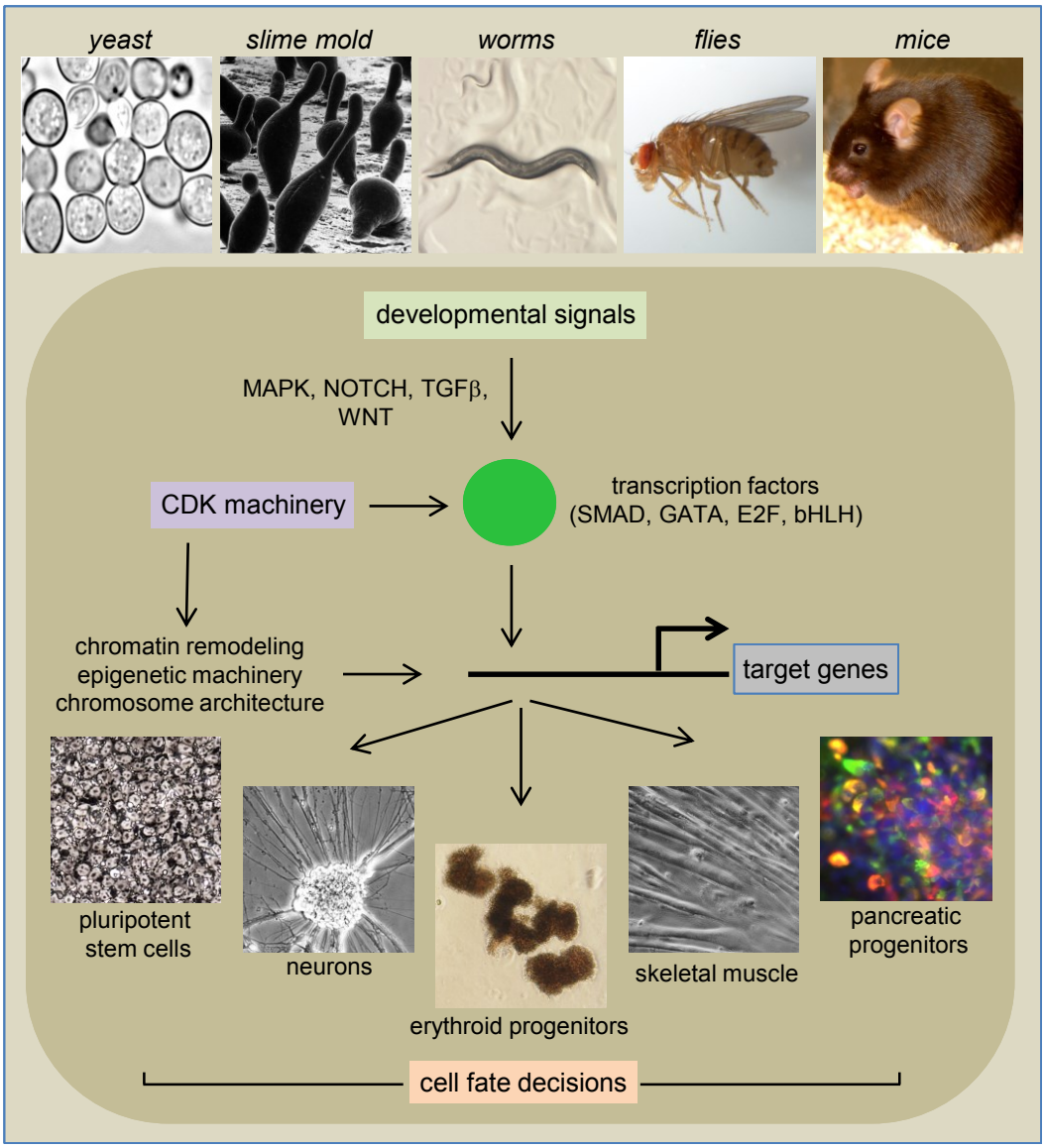
Figure legends

Fig. 1. The cell cycle controls developmental decisions. The intersection between cell cycle control and cell fate determination mechanisms involves developmental signals and cyclin-dependent protein kinases (CDKs) targeting transcription factors that control developmental genes. CDKs also work in parallel to this pathway by modulating the epigenetic landscape and chromosome architecture around developmental genes. The activation of certain target genes determines important cell fate decisions and subsequent lineage commitment.

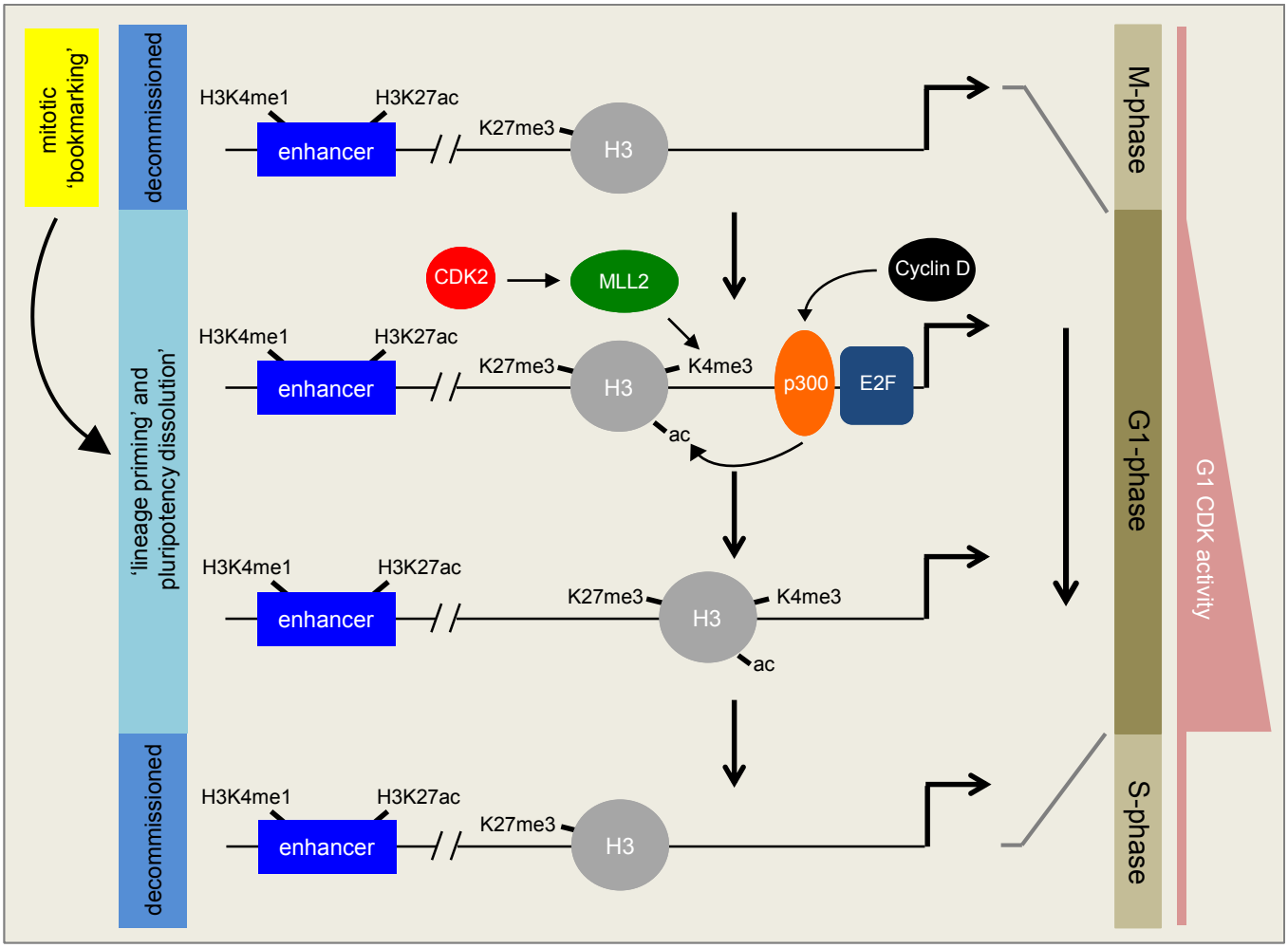
Fig. 2. Mechanisms of lineage-priming and pluripotency dissolution in the G1-phase of pluripotent stem cells (PSCs). As PSCs exit M-phase, G1-CDK activities are activated and in concert with developmental signals act through transcription factors which load onto developmental target genes. Developmental genes are 'bookmarked' epigenetically in mitosis for rapid activation in the upcoming G1-phase. In conjunction with this, epigenetic modifiers, such as MLL2, modify the local epigenetic landscape around developmental genes in G1-phase and Cyclin D recruits co-repressors and co-activators. Chromosome loops are then formed, recruiting enhancers to the proximal promoter, thereby establishing the lineage-primed state. Before and after G1-phase, developmental genes are decommissioned due to the erasure of some epigenetic marks and chromosome loops. Outside of G1-phase, the pluripotency network is stabilized by S-phase and G2-phase regulators that block pluripotency dissolution. Dissolution of pluripotency and lineage-priming work in concert to orchestrate exit from pluripotency and initiate cell fate decisions in G1-phase.

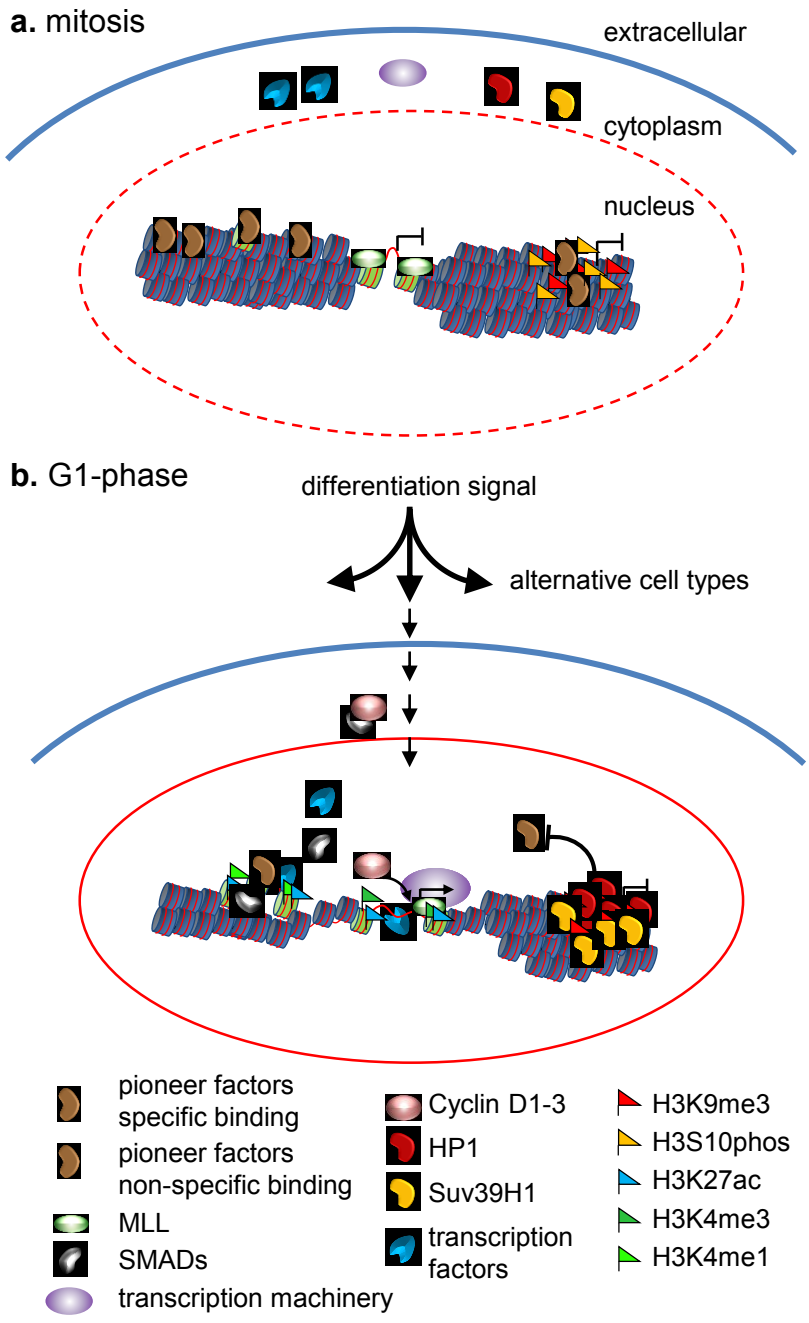
Fig. 3. Mitotic 'bookmarking' and entry into the lineage-primed state in G1-phase. (A) During mitosis the nuclear membrane is broken down and chromatin is highly condensed, as depicted by densely packed nucleosomes. Transcription then halts, coinciding with exclusion of the transcription machinery and most transcription factors from the nucleus. Pioneer factors are retained in mitotic chromatin both specifically and non-specifically. Chromatin modifiers such as MLL are retained by mitotic chromatin and 'bookmark' promoters in preparation for activation in G1-phase. A subset of histone modifications, such as H3K9me3, are also retained in mitosis. H3 is specifically

phosphorylated at S10 by the mitotic kinase AURORA B, resulting in the eviction of HP1. SUV39H is also phosphorylated during mitosis and dissociates from chromatin. (B) Upon mitotic exit, cells respond to differentiation signals, which are transduced to the nucleus through the action of Cyclin D, SMADs and other effectors. SMADs for example, bind with other transcription factors to sites specifically 'bookmarked' by pioneer factors during mitosis or primed by other transcription factors during early G1-phase. Chromatin modifiers such as MLL or newly recruited transcription factors and CDK components re-establish histone modifications at enhancers and promoters and developmental genes are reset to the lineage-primed state. Also in G1-phase, H3S10 phosphorylation is lost and HP1 and SUV39H bind to H3K9me3-enriched chromatin to re-establish heterochromatin, blocking access to transcription factors in these regions.


yeast
slime mold
worms
flies
mice
developmental signals
MAPK, NOTCH, TGFβ, WNT
CDK machinery
transcription factors (SMAD, GATA, E2F, bHLH)
chromatin remodeling epigenetic machinery chromosome architecture
target genes
pluripotent stem cells
neurons
erythroid progenitors
skeletal muscle
pancreatic progenitors
cell fate decisions

Soufi and Dalton Figure 2




a. mitosis
extracellular
cytoplasm
nucleus
b. G1-phase
differentiation signal
alternative cell types
pioneer factors specific binding
pioneer factors non-specific binding
MLL
SMADs
transcription machinery
Cyclin D1-3
HP1
Suv39H1
transcription factors
H3K9me3
H3S10phos
H3K27ac
H3K4me3
H3K4me1