

REVIEW

DNA replication and mitotic entry: A brake model for cell cycle progression

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The core function of the cell cycle is to duplicate the genome and divide the duplicated DNA into two daughter cells. These processes need to be carefully coordinated, as cell division before DNA replication is complete leads to genome instability and cell death. Recent observations show that DNA replication, far from being only a consequence of cell cycle progression, plays a key role in coordinating cell cycle activities. DNA replication, through checkpoint kinase signaling, restricts the activity of cyclin-dependent kinases (CDKs) that promote cell division. The S/G2 transition is therefore emerging as a crucial regulatory step to determine the timing of mitosis. Here we discuss recent observations that redefine the coupling between DNA replication and cell division and incorporate these insights into an updated cell cycle model for human cells. We propose a cell cycle model based on a single trigger and sequential releases of three molecular brakes that determine the kinetics of CDK activation.

Introduction

Pioneering autoradiography studies in the 1960s revealed that the events preceding cell division involved a sequence of distinct metabolic states, called cell cycle phases. These were characterized by a period of DNA synthesis (S-phase) surrounded by periods in which no DNA synthesis was detected (G1- and G2-phase; [Baserga, 1965](#)). Decades later, molecular activities that drive cell cycle progression were identified, and a central role for cyclins in complex with cyclin-dependent kinases (CDKs) was established ([Nurse, 1990](#); [Stern and Nurse, 1996](#)). D-type cyclins (which assemble with CDK4 or CDK6) and E-type cyclins (which assemble with CDK2) function as molecular triggers for cell cycle entry ([Sherr, 1993](#)). D- and E-type cyclins generate positive feedback loops that increase cyclin expression, thereby producing a surge in cyclin-CDK activity that irreversibly leads to cell cycle commitment ([Bertoli et al., 2013](#)). Overexpression of D- or E-type cyclins alleviates growth factor dependence, overrides G1 arrest, and advances S-phase entry ([Brewer et al., 1999](#); [Ohtsubo and Roberts, 1993](#); [Pagano et al., 1994](#); [Quelle et al., 1993](#); [Resnitzky et al., 1994](#)). The kinetics of cell cycle commitment is influenced by external stimuli (e.g., growth factors), genetic context (e.g., oncogenes), and factors inherited from previous cell cycles (e.g., DNA damage signals; [Arora et al., 2017](#); [Barr et al., 2017](#); [Moser et al., 2018](#); [Pardee, 1989](#); [Schwarz et al., 2018](#); [Spencer et al., 2013](#); [Yang et al., 2017](#)).

Once committed to the cell cycle, E- and A-type cyclins complex with CDK2 to drive DNA replication. These cyclin-CDK2

complexes are later joined in by cyclin A-CDK1 and cyclin B-CDK1, ensuring a rise in CDK activity. As the cyclin-CDK complexes transit from low activity to high activity, they phosphorylate thousands of residues in various target proteins to initiate mitosis ([Dephoure et al., 2008](#); [Ly et al., 2017](#); [Ohta et al., 2016](#); [Olsen et al., 2010](#); [Swaffer et al., 2018](#)). Cyclin-CDK activity is controlled at multiple levels, involving complex formation of cyclin-CDKs, direct binding of accessory/inhibitory proteins, posttranslational modifications, and regulated activity of phosphatases that reverse cyclin-CDK-mediated phosphorylation ([Hégarat et al., 2016](#); [Malumbres, 2014](#); [Nilsson, 2019](#)). Many regulators of cyclin-CDK activity are also direct or indirect targets of cyclin-CDK activity, creating positive feedback loops that ensure that cyclin-CDK activity continues to rise once activated ([Hégarat et al., 2016](#); [Lindqvist et al., 2009](#); [Pomerening, 2009](#)). One such regulator is the kinase PLK1, which, apart from activating cyclin-CDK, plays a key role in mitotic entry and progression ([Combes et al., 2017](#); [Joukov and De Nicolo, 2018](#); [Pintard and Archambault, 2018](#)). Here we discuss how spiraling cyclin-CDK activities are kept in check and coordinated with genome duplication. We incorporate these insights into an updated cell cycle model based on three molecular brakes that control the level of CDK activation and thus the timing of cell division.

DNA replication and mitosis: From a gap to a link

What triggers cell division has remained a key unanswered question for cell cycle research ([Mchedlishvili et al., 2015](#)). In

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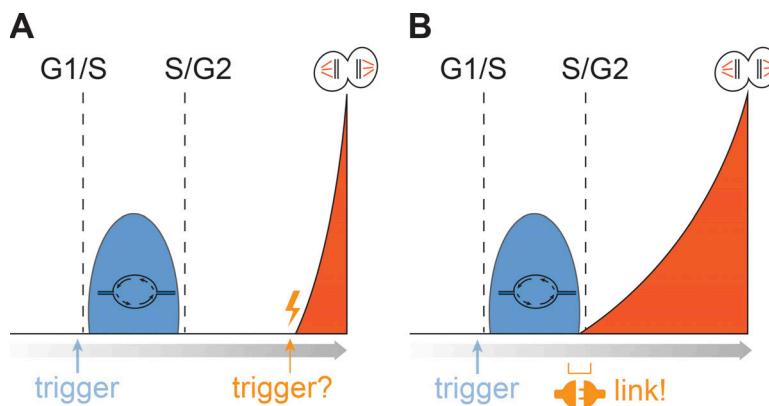


Figure 1. Models on DNA replication and mitotic triggers. Gray arrow depicts time toward mitosis, and dashed lines indicate G1/S and S/G2 transitions. **(A)** Model 1 is based on two independent triggers: one trigger for DNA replication (blue) and one trigger for mitotic kinase activation (orange). **(B)** Model 2 is based on a single trigger for DNA replication (blue) and mitotic kinase activation (orange), yet a signaling cascade links both processes and separates the two events in time. Kinases that drive mitotic entry are activated when bulk DNA replication is completed at the S/G2 transition.

eukaryotic cells, DNA replication and cell division are separated by the intervening G2-phase. Due to the several-hour duration of G2 in somatic cells, DNA replication and cell division were seen as largely independent events, which suggested that the trigger of cell division was not directly coupled to DNA replication (Fig. 1 A). Accordingly, much effort has gone into identifying what triggers cell division at the end of G2-phase. Although many proteins are involved (Hégarat et al., 2016; Lindqvist et al., 2009), recent data suggest a particular involvement of cyclin A-CDK and PLK1 in initiating mitotic entry. Apart from directly phosphorylating targets that promote mitosis, cyclin A in complex with CDK1 or CDK2 phosphorylates the Aurora A cofactor Bora to stimulate activation of PLK1 (Ghegiani et al., 2017; Lindqvist et al., 2009; Macůrek et al., 2008; Seki et al., 2008; Thomas et al., 2016; Vigneron et al., 2018). However, cyclin A-CDK complexes are active from the onset of S-phase (Fotedar et al., 1996; Girard et al., 1991; Minshull et al., 1990), raising the question, what determines the timing of mitosis?

Recent advances have shown that while DNA replication and cell division occur at different times, from a signaling perspective, both processes are heavily connected. While CDK1 and PLK1 are classified as mitotic kinases, both are active before mitosis. In fact, mass spectrometry studies indicated that >100 mitotic phosphorylation sites are already phosphorylated in G2-phase (Ly et al., 2017). Quantitative immunofluorescence approaches also showed that Lamin A/C and TCTP (substrates of CDK1 and PLK1, respectively) are increasingly phosphorylated through G2-phase. Importantly, these phosphorylated residues are first detected at the S/G2 transition, implying a molecular link between DNA replication and mitotic entry (Akopyan et al., 2014; Cucchi et al., 2010; Lemmens et al., 2018). A live-cell sensor that responds to PLK1 activity confirmed these observations and showed nuclear PLK1 target phosphorylation at the moment cells completed DNA replication (Akopyan et al., 2014). Similarly, a live-cell sensor based on the CDK1 phosphorylation motif in SLBP was degraded the moment cells finished bulk DNA synthesis (Bajar et al., 2016). Thus, both PLK1 and CDK1 start to phosphorylate target proteins at the S/G2 transition, suggesting a direct link between S-phase completion and mitotic kinase activation (Fig. 1 B).

Mitotic entry: From a trigger to a brake

The tight temporal connection between completion of DNA replication and activation of mitotic kinases raises several

fundamental questions. What triggers the onset of mitotic kinase activities at the S/G2 transition? And if mitotic kinases can be activated hours before mitosis, why are they not activated earlier? In fact, inhibition of phosphatases can induce mitosis in S-phase without production of new proteins (Yamashita et al., 1990). Similarly, inhibition of WEE1, a negative regulator of CDK1 and CDK2, can promote premature mitotic entry in S-phase (Aarts et al., 2012; Sørensen and Syljuåsen, 2012). Thus, mitotic kinases can, in principle, be activated in S-phase, but are kept suppressed until the S/G2 transition.

To explain the notion that eukaryotic cells are able to proliferate with high fidelity, Hartwell and Weinert (1989) theorized that human cells might possess surveillance mechanisms that allow cell division only when DNA replication is complete. While this idea was postulated decades ago, direct empirical evidence proved hard to obtain. Recent data show that human cells that cannot initiate DNA replication prematurely activate PLK1 and CDK1 and promptly enter mitosis, demonstrating that DNA replication itself is a key regulator of mitotic kinases (Lemmens et al., 2018). The absence of DNA replication causes immediate CDK hyperactivation upon S-phase entry and is sufficient to trigger premature mitotic entry, showing that the process of DNA replication generates a brake signal that controls the timing of mitosis (Lemmens et al., 2018).

While the absence of DNA replication advances mitotic entry (Lemmens et al., 2018), perturbation of ongoing replication postpones mitotic entry (Bajar et al., 2016; Boddy et al., 1998; Darzynkiewicz et al., 2011; Lemmens et al., 2018; Sørensen et al., 2003; Zineldeen et al., 2017). The latter could be explained by delayed completion of DNA replication, but altered replication fork progression typically induces immediate DNA damage, which obscures the role of unperturbed DNA replication in cell cycle regulation (Darzynkiewicz et al., 2011). DNA damage is a potent and well-studied inhibitor of mitotic entry (Harrison and Haber, 2006; Sancar et al., 2004). Stalled replication forks halt the cell cycle primarily through the action of ATR and CHK1 kinases, and several studies show that the ATR-CHK1 axis also plays an important role during an unperturbed S-phase (Petermann and Caldecott, 2006; Saldivar et al., 2017; Sørensen and Syljuåsen, 2012). ATR and CHK1 are essential proteins with highly conserved functions in cell cycle regulation (Brown and Baltimore, 2000; Cliby et al., 1998; Eykelenboom et al., 2013; Hekmat-Nejad et al., 2000; Niida et al., 2005;

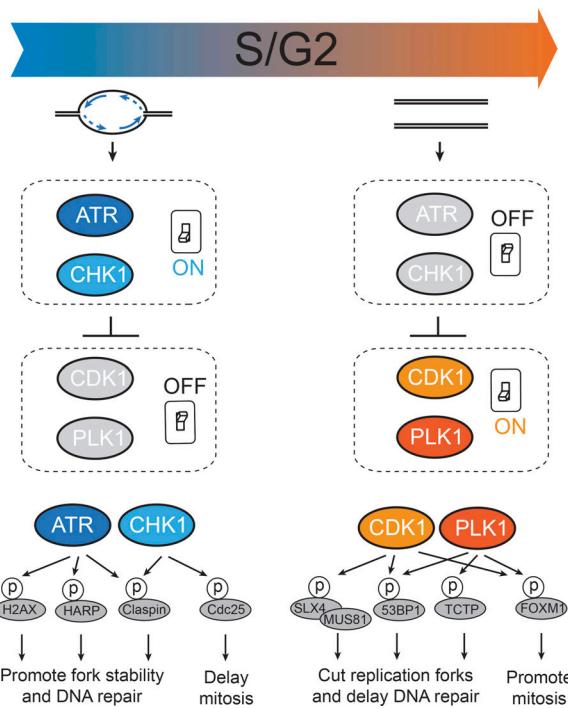


Figure 2. Molecular switches at the S/G2 transition. The S/G2 transition is dictated by DNA replication status. DNA replication in S-phase activates ATR/CHK1 signaling, which represses CDK1 and PLK1 activity and promotes fork stabilization and DNA repair. Completion of bulk DNA replication allows CDK1 and PLK1 activation, which promotes mitotic entry and processing of persistent replication intermediates. Lower panel depicts examples of phosphorylation targets of ATR/CHK1 or CDK1/PLK1. The ATR/CHK1 axis inhibits mitotic entry (e.g., via Cdc25) and promotes fork stability (e.g., via H2AX, HARP, and Claspin), while the CDK1/PLK1 axis promotes mitotic entry (e.g., via FOXM1) and alters DNA repair (e.g., via 53BP1, SLX4, and TCTP).

Petermann et al., 2006; Schmitt et al., 2006; Sørensen et al., 2003; Sørensen et al., 2004). In particular, multiple targets of ATR and CHK1 can stabilize DNA replication forks and promote DNA repair (Fig. 2; Kumagai and Dunphy, 2003; Scorah and McGowan, 2009; Uto et al., 2004; Ward and Chen, 2001; Yuan et al., 2009). However, although inhibition of ATR is toxic to cells, it can be rescued by simultaneous depletion of the CDK-activating phosphatase Cdc25A, suggesting that a key function of ATR is to counteract CDK activity (Ruiz et al., 2016). In line with this finding, inhibition of CHK1 was shown to lead to premature activation of CDK1 and PLK1 in S-phase (Lemmens et al., 2018; Saldivar et al., 2018; Syljuåsen et al., 2005). Importantly, a CDK1-dependent transcriptional program of mitotic regulators was found to be prematurely initiated upon inhibition of ATR in S-phase (Saldivar et al., 2018). These observations point toward a model in which mitotic kinases are actively suppressed during DNA replication (Sørensen and Syljuåsen, 2012). As long as DNA replication is ongoing, ATR-CHK1 inhibits CDK1 and PLK1, ensuring that DNA replication and mitosis are separated in time (Fig. 2). The phosphorylation events triggered by ATR-CHK1 are likely complemented by other pathways, including ubiquitin-mediated processes. For instance, the SCF E3 ligase component cyclin F shows synthetic lethality with Chk1 inhibition (Burdova et al., 2019) and, similar to ATR, can attenuate the

transcriptional response that drives mitosis (Burdova et al., 2019; Clijsters et al., 2019; Klein et al., 2015; Mavrommatis et al., 2018).

Although the exact nature of the replication intermediate controlling CDK activity during an unperturbed S-phase is unknown, a recent study suggests it involves single-stranded DNA (Saldivar et al., 2018). Whether the presence of single-stranded DNA is complemented by another DNA replication intermediate, signaling from active replication forks, DNA topological stress, and/or low levels of stochastic DNA damage, the process of DNA replication constitutes an integral signaling component of the human cell cycle. Completion of DNA replication removes the substrates for ATR-CHK1 activity and hence allows the immediate activation of mitotic kinases at the S/G2 transition (Lemmens et al., 2018; Saldivar et al., 2018). Thus, changing from an S-phase state to a G2-phase state requires release of a brake, rather than the generation of a new trigger (Fig. 2).

Better to brake than to break

Mitosis is sensitive to persisting replication intermediates that can prevent sister chromatid separation. Hence, persisting replication intermediates are cut by structure-specific nucleases to promote faithful sister chromatid disjunction (Chan and West, 2018; Naim et al., 2013; Ying et al., 2013). While cleavage of a few remaining replication intermediates during mitosis might be beneficial, such activities in S-phase would cause genome-wide havoc. Different mechanisms have evolved to restrict endonucleases to mitosis. Whereas the GEN1 nuclease depends on nuclear envelope breakdown for access to DNA (Chan and West, 2018), the Mus81-Slx4 nuclease depends on PLK1 and CDK1 activity for assembly (Svendsen et al., 2009; Cucchi et al., 2010; Duda et al., 2016; Wyatt et al., 2017). Interestingly, PLK1 and CDK1 also actively suppress DNA double-strand repair during mitosis (Benada et al., 2015; Orthwein et al., 2014), possibly preventing immediate religation of cut chromosomes and thus deleterious chromosome entanglements. Reinstating double-strand break repair during mitosis is highly toxic and causes telomere fusions (Orthwein et al., 2014).

The fact that CDK1 and PLK1 suppress DNA repair and activate endonucleases makes them tailored for mitosis but dangerous in S-phase (Fig. 2; Chen et al., 2009; Cucchi et al., 2010; Duda et al., 2016; van Vugt et al., 2010; Zhang et al., 2012). In addition, CDK deregulation can lead to various DNA replication stresses, including faulty origin firing, nucleotide imbalances, and RPA exhaustion (Gaillard et al., 2015; Hills and Diffley, 2014; Toledo et al., 2017). Indeed, inhibition or depletion of the WEE1 kinase, a negative regulator of CDK1 and CDK2, leads to activation of mitotic kinases in S-phase, DNA damage, and altered DNA repair (Beck et al., 2010, 2012; Heald et al., 1993; Krajewska et al., 2013). Mutation of CDK1 so that it can no longer be targeted by WEE1 leads to embryonic lethality in mice, where cells arrest in S-phase with high levels of damaged DNA (Szmyd et al., 2019). Similarly, inhibition of CHK1 causes immediate DNA damage in replicating cells (Lemmens et al., 2018; Syljuåsen et al., 2005). In all three cases, DNA damage could be prevented by simultaneous CDK inhibition, revealing unrestrained CDK activity as the cause of genome instability. A brake on PLK1

and CDK1 activity during S-phase is therefore instrumental for maintaining DNA integrity.

When cells experience DNA damage, an elaborate DNA damage response (DDR) is triggered, which inhibits mitotic kinase activities and cell cycle progression (Harrison and Haber, 2006; Sancar et al., 2004). The DDR buys time for DNA repair and prevents propagation of damaged genomes. A common form of DNA damage during S-phase is replication stress, which is due to faulty DNA replication. Replication stress can be caused by many factors, including deregulation of CDK activity by oncogenes such as cyclin E and Cdc25A (Beck et al., 2010; Gaillard et al., 2015; Macheret and Halazonetis, 2015; Toledo et al., 2017). As treatments that induce premature CDK activation cause DNA damage, they are expected to trigger a feedback loop in which the DDR counteracts the rising CDK activities. Indeed, fluctuation of CDK2 activity has been observed upon spontaneous and ectopic DNA replication stress, and a subset of cells fails to enter mitosis upon WEE1/CHK1 inhibition (Daigh et al., 2018; Lemmens et al., 2018). Given that PLK1 and CDK1 control structure-specific DNA nucleases (Chan and West, 2018; Wyatt et al., 2017), DNA replication intermediates can act as downstream substrates as well as upstream regulators of mitotic kinase activity. Interestingly, forced mitotic entry by WEE1 inhibition requires the structure-specific DNA nuclease Mus81, suggesting that stalled forks need to be processed to allow premature mitotic entry (Duda et al., 2016). Thus, high CDK activity during S-phase leads to replication stress, which in turn limits CDK activity, revealing a delicate balancing mechanism that brakes CDK activities in S-phase.

A cell cycle model based on brakes

The quantitative threshold model for cell cycle progression, originally introduced by Stern and Nurse (1996) to explain that a single cyclin-CDK can drive the fission yeast cell cycle, has for decades stood the test of time (Coudreuse and Nurse, 2010; Swaffer et al., 2016). The quantitative model focuses on the amount of CDK activity rather than the identity of different cyclin-CDK complexes. In this model, S-phase requires relatively low cyclin-CDK activity, whereas mitosis only occurs above a much higher threshold of cyclin-CDK activity. Although likely complemented with different specificities of various cyclin-CDK complexes in other eukaryotes, this model explains why DNA replication commences before cell division (Hochegger et al., 2008; Uhlmann et al., 2011). However, it raises the question of how the cell cycle control system is wired to ensure that cyclin-CDK activity does not rise too quickly. As discussed earlier, DNA replication is sensitive to high CDK activity and needs to be completed before cell division.

Increasing cyclin-CDK activity can in principle be accomplished by triggering additional sets of engines that push cyclin-CDK activity to higher levels. Accordingly, the road to mitosis is sometimes envisioned as a mountain. Approaching mitosis, the slope of the mountain is very steep, reflecting the high levels of CDK activity required for cell division (Fig. 3 A). The increasing CDK activity observed in cycling cells is primarily due to the presence of multiple positive feedback loops (Lindqvist et al., 2009; Medema and Lindqvist, 2011; Pomerening, 2009). These

feedback loops ensure that once CDK activation is initiated, it will autonomously continue to increase in an exponential fashion, similar to a ball rolling down a mountain picking up speed without the need for extra engines. As discussed, progressing from an S-phase state to a G2-phase state requires the release of a brake (DNA replication), rather than the generation of a new trigger. Similarly, deficiencies in WEE1 or ATR/CHK1 advance mitotic entry, revealing a poised state toward cell division that needs to be restrained. Thus, mitotic entry per se does not require an extra engine or trigger in G2-phase, but instead might involve a single trigger in G1-phase and a set of counteracting brakes. These molecular brakes ensure that CDK activation occurs in a stepwise manner and on par with the processes required for faithful cell division. For instance, CDK-dependent FOXM1 phosphorylation is initiated early but plateaus during S-phase, which postpones full activation of the promitotic transcriptional program until after genome duplication (Saldivar et al., 2018). We believe recent discoveries fit a model in which the road to mitotic entry, instead of a steep climb, resembles a well-monitored descent (Fig. 3 B). We here propose a model in which the descent is controlled by three brake modules that collectively determine CDK activity output and thus the timing of mitosis (Fig. 3 C).

Instead of focusing solely on the source and amount of CDK activity, we propose that it is more informative to think of the cell cycle in terms of an energy landscape, as is frequently done for chemical reactions, and for cell biology has been made popular by the cellular route to differentiation (Takahashi, 2012; Waddington, 1957). Because the signaling landscape in which a given CDK activity acts is critical to its outcome, we propose that the kinetics of the cell cycle relies on the condition of the road and strength of the brakes rather than the power of the engine. We discriminate a G1/S brake, a S/G2 brake, and an M-entry brake. While all three brakes regulate CDK activity, they have different effectors and act at different stages of the cell cycle. In unchallenged conditions, the release of each brake corresponds to a cell cycle transition.

G1/S brake

The G1/S brake prevents G1/S transition and is mainly controlled by the anaphase-promoting complex/cyclosome (APC/C) ubiquitin ligase. Activation of APC/C in mitosis triggers the separation of duplicated chromosomes and resets the cell cycle. The APC/C complex triggers the degradation of many CDK activators (including cyclin A, cyclin B, and PLK1) and thus constitutes a major brake to cell cycle progression (Alfieri et al., 2017; Watson et al., 2019). Recent data indicate that inactivation of the APC/C complex occurs in a switch-like manner and precedes S-phase entry (Barr et al., 2016; Cappell et al., 2016, 2018; Yuan et al., 2014). Rising CDK activity in G1-phase, driven by D- and E-type cyclins that are not targeted by APC/C, ultimately shuts off the G1/S brake. CDK inactivates APC/C both directly (through inactivating phosphorylations on CDH1, an activator of APC/C) and indirectly (through induced expression of EMIL, a negative regulator of APC/C; Hsu et al., 2002; Kramer et al., 2000; Lukas et al., 1999). A dual negative feedback between APC/C and EMIL generates a bistable switch: once a threshold level of CDK

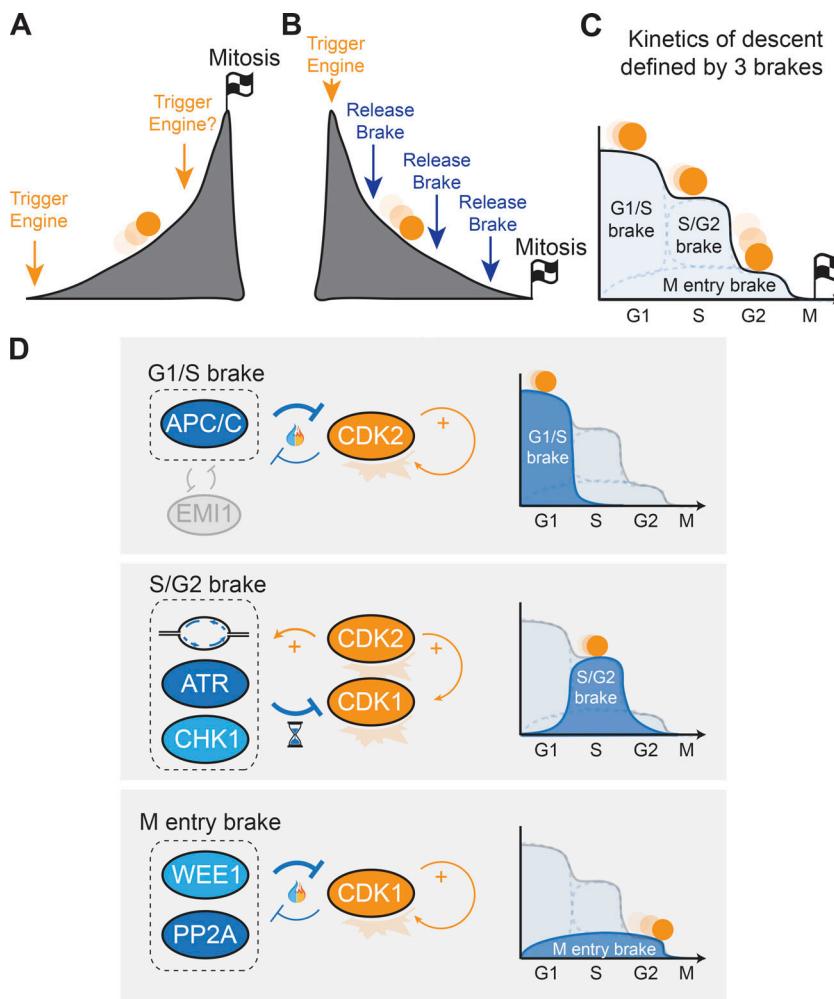


Figure 3. A brake model for cell cycle progression. **(A)** Model 1 is based on multiple independent triggers that initiate cell cycle engines and push the cell into mitosis, akin to pushing a ball up a mountain. **(B)** Model 2 is based on a single trigger and multiple brakes that restrain cell cycle engines and mitotic entry, akin to braking a ball rolling down a mountain. **(C)** At least three brake modules collectively determine cell cycle progression. Visualized as an energy landscape, the brakes define the slope of the descent and thus the speed of the ball rolling toward mitosis. **(D)** Basic signaling circuits of the three brake modules controlling CDK activity (left) and the timing of the respective brakes during the cell cycle (right). The G1/S brake and M entry brake are wired to CDK via double-negative feedback loops, and eventually are overruled by self-amplifying CDK activity. The S/G2 brake is wired to CDK via a transient incoherent feed-forward loop: CDK2 activity triggers DNA replication, which inhibits CDK1, but the brake is inherently transient because it resolves the moment the cell completes genome replication.

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activity is reached, the G1/S brake is turned off in an irreversible manner (Barr et al., 2016; Cappell et al., 2016, 2018). Linking spiraling CDK activation to dual negative feedback loops ensures full commitment into S-phase (Fig. 3 D).

S/G2 brake

The S/G2 brake requires DNA replication (Lemmens et al., 2018) and acts via various substrates of ATR/CHK1 that counteract CDK activation (Lemmens et al., 2018; Saldívar et al., 2018; Sørensen et al., 2003). While the G1/S brake is controlled by shifting double-negative feedback loops, the S/G2 brake is manifested through a transient incoherent feed-forward loop. Rising CDK2 activity promotes CDK1 activity and triggers the initiation of DNA replication, which by itself constitutes a brake on CDK1 activation. In other words, the cell pushes the gas and brake at the same time, yet the brake is inherently transient. Completion of DNA replication at the S/G2 transition resolves the brake signal and allows activation of CDK1 and PLK1 (Fig. 3 D).

Current models suggest that ATR/CHK1 postpones the activation of CDK1 through Cdc25/Wee1 regulation, similar to the response to DNA damage (Goto et al., 2019; Sørensen and Syljuåsen, 2012). How PLK1 is suppressed during unperturbed proliferation is not clear, but after DNA damage, PLK1 activity is

limited by dephosphorylation, disruption of Aurora A-Bora interaction, and direct methylation (Bruinsma et al., 2017; Hu et al., 2018; Li et al., 2019). In addition, PLK1 and CDK1 activity are tightly interlinked, and suppression of one can affect the other (Ghegian et al., 2017; Lindqvist et al., 2009; Macurek et al., 2008; Seki et al., 2008; Thomas et al., 2016; Vigneron et al., 2018). How quickly mitotic kinase activity builds up at the S/G2 transition is likely linked to the kinetics of DNA replication completion: a cell that completes DNA replication efficiently is expected to activate mitotic kinases promptly, whereas a cell sustaining stalled forks is expected to activate CDK1 and PLK1 relatively slowly, providing extra time to resolve DNA replication issues before mitotic entry.

M-entry brake

Finally, cells are equipped with an M-entry brake that prevents premature mitosis throughout interphase and is mostly controlled by WEE1 and PP1/PP2A activities. The M-entry brake is a composite brake module that acts both on CDK itself and on its substrates. Similar to the G1/S brake, the M-entry brake is controlled by dual negative feedback loops (Fig. 3 D). Both WEE1 and the PP1/PP2A phosphatases counteract CDK activity but are also restrained by CDK activity. WEE1 inhibits CDK1 by direct phosphorylation, but elevated levels of CDK1 activity (together

with PLK1 activity) target WEE1 for degradation (Parker and Piwnica-Worms, 1992; Watanabe et al., 2005). Similarly, the PP2A-B55 phosphatase effectively counteracts CDK1 target phosphorylation, yet elevated levels of CDK1 activity reduce PP2A-B55 activity through the Greatwall kinase and the PPI phosphatase (Dohadwala et al., 1994; Gharbi-Ayachi et al., 2010; Grallert et al., 2015; Kwon et al., 1997; Mochida et al., 2010). The M-entry brake thus functions as a constitutive counterweight to the spiraling CDK activity, which raises the threshold for mitotic entry and supports full commitment after the G2/M transition.

Together, these three brakes ensure a controlled stepwise descent toward mitosis: the G1/S brake restricts CDK activity in G1-phase, the S/G2 brake restricts CDK activity in S-phase, and the M-entry brake suppresses CDK activity throughout interphase and determines the final kinetics of mitotic entry (Fig. 3). Defects in any of these brakes cause unrestrained CDK activation, which leads to hasty cell cycle progression and thus severe errors in DNA replication and/or chromosome segregation (Aarts et al., 2012; Beck et al., 2012; Brown and Baltimore, 2000; Cappell et al., 2016; Labib and De Piccoli, 2011; Lemmens et al., 2018; Niida et al., 2005; Sørensen and Syljuåsen, 2012; Yamashita et al., 1990).

From “intrinsic checkpoint” to “intrinsic brake”

Given that the G1/S brake, S/G2 brake, and M-entry brake are required for cell survival and rely on cues inherent to cell proliferation (i.e., DNA replication and CDK activity oscillations), we refer to these brakes as “essential” or “intrinsic” brakes. These intrinsic brakes can also be called “checkpoints” by the original definition given by Hartwell and Weinert (1989) or as redefined by Elledge (1996). The word “checkpoint,” however, has led to confusion in the field because it traditionally refers to a predefined point or barrier on a street or path that can only be passed if certain conditions are fulfilled. Using “checkpoints” to describe cell cycle regulation thus implies that cellular status is checked at defined moments (e.g., just before cell cycle transitions) and that the approach leading up to these checkpoints is not monitored. For example, the terms “S-M checkpoint” or “S/G2 checkpoint” could imply that DNA replication status is monitored just before mitotic entry or S/G2 transition, respectively (Eykelenboom et al., 2013; Saldivar et al., 2018). However, the factors that enforce the cell cycle delay (i.e., ATR/CHK1) are active throughout S-phase and cause oscillating CDK activities well before S/G2 transition (Daigh et al., 2018; Lemmens et al., 2018; Michelena et al., 2019; Saldivar et al., 2018). We therefore favor the use of “brake” terminology, instead of “checkpoint” terminology. The timing of cell cycle transitions can be explained by the sequential release of three essential brake signals that counteract CDK activation (Fig. 3).

Extra brakes in case of emergency

Thus far we have mainly focused on the intrinsic signaling circuits that determine cell cycle progression, but cell proliferation is highly sensitive to external cues. A multitude of developmental or experimental conditions can slow down or arrest the cell cycle (Edgar and Lehner, 1996; Elledge, 1996; Kipreos and van den Heuvel, 2019). As a result, the term “checkpoint” has

gained a second meaning in cell biology, referring to a halt in cell cycle progression due to external stresses. Most famous among this second class of checkpoints are the DNA damage checkpoints, which prevent G1/S and G2/M transition upon DNA damage (Cuadrado et al., 2009; Falck et al., 2001; Gutierrez et al., 2010; Jackson and Bartek, 2009; Mirzayans et al., 2012; Privette and Petty, 2008; Zarubin and Han, 2005). Examples of effectors of DNA damage checkpoints are ATM and the p53-p21 axis, which are critical for maintaining genome integrity but are not required for cell cycle progression per se (Bartkova et al., 2006; Sancar et al., 2004; Zhou and Elledge, 2000). Although a DNA damage checkpoint can be induced by a single DNA break (van den Berg et al., 2018), low levels of DNA damage are often not sufficient to sustain a cell cycle arrest (Deckbar et al., 2007, 2010; Syljuåsen et al., 2006). In G2-phase, this depends on the self-amplifying properties of promitotic signaling, which eventually overcomes checkpoint signaling (Jaiswal et al., 2017; Liang et al., 2014). Apart from strengthening CDK activity, in particular PLK1 can by multiple means counteract checkpoint kinases (Mailand et al., 2006; Mamely et al., 2006; Peschiaroli et al., 2006; Syljuåsen et al., 2006; van Vugt et al., 2004, 2010). The G2 DNA damage checkpoint therefore delays rather than blocks mitosis, arguing that also DNA damage checkpoints can function as brakes rather than strict checkpoint barriers. The cell cycle thus is controlled by two distinct classes of brakes: (a) “essential” brakes that are inherent to the cell cycle and (b) “emergency” brakes that are conditional and come into play upon stress. Examples of brake effectors for each class are depicted in Fig. 4. The process of DNA replication can modulate the timing and amplitude of both types of brakes. For instance, DNA replication triggers ATR/CHK1 signaling (Lemmens et al., 2018; Saldivar et al., 2018; Sørensen et al., 2003) but also p21 degradation (Abbas et al., 2008; Coleman et al., 2015), substantiating a tight link between genome duplication and cell cycle progression.

Concluding remarks

The activities capable of copying a living cell have intrigued scientists for decades, and through the years many fundamental concepts of the cell cycle have been exposed (Baserga, 1965; Dephoure et al., 2008; Nurse, 1990; Ohta et al., 2016; Stern and Nurse, 1996). One key feature of cycling cells is the distinct surge of DNA incorporation in S-phase, in which the cell duplicates its genome before cell division. It is now clear that DNA replication is not just an output of the cell cycle, but in fact feeds back into the signaling networks controlling mitotic entry. Linking DNA replication to the mitotic entry network dismisses the need for separate triggers while allowing temporal separation. We propose a cell cycle model based on a single trigger, which together with a set of three molecular brakes generates distinct waves of protein activities. This brake model can explain gradual cyclin-CDK activation and distinct phase transitions, as well as commitment to complete the cell cycle once initiated.

Many aspects of this model require further study. Are there situations in which additional triggers are needed? What is the molecular identity of the DNA replication intermediate postponing mitosis? How can DNA replication impose differential regulation on different cyclin-CDK complexes? What systems

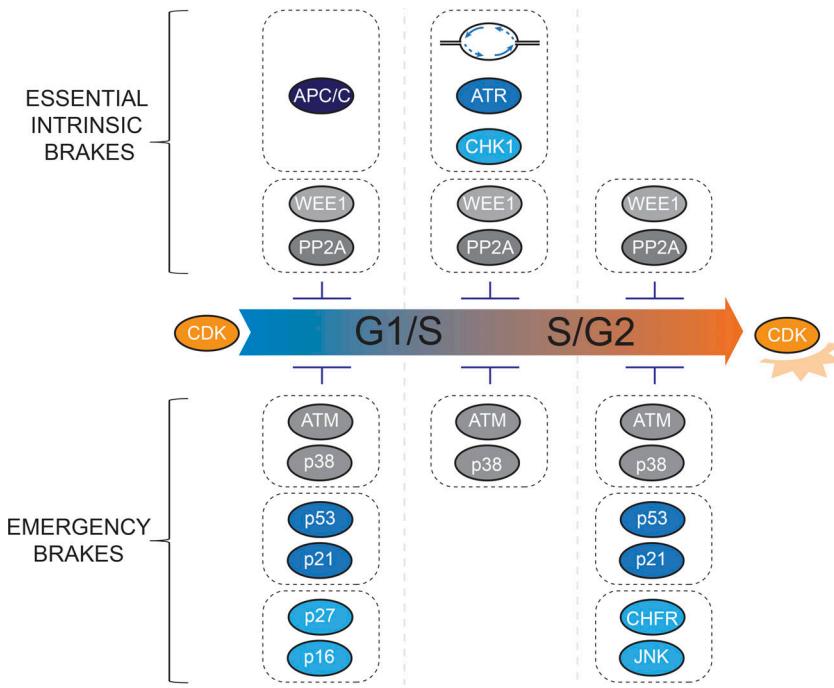


Figure 4. Two types of brakes control cell cycle timing. Intrinsic brakes are essential to life and ensure cell cycle phase dependence, while emergency brakes delay or arrest cell cycle progression upon external cues and stresses. The APC/C complex, ATR/CHK1, and WEE1/PP2A represent intrinsic brakes required to restrain CDK activation during the unchallenged cell cycle (top). In contrast, signaling factors such as ATM, p38, p27, p16, p53, p21, CHFR, and JNK are not essential for cell proliferation, per se, but effectively inhibit CDK activation upon stress (lower panel).

properties are emerging for the S/G2 transition, and how does that affect signaling sensitivity and stability? Moreover, while we propose three basic brake circuits controlling CDK activation, more work is needed to determine their exact wiring. Understanding the nature of these brake modules will help explain and predict the kinetics and behaviors of the different cell cycle transitions. The current pace of discoveries in the cell cycle field is encouraging, and the continuous development of time-resolved technologies at single-cell resolution will surely further our understanding of how cells establish concerted waves of protein activities to control the road to cell division.

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