Trends in
Biochemical Sciences

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Review

Coordinating gene expression during the cell cycle

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Cell cycle-dependent gene transcription is tightly controlled by the retinoblastoma (RB):E2F and DREAM complexes, which repress all cell cycle genes during quiescence. Cyclin-dependent kinase (CDK) phosphorylation of RB and DREAM allows for the expression of two gene sets. The first set of genes, with peak expression in G1/S, is activated by E2F transcription factors (TFs) and is required for DNA synthesis. The second set, with maximum expression during G2/M, is required for mitosis and is coordinated by the MuvB complex, together with B-MYB and Forkhead box M1 (FOXM1). In this review, we summarize the key findings that established the distinct control mechanisms regulating G1/S and G2/M gene expression in mammals and discuss recent advances in the understanding of the temporal control of these genes.

Highlights

It was recently demonstrated that cell cycle-dependent gene expression can be separated into two major subgroups: those predominantly expressed and largely functional during either the G1/S or G2/mitosis (M) phases of the cell cycle.

G1/S genes are primarily repressed by the retinoblastoma (RB):E2F complex with contributing effects by DP, RB-like, E2F4, and multi-vulval class B or MuvB (DREAM), and are activated by E2F transcription factors.

In contrast, G2/M genes are primarily repressed during G0 by DREAM, with indirect effects possibly elicited through the repression of G1/S genes by RB:E2F, and are activated by the MuvB complex and B-MYB and Forkhead box M1 (FOXM1) transcription factors.

Regulation of the mammalian cell cycle

The cell division cycle is at the heart of all multicellular growth and comprises four phases: **mitosis** (M; see Glossary), DNA synthesis (**S**), **G1**, and **G2**. A multilayered control system, which is conserved in most eukaryotic cells, ensures a precisely timed one-way transition through the cell cycle when prompted by external stimuli and permitted by internal checkpoints. The tasks of replicating billions of DNA base pairs in mammalian cells during S phase and then accurately separating chromosomes and cell organelles into two daughter cells during mitosis require hundreds of highly specialized proteins encoded by cell cycle-dependent genes, the activity of which must be tightly regulated. Growth stimuli elicit cellular signaling cues that lead to activation of **CDKs**, serine/threonine kinases that enable cell cycle entry and coordinate precisely timed cell cycle progression [1]. Degradation of specific cell cycle proteins through the ubiquitin system provides an additional layer of control [2,3]. The levels of most proteins necessary for cell cycle progression are regulated through the periodic expression of their encoding mRNAs during the cell cycle [4,5]. The periodic expression of cell cycle regulators occurs in two broad waves of expression, which peak during the G1/S and G2/M phase transitions, and is coordinated by a series of evolutionarily conserved, interconnected complexes containing the **E2F**, **B-MYB**, and **FOXM1** transcription factors, as well as the **RB tumor suppressor or pocket protein family** of transcriptional repressors. Importantly, these control mechanisms regulate one another and generate feedback loops and redundancies to provide an almost fail-safe system of progression from G1 through S and G2 phases and into mitosis followed by cell division.

A corrupted cell cycle control system can give rise to growth-associated diseases, including cancer, and cell cycle genes and their encoded proteins serve as proliferation markers that display increased expression in many cancers [6], including the well-established proliferation marker Ki67 [7]. For example, in a pan-cancer analysis across ~18 000 cancers, expression of the cell cycle gene-encoded TF FOXM1 and its co-expressed network displayed the strongest

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correlation with poor prognosis [8]. Here, we review recent findings on cell cycle gene regulation that have increased our understanding of cell cycle control.

Cell cycle genes: G1/S and G2/M

The first high-throughput identification of cell cycle-regulated genes classified them into five groups based on peak expression profiles during G1/S, S, G2, G2/M, and M/G1 [9]. More recent analyses suggested that a classification using only two groups may better reflect their transcriptional control [10]. In-depth analysis of these two groups revealed one group, 'G1/S', comprising genes that regulate several key processes required for DNA synthesis, including DNA metabolism, DNA replication, and DNA repair. Moreover, **G1/S genes** were enriched for harboring the evolutionarily conserved **E2F binding motif** (5′-TTSSCGC-3′). The second group, 'G2/M', encoded proteins with functions in mitosis, including spindle assembly, chromosome segregation, and cytokinesis. **G2/M genes** were enriched for harboring the conserved **cell cycle genes homology region (CHR) motif** (5′-TTYRAA-3′) bound by TFs largely distinct from those binding to the E2F motif (see below) [10]. Thus, the presence of two distinct promoter DNA motifs, namely E2F and CHR, and the TFs binding to these sites, offers an explanation for most characteristics of G1/S and G2/M cell cycle gene transcription [11]. Together, these findings established that cell cycle genes can be separated into two major subgroups, which are predominantly expressed and largely function in either G1/S or G2/M phases of the cell cycle.

Transcriptional cell cycle regulators

The E2F and RB families

Cell cycle regulators display substantial evolutionary conservation in vertebrates, invertebrates, and plants. In jawed vertebrates, the **E2F family** includes eight members (E2F1–E2F8) [12], and the RB pocket protein family includes RB (*RB1*), p107 (*RBL1*), and p130 (*RBL2*) [13]. All E2F TFs can directly bind to DNA through E2F recognition motifs typically found in promoters proximal to the transcription start site (TSS). E2F1–E2F6 bind to DNA as heterodimers with their dimerization partner DP1 or DP2. By contrast, E2F7 and E2F8 contain two DNA-binding domains and do not dimerize with DP1/2 [14]. Furthermore, the E2F family contains canonical and non-canonical TFs. Canonical E2Fs can be divided into transcriptional activators (E2F1, E2F2, and E2F3a) and repressors (E2F3b, E2F4, and E2F5), although recent studies revealed that E2F4 can also broadly activate transcription [15]. The activating E2F **transactivation domain** recruits chromatin modifiers, including the histone acetyltransferase complexes Tip60 and PCAF/GCN5, to activate target gene expression during late G1 and S [16,17]. All RB family members interact with canonical E2F proteins to form transcriptional repressor complexes [12,13]. By contrast, the non-canonical E2Fs (E2F6, E2F7, and E2F8) predominantly serve as transcriptional repressors. They do not bind to RB family members but can repress G1/S gene expression independent of the RB family [12]. E2F6 was demonstrated to repress G1/S gene expression [18], with recent analyses revealing that E2F6 can bind to, and regulate, G2/M genes [10,19,20] as well as MYC targets [19,21]. Moreover, E2F6 serves as a component of the polycomb repressor complex 1 subtype 6 (PRC1.6), which can repress target genes during **quiescence** [19–21]. While PRC1.6 appears to regulate many cell cycle genes, it remains to be determined whether it affects cell cycle-dependent gene expression.

RB represses target genes by masking E2F transactivation domains and by recruiting repressive chromatin modifiers through its LxCxE binding cleft to reduce acetylation and increase methylation at nucleosomes [22]. As noted previously, RB can interact with all canonical E2Fs, whereas p107 and p130 specifically interact with E2F4 and E2F5 [12,13,23] (Figure 1A, left). Thus, RB has the unique ability to bind to, and inhibit, the activators E2F1–3a [24,25], which offers an explanation for why RB tends to be a stronger tumor suppressor compared with p107 and p130 [26].

Glossary

B-MYB: TF that activates G2/M gene expression.
Cell cycle genes homology region (CHR) motif: enriched in promoters of G2/M genes; 5′-TTYRAA-3′.
Cyclin D:CDK4/6: kinase complex comprising cyclin D and CDK4 or CDK6, which is activated to transition from quiescence to early G1 phase; phosphorylates many targets, including pocket proteins RB, p130, and p107.
Cyclin E:CDK2: kinase complex activated in mid-G1; phosphorylates many targets, including hyperphosphorylation of RB and p103/p107.
Cyclin-dependent kinase (CDK): serine/threonine kinase that promotes cell cycle progression.
DP, RB-like, E2F4/5, and MuvB (DREAM) repressor complex: binds to G1/S and G2/M genes in G0 and early G1.
DYRK1A: kinase that phosphorylates LIN52, necessary for DREAM complex formation.
E2F family: TFs that bind to E2F motifs in promoters of cell cycle genes.
E2F-binding motif: enriched in promoters of G1/S genes; 5′-TTSSCGC-3′.
Forkhead Box M1 (FOXM1): TF that activates G2/M gene expression.
G1 phase: gap phase of cell growth; phase between mitosis or quiescence before the onset of DNA synthesis (S phase).
G1/S genes: genes enriched with E2F-binding motifs that display a maximal expression during S phase; activated by activator E2Fs; encode many factors required for DNA synthesis.
G2 phase: gap phase of quality control; phase between S phase and mitosis.
G2/M genes: genes enriched with CHR motifs that display maximal expression during G2 and M phases; activated by B-MYB and FOXM1 TFs; encode many factors necessary for mitosis.
Mitosis (M) phase: cell cycle phase in which chromosomes are segregated; between G2 phase and cytokinesis.
MuvB complex: comprises LIN9, LIN37, LIN52, LIN54, and RBBP4; binds to CHR motifs via LIN54.
MYB:MuvB (MMB):FOXM1 complex: activating complex comprising MuvB, B-MYB, and FOXM1; activates G2/M gene expression.
p53-p21 pathway: activation of tumor suppressor p53 stimulates expression of

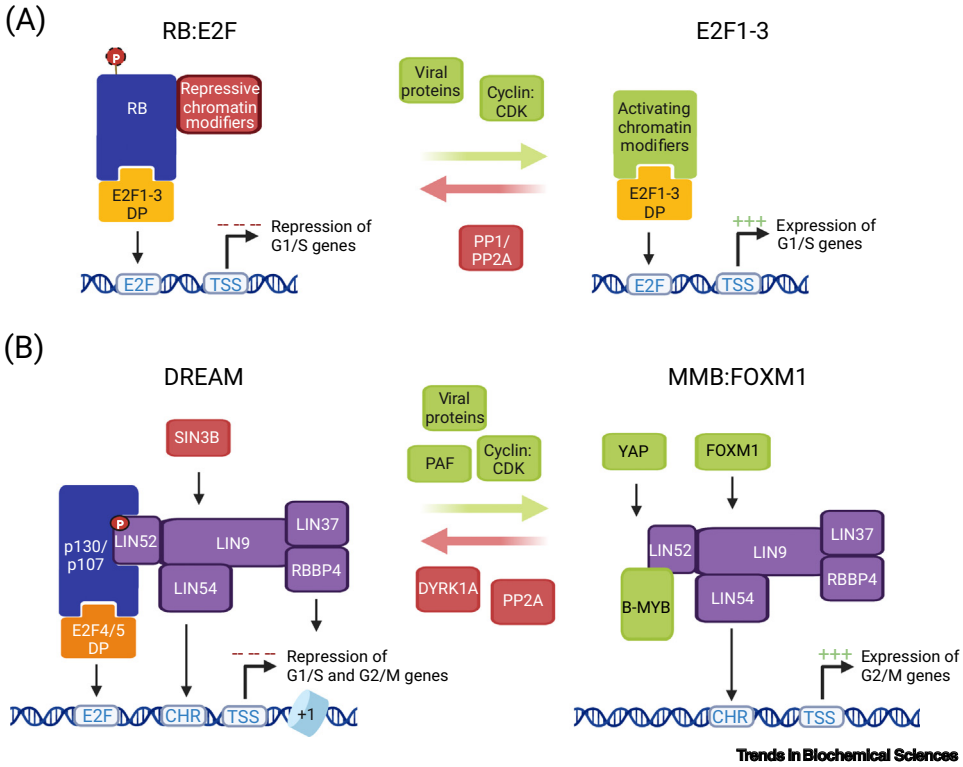

Figure 1. RB:E2F, DREAM, and MMB:FOXM1 complexes. Components essential for the formation and function of transcriptional repressors are shown in red, while activators are highlighted in green. (A) Expression of G1/S genes by E2F1-3:DP is repressed by mono- or unphosphorylated RB. RB masks the transactivation domains of E2F1–3 and recruits repressive chromatin modifiers. Dephosphorylation by PP1 and PP2A maintains active RB [125]. RB:E2F repressor complexes can be disrupted by cyclin:CDKs and viral oncoproteins. Then, E2F1-3:DP together with activating chromatin modifiers stimulate gene transcription in G1/S. (B) The MuvB core (LIN9, LIN54, LIN52, LIN9, LIN37, and RBBP4) assembles with p130/p107, E2F4/5, and DP to form the transcriptional repressor DREAM. Un- or hypophosphorylated p130/p107 bind to LIN52 phosphorylated at S28 by DYRK1A. Dephosphorylation of p130/p107 is driven by PP2A. DREAM binds G1/S genes through E2F4/5:DP interacting with E2F promoter sites, while the complex is recruited to CHR elements in G2/M gene promoters via LIN54. Mechanistically, DREAM represses genes by stabilizing the +1 nucleosome downstream of the TSS through interaction with RBBP4, and by recruiting the co-repressor SIN3B. DREAM can be disrupted by viral oncoproteins competing with LIN52 for binding to p130/p107. Cyclin:CDK-dependent phosphorylation of p130/p107 and the interaction with PAF also results in disassembly of DREAM. The MMB complex forms when B-MYB binds to LIN52 and LIN9. Interaction of YAP with B-MYB stimulates formation of MMB. Binding of B-MYB to MuvB is necessary for recruiting FOXM1. MMB:FOXM1 contacts CHR promoter sites via LIN54 to activate G2/M genes. Abbreviations: CDKs, cyclin-dependent kinases; CHR, cell cycle genes homology region; DREAM, DP, RB-like, E2F4, and multi-vulval class B or MuvB; FOXM1, Forkhead box M1 (FOXM1); M, mitosis; MMB, MYB:MuvB; PP, protein phosphatase; RB, retinoblastoma; TSS,transcription start site.

CDK inhibitor p21 to halt cell cycle progression.
Quiescence: G0; reversible cell cycle exit, characterized by low cell cycle and cell growth gene expression.
RB:E2F: activator E2F in complex with repressive RB protein.
Retinoblastoma (RB) tumor suppressor or pocket protein family: pocket proteins; RB, p130 (RBL2), and p107 (RBL1); contain LxCxE binding cleft.
S phase: phase of cell cycle in which DNA is replicated; between G1 and G2 phases.
Transactivation domain: protein domain in TFs that facilitates recruitment of RNA polymerase II to upregulate gene expression; often recruits co-factors, such as histone modifiers.

Interestingly, CDKs generate two major forms of phospho-RB. **Cyclin D:CDK4** can mono-phosphorylate RB on any one of 14 known CDK sites. By contrast, CDK2 and CDK1 contribute to the multi- or hyperphosphorylation of RB [27]. Hyper-phosphorylated RB dissociates from activator E2Fs, resulting in exposure of their transactivation domains and enabling recruitment of chromatin remodelers (Figure 1A, right). By contrast, monophosphorylated RB can retain binding to E2F. Intriguingly, stress-activated protein kinases of the p38 family can also phosphorylate RB. In contrast to CDK-dependent phosphorylations, these modifications lead to increased affinity to E2F1 and the presence of hyperphosphorylated RB in **RB:E2F** transcriptional repressor complexes, which allow cells to slow proliferation and increase survival upon cellular stress [28].

The LxCxE binding cleft of RB was originally defined by its ability to bind to the viral oncoproteins adenovirus E1A, SV40 Large T antigen, and human papillomavirus E7 [29–31]. Viral protein binding to RB disrupts binding to E2F as well as a variety of cellular proteins that contribute to RB-mediated tumor suppression [32] (Figure 1A, right). While the LxCxE cleft in RB recruits repressive chromatin modifiers through LxCxE motifs, the LxCxE binding cleft of p107 and p130 binds specifically to the phosphorylated LxSxE motif of LIN52 to form the **DP, RB-like, E2F4, and multi-vulval class B or MuvB (DREAM) repressor complex** (Figure 1B, left), although RB itself is not capable of binding to LIN52 and the **MuvB complex** and does not form a DREAM complex [33].

DREAM complexes

MuvB complexes are evolutionary conserved and, in all animals, contain orthologs of mammalian LIN9, LIN37, LIN52, LIN54, and RBBP4 (Figure 1B, left) [4,5,34–36]. All MuvB complexes contain LIN54, which specifically recognizes CHR motifs located proximal to the TSS through its DNA-binding domain [37–39]. Repressive DREAM complexes form through the interaction of hypophosphorylated p107/p130 with MuvB through phosphorylated LIN52 [33,40]. LIN52 phosphorylation is driven by the **DYRK1A** kinase at S28 [40]. DREAM incorporates p107 or p130 depending on cellular conditions and cell types. In quiescent cells, p130 is the most abundant pocket protein, while p107 is essentially absent. Consequently, DREAM extracted from quiescent cells predominantly contains p130 [41–43]. In quiescent cells lacking p130 or RB, increased levels of p107 lead to its incorporation into DREAM [41,43]. During S phase, when p107 levels normally peak, it may bind to MuvB during DNA damage conditions [43]. p130/p107 recruits E2F4:DP to the MuvB core, which enables DREAM to bind to E2F promoter elements of G1/S genes [11]. Notably, p107 can also bind to MuvB without engaging E2F4:DP in G1 [42]. Although p107 contributes to DREAM-dependent gene repression during DNA damage [43], any functional differences between p130 and p107-containing DREAM complexes remain unresolved. In general, little is known about how DREAM represses gene expression (Box 1).

MMB:FOXM1 complexes

CDK phosphorylation disrupts DREAM with release of p130 and E2F4:DP1 from MuvB. Subsequently, MuvB sequentially interacts with the activating TFs B-MYB and FOXM1 to form the **MYB:MuvB (MMB):FOXM1 complex**, which binds via LIN54 to TSS proximal CHR motifs in cell cycle genes [37–39], particularly those expressed in G2 phase and mitosis (Figure 1B, right). LIN52 is essential for the interaction of B-MYB with MuvB; B-MYB contacts both LIN52 and LIN9 via its

Box 1. Mechanisms of transcriptional repression by DREAM

A long-standing question concerns how DREAM represses its target genes. Given that the LxCxE cleft of p130 is bound by LIN52 [33], LxCxE-mediated recruitment of chromatin modifiers similar to RB appears unlikely. However, the MuvB complex has a key role in DREAM-mediated repression. LIN37 is essential for DREAM-dependent gene repression, but its mode of action remains unknown [58,64,112–114]. Interestingly, it is dispensable for the integrity of DREAM and transcriptional activation mediated by MMB:FOXM1 [58,64]. LIN37 and RBBP4 interact with the N terminus of LIN9, while LIN54 and LIN52 bind to the C terminus of LIN9 [33,44,113] indicating that LIN9 forms a central scaffold within the MuvB core (see Figure 1 in the main text). Recent analyses showed that the LIN9 N terminus contacts RBBP4, creating a binding surface for LIN37. RBBP4 is a component of several chromatin-modifying complexes and can bind histones H3 and H4. When bound to LIN9, the binding site of RBBP4 for histone H3, but not H4, is accessible, and a reconstituted LIN9-LIN37-RBBP4 complex was found to bind nucleosomes. In quiescent cells, DREAM primarily associates with the +1 nucleosome near TSS, offering an explanation for gene repression by stabilizing the +1 nucleosome [113]. Interestingly, PAF/PCLAF was recently reported to bind to RBBP4, thereby inhibiting DREAM formation and function [115].

SIN3B, a scaffold protein found in various chromatin repressor complexes, bound MuvB independent of pocket proteins, and loss of SIN3B derepressed DREAM targets in serum-starved cells [110], suggesting that DREAM can recruit chromatin modifiers through its MuvB core as an additional mechanism to achieve target gene repression.

C terminus, although it binds to a different surface compared with p130/p107 [44] (Figure 1B). Interestingly, MuvB complexes that contain both p130 and ectopically expressed B-MYB have been detected [33]. Similarly, p107-containing MuvB complexes were found in proliferating cells and B-MYB can coprecipitate with p107 [42], suggesting that complexes exist that contain both p107/p130 and B-MYB proteins, but the context in which they form and their functions remain elusive.

MMB:FOXM1 forms and disassembles in three temporal steps or subcomplexes that remain to be fully understood from a mechanistic perspective: (i) B-MYB binding to MuvB forms the MMB subcomplex during S phase [45,46]; (ii) FOXM1 is subsequently recruited to MMB, forming the MMB:FOXM1 complex during S/G2 transition [45,47,48]; and (iii), during G2, B-MYB is proteolytically degraded, while MuvB and FOXM1 persist at the DNA into mitosis [45]. Formation of MMB is essential for recruiting FOXM1 [45,47,48] and the interaction of the MuvB core with both B-MYB and FOXM1 contributes to activation of the CHR-containing genes. Given that CHR motifs are primarily enriched at target gene promoters, while canonical MYB and forkhead DNA recognition motifs are less abundant [39], it is likely that both proteins stabilize MuvB complexes at CHR elements by contacting the DNA largely in a nonsequence-specific manner [48,49].

Repression of cell cycle genes to halt and exit the cell cycle

Cycling cells can exit the cell cycle when mitogenic signals are reduced, upon receiving differentiation signals, or under stress conditions. When mitogenic signals are insufficient, cells exit the cell cycle and enter quiescence (G0), a state of growth cessation that preserves the capacity to proliferate. Historically, the canonical model describing cell cycle entry begins by starting in quiescence with progression into early G1 phase as cells sense growth signals to pass a restriction point in late G1 phase followed by commitment to a full cell division cycle [27,50–52]. However, recent studies provided evidence that the canonical model does not fully account for cell cycle commitment decisions taken by cycling cells and instead suggest a continuous sensing of growth signals throughout the cell cycle, with information from the mother cell passed on to the two daughter cells [53–57].

Pocket proteins, in particular RB, are at the heart of most models that describe proliferation–quiescence decisions. Both DREAM and RB cooperate to repress cell cycle gene expression during quiescence [58,59] and it has been shown that activity of PP2A phosphatase during late G2 and mitosis is required for cells to both activate RB and form the DREAM complex to enable entry into quiescence [54]. While PP2A removes CDK-mediated phosphorylation, the DYRK1A kinase phosphorylates LIN52 on Ser28 to promote DREAM complex formation, which is important for a cell to enter and remain in quiescence [40,60,61] (Figure 1). Additionally, even when growth signals are maintained, a subpopulation of cycling cells can withdraw from the cell cycle due to increased p21 levels inherited from replication stress-induced DNA damage during the preceding S phase [57,62,63]. Activation of p21 and inhibition of CDKs leads to the reformation and activation of DREAM and RB and their subsequent downregulation of cell cycle gene expression [10,43,64–68]. Furthermore, in response to the tumor suppressor and stress sensor p53, which induces p21 (the **p53–p21 pathway)**, DREAM and RB halt the cell cycle [43,64]. Stress can induce senescence, which shares many characteristics with quiescence, including repression of G1/S genes by RB and G2/M genes by DREAM. In sum, both DREAM and RB have important roles in promoting and maintaining cellular senescence, which is regulated, in part, through signaling from the LATS2 kinase to DYRK1A and ultimately to cell cycle gene repression by DREAM and RB [40,69]. Quiescence is a prerequisite for many cell differentiation events and, thus, cell differentiation is affected and supported by both DREAM and RB [5].

Given that RB:E2F and DREAM complexes have some redundant functions, it has been a long-standing challenge to dissect their unique contributions to the regulation of cell cycle genes. Recent studies revealed that G1/S and G2/M cell cycle genes are differentially repressed by RB:E2F and DREAM complexes. For example, senescent lung fibroblasts, in which cell cycle genes are repressed, display upregulation of G1/S but not G2/M genes upon knockdown of RB (Figure 2A) [70,71]. Similarly, doxorubicin-treated foreskin fibroblasts display upregulation of G1/S but not G2/M genes upon RB knockout, while knockout of p130 had little effect on both gene groups (Figure 2B) [43]. Similarly, when p53 or p21 (CDKN1A) was expressed in SaOS2 cells, an osteosarcoma cell line with loss-of-function mutations in RB and p53, G2/M but not G1/S genes were downregulated (Figure 2C) [43,72]. While RB has a particularly important role in the regulation of G1/S genes, G2/M genes were more sensitive to the availability of the

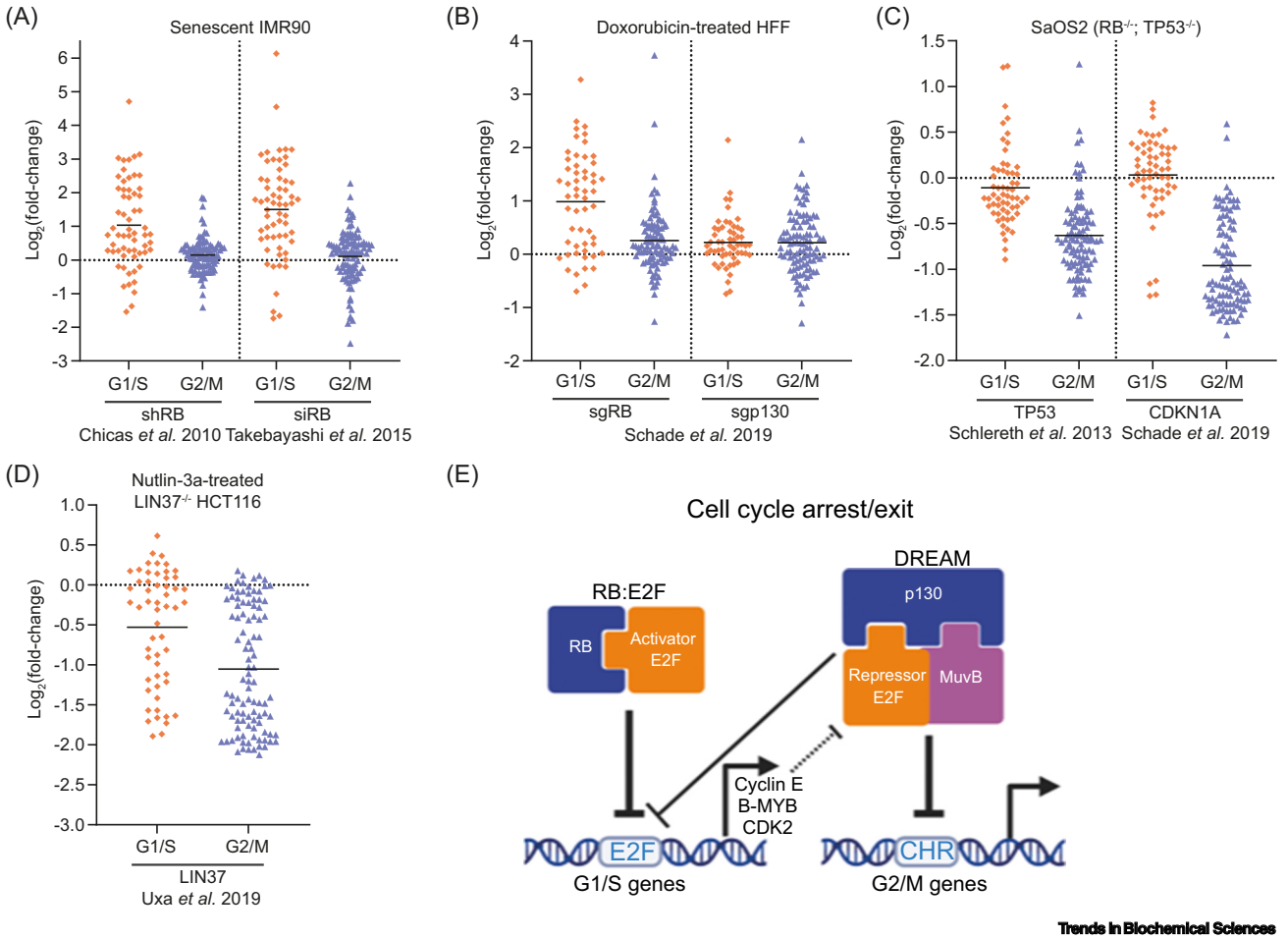

Figure 2. Differential regulation of G1/S and G2/M genes. (A) G1/S but not G2/M genes are upregulated upon RB depletion in senescent IMR90 primary human lung fibroblasts. (B) Upregulation of G1/S genes in response to RB depletion in doxorubicin-treated primary human foreskin fibroblasts (HFF) is more pronounced compared with G2/M genes. (C) G2/M but not G1/S genes are downregulated in response to p53 (TP53) or p21 (CDKN1A) expression in RB and TP53-negative SaOS2 osteosarcoma cells). (D) Downregulation of G2/M genes in response to LIN37 re-expression in LIN37-deficient HCT116 colorectal cancer cells is more pronounced compared with G1/S genes. (A–D) Classification of G1/S and G2/M cell cycle genes taken from [10]. (E) During cell cycle arrest or exit, RB serves principally to repress G1/S genes through E2F DNA motifs, while p130/p107 DREAM complexes repress G2/M genes through CHR motifs. In addition to binding to CHR motifs, DREAM has the ability to bind to E2F motifs and contribute to repressing G1/S genes. Many cell cycle regulators that can destabilize DREAM are encoded by G1/S genes and offer an explanation for how RB can indirectly contribute to the repression of G2/M genes. Abbreviations: CHR, cell cycle genes homology region; DREAM, DP, RB-like, E2F4, and multi-vulval class B or MuvB; M, mitosis; RB, retinoblastoma. Data from [70,71] (A), [43] (B,C), [72] (C), and [64] (D).

DREAM components p130/p107 and LIN37 (Figure 2D) [43,64]; cells lacking both p130 and p107 or LIN37 were unable to repress G2/M genes during cell cycle arrest. Although loss of p130 and p107 did not dysregulate G1/S cell cycle gene expression during cell cycle entry [59], loss of LIN37 partially impaired their repression during quiescence [58]. The DREAM complex does repress G1/S gene expression when RB is lost, although it is not sufficient to fully compensate [58,59]. Distinguishing between effects elicited by RB and DREAM is further complicated not only by possible redundancies in their function at gene promoters, but also by indirect effects caused through their target genes. For instance, RB-controlled G1/S genes comprise multiple potent cell cycle regulators, such as cyclin E and B-MYB, which can mediate further transcriptional consequences [10,43,64]. Together, it has become evident that G1/S genes are primarily repressed by RB:E2F, with contributing effects of DREAM. By contrast, G2/M genes are primarily repressed during G0 by DREAM, with indirect effects possibly elicited through the repression of G1/S genes by RB:E2F [43,64] (Figure 2E). Combined loss of DREAM and RB renders cells unable to shut down cell cycle gene expression, which compromises the G1 checkpoint, resistance to CDK4/6 inhibitors, and cell cycle arrest in response to other growth-restricting conditions [43,58,59,64].

Coordinating G1/S gene expression

The progression of cells from G0/G1 into S phase is largely driven by the expression of the G1/S, E2F-dependent, cell cycle genes. As described previously, in G0, G1/S genes are repressed by RB binding to activator E2Fs as well as by DREAM complex-containing repressor E2Fs binding to E2F DNA recognition elements (Figure 3, quiescence/G0). To relieve this block, mitogen stimulation leads to FOS and JUN expression and their transcriptional activation of cyclin D and subsequent active cyclin D:CDK4/6 complexes [73]. The initial phosphorylation of RB depends on an interaction with cyclin D:CDK4/6 [27,74], and the cyclin D:CDK4 monophosphorylation code controls how RB interacts with its binding partners, enabling specific transcriptional outputs in G1 [75], but the exact mechanisms of distinct monophosphorylation events remain unknown. Monophosphorylation of RB modestly reduces repression of activator E2Fs and G1/S gene expression (Figure 3, early G1).

Concurrently with RB phosphorylation, the DREAM complex is disrupted through phosphorylation of p130 [33] (Figure 3, early G1). DREAM is disrupted in two steps during cell cycle entry, with cyclin D:CDK4/6-driven loss of p130:E2F4 binding preceding loss of p130:MuvB binding [59]. Since DREAM associates with G1/S gene promoters via E2F4, cyclin D:CDK4/6 phosphorylation of p130 results in loss of DREAM complex binding to G1/S genes. The net effect of cyclin D:CDK4/6 activation on G1/S gene expression is the loss of DREAM repression and partial loss of RB repression of activator E2Fs, resulting in an increase in activator E2F-driven G1/S gene expression [59].

G1/S gene expression is directly promoted by the activator E2Fs (E2F1-3a), which bind with their dimerization partner DP1/2 and drive transcription through specific binding to E2F elements [76,77]. In mid G1, activator E2F-driven expression of G1/S genes is further promoted by a positive feedback loop with expression of cyclin E and E2F1-3a. **Cyclin E:CDK2** mediates hyperphosphorylation of RB and p130 and the complete inactivation of the DREAM complex (Figure 3, late G1/early S). Hyperphosphorylated forms of RB and p130 do not interact with E2Fs or MuvB, and p130 is degraded upon ubiquitination by SCF:SKP2 and SCF:cyclin F complexes [78–80]. Furthermore, B-MYB, a component of the MMB:FOXM1 complex, is encoded by a G1/S cell cycle gene and its high expression promotes DREAM complex dissociation [81]. Cyclin E:CDK2 phosphorylation of MuvB components and E2F4/5 may contribute to further DREAM complex disruption in S phase, although their specific roles during cell cycle entry are

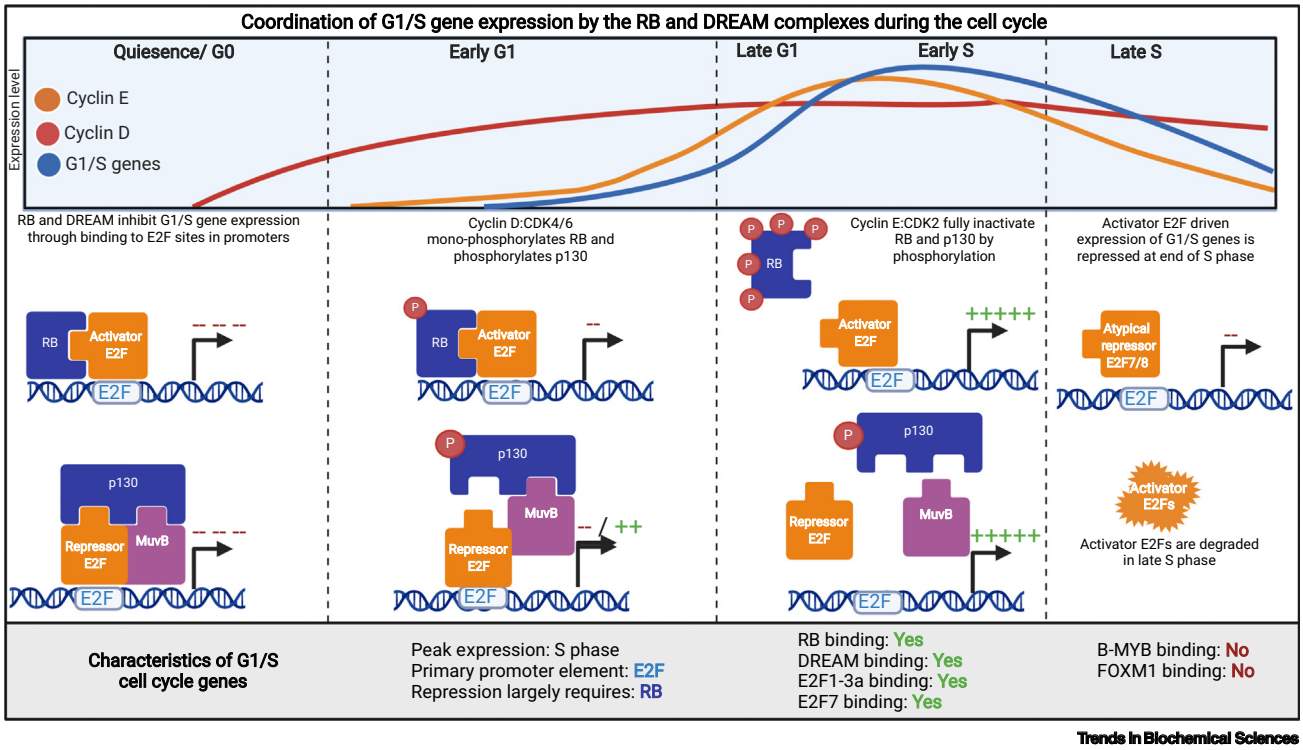

Figure 3. Coordination of G1/S gene expression by RB:E2F and DREAM. In G0, unphosphorylated RB and p130:DREAM repress G1/S gene expression through binding to E2F sites in promoters, resulting in low expression of G1/S genes (left panel). In early G1, increased levels of cyclin D enables cyclin D:CDK4/6 complexes to phosphorylate RB and p130, resulting in modest G1/S gene expression (including cyclin E) and release of p130:MuvB from chromatin (center-left panel). In late G1 and early S phase, cyclin E:CDK2 complexes fully inactivate RB and p130 by phosphorylation, allowing for maximal G1/S gene expression (center-right panel). In late S phase, levels of G1/S genes are reduced when activator E2Fs are degraded and the atypical repressors E2F7 and E2F8 are expressed (right panel). Abbreviations: CDK, cyclin-dependent kinase; DREAM, E2F and DP, RB-like, E2F4, and multi-vulval class B or MuvB; RB, retinoblastoma.

unknown [82–84]. While high levels of B-MYB can disrupt DREAM, this effect may be caused by an accumulation of unphosphorylated LIN52 rather than by a direct competition of B-MYB and p130 for binding to MuvB [81,85].

G1/S gene expression peaks in late G1 to early S phase and tapers off by the end of S phase (Figure 3). Peak expression at this point is necessary for progression through S phase, with a whole class of genes responsible for DNA replication and origin firing [10,86,87]. Activator E2F-driven expression of G1/S genes is repressed during late S and in G2 phases of the cell cycle. Loss of activator E2F activity in S phase is necessary to reset G1/S expression to prevent unscheduled re-entry into the next S phase [88]. During the S/G2 transition, activator E2Fs are marked for degradation by SCF:cyclin F [89,90]. G1/S gene expression is then repressed by E2F7 and E2F8 [88,91,92].

Coordinating G2/M gene expression with cell cycle entry and DNA replication

During quiescence, B-MYB and FOXM1 levels are restricted transcriptionally by RB:E2F and DREAM complexes and post-translationally by protein degradation. This is of particular importance since elevated expression of B-MYB is sufficient to disrupt the DREAM complex [81], and the presence of FOXM1 in G1 promotes entry into S phase [93]. FOXM1 is degraded by the APC/C:FZR1 complex in G1 [93,94], while B-MYB is degraded in confluent cells by pVHL:CUL2 complexes [95] (Figure 4, confluent G0/G1). Receptor tyrosine kinase-dependent

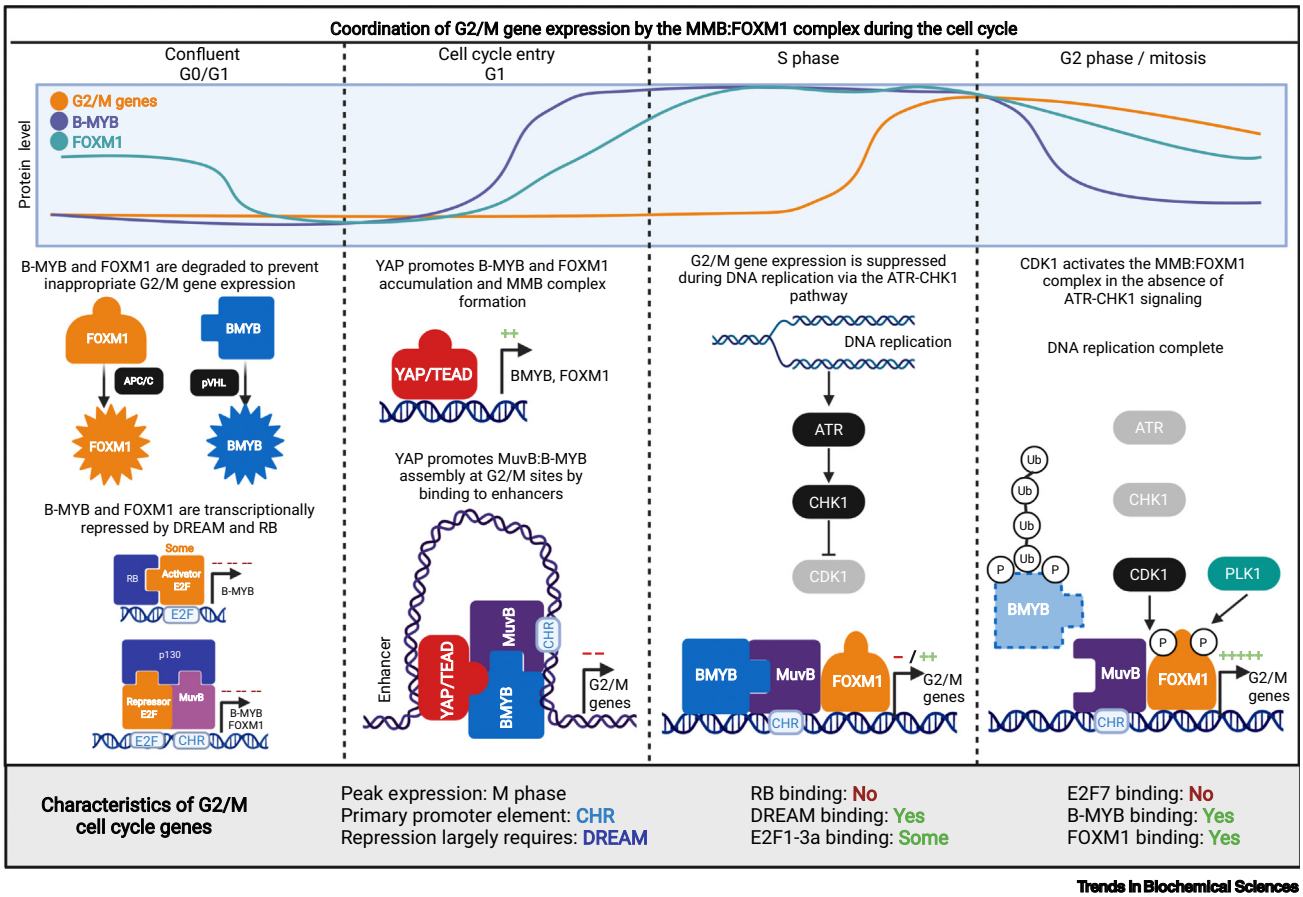

Figure 4. Coordination of G2/M gene expression with cell cycle entry and DNA replication by MMB:FOXM1. In quiescent or confluent cells, G2/M gene expression is repressed by the DREAM complex, and activators B-MYB and FOXM1 are degraded (left). During cell cycle entry, YAP contributes to B-MYB and FOXM1 expression (center left) and facilitates the activation of the MMB complex through enhancer–promoter interactions. G2/M gene expression is suppressed during S phase (center right) by the ATR-CHK1 pathway, restricting CDK1-dependent activation of the MMB:FOXM1 complex. Upon completion of DNA replication and progression in G2 (right), CDK1 and PLK1 activate the MMB:FOXM1 complex, driving up G2/M gene expression, while B-MYB is lost through proteasomal degradation as G2/M gene expression peaks. Abbreviations: CDK1, cyclin-dependent kinase 1; FOXM1, Forkhead box M1; M, mitosis; MMB, MYB:MuvB.

phosphorylation of B-MYB at Y15 blocked degradation by pVHL:CUL2 [95], further linking these degradation events with proliferative signaling.

Recently, the Yes-associated protein 1 (YAP) transcription co-factor was shown to promote B-MYB and FOXM1 gene expression in response to mitogenic signaling (Figure 4, cell cycle entry G1) [96–98]. YAP is normally sequestered in the cytoplasm by Hippo pathway growth-inhibitory signals mediated by confluency and contact arrest. In response to mitogenic signaling, such as EGF signaling, YAP undergoes nuclear translocation and binding to the TEAD1-4 family of TFs [99]. The stimulation of B-MYB and FOXM1 expression by YAP:TEAD complexes may facilitate the accumulation of MMB:FOXM1 in coordination with the disassembly of DREAM by cyclin:CDK activity. In addition, YAP:TEAD physically interacts with MMB and FOXM1 and promotes the expression of an overlapping set of genes with the MMB:FOXM1 complex (Figure 4, cell cycle entry G1) [96–98,100]. In this model, long-range interactions on chromatin are facilitated between YAP:TEAD at enhancers and MMB:FOXM1 at promoters. An interaction between LIN9 and YAP was observed in G1, suggesting a role for YAP:TEAD in promoting MMB complex

formation at G2/M promoters before FOXM1 recruitment during the S/G2 transition. While the specific role of YAP in binding to B-MYB and FOXM1 remains to be deciphered, activation of the YAP-FOXM1 axis in lung cancer contributes to EGFR inhibitor resistance [98] and highlights a functional link between YAP-dependent activation of MMB:FOXM1 and cellular proliferation.

The ATR-CHK1 pathway, as part of the S-M checkpoint (Box 2), coordinates G2/M gene expression with DNA replication to prevent premature entry into mitosis by suppressing a positive feedback loop between MMB:FOXM1 and CDK1 (Figure 4, S phase). Increased ATR-CHK1 activity during S phase restricts CDK1-dependent phosphorylation of FOXM1 at T600 and prevents the premature expression of G2/M genes, including those encoding CDK1, cyclin B1, cyclin A2, and CDC25B [101,102]. Phosphorylation of B-MYB can be detected after ATR and CHK1 inhibition, but the functional consequence of this modification is unknown. Notably, phosphorylation of FOXM1, but not B-MYB, after CHK1 inhibition is impaired in LIN54 knockout cells [102], highlighting the sequential nature of B-MYB and FOXM1 activation, with full activation of FOXM1 requiring an intact MMB complex. MMB:FOXM1 complex activity is required for sensitivity to ATR and CHK1 inhibition in multiple cancer types [102–104]. Inhibition in CCNE1-amplified cells of PKMYT1, a kinase that restricts CDK1 activity by phosphorylating T14 on CDK1 [105], triggers unscheduled activation of cyclinB:CDK1 and entry in mitosis because high cyclin E levels induce high basal levels of MMB:FOXM1 activity [106]. Perturbation of the MMB:FOXM1 complex limits cyclin B1 accumulation in S/G2 and confers resistance to PKMYT1 inhibition [106], suggesting that the CDK-dependent activation of the MMB:FOXM1 complex and subsequent G2/M gene expression have an important role in the transition from DNA replication to mitosis.

CDK-dependent phosphorylation of B-MYB and FOXM1 during S/G2 may facilitate activation of MMB:FOXM1 (Figure 4, G2/mitosis). CCNE1 overexpression promotes B-MYB phosphorylation and G2/M gene expression [106], highlighting the link between CDK phosphorylation and MBM:FOXM1 activity. For B-MYB, phosphorylation at CDK sites facilitates an interaction with PIN1 as well as PLK1-dependent phosphorylation, which promotes gene expression [107]. Similarly, sequential phosphorylation of FOXM1 by CDK1 and PLK1 triggers a conformational change in

Box 2. ATR-CHK1 signaling regulates the G1/S–G2/M transition

The ATR-CHK1 pathway manages the transition from S phase to mitosis as part of the S–M checkpoint by limiting cell cycle progression until DNA replication is complete [116–118]. Similarly, the transition from G1/S to G2/M gene expression needs to be coordinated with DNA replication because expression of G2/M genes, such as cyclin B, can disrupt DNA replication [119]. The ATR-CHK1 pathway coordinates the transition from G1/S gene expression to G2/M gene expression with DNA replication by sustaining E2F activity and restricting MMB-FOXM1 activity during S phase, because inhibition of CHK1 during S phase leads to decreased G1/S and increased G2/M gene expression [101,102]. ATR is recruited to single-stranded DNA structures and activated by interactions with RPA and ETAA1 [101,118]. Once active, ATR proceeds to derepress CHK1 by phosphorylating CHK1 at SQ sites [118,120]. Activation of CHK1 during S phase likely limits E2F1 degradation because CHK1 inhibition led to SCF:cyclin F-dependent degradation of E2F1 [90]. Furthermore, CHK1 limits the repression of E2F activity because CHK1 phosphorylates repressive E2F6 to promote its release from E2F promoters [121] and phosphorylates repressive E2F7/8 to facilitate their sequestration by 14-3-3 [122] when DNA replication is disrupted by hydroxyurea treatment. Thus, the ATR-CHK1 pathway prevents the repression of E2F activity, leading to sustained G1/S gene express during DNA replication.

The ATR-CHK1 pathway restricts G2/M gene expression during DNA replication by suppressing CDK1 activity to prevent activation of a CDK1-MMB:FOXM1 positive feedback loop that drives G2/M gene expression. To limit CDK1 activity, CHK1 phosphorylates WEE1 and CDC25B/C, facilitating interactions with 14-3-3 scaffolding proteins, which leads to the tyrosine phosphatases CDC25B/C being sequestered by 14-3-3 [123] and WEE1 tyrosine kinase activity being promoted by the 14-3-3 interaction [124], resulting in accumulation of the inhibitory phospho-Y15 mark on CDK1. Restriction of CDK1 activity by ATR-CHK1 during DNA replication (see Figure 4 in the main text) prevents premature MMB-FOXM1 activation, as denoted by phospho-T600 FOXM1, G2/M gene expression in S phase, and premature progression in mitosis, even in DNA-replicating cells [101,102], indicating that the ATR-CHK1 pathway suppresses G2/M gene expression and favors G1/S gene expression during DNA replication to regulate the transition from DNA replication to mitosis.

the transactivation domain, allowing FOXM1 to interact with CBP/p300 [108]. Notably, B-MYB phosphorylation during G2 phase coincides or immediately precedes B-MYB proteasomal degradation (Figure 4, G2/mitosis) [45,109]. However, the specific role of B-MYB phosphorylation and degradation in MMB:FOXM1 activity remains to be elucidated.

In general, the role of B-MYB in the MMB complex is not fully understood. Given that B-MYB is degraded as G2/M gene expression peaks [45], it might be that B-MYB has only a limited role in the activation of MuvB target genes and instead mainly functions to cooperate with MuvB to recruit FOXM1 to the G2/M promoters. SIN3B binding to MMB may contribute to repression of G2/M genes until B-MYB is degraded [110]. Furthermore, how G2/M gene expression is coordinated by B-MYB and FOXM1 CDK phosphorylation and YAP signaling has not yet been studied [96–98,100,107,108]. Integration of these signaling pathways by understanding their full impact on the regulation of other MMB:FOXM1 complex components may yield important insights into the coordination of G2/M gene expression by the MMB:FOXM1 complex.

Concluding remarks

Classification of cell cycle genes into two groups, namely G1/S and G2/M, based on their transcriptional expression and regulation profiles, has provided a new perspective that enables a better understanding of cell cycle-dependent gene regulation and distinct functions of transcriptional cell cycle regulators, such as RB and DREAM. While this perspective is a simplification that naturally does not reflect the precise temporal expression of every cell cycle gene, it helps to distill that short DNA recognition sequences, namely the E2F and CHR motifs located proximal to the TSS of the respective genes, determine the regulation of G1/S and G2/M gene groups at large. These promoter elements recruit distinct combinations of TFs that coordinate the temporal expression of G1/S and G2/M cell cycle genes. To quickly retrieve information on whether and how any human gene of interest is regulated by the cell cycle, we established a web-atlas[i] [10,111].

As reflected in this review, the study of cell cycle-dependent gene regulation has largely focused on genes that display maximal expression in early S phase (G1/S genes) or in G2 and mitosis (G2/M genes) and that encode proteins with important functions in DNA synthesis and mitosis, respectively. At the same time, there has been a paucity of research on genes that display maximal expression during quiescence or early G1 phase, which include the genes encoding the CDK inhibitor p27 and cyclin D, which contribute to the regulation of RB and DREAM. We believe that there is a need to better map the genes that are predominantly expressed during G0 and early G1, and to gain deeper insights into the mechanisms that underlie their transcriptional regulation through state-of-the-art transcriptome studies. Although our understanding of G1/S and G2/M gene regulation has deepened considerably over the past two decades, many questions remain (see Outstanding questions). For instance, the many members of the E2F and RB families can form multiple complexes that display overlapping and distinct functions. Although both RB:E2F and DREAM complexes can bind to E2F promoter motifs, we have begun to understand that RB:E2F complexes have a predominant role over the DREAM complex in regulating G1/S genes during senescence or in response to p53 signaling (Figure 2). However, there are multiple different RB:E2F and DREAM complexes, such as RB:E2F1, RB:E2F2, RB:E2F3, p130:DREAM, and p107:DREAM, all of which potentially bind to, and regulate, E2F-containing gene promoters. In addition, the E2F6-containing PRC1.6 complex has been found to regulate gene expression through E2F motifs. It appears likely that these many complexes did not evolve to generate redundancies in the regulation of E2F-responsive genes but rather to serve in specific contexts. Over the next few years, the most challenging questions in the field may concern the identification of the different contexts in which the distinct complexes have their main role.

Outstanding questions

How do DREAM and RB:E2F complexes coordinate their binding to E2F promoter motifs? From a biochemical and steric viewpoint, it is unlikely that they can bind together. Can both complexes reside at the chromatin, leading to an interchangeable binding? What factors affect their binding dynamics? In RB-mutated cancer, do activating E2F1–3 compete with the DREAM complex for binding to the E2F promoter site?

What role does the E2F6-containg PRC1.6 complex have in cell cycle-dependent gene expression? How does it coordinate binding to E2F promoter motifs with RB:E2F and DREAM complexes and what overlapping and distinct roles does PRC1.6 have compared with RB:E2F and DREAM?

Do the p130:DREAM and p107:DREAM complexes have distinct functions?

What additional post-translational modifications and interaction partners regulate the functions and composition of MuvB complexes?

How does FOXM1 get recruited to MMB and what exact role does B-MYB have in this?

What role does B-MYB degradation have in MMB:FOXM1 complex activation? Is it regulated by CDK phosphorylation?

Acknowledgments

This work was supported, in part, by the US Public Health Service grants R35CA232128 and P01CA203655 to J.A.D. and American Cancer Society Postdoctoral Fellowship (PF-22-040-01-ET) to A.S. Funding for open access charge: Leibniz Institute on Aging – Fritz Lipmann Institute (FLI), Jena, Germany. The FLI is a member of the Leibniz Association and is financially supported by the Federal Government of Germany and the State of Thuringia. Figures created with BioRender.com.

Declaration of interests

J.A.D. has received research support from Rain Therapeutics. The remaining authors declare no competing interests.

Resources

[i]www.TargetGeneReg.org

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