Control of cell cycle transcription during G1 and S phases

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Abstract | The accurate transition from G1 phase of the cell cycle to S phase is crucial for the control of eukaryotic cell proliferation, and its misregulation promotes oncogenesis. During G1 phase, growth-dependent cyclin-dependent kinase (CDK) activity promotes DNA replication and initiates G1-to-S phase transition. CDK activation initiates a positive feedback loop that further increases CDK activity, and this commits the cell to division by inducing genome-wide transcriptional changes. G1–S transcripts encode proteins that regulate downstream cell cycle events. Recent work is beginning to reveal the complex molecular mechanisms that control the temporal order of transcriptional activation and inactivation, determine distinct functional subgroups of genes and link cell cycle-dependent transcription to DNA replication stress in yeast and mammals.

Ubiquitin ligase
An enzyme that recognizes Lys residues on a target protein and causes the attachment of ubiquitin to these residues.

RB
A protein that binds activator E2F proteins to inhibit transcription outside of G1–S in animals. RB is an oncoprotein that is dysfunctional in several major cancers.

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The eukaryotic cell cycle is controlled by a regulatory network, the general features of which are conserved from yeast to humans[1]. It proceeds through tightly regulated transitions to ensure that specific events take place in an orderly manner. The discovery of cyclins and cyclin-dependent kinases (CDKs), the elucidation of the mechanisms underlying transcriptional control and checkpoint signalling and the characterization of ubiquitin ligase regulatory pathways have revealed that general cell cycle regulatory principles are shared across eukaryotes.

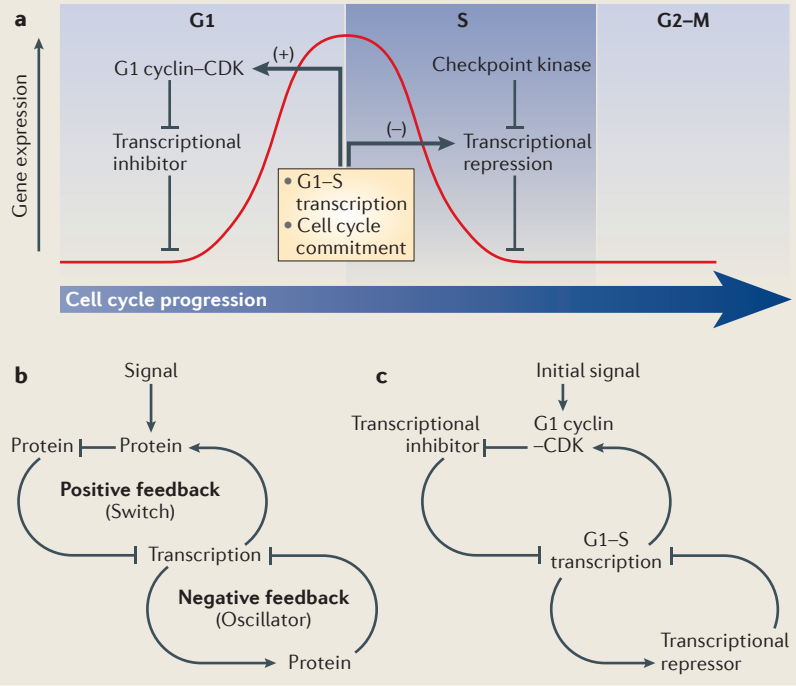
Two crucial aspects of cell cycle regulation are the existence of DNA structure checkpoints, which arrest the cell cycle in response to DNA damage or incomplete replication, and the existence of a 'commitment point'. This point is known as the 'restriction point' in animal cells and 'start' in yeast and is defined as the point after which a cell becomes committed to enter the cell cycle and progress through it independently of signals from the environment. The importance of DNA checkpoints and commitment point control for proper cell division is illustrated by the high frequency of mutations found in their constituent regulatory proteins during oncogenesis[2]. One notable regulatory protein that is often mutated in cancer is the tumour suppressor protein RB[3]. RB is a potent inhibitor of G1–S transcription (that is, a transcriptional wave that initiates during G1 and is subsequently inactivated during S phase), and its discovery over 20 years ago first suggested the dependency of cell cycle commitment on transcriptional regulation in G1 (REFS 4–6). Subsequent studies showed that the broad mechanisms of eukaryotic G1 cell cycle control are highly conserved[7–9,10]. Intriguingly, recent work demonstrated that DNA checkpoint control depends on the same transcription factors responsible for commitment point regulation[11].

The dynamic changes in gene expression as a function of cell cycle progression are regulated by specific CDK activities. These variations in gene expression levels control the accumulation of several cyclins and thereby regulate CDK activity, thus driving cell cycle progression. Genes regulated during the cell cycle encode several proteins that function in the subsequent phase of the cell cycle. In most eukaryotes, cell cycle-regulated transcription can be grouped into three main waves[12]. These waves of transcription coincide with the different transition points during the cell cycle, namely G1-to-S, G2-to-M and M-to-G1. Although all three cell cycle transcript waves are well-characterized in yeast, transcription that occurs during the M-to-G1 phase transition in human cells is less well-defined[13]. Largely on the basis of work carried out in the budding yeast *Saccharomyces cerevisiae*, it is thought that the subsequent waves of transcription form a continuous regulatory network in which each wave is activated by the previous one and contains activators of the following wave[14]. Of the cell cycle transcriptional waves, G1–S transcription has been the most studied because of its important role in the tight regulation of G1-to-S phase transition. Derepression of G1–S transcription allows cells to progress into S phase in an unrestrained fashion, a hallmark of cancer. Along with the recently established link of the cell cycle checkpoint

Box 1 | **Cell cycle-regulated transcription during the G1 and S phases**

The G1–S transcriptional network is involved in two crucial aspects of cell cycle regulation: cell division cycle control and maintenance of genome stability. Phosphorylation of transcriptional inhibitors by cyclin-dependent kinase (CDK) releases them from transcription factors to activate G1–S genes, including G1 cyclins (see the figure, part **a**). This reinforces a positive feedback loop, further committing the cell to a new division cycle and activating G1–S transcription. Negative feedback loops subsequently inactivate transcription, which terminates a wave of gene expression (indicated by the red curve) that peaks at the transition from G1 to S phase. A recently identified negative autoregulatory feedback loop involves transcriptional repressors that are G1–S targets themselves. These repressors accumulate and bind to G1–S gene promoters to turn off transcription when cells progress to S phase. In addition, these transcriptional repressors are directly targeted by the DNA replication checkpoint protein kinases to maintain G1–S transcription during a checkpoint arrest.

The fundamental regulatory pathways that drive changes in cell cycle-regulated gene expression during the G1 and S phases of the cell cycle are conserved from yeast to humans. Transcriptional regulators involved in this regulation in various eukaryotic systems are listed in TABLE 1. Conserved systems level properties are involved in G1–S transcriptional regulation across eukaryotes (see the figure, part **b**). Linking a positive feedback mechanism to a negative feedback loop ensures that a switch-like commitment to activation results in timely inactivation via an oscillator. The particular network wiring required for G1-to-S phase transition involves a transcriptional inhibitor and cell cycle-regulated transcription of G1 cyclins and transcriptional repressors (see the figure, part **c**).



response to replication stress, this unrestrained growth illustrates the importance of cell cycle-regulated transcription, which is both driven by and a driving force for cell cycle progression.

Here, we review recent progress in determining the simple but elegant mechanisms by which cells regulate their G1–S phase transcriptional network to control the commitment to cell division and the DNA replication checkpoint response. Although most work has focused on the role of transcriptional activation during cell cycle progression from G1 to S and the genome-wide changes in the transcriptional programme[14–16], recent work has uncovered many new insights into the regulation of commitment to cell division, the temporal confinement of G1–S transcription and the response to DNA replication stress (BOX 1). These systems-level properties are conserved across eukaryotes despite frequent lack of protein sequence homology of transcriptional regulators (BOX 1; TABLE 1). We briefly discuss G1–S transcriptional regulation in the context of other cell cycle pathways, such as cyclins and CDKs, checkpoint signalling and the ubiquitin ligase regulatory pathways, but we also refer readers to more comprehensive reviews on these specific topics[17–22]. Finally, we share our views on how current understanding of the regulation of G1-to-S phase transition may provide a blueprint for future research into the fundamental regulatory mechanisms controlling cellular decision-making processes, dynamic changes in gene expression and checkpoint-dependent rewiring of transcriptional networks.

Activation of G1–S transcription

Cells commit to enter a new cell cycle during G1 by activating cyclin–CDK-dependent transcription (FIG. 1). G1–S transcriptional activation during late G1 promotes entry into S phase after which expression is turned off. This creates a wave of transcription, which peaks at the G1-to-S transition (BOX 1). The mechanism of G1–S transcriptional activation has been well-established and is conserved from yeast to humans.

E2F family and pocket proteins. In human cells, G1–S transcription depends on the E2F family of transcription factors and their dimerization partner proteins. Misregulation of E2F function is frequently found in cancer, which further supports the role of G1–S transcription in oncogenesis[23–28]. E2F family members are generally associated with either transcriptional activation (E2F1, E2F2 and E2F3A) or repression (E2F3B, E2F4, E2F5, E2F6, E2F7 and E2F8). However, recent findings revealed a more complex scenario in which activator E2F proteins can act as repressors and repressor E2F proteins can activate transcription[29–31]. In addition to E2F proteins, confining transcription to the late G1 and S phases of the cell cycle requires regulation by pocket proteins, including RB, p107 and p130, which bind and inhibit the expression of E2F-regulated genes[6,32–36].

G1–S transcriptional activation in mammalian cells. E2F family members, their DNA binding partners (dimerization partner proteins) and pocket proteins bind cell cycle gene promoters at different stages of the mitotic cell cycle to ensure the proper temporal expression of target genes[4,37] (BOX 2). The association of dimerization partner proteins enhances the DNA-binding affinity of E2F family members so that they can function as transcriptional regulators[38]. During early G1, activator E2F proteins are bound and inhibited by RB[6], whereas E2F4 (and presumably E2F5) bind p130 and p107 at promoters to repress transcription[39–42] (FIG. 1b). E2F4 and E2F5 depend on pocket protein binding for nuclear localization. When pocket proteins are phosphorylated by G1 cyclin–CDKs during G1-to-S phase transition, E2F4 and E2F5 are released, shuttled into the cytoplasm[4,37,43] and replaced at

Pocket proteins
Family of proteins, including RB, p107 and p130, that associates with members of the E2F transcription factor family to inhibit transcription. The pocket domain is essential for tumour suppressing activity.

Table 1 | **Conservation of cell cycle regulatory proteins***

Regulator type	*Saccharomyces cerevisiae*	*Schizosaccharomyces pombe*	*Drosophila melanogaster*	*Homo sapiens*
G1–S transcriptional regulators				
Activators	SBF (Swi6–Swi4)	MBF (Cdc10–Res1–Res2)	E2f1	E2F1, E2F2, E2F3
Repressors	MBF (Swi6–Mbp1)		E2f2	E2F4, E2F5, E2F6, E2F7, E2F8
Inhibitors	Whi5	Possibly Whi5	Rbf1	RB
Co-repressors	Nrm1	Nrm1, Yox1	Rbf2	p107, p130
Cyclin–CDK				
G1 phase regulator	Cdc28–Cln3	Cdc2–Puc1	Cdk4–cyclin D	CDK4–cyclin D, CDK6–cyclin D
G1–S phase regulator	Cdc28–Cln1, Cdc28–Cln2	Cdc2–Puc1 Cdc2–Cig1	Cdk2–cyclin E	CDK2—cyclin E
S phase regulator	Cdc28– Clb5, Cdc28–Clb6	Cdc2– Cig1, Cdc2– Cig2	Cdk2–cyclin E, Cdk1–cyclin A, Cdk2–cyclin A	CDK2—cyclin E, CDK1–cyclin A, CDK2–cyclin A
M phase regulator	Cdc28– Clb1, Cdc28–Clb2, Cdc28–Clb3, Cdc28–Clb4	Cdc2–Cdc13	Cdk1–cyclin B	CDK1–cyclin B
Checkpoint protein kinases				
Sensor and/or transducer	Mec1	Rad3	ATR	ATR
	Tel1	Tel1	ATM	ATM
Effector	Chk1	Cds1	Chk1	CHK1
	Rad53	Chk1	Chk2	CHK2

ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia and Rad3-related protein; CDK, cyclin-dependent kinase; CHK, checkpoint kinase; MBF, MCB-binding factor; Mec1, mitosis entry checkpoint 1; Rbf, retinoblastoma family; SBF, SCB-binding factor; Tel1, telomere length regulation 1.
*Listed are the functional orthologues between yeast, flies and humans of G1–S phase transcriptional regulators, cyclin-CDKs and checkpoint protein kinases. Although the functional orthologues of cyclins, CDKs and the checkpoint protein kinases share significant sequence homology, there is a total lack of sequence homology between yeast and the higher eukaryotic G1–S transcriptional regulators.

promoters by activator E2F family members (E2F1, E2F2 and E2F3A) that initiate transcription[40,41]. This classic paradigm of the role of activator E2F proteins in inducing G1–S gene expression and driving cell cycle entry derives mainly from studies using cultured cells and flies. More recent evidence from *in vivo* studies in knockout mice has revealed a more complicated picture[29,44]. The ablation of all activator E2F proteins, E2F1, E2F2 and E2F3, does not prevent normal proliferation of embryonic stem (ES) cells and intestinal and retinal progenitor cells, suggesting that these proteins are dispensable for proliferation in this context. However, an increase in DNA damage and apoptosis is observed in these triple-knockout cells, which suggests a role for transcriptional control by the activator E2F proteins.

A large number of studies used cultured cells re-entering the cell cycle after serum removal to analyse transcriptional activation in G1. After prolonged serum withdrawal, cells enter a condition known as quiescence, which is different from the G1 phase of cycling cells and has been defined as the G0 phase of the cell cycle[135,136]. Both during G0 and early G1, binding of pocket proteins to E2F proteins prevents transcription. The interaction is disrupted by CDK-dependent phosphorylation in mid-G1, resulting in transcriptional activation. In G0, most E2F-responsive promoters are bound and repressed by p130 together with E2F4 (REFS 40,45), whereas in early G1 of cycling cells, p107 also interacts with DNA-bound E2F4 to repress transcription in a similar manner[41]. The relative importance of pocket proteins for transcriptional repression during different phases of the cell cycle largely correlates with their protein levels, as pocket proteins function in similar ways to repress transcription[4]. Although p130 seems to be the most abundant pocket protein during quiescence, its levels are greatly reduced during proliferation[4]. By contrast, RB and p107 are barely detectable in quiescent cells but are found at higher levels in cycling cells; this is likely to be due to E2F-dependent transcription. Recent chromatin immunoprecipitation (ChIP) data suggests that RB is present at promoters during quiescence and in cycling cells, as was commonly assumed[46], as well as in senescent cells, in which it represses G1–S genes. Intriguingly, the permanent exit from the cell cycle in differentiating cells requires activator E2F proteins in complex with RB to repress cell cycle genes[29]. It is possible that this mechanism of active repression promotes cell cycle exit and is then followed by a more stable repression mediated by E2F4 and E2F5 together with p130 (REF. 29).

Conserved mechanisms govern transcriptional activation. Although there is no conservation at the protein level, a regulatory mechanism for G1–S transcriptional activation similar to that established for mammalian cells was recently found in yeast[10,47,48] (FIG. 1c).

In *S. cerevisiae* many of the genes involved in G1-to-S phase transition are regulated by one of two transcription factor complexes, SBF (SCB-binding factor) or

MBF (MCB-binding factor). SBF comprises of a Swi4 DNA-binding component and Swi6 and is required to activate G1–S transcripts during G1. MBF is composed of an Mbp1 DNA-binding component and Swi6 and is required to repress G1–S transcripts outside of G1. Swi4 and Mbp1 bind to specific G1–S target promoters through the SCB (Swi4 cell cycle box) and MCB (MluI cell cycle box) recognition sequences, respectively. In mammalian cells, G1–S genes are regulated by several E2F transcription factor complexes at different stages of the cell cycle. In yeast, G1–S transcripts can be roughly divided into two groups: SBF- or MBF-dependent genes. However, it has been shown that in the absence of the either factor, SBF and MBF may be found at each other's consensus DNA-binding motif[49,50]. Although the temporal gene expression pattern induced by either SBF or MBF is similar, the regulatory mechanism is distinct. SBF-regulated genes remain switched off in cells that lack the DNA-binding component Swi4, suggesting that SBF is required for transcriptional induction during G1. Conversely, the repressor complex MBF is required to repress transcription outside of G1, as MBF-regulated genes remain activated in cells lacking DNA-binding component Mbp1. Thus, although SBF acts more like activator E2F proteins, MBF proteins resemble repressor E2F proteins (FIGS 1,2); however, they share no sequence homology with E2F proteins[49,51,52]. In addition, the *S. cerevisiae* transcriptional inhibitor Whi5 is functionally orthologous to pocket proteins despite a complete lack of sequence homology[10]. During G1, binding of Whi5 to SBF inhibits the activity of SBF, much like pocket proteins inhibit E2F activity (FIG. 1). Whi5 is phosphorylated by G1 cyclin–Cdk, leading to its release from SBF at promoters, export from the nucleus[53] and inactivation. This ensures SBF-dependent transcriptional activation[47,48].

A positive feedback switch ensures commitment. Activation of G1–S transcription by a positive feedback loop creates an 'all-or-none switch' that results in the commitment of cells to enter the cell cycle[54,55] (FIG. 1a). The point at which cells commit to enter a new cell cycle, after which it will progress independently of signals from the environment, is known as the restriction point in mammals and start in yeast. Increased cyclin–CDK activity and the corresponding phosphorylation and inactivation of pocket proteins in mammals, or export and inactivation of Whi5 in *S. cerevisiae*, allows an initial activation of G1–S transcription factors. The genes encoding the G1 cyclins CLN1 and CLN2 in yeast and cyclin E in mammals are some of the first G1–S genes to be transcribed[56].Through positive feedback, G1 cyclins increase their own transcription to produce a rapid increase in cyclin–CDK activity that irreversibly leads to cell cycle commitment. In addition to defining the point at which cells commit, the rapid increase in CDK activity driven by this positive feedback results in the timely and coherent activation of the entire G1–S regulon[54] (BOX 1; FIG. 1).

Positive feedback loops other than the G1 cyclins loop have also been implicated in mammalian cells, including, but not limited to, the accumulation

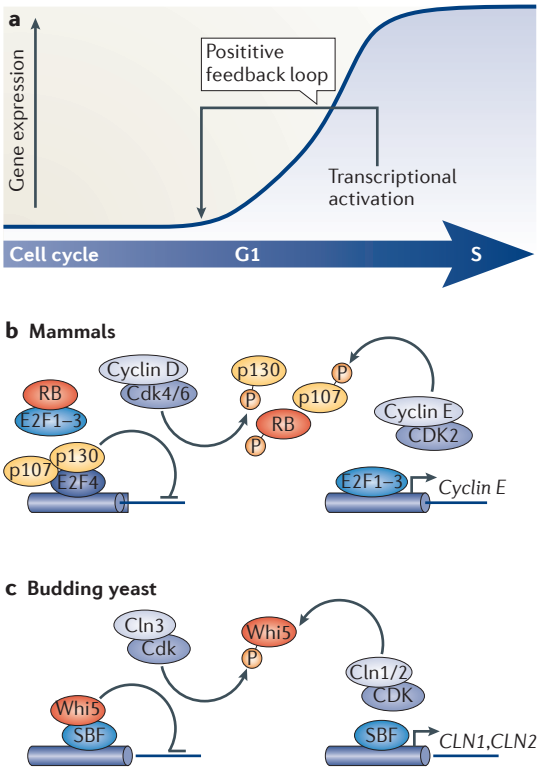


Figure 1 | **G1–S transcriptional activation.** **a** | Schematic showing how the G1–S transcriptional programme, once initiated, is reinforced by a positive feedback loop. **b** | In mammalian cells, the transcriptional repressors RB, p107 and p130 (collectively known as pocket proteins) are bound to E2F transcription factors to repress expression during early G1. Pocket proteins either prevent activator E2F proteins (such as E2F1, E2F2 and E2F3) to activate transcription or function as co-repressors for repressor E2F proteins (such as E2F4). Phosphorylation of pocket proteins by cyclin D– cyclin-dependent kinase 4 (CDK4) and cyclin D–CDK6 probably releases them from the E2F transcription factors. This induces the transcription of G1–S target gene, including the gene encoding cyclin E. Cyclin E–CDK2 phosphorylates pocket proteins, thereby providing a positive feedback loop. **c** | Model depicting G1–S transcriptional activation in budding yeast. In early G1, transcription is inhibited by Whi5 binding to the SBF (SCB-binding factor) complex at target promoters. Cln3–Cdk relieves transcriptional inhibition by phosporylating Whi5, which induces its nuclear export and thereby G1–S transcription. Activation of transcription results in the accumulation of Cln1 and Cln2, which in complex with Cdk, further inactivate Whi5 through phosphorylation. This provides positive feedback that results in cell cycle commitment.

of activator E2F proteins[57–59]. It is worth noting that although the molecular link between the commitment point and positive feedback activation has been firmly established in yeast[55], it is more contentious in mammalian tissue culture cells, in which the application of temporal high-resolution imaging approaches, to temporally link transcriptional regulation to cell cycle commitment in single cells, has been more limited[60]. In fact, some data obtained from single-cell measurements

Whi5
An inhibitor of SBF (SCB-binding factor)-dependent transcription during early G1 in yeast.

Regulon
A collection of genes under the control of the same regulatory protein.

Box 2 | **Mammalian cell cycle transcriptional regulation is dependent on E2F and pocket proteins**

The E2F family of transcription factors and their dimerization partner proteins act as transcriptional regulators of G1–S transcription.

E2F1, E2F2 and E2F3

These proteins are found in complex with RB during G1[121,122]. They can be detected at E2F target gene promoters by chromatin immunoprecipitation (ChIP) predominantly during G1-to-S transition, which corresponds with transcriptional induction of G1–S cell cycle genes[40,41]. As they are E2F targets, E2F1, E2F2 and E2F3 accumulate outside of G1 but are detected, to a significantly lesser extent, in G0 and G1 (see the figure).

E2F4 and E2F5

They are found in complex with p130 in G0 and p107 and p130 in G1 (REFS 40–42,123,124). E2F4 can be detected at E2F target promoters by ChIP predominantly during G0, which corresponds with transcriptional repression, but also during G1 (REFS 40,41). E2F4 is shuttled into the cytoplasm during G1-to-S phase transition when pocket proteins disassociate in response to CDK-dependent phosphorylation[43,125]. Upon return to interphase, dephosphorylated p107 and p130 associate with E2F4, promoting its transport into the nucleus and binding to target promoters[41]. Like E2F4, E2F5 is found in complex with p130 (REF. 126) and p107 (REF. 127). The timing at which it binds to target promoters has not been well-established, but it is assumed that binding mirrors that of E2F4 (REF. 39).

E2F6, E2F7 and E2F8

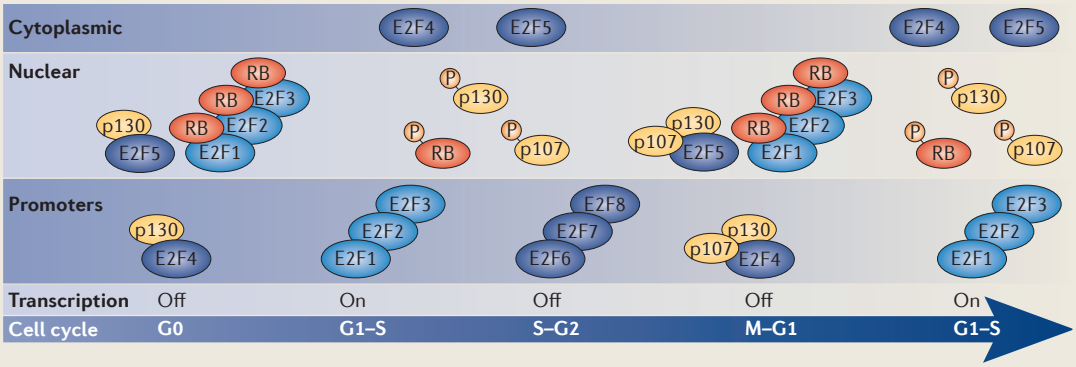
This subset of E2F proteins do not require binding to pocket proteins for its repressor function[92,96]. E2F6 and E2F7 accumulate during the G1-to-S phase transition and bind to target promoters, which coincides with transcriptional inactivation during S phase (REFS. 89, 98, 99, and C.B. and R.A.M. d-B., unpublished data). The timing of target-promoter binding by E2F8 has not been established, but it is assumed that binding is similar to that of E2F7. E2F8 and E2F7 form homo- and heterodimers to repress transcription[88,91,128,129].

RB

This protein is found in complex with E2F1, E2F2 and E2F3 in asynchronous cycling cells[46]. It can be detected by ChIP at target promoters in asynchronous cell cultures and, to a lesser extent, in quiescent cells[46]. RB accumulates outside of G1.

p107 and p130

These two pocket proteins are found in complex with E2F4 and E2F5 (REFS 35,41,127). p107 accumulates during the G1-to-S phase transition and is detected at target promoters by ChIP during G1 (REF. 41). p130 is predominately detected at target promoters by ChIP in G0 and to a lesser extent in G1 (REFS 40,41). High levels of p130 are detected in G0 and low levels throughout the cell cycle[130].



Dimerization partner proteins are omitted for simplicity. Hyperphosphorylation is indicated by 'P'. Promoter binding for individual E2F family members is only indicated when binding has been established during the cell cycle.

suggests a model that places the restriction point well before RB phosphorylation and E2F-dependent transcriptional activation occur[61,62].

DNA replication switch and ordering of cell cycle events. The Cdk inhibitor Sic1 causes a delay between the activation of the transcriptional positive feedback loop and the initiation of DNA replication[63,64]. In a two-step process, G1 cyclin–Cdk activity is required for Sic1 phosphorylation, which primes Sic1 for subsequent Clb-dependent phosphorylation leading to its degradation[65]. The mutual inhibition of the S phase cyclins and Sic1 form the basis of an ultrasensitive DNA replication switch that depends on the strong binding affinity of Sic1 for the Clb–Cdk complex[65]. Once activated, the S phase cyclins Clb5 and Clb6 in complex with Cdk phosphorylate Sld2 and Sld3 to initiate the formation of the Sld2–Sld3–Dpb11 complex. This complex mediates the activation of DNA replication[66–68].

Cyclin specificity has an important role in ordering cell cycle events[18]. In particular, the hydrophobic patch on Clb5 binds to RXL motifs, which is important for its specific function in initiating DNA replication[69]. Similarly, the G1 cyclin Cln2 docks a distinct LP motif, which makes Cln2 highly specific for the transcriptional inhibitor Whi5, and Cln3 binds to another, currently unknown motif[70,137]. The later B-type cyclin Clb2, although able to initiate replication, lacks the specific

docking ability of earlier cyclins. Rather, Clb2 is characterized by a higher intrinsic kinase activity[71]. Thus, earlier cyclins have weaker intrinsic activity but this is compensated by specific binding motifs, whereas the later mitotic cyclin Clb2 are characterized by a higher intrinsic activity. This yields an updated quantitative model of Cdk activity that progressively targets an increasing number of substrates through a 'hand-off' from specificity to higher intrinsic activity[70,72]. Moreover, recent work has demonstrated that a range of complex signal processing functions can be performed by combinatorial multi-site phosphorylation events on individual Cdk substrates[65].

Negative feedback turns off transcription

Upon G1–S transcriptional activation, cells progress to S phase, initiate DNA replication and subsequently inactivate transcription. It has become increasingly clear that the mechanism of G1–S transcriptional inactivation in both yeast and mammals involves negative feedback loops (FIG. 2). Several feedback mechanisms target the transcriptional activators that drive the G1-to-S phase transition for inactivation. Moreover, recent work shows that the accumulation of transcriptional repressors during S phase also has an important role in turning off transcription (FIG. 2a).

Inactivation of transcriptional activators. In *S. cerevisiae*, transcriptional inactivation of SBF targets upon exit from G1 requires the activity of the mitotic cyclin Clb in complex with Cdk1 (also known as Cdc28), which leads to dissociation of SBF from promoters[73–76]. Clb is initially inhibited during late G1 by the Cdk inhibitor Sic1 (FIG. 2c). The genes encoding G1 cyclins, *CLN1* and *CLN2*, and the Clb cyclins, *CLB5* and *CLB6*, are G1–S target genes, the protein products of which participate in the destruction of Sic1 and the activation of B-type cyclin activity[77]. Thus, the regulation of Cln and Clb levels by G1–S transcription forms a negative feedback loop through which SBF participates in its own inactivation.

As in yeast, inactivation of G1–S transcription in mammals involves multiple negative feedback loops (FIG. 2b). Similarly to the CDK-dependent inactivation of the transcriptional activator SBF, CDK activity has been proposed to inactivate E2F-mediated transcription during S phase in mammalian cells. This hypothesis was based on the observation that E2F1 is bound and phosphorylated by cyclin A–CDK2 both *in vitro* and *in vivo*, and that this promotes the dissociation of E2F1 from DNA and the inactivation of E2F1 target genes[78–80]. Because the gene encoding cyclin A is itself an E2F target, this constitutes a negative feedback loop. Furthermore, the activity of cyclin A–CDK2 is inhibited by the CDK inhibitor p27, which is targeted for degradation by cyclin E–CDK2 and cyclin A–CDK2; the genes encoding cyclin E and cyclin A are both targets of E2F proteins, thus contributing to the negative feedback loop[81,82]. In addition, the ubiquitin ligase regulatory SCF (S phase kinase-associated protein 2 (SKP2)–cullin 1–F-box protein) complex has been proposed to regulate the stability of E2F1 during S phase and G2 (REFS 83–85). As the SCF regulatory subunit SKP2 is encoded by an E2F target gene, this also represents a negative feedback loop.

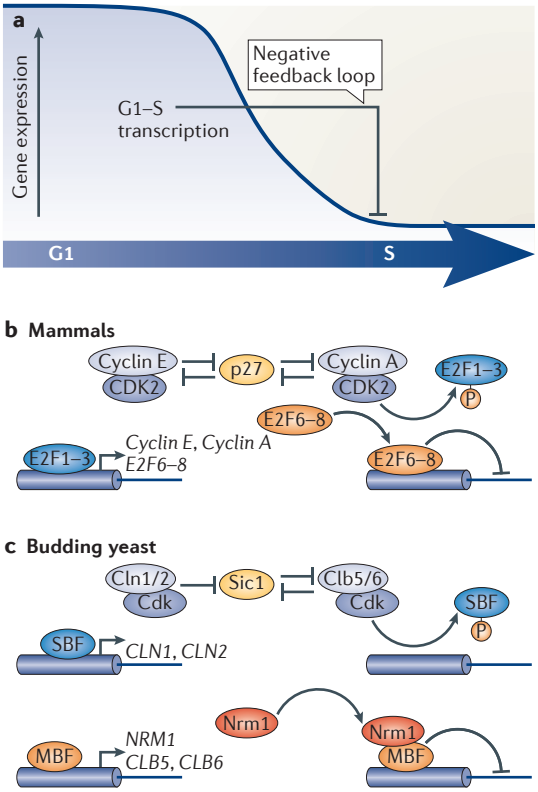


Figure 2 | **G1–S transcriptional repression. a** | Inactivation of E2F-dependent cell cycle transcription involves multiple negative feedback mechanisms. **b** | In mammalian cells, G1 cyclin–cyclin-dependent kinase (CDK) (cyclin E–CDK2) together with S phase cyclin–CDK (cyclin A–CDK2) targets the S phase cyclin-specific inhibitor p27 for degradation. The subsequent increase in CDK2 activity results in phosphorylation and release of the activator E2F1, E2F2 and E2F3 transcription factors from gene promoters, thus inactivating transcription. In addition, the E2F targets E2F6, E2F7 and E2F8 accumulate when cells progress to S phase, and they repress transcription when bound to target promoters. The negative feedback mechanism involving the E2F target S phase kinase-associated protein 2 (SKP2), which has a role in targeting E2F1 for degradation via the SCF (SKP2–cullin 1–F-box protein) ubiquitin ligase pathway, has been omitted for simplicity. **c** | Activation of G1–S transcription in budding yeast results in the accumulation of ~300 gene products, including Nrm1, Cln1, Cln2, Clb5 and Clb6. Some of these proteins are directly or indirectly involved in turning off transcription, thereby forming a negative feedback loop. Cln1–Cdk and Cln2–Cdk prime the Clb–Cdk-specific inhibitor Sic1 for Clb-Cdk phosphorylation, which targets it for degradation (not shown). Clb–Cdk-dependent phosphorylation of SBF (SCB-binding factor) components releases SBF from promoters, and this leads to the inactivation of transcription. MBF (MCB-binding factor)-dependent transcription is inactivated through binding of the MBF-associated co-repressor Nrm1.

Inactivation by transcriptional repression. The mechanism of inactivation of MBF-dependent transcription is different from that of SBF-dependent transcriptional inactivation and involves a negative feedback loop based on co-repressors. MBF functions as a cell cycle transcriptional repressor in a similar way to the mammalian E2F4, E2F5, E2F6, E2F7 and E2F8. It most closely resembles E2F4 and E2F5, which repress transcription with co-repressor pocket proteins. However, repressor E2F proteins only bind to promoters during specific phases of the cell cycle, whereas MBF is bound to promoters during the entire cell cycle. Temporally confining MBF activity so that transcription is switched on during G1 and switched off in S phase depends on the co-repressor proteins Nrm1 in *S. cerevisiae* and Nrm1 and Yox1 in the fission yeast *Schizosaccharomyces pombe*. Because they are both MBF targets, these proteins are involved in a negative feedback loop[49,86] (FIG. 2c). Nrm1 is expressed at low levels during early G1, and it is a target of the APC/C (anaphase-promoting complex; also known as the cyclosome) and is degraded at mitotic exit[49,87]. Thus, co-repressors accumulate progressively once MBF-regulated gene transcription is activated, leading to binding of the co-repressors to promoter-bound MBF and concomitant repression of G1–S transcription during S phase. Although Nrm1 and Yox1 seem to have a similar function as pocket proteins in mammalian cells, they actually differ in their ability to repress transcription: whereas Nrm1 and Yox1 function as transcriptional co-repressors during S phase, mammalian pocket proteins do not seem to be involved in turning off transcription during S phase when Cdk activity is high.

Finally, additional negative feedback loops that lead to transcriptional repression dependent on E2F6, E2F7 or E2F8, or a combination of these repressors, have been proposed[88,89] (FIG. 2b). The genes encoding these E2F proteins are themselves E2F targets and accumulate during the G1-to-S transition[90,91]. E2F6, E2F7 and E2F8 do not require pocket proteins for their repressor activity[88,92–96]. As a result, they should be able to repress transcription during S phase and the latter part of the cell cycle when pocket proteins are inhibited by Cdk activity[97]. Consistent with this view, E2F6 was recently found to repress G1–S transcription in late S phase[98] (FIG. 2b). E2F6 accumulates when cells progress to S phase and inactivates transcription in a timely manner by replacing activator E2F proteins at target promoters. Although deletion of E2F6 alone does not affect G1–S transcription in mouse embryonic fibroblasts (MEFs)[99], it was shown that, in the absence of E2F6, E2F4 binds to promoters normally bound by E2F6 during S phase, suggesting a compensatory role. Accordingly, depletion of E2F4 in E2F6-knockout MEFs leads to derepression of G1–S genes during S phase. Moreover, E2F7 and E2F8 are likely to be involved in transcriptional inactivation, given that deletion of both E2F7 and E2F8 in MEFs and overexpression of E2F7 in HeLa cells causes activation and inhibition, respectively, of some E2F target genes characteristic of G1-to-S phase transistion[88,89]. Overall, it seems likely that negative feedback through atypical repressor E2F proteins is required to turn off G1–S transcription.

Nrm1 and Yox1
Nrm1 in budding yeast and Nrm1 and Yox1 in fission yeast bind MBF (MCB-binding factor) to inhibit transcription once cells transit into S phase.

Subgroups within G1–S transcripts

Single-gene studies in the 1980's identified subsets of genes differentially regulated during the cell cycle and high-throughput microarray data in the late 1990's revealed the full extent of cell cycle-dependent gene expression[15,100]. These and subsequent studies have revealed subgroups of genes, the function and expression timing of which may be correlated.

Subgroups based on function. In *S. cerevisiae*, over 200 G1–S genes depend on the transcriptional activator SBF and/or the transcriptional repressor MBF. A large body of research has revealed that most of these genes can be grouped into SBF-, MBF-, SBF- and MBF-regulated and 'switch' genes (which are regulated by both SBF and MBF at different times during the cell cycle)[51,100,101].

Genes that are under the control of either SBF or MBF can also loosely be classified on the basis of their function. Genes involved in driving cell cycle progression, such as the G1 cyclins *CLN1* and *CLN2*, are more likely to be regulated by SBF, whereas MBF-targets are enriched for genes involved in DNA replication, DNA repair and other essential genes.

Controlling essential genes that do not dictate cell cycle timing with a transcriptional repressor such as MBF may constitute a selective advantage in case of MBF absence, as the removal of MBF at any specific promoter results in derepression of those genes rather than lack of expression. Conversely, constitutive derepression of genes involved in cell cycle timing may cause uncontrolled cell proliferation and would be 'safer' under the control an activator such as SBF. In addition, the finding that MBF-dependent genes seem to be involved in DNA replication may be a consequence of the transcriptional activation of MBF- but not SBF-dependent genes during replication stress[101–103]. A detailed description of how MBF-dependent transcription is regulated in response to replication stress is discussed below. Recently, another subgroup of genes regulated by an SBF-to-MBF switch during G1-to-S phase transition was identified[103]. Interestingly, these switch genes are enriched for G1–S genes that cause a cell cycle progression defect when overexpressed but are upregulated in response to replication stress. The dependency of switch genes on SBF during G1 and MBF outside of G1 prevents them from being constitutively expressed in the event of MBF malfunction, yet renders them responsive to replication stress. Interestingly, whereas only a small number of G1–S targets are regulated by the SBF-to-MBF switch in *S. cerevisiae*, E2F switching at G1–S target promoters in mammalian cells seems to be the norm. Overall, the use of two distinct transcription factors allows budding yeast to implement combinatorial control of its G1–S transcriptional programme in response to replication stress.

Subgroups based on timing. There is great variation in the expression timing of G1–S genes[56]. Genes that encode proteins involved in the positive feedback loop, such as those encoding G1 cyclins, are activated before other SBF, MBF and E2F target genes. This results in the decision to divide, which is coincident with the transcriptional activation of positive feedback elements, preceding a genome-wide

change in transcription. In other words, the decision to enter a new cell cycle precedes the activation of the genome-wide change in transcription despite the fact that target genes of both processes are regulated by the same transcription factors. In addition, the yeast co-repressor Nrm1, which is involved in a negative feedback, is one of the latest genes to be activated.

The importance of 'positive feedback first' and 'negative feedback last' was demonstrated when placing *CLN2* under control of the *NRM1* promoter, which resulted in uncoordinated cell cycle commitment and cell death. Thus, the timing of gene activation involved in feedback regulation to temporally confine G1–S transcription follows a logical order, starting with the robust activation of transcription (dependent on the upregulation of *CLN1* and *CLN2*) in G1 which is finally turned off during S phase (dependent on the upregulation of *NRM1*). The establishment of this order is not clearly linked to the specific transcription factors that control gene activation and requires further investigation.

Positive feedback first regulation is the most robust feature of transcriptional timing. Even though the timing of expression of nearly all G1–S genes significantly changes when cell cycle synchrony is established by arresting cells either in G1 phase or mitosis, G1 cyclins that affect positive feedback first regulation are among the earliest activated genes in both cases of cell cycle arrest[56]. The temporal subdivision into blocks of genes across the cell cycle revealed that when cells enter the cell cycle from mitosis, SBF targets are activated before MBF targets. Conversely, when cells re-enter the cell cycle from G1, the order is reversed and MBF targets are activated before SBF targets. Interestingly, genes that encode proteins involved in the positive feedback loop are likely to be under the control of both the SBF and MBF transcription factors. The dually regulated SBF and MBF targets are activated by either of these two factors (the first one activated), which ensures these genes are activated early in the cell cycle. Thus, for the genes that can be activated by either SBF or MBF, the induction of either factor is sufficient to initiate transcription and functions as a logical 'or' gate, whereby SBF 'or' MBF will activate transcription irrespective of the previous cell cycle. This reveals an important mechanistic aspect of transcriptional activation.

G1–S transcript subgroups in mammalian cells. As in yeast, the activation timing of individual genes within the G1–S wave of transcription varies greatly in mammalian cells with positive feedback-associated genes being activated first[16,56]. This suggests that the positive feedback first principle is conserved in eukaryotes. By contrast, cell cycle-dependent transcriptional regulation of specific target genes is very poorly conserved across eukaryotes[104].

Similarly to SBF and MBF regulating distinct sets of genes in *S. cerevisiae*, distinct E2F proteins and pocket proteins, perhaps interacting with other co-regulators, might bind to specific promoters to define subgroups. Consistent with this model, many studies suggest that in addition to largely overlapping targets, individual members of the E2F family may display different DNA sequence specificities[105,106]. However, single-gene analyses indicate similar temporal sequences of E2F and pocket protein binding to the examined genes. It will be interesting to see whether and how the composition of E2F target subgroups emerges.

G1–S transcription and genome stability

When cells become committed to a division cycle, they initiate DNA replication and progress to S phase. Such cells rely on two DNA structure checkpoints (BOX 3) — the DNA damage checkpoint and the DNA replication checkpoint — to protect themselves from irreversible DNA damage. These checkpoints delay mitotic entry and initiate a specific transcriptional programme. The importance of DNA

Box 3 | **The DNA structure checkpoints**

To properly replicate the genome and prevent tumorigenesis, cells rely on the DNA structure checkpoints, an evolutionarily conserved set of signalling pathways that monitor DNA damage and the loss of DNA replication fork integrity. These checkpoints delay mitotic entry and initiate a specific transcriptional programme. The signalling pathways involved rely on evolutionarily conserved protein kinases, including the sensor molecules that detect damage or replication stress, that in turn activate transducer proteins that relay the signal to downstream effector proteins required to initiate the full response. DNA structure checkpoints are mediated via the ATM (ataxia-telangiectasia mutated) and ATR (ataxia-telangiectasia and Rad3-related protein) protein kinases and their downstream targets checkpoint kinase 1 (CHK1) and/or CHK2. The nature of the DNA structure triggering the checkpoint response determines the activity of downstream effector kinases: CHK1 is activated by replication fork arrest during S phase, whereas CHK2 is activated by damaged DNA detected during interphase[19–21,107,131,132]. On this basis, the DNA structure checkpoint can be divided into the DNA replication checkpoint and the DNA damage checkpoint. The DNA replication checkpoint is essential to prevent DNA damage in response to replication stress during S phase, whereas the DNA damage checkpoint is required to detect and resolve DNA damage during interphase[131,133]. Both checkpoint signalling cascades arrest cell cycle progression, mostly through the regulation of key regulators of cyclin–cyclin-dependent kinase (CDK) activity, such as the phosphatase CDC25 and the CDK inhibitor p21 (REFS 21,131,132,134). CDC25 removes inhibitory phosphorylation from CDK1 and CDK2 to promote CDK activity and therefore mitotic entry. When the checkpoint is engaged, CDC25 becomes phosphorylated and is subsequently degraded to prevent progression through mitosis. Cell cycle arrest ensures that DNA damage can be avoided or that the detected damage can be repaired before division to limit heritable mutation. Although both checkpoints delay progression of mitosis through largely overlapping mechanisms, they induce distinct responses to specific stresses. Two other important checkpoint responses are the transcriptional response and the initiation of programmed cell death when the damage cannot be resolved. The transcriptional response differs between the replication and damage checkpoints, as, for instance, G1–S transcription is only regulated by the replication checkpoint. Programmed cell death is of particular importance in multicellular organisms and is predominately associated with the DNA damage checkpoint response[21].

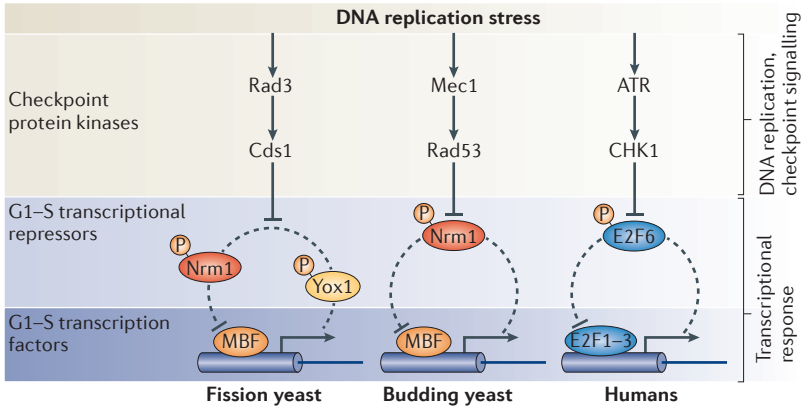


Figure 3 | **G1–S phase transcription and genome stability.** The mechanism by which the DNA replication checkpoint maintains high levels of G1–S transcription in response to replication stress is conserved from yeast to humans. This mechanism involves the inactivation of a transcriptional repressors and/or co-repressors (Nrm1 and Yox1 in yeast and E2F6 in human cells) involved in an autoregulatory negative feedback loop. The downstream effector checkpoint protein kinase (Cds1 in fission yeast, Rad53 in budding yeast and checkpoint kinase 1 (CHK1) in mammals) inactivates the transcriptional repressors Nrm1, Yox1 and E2F6 through phosphorylation to maintain high levels of G1–S transcription. The DNA replication checkpoint protein kinases are conserved from yeast to humans, but the G1–S transcriptional repressors and transcriptional activators are not. MBF, MCB-binding factor.

checkpoint signalling is illustrated by the conservation of the subfamily of checkpoint protein kinases (TABLE 1).

DNA replication checkpoint. In this Review, we focus on DNA replication checkpoint signalling because recent work has linked this pathway to G1–S transcription. DNA replication stress is defined as inefficient DNA replication that causes DNA replication forks to progress slowly or stall. The DNA replication checkpoint prevents the accumulation of DNA damage as a result of replication stress by stabilizing stalled replication forks, preventing late origins from firing and enabling replication to resume once the stress has been resolved[107].

During DNA replication, cells are particularly vulnerable to genomic instability, as replication forks are prone to stall and collapse when encountering replication blocks or damaged DNA templates. The DNA replication checkpoint transcriptional response probably maintains G1–S transcription in order to prevent genomic instability[98,101,102,108] (FIG. 3).

The replication checkpoint induces G1–S transcription. In both fission and budding yeast, genes that are activated during G1 and that depend on MBF to be inactivated outside of G1 are induced in response to DNA replication stress[11,103,109]. Activation of these MBF-responsive genes results from checkpoint signalling-dependent inhibition of the negative feedback loop that turns off G1–S transcription during G1-to-S phase transition. The checkpoint effector kinases Rad53 and Cds1 directly target and inhibit the main G1–S transcriptional co-repressor Nrm1 in budding yeast and Nrm1 and Yox1 in fission yeast[101,102,110–114] (FIG. 3). Upon phosphorylation, these repressors are no longer able to bind MBF, allowing for continuous expression of MBF-regulated genes.

Maintenance of MBF-regulated gene expression is important for cell survival in response to replication stress because many MBF-targets function in replication, DNA repair and nucleotide synthesis. Consequently, the deletion of Nrm1 and/or Yox1 in fission yeast improves the survival of mutants defective in the DNA replication checkpoint in response to replication stress and, conversely, overexpression of stabilized Nrm1 in budding yeast increases sensitivity to replication stress.

Conservation of the checkpoint transcriptional response. Similarly to the induction of MBF targets by replication stress in yeast, E2F-dependent transcription is induced in mammals by a closely related mechanism[98] (FIG. 3). In response to replication stress, checkpoint kinase 1 (CHK1) phosphorylates and inhibits E2F6, which is responsible for inactivating G1–S transcription during the mitotic cell cycle. Inactivation of E2F6 leads to its release from promoters and allows G1–S transcription to persist. The ability of cells to activate G1–S transcription is crucial for survival upon hydroxyurea treatment, probably because E2F targets include proteins that prevent replication fork collapse and DNA damage. This transcriptional response seems to be specific for replication stress, which, in contrast to the DNA damage response, does not induce apoptosis. Interestingly, the DNA damage response can induce pro-apoptotic E2F1 targets[115–120]. However, these targets are distinct from those normally regulated by E2F1 during the mitotic cell cycle. Despite a distinct lack of conservation of the proteins affecting this regulation, the conservation of the transcriptional response to replication stress underscores the conservation of G1–S control systems-level features across eukaryotes.

Conclusion and perspective

In this Review, we emphasized recent work uncovering simple yet elegant mechanisms of gene expression control during G1 and S phases that regulate commitment to cell division, temporally confine G1–S transcription and respond to replication stress. This work has revealed many new insights into how transcription of different G1–S cell cycle genes is restricted to G1 and how this is regulated by DNA replication checkpoint protein kinases as part of the checkpoint transcriptional response. Despite frequent lack of sequence homology, conservation of systems-level properties across eukaryotes is an emerging theme of cell cycle control. Indeed, the conserved regulation of G1–S transcripts by the replication checkpoint suggests a central role for this transcriptional wave in the maintenance of genome stability.

Genome-wide transcriptional changes are a general feature of cellular transitions. Our knowledge of the transcriptional regulation of the G1-to-S phase transition may be widely applicable to the study of other such transitions. Resulting from decades of concerted effort, our deep understanding of the G1-to-S phase transition provides a blueprint for future research to investigate fundamental regulatory mechanisms controlling cellular decision-making processes, dynamic changes in gene expression during cellular transitions and context-specific rewiring of transcriptional networks.

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