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Journal

Oncogene, 41(21)

ISSN

0950-9232

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Publication Date

2022-05-20

DOI

10.1038/s41388-022-02321-x

Peer reviewed

Published in final edited form as:

Oncogene. 2022 May ; 41(21): 2909–2919. doi:10.1038/s41388-022-02321-x.

Structure and Function of MuvB Complexes

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Abstract

Proper progression through the cell-division cycle is critical to normal development and homeostasis and is necessarily misregulated in cancer. The key to cell-cycle regulation is the control of two waves of transcription that occur at the onset of DNA replication (S phase) and mitosis (M phase). MuvB complexes play a central role in the regulation of these genes. When cells are not actively dividing, the MuvB complex DREAM represses G1/S and G2/M genes. Remarkably, MuvB also forms activator complexes together with the oncogenic transcription factors B-MYB and FOXM1 that are required for the expression of the mitotic genes in G2/M. Despite this essential role in the control of cell division and the relationship to cancer, it has not been well understood how MuvB complexes inhibit and stimulate gene expression. We review here recent discoveries of MuvB structure and new molecular interactions, including with nucleosomes and other chromatin-binding proteins, which have led to the first mechanistic models for the biochemical function of the complex.

Keywords

cell cycle; gene expression; transcription; G1/S; G2/M; DREAM; B-MYB; FOXM1

Introduction: Role of MuvB complexes in cell cycle-dependent gene transcription and cancer

Cell cycle-dependent gene expression is central to the coordination of proliferation, cell-cycle arrest, and cell-cycle exit. These processes are essential for development and homeostasis and are misregulated in cancer. Two distinct sets of genes exhibit peak expression in either late G1 and S phase (G1/S genes), or in G2 and mitosis (G2/M genes), respectively (Fig. 1). G1/S genes largely encode for proteins controlling processes connected to DNA synthesis and S phase progression, while the products of G2/M genes

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Author Contributions

G.A.M., A.A., and S.M.R. wrote and edited the manuscript.

Competing Interests

The authors declare no competing interest.

regulate mitosis and cytokinesis [1]. The timed synthesis and degradation of these proteins are essential for proper cell division and are controlled by several layers of regulation: (I) mRNA transcription in G1/S or G2/M, (II) post-translational modifications, and (III) degradation through ubiquitin-dependent pathways. These tightly concerted processes ensure that cells can enter the cell cycle upon sensing mitogenic stimuli, progress unidirectionally through G1, S, G2, and M, and halt or exit the cell cycle in response to growth-limiting signals [2]. Defects in the networks controlling the cell cycle can have dramatic effects on the survival of cells and organisms. Aberrant expression of cell-cycle genes caused by loss of repressors or hyperactivity of activators can stimulate uncontrolled proliferation and can compromise cell-cycle arrest or exit [3, 4]. Such defects represent an important step in oncogenic transformation.

The differential transcription of G1/S and G2/M genes mainly depends on four groups of proteins (Fig. 1) [5, 6]: (I) retinoblastoma family proteins (RB, p107, p130), (II) E2F transcription factors (the activator E2Fs 1–3a, the repressor E2Fs 3b-5, the non-canonical E2Fs 6–8, and the dimerization partners DPs 1/2), (III) proteins forming the MuvB complex (LIN9, LIN37, LIN52, LIN54, and RBAP48), and (IV) the transcriptional activators B-MYB and FOXM1. Both G1/S and G2/M genes are repressed in quiescent cells by the MuvB containing DREAM (DP, RB-like, E2F, and MuvB) complex. DREAM binds E2F promoter sites in G1/S genes and CHR promoter elements in G2/M genes. Expression of G1/S genes can also be suppressed by RB-E2F complexes. When a cell receives mitogenic stimuli, cyclin-dependent kinases (CDKs) are activated and phosphorylate the pocket proteins, which disrupts the repressor complexes. Activation of G1/S genes is induced by E2F1–3a, while expression of G2/M genes is stimulated later in the cell cycle by the MuvB complexes MMB (B-MYB-MuvB) and FOXM1-MuvB (Fig. 1).

In contrast to RB, which has long been known to be inactivated in most cancer types [7], direct evidence for the role of DREAM in cancer development has been found just recently [8–10]. The reasons why RB appears to be a stronger tumor suppressor than DREAM are not yet completely understood, but possibilities include the unique ability of RB to bind and inhibit E2F1–3 and RB functions unrelated to cell-cycle control such as genome maintenance [11, 12]. However, multiple oncogenesis-supporting upstream mechanisms, like the expression of viral oncogenes, overexpression of cyclins, and loss of p53 or CDK inhibitors, interfere with the formation of both RB-E2F and DREAM complexes [5], and only the combined loss of RB and DREAM function results in a complete absence of the G1/S checkpoint [13, 14]. Thus, DREAM has important tumor suppression functions and can at least partially substitute for inactivated RB.

Components of activator MuvB complexes have been frequently associated with cancer formation and progression. FOXM1 had been known to have oncogenic properties long before the interaction with MuvB had been described. FOXM1 is overexpressed in a multitude of cancer types [15], and gene amplification was found in a high percentage of testicular germ cell tumors, high-grade serous ovarian cancer, and basal breast cancer [16]. Likewise, overexpression of the proto-oncoprotein B-MYB promotes the progression of a large number of solid cancers and correlates with poor prognosis [17]. Interestingly, *MYBL2* (encodes for B-MYB) amplification and overexpression are sufficient to disrupt

DREAM, leading to a global de-repression of DREAM target genes [18, 19]. More recently, amplification and overexpression of the MuvB protein LIN9 were connected to breast cancer development, and a high expression of LIN9 correlates with lower relapse-free and overall survival [20, 21]. Furthermore, upregulation of LIN9 together with other MuvB components was shown in esophageal adenocarcinoma [22] and hepatocellular carcinoma [23].

Taken together, increasing evidence supports a central role of MuvB complexes in cancer, which is further highlighted by the observation that many pan-cancer genes connected to poor survival are direct targets of MuvB [24]. While the mechanisms of transcriptional regulation by RB-E2F complexes have been studied extensively, insights into the structure and function of MuvB complexes have emerged just recently. Here, we describe the architecture of MuvB complexes and discuss models for how they repress and activate genes in different phases of the cell cycle.

Structure and architecture of the MuvB core complex

The mammalian MuvB complex consists of the protein subunits LIN9, LIN37, LIN52, LIN54, and RBAP48 (also known as RBBP4) (Table 1). Depending on additional interaction partners, MuvB can form the transcriptional repressor complex DREAM or the activator complexes MMB and FOXM1-MuvB. The overall molecular weight of the MuvB core is ~230kDa, yet only ~57% is predicted to be structured based on sequence analysis. This considerable disorder may account for why high-resolution structural studies of the entire complex have been challenging, and our knowledge to date of subunit structures and how they assemble comes from x-ray crystal structures of subcomplexes and individual domains (Fig. 2A). Using these available data and structure predictions from AlphaFold, we present a model for the overall structure of the complex (Fig. 2B).

Structural and genetic data implicate LIN9 as the central scaffold protein that is essential for assembling the other subunits of the core MuvB complex and for binding additional protein partners [25–29]. The structures of two domains in LIN9 have been determined (Fig. 2A). The sequence from residues 94–278 ($LIN9^{DIRP}$), which has been called the DIRP domain (domain in RB-related pathway) [30], is the domain that binds RBAP48 and LIN37 [25]. A small helical bundle toward the C-terminus of LIN9 ($LIN9^{MybBD}$, residues 333–421) recruits LIN52 into MuvB and directly contacts B-MYB [26, 29].

Our proposed model for the overall architecture of the complex organizes MuvB into two subcomplexes, which we call MuvBN and MuvBC based on the presence of N-terminal or C-terminal fragments of LIN9 (Figs. 2A and 2B). The structural model presented here, which includes predicted unknown structural elements from AlphaFold, supports this organization and reveals potential for the two subcomplexes to associate across different interfaces (Fig. 2B). MuvBN contains $LIN9^{DIRP}$, RBAP48, and LIN37, while MuvBC contains $LIN9^{MybBD}$ and $LIN9^{C}$, LIN52, and LIN54. In the next sections, we detail the known structures within these subcomplexes.

MuvBN

RBAP48 is the only MuvB subunit that has been found in other protein complexes. These complexes, including chromatin assembly factor (CAF-1), Polycomb repressive complex 2 (PCR2), and the nucleosome remodeling and deacetylase (NuRD) complex, all function in chromatin maintenance and regulation of gene expression [31–33]. While knowledge of the function of RBAP48 in these complexes is not complete, it clearly plays an assembly role, interacting with multiple protein subunits, and it also has histone binding activity. RBAP48 is comprised of a WD β-propeller domain, which consists of seven 4-stranded β-sheets, and an N-terminal helix. There are two well-characterized protein binding sites in the domain. One site, which lies across the face of the propeller, notably binds the histone H3 tail [31]. The second site is adjacent to the N-terminal helix alongside the propeller and binds histone H4 [32].

The crystal structure of MuvBN revealed that LIN9 and LIN37 both directly bind RBAP48 in assembling the MuvB complex [25]. Six helices from the LIN9^{DIRP} domain form an extensive interface with RBAP48 focused around the RBAP48 N-terminal helix. LIN9 contacts and blocks the H4 peptide binding site, but the H3 binding site is largely accessible in the MuvBN structure. LIN9 and LIN37 both make several contacts with a long insertion in the first RBAP48 propeller blade that is not ordered in many other structures of RBAP48 complexes. The insertion contains a tyrosine (Y98) that stabilizes a network of interactions involving all three proteins. Notably, this tyrosine is not conserved in the otherwise nearly identical paralog RBAP46/RBBP7, explaining why RBAP46 does not assemble into MuvB [25].

Binding assays in cells and with purified proteins confirm that MuvB binds nucleosomes and that MuvBN is sufficient for this association. The nature of the nucleosome binding interface remains uncertain. While recombinant MuvBN and MuvB both bind histone H3 tail peptides, these complexes also bind reconstituted nucleosomes with histones that lack these tails [25]. This result that H3 tails are sufficient but not necessary for MuvB binding suggests the possibility that MuvB makes multiple interactions with the nucleosome.

The LIN9^{DIRP} domain also contains a Tudor domain. Tudor domains are protein-protein interaction modules commonly found in chromatin-binding proteins, and several Tudor domains bind specific marks in histone tails [34]. While it is tempting to speculate that the LIN9 Tudor binds histone tails, there have been no reports correlating the presence of LIN9 or MuvB to specific chromatin marks. Moreover, the structure of the LIN9 Tudor suggests that the typical histone binding site is occluded and lacks the full complement of aromatic residues that bind methylated lysines or arginines [25].

With respect to structure and function, LIN37 is among the least well-characterized subunits of the MuvB complex. The structure of a small region from residue ~95–126 was determined as part of the MuvBN complex with RBAP48 and LIN9^{DIRP} [25]. This domain, which was named CRAW after the four conserved amino acids, consists of a β-hairpin and two small helices. Notably, the CRAW domain precisely corresponds to the best-conserved sequence in LIN37, and mutations in the region inhibit LIN37 association with the MuvB

core and LIN37 function in gene repression [13, 25]. Although the LIN37 CRAW domain contacts both RBAP48 and LIN9 in the MuvBN structure, the MuvB core and DREAM can still assemble even in the absence of LIN37 [13].

MuvBC

LIN54 is the MuvB subunit that directly binds DNA [35–37]. Chromatin immunoprecipitation and DNA-affinity purification experiments with MuvB in cell extracts revealed association with a DNA sequence motif, named the cell-cycle genes homology region (CHR), which appears in the promoters of a large set of genes that show maximum expression in G2/M [28, 38, 39]. The DNA consensus motif bound by LIN54 was further defined by affinity measurements with purified protein as TT(C/T)(G/A)AA [35]. The LIN54 DNA binding domain (DBD) is comprised of two tandem Tesmin or CxC subdomains; each subdomain uses 9 cysteines to coordinate 3 Zn atoms critical for their fold. The crystal structure of the DBD with a consensus DNA oligonucleotide reveals that the two subdomains bind along the same side of the DNA helix [35]. Each subdomain interacts with half the consensus primarily through insertion of a highly conserved tyrosine into the minor groove. Point mutations introduced into the CxC domain that abolish DNA binding increased cytoplasmic localization, suggesting that DNA binding is essential for nuclear retention of LIN54 [37].

Beyond the DBD, the structure of LIN54 has been poorly characterized. The N-terminal ~500 amino acids are not well conserved and are predicted to be disordered (Fig. 2B). Two nuclear localization signals were shown to cooperate in nuclear import (NLS1: KKPR 231–234, NLS2: RPRK 520–523) [37]. In contrast, the sequence C-terminal to the DBD is more conserved and has predicted structure. We suspect this region is used by LIN54 to associate with the other subunits in MuvBC (see $LIN54^{C}$ in Fig. 2). This idea is supported by the recent finding that a LIN54 truncation missing the C-terminal 100 amino acids cannot compensate for loss of LIN54 in embryonic stem cells [40]. Interestingly, LIN54-related proteins also play an essential role in the germline of multiple organisms. LIN54/Mip120 is essential for oogenesis in *Drosophila* [41], and another CxC domain-containing protein - tombola - has been identified as a component of the MuvB-related tMAC complex and is essential for spermatogenesis [42, 43]. More recently, it was shown that loss of Tesmin, a testis-specific paralog of LIN54, leads to meiotic arrest during spermatogenesis and infertility in mice, suggesting that it may have comparable functions to LIN54 in the meiotic cell cycle and potentially forms MuvB-related complexes [44]. Indeed, Tesmin interacts with LIN9 through the C-terminal 443–475 residues, and knock-in mice expressing a truncated Tesmin mutant that cannot interact with LIN9 phenocopy the defects observed in Tesmin knockout mice [45].

The LIN52 subunit appears most critical for organizing and regulating MuvB interactions with other transcription factors [26, 46, 47]. The C-terminus of LIN52 ($LIN52^{MybBD}$) associates with the $LIN9^{MybBD}$ to form what we predict is the core of the MuvBC subcomplex [26]. The $LIN9^{MybBD}$-$LIN52^{MybBD}$ heterodimer structure consists of an antiparallel three-helix bundle with one helix from LIN52 and two from LIN9 (Fig. 2). The bundle forms the binding site for B-MYB – an interaction essential for the assembly

of the MMB complex [26, 48]. A short sequence within the B-MYB C-terminus (residues 657–688) folds into two short helices and makes critical interactions with both LIN9 and LIN52 [26, 48, 49]. LIN52 is also the MuvB subunit responsible for assembling MuvB into the DREAM complex. Phosphorylation of LIN52 at Ser28 by the kinase DYRK1A induces a direct association between the disordered N-terminus and the pocket domain of p107 and p130 [46, 47]. Near Ser28, LIN52 contains an LxSxE sequence (residues 18–24) that is a suboptimal version of a well-characterized RB-family binding motif, present in a large number of viral and cellular proteins, known as the LxCxE motif. The weaker affinity of the LxSxE interaction sensitizes the association such that phosphorylation can act as a switch for complex formation. Moreover, the absence of the phosphate-binding pocket in RB explains why p130 and p107 but not RB are found in DREAM complexes [28, 46].

A remarkable feature of MuvB is that it functions in both gene repression and activation. In mammalian cells, these opposing activities are distinguished by the timing in the cell cycle, with repression occurring during quiescence and activation occurring in S/G2/M, and by which transcription factors are associated with the core MuvB complex (Fig. 1). We describe in the next sections the biochemical and genetic evidence supporting the distinct roles of core MuvB proteins and the larger DREAM and MMB complexes in the regulation of transcription.

The DREAM complex represses transcription of G1/S and G2/M genes

The identification of DREAM as a transcription repressor followed from the initial discoveries in multiple species that the complex contains orthologs of the retinoblastoma (RB) tumor suppressor protein, which was a well-known inhibitor of the cell cycle and E2F target gene expression [27, 28, 50–53]. In addition to the MuvB core, the mammalian DREAM complex primarily contains the RB family member p130, although p107 has also been found associated with MuvB, especially under conditions of low abundance of p130 [28, 52–54]. The complex also contains E2F4 or E2F5 (E2F4/5), which are E2F family members primarily associated with gene repression [55]. Together with their dimerization partners DP1 or DP2, E2F4/5 bind to E2F promoter elements to recruit DREAM to G1/S genes [56]. Thus, DREAM can repress G1/S as well as G2/M genes by binding to E2F promoter elements or to CHR sites via LIN54 (Fig. 1) [57].

Deletion or knockdown of the DREAM-specific protein components in various animal and cell models leads to increased expression of known DREAM target cell-cycle genes. For example, in mammalian cells, p107 and p130 deletion causes defects in cell-cycle gene repression in response to serum starvation or DNA damage [14, 58, 59]. Specific disruption of DREAM by breaking the interaction between the pocket protein and LIN52 also results in loss of repression of target genes [54, 60]. In mice, this DREAM disruption leads to bone development defects caused by the failure of chondrocyte cells to arrest [54]. This phenotype is similar to that observed in a p107 and p130 double knockout model, suggesting a significant part of p107 and p130 function is via their role in DREAM [61].

Loss of RBAP48 in flies and LIN37 in human and mouse cells has no effect on the expression of G2/M genes, but results in a specific defect in the repression of DREAM

target genes [13, 62–64]. Mammalian cells in which LIN37 is knocked out are deficient in repressing cell-cycle genes upon serum starvation or activation of the p53-p21 pathway in a manner that resembles cells depleted of p107 and p130 [13, 63]. Notably, re-expression of LIN37 with specific mutations in the CRAW domain fails to rescue the repression defect compared to re-expression of WT LIN37 [13]. Integrating these genetic data with the structural model for MuvB, we propose that the MuvBN subcomplex plays a critical role in MuvB core repressive function within DREAM but is dispensable for the activation of genes in the context of activating MuvB complexes.

In contrast to RBAP48 and LIN37, the MuvB subunits LIN52, LIN54, and LIN9 are critical for both gene repression and activation, which is due to their roles in recruiting transcription factors such as B-MYB, in binding DNA, and in providing an overall scaffold for the core complex. When LIN9 is knocked-down and cells are arrested through serum starvation, they show defects in cell-cycle gene repression, which reflects the loss of DREAM activity [28]. However, knockout of LIN9 in mice leads to an embryonic lethal phenotype that results from defective inner cell mass proliferation and that is similar to the phenotype observed from deletion of B-MYB [65, 66]. Also similar to observations made for B-MYB, cells lacking LIN9 show proliferation defects and decreased expression of G2/M genes [28, 29, 65, 67]. A requirement of functional LIN54 and LIN52 for proliferation and mitotic gene expression has also been observed [37, 68, 69]. We interpret these results as reflecting the important role of the LIN9, LIN52, and LIN54 subunits in the formation and activity of the MMB and FOXM1-MuvB complexes, which we discuss next.

The MMB and FOXM1-MuvB complexes activate transcription of G2/M genes

The B-MYB-MuvB (MMB) complex is required for proliferation and is essential for activation of the mitotic transcription program during G2/M [29, 52, 53, 70–72]. The specific biochemical activities of B-MYB and MuvB in this complex are still being understood. Like all members of the MYB protein family (A-MYB, B-MYB, C-MYB), B-MYB contains a DNA binding domain (DBD). *In vitro* DNA-binding assays have shown that all MYB proteins can contact DNA via MYB binding sites (MBS), which contain the minimal sequence (C/T)AAC(G/T)G, and overexpression can activate the same reporter constructs [73]. However, the groups of genes regulated by the three members are largely different [74]. Several key amino acids sequences in the MuvB-binding domain of B-MYB are not conserved in A-MYB or C-MYB, and only B-MYB makes a high affinity interaction with MuvB *in vitro* [26]. B-MYB, but not A-MYB or C-MYB, could be co-immunoprecipitated with LIN9 [53], although proteomic analysis by mass spectrometry detected minor populations of A-MYB-MuvB complexes [28]. Chromatin immunoprecipitation experiments demonstrate that B-MYB binding to G2/M gene promoters correlates with the presence of the CHR element and that B-MYB association with these genes requires MuvB [39, 70]. Furthermore, the CHR element is sufficient for MMB binding to DNA probes, and evolutionary conserved CHRs are highly enriched in MMB target genes [39]. These data suggest that MuvB recruits B-MYB to CHR sites within cell-cycle gene promoters, although some of these promoters do contain B-MYB

recognition elements [75]. Conversely, B-MYB knockdown led to loss of MuvB from two G2/M gene promoters [70], which supports a model that binding of MMB to CHR sites may be stabilized by an interaction of B-MYB with MBSs or non-sequence-specific DNA.

Interactions with additional transcription factors have an important role in gene activation by the MMB complex. It was recently shown that the transcriptional co-activator YAP cooperates with MMB to activate a set of mitotic genes [76–78]. YAP together with TEAD binds to distant enhancer sites and through interaction via DNA looping, increases the stability of the MMB complex on promoters [76]. Evidence implicates a direct interaction between the WW domain in YAP and a region between aa 80–241 containing a PPxY motif in B-MYB as critical for the YAP-MMB complex association [77]. In a potentially overlapping but indirect mechanism, YAP induces expression of the kinase UHMK1, which interacts with B-MYB to increase its nuclear localization [78]. Furthermore, YAP stimulates the expression of both B-MYB and another critical interaction partner of MMB: the oncogenic transcription factor FOXM1 [76, 79, 80].

FOXM1 binding to MuvB is essential for expression of late cell-cycle genes during G2/M [70, 81–83]. The structure, function, and regulation of FOXM1 is remarkably similar to B-MYB. Both proteins regulate the expression of a similar set of mitotic genes [84]. FOXM1 contains a transactivation domain and a regulatory domain that like B-MYB is activated through CDK and PLK1 phosphorylation [85, 86]. Also similar to B-MYB, FOXM1 contains its own DNA binding domain, yet binding to G2/M gene promoters highly correlates with CHR sequences and not with canonical forkhead binding sites [70, 81]. These data suggest that like B-MYB, FOXM1 is recruited to promoters through MuvB. However, a subset of G2/M genes showed reduced promoter occupancy and gene activity when FOXM1 mutants with a non-functional DBD were expressed, suggesting that FOXM1-DNA interactions may stabilize FOXM1-MuvB binding to CHR sites in a non-sequence specific manner [87].

Structural details regarding how FOXM1 binds MuvB are not known, and there has been no reported reconstitution of the complex *in vitro* from purified proteins. In one report, FOXM1 binds LIN9 directly [22], but others have found that B-MYB is required for association of FOXM1 with MuvB and binding of all the complex components to DNA [70, 81, 82].

Switching between repressor and activator MuvB complexes

The formation of repressive and activating MuvB complexes is tightly connected to cell-cycle entry, progression, and exit. Blocking of cell-cycle gene expression by DREAM and E2F-RB complexes is a prerequisite for cell-cycle arrest [13, 14, 54, 59, 63]. Formation of DREAM occurs in the presence of hypo- or unphosphorylated p130/p107 [46, 53, 59] and LIN52 phosphorylated at S28 by DYRK1A [47]. Phosphorylation of the RB-related proteins is inhibited when CDK-cyclin complexes are inactivated, either by the up-regulation of CDK inhibitors of the CIP/KIP and INK4 families or by a reduced expression of cyclins [88]. Furthermore, inactivating phosphates can be removed from p130/p107 by PP2A [89]. The phosphatase dephosphorylating LIN52 is unknown. CDK-dependent phosphorylation of p130/p107 disrupts DREAM in G1 [46, 53, 59], and this mechanism can be supported and

maintained by binding of the G1/S protein PAF to MuvB [8], and by a ubiquitin-dependent degradation of p130 [90, 91]. Furthermore, viral oncoproteins like HPV E7 compete with LIN52 for binding to the LxCxE binding cleft of p130/p107 to disrupt DREAM and stimulate cell proliferation [92, 93].

The sequential formation of MMB, MMB-FOXM1, and FOXM1-MuvB activates mitotic genes during G2/M [70]. MMB forms when DREAM is disrupted and B-MYB expression is induced in late G1. FOXM1 synthesis begins in S phase, but its levels are kept low until G2/M by DCAF1-mediated degradation [94]. B-MYB and FOXM1 are both DREAM targets [13, 63], which suggests that loss of DREAM repression stimulates subsequent activator complex formation. B-MYB is phosphorylated by CDK2 and PLK1, ubiquitinylated, and degraded by the proteasome in G2 [95, 96]. FOXM1 first associates with MMB, and following B-MYB loss from the complex, FOXM1 binding to MuvB persists and coincides with maximum expression of G2/M genes [70]. FOXM1 is finally degraded in a ubiquitin-dependent manner during exit from mitosis [97, 98]. DREAM reforms at the beginning of G1 or during cell-cycle exit when CDK activity decreases.

Although DREAM and MMB are thought of as distinct complexes in mammalian cells, p130 or p107 (p130/p107) and B-MYB can simultaneously associate with MuvB *in vitro,* and p130 can immunoprecipitate with ectopically expressed B-MYB [26]. Furthermore, p107 can bind to MuvB during S phase independent of E2F4/5 [52], and direct interactions between B-MYB and p107 have been shown [99, 100]. Indeed, DREAM complexes with MYB have been observed in flies [50, 51], but the functions of MuvB complexes potentially containing pocket proteins and B-MYB simultaneously in mammalian cells remains to be elucidated.

Models for MuvB function in repressing and activating target genes

We propose several mechanistic models to account for how MuvB regulates gene expression that integrate the knowledge of MuvB genetics, structure, and known interaction partners reviewed above (Fig. 3). One well-defined function of MuvB is that it serves as a platform in the assembly of transcription factors, co-activators, and co-repressors near the transcription start site (TSS) of cell-cycle genes. It is notable that the chromatin association of both repressive (E2F4-p130) and activating (B-MYB, FOXM1) transcription factors require association with MuvB. Because MuvB is the factor that binds the CHR element present in cell-cycle gene promoters, it is essential for recruiting the needed transcription factor at the proper time in the cell cycle. Additional MuvB-binding proteins have been identified (Table 1) and are important to consider toward developing models for how MuvB regulates transcription. While none of the core MuvB components have known repressive enzymatic activity, there have been reports that such enzymatic activities are recruited to promoters. Recruitment of co-activators and repressors likely plays an important role in MuvB-dependent regulation of transcription (Fig. 3).

There are several potential mechanisms by which p130/p107-E2F4/5, recruited by MuvB to form the DREAM complex, can contribute to gene repression. The presence of the inhibited E2F complex bound at the E2F promoter site is expected to prevent gene

transactivation, and it is also possible that p130/p107 recruits other transcriptional repressive machinery including histone deacetylases [101]. However, association of pocket proteins with co-repressors is thought to be mediated through the LxCxE binding site, and p130/p107 already uses this site to bind LIN52 in assembling DREAM [46]. In fact, mass spectrometry analyses of p130 and E2F4 interacting partners did not robustly detect chromatin modifiers such as HDACs [28, 102]. Although possible, it seems less likely that co-repressor complexes are recruited through the pocket protein, and if so, recruitment occurs through atypical mechanisms. Thus, the main role of the p130/p107-E2F4/E2F5 module that reconciles these data might be to recruit the MuvB complex to E2F sites in G1/S gene promoters. Notably, even G2/M genes that contain CHR sites but no E2F binding sites are derepressed when p130 and p107 are knocked out [14], suggesting that the pocket proteins are either required to stabilize MuvB binding to CHR promoters or for MuvB function in repression. Given that we currently lack evidence that p130 recruits any co-repressors to DREAM, we favor the idea that p130/p107, by bridging to E2F4/5, plays a role in stabilization of promoter binding.

In fact, the only known co-repressor that has been shown to associate with DREAM is recruited independently of p130/p107. SIN3B co-immunoprecipitates with DREAM and MMB, and its depletion results in loss of repression of DREAM target genes in serum-starved T98G cells [103]. SIN3B forms a complex with HDACs, and an increase of activating histone H3 acetylation was detected at two G2/M genes upon SIN3B loss. How SIN3B binds MuvB is not clear, but the facts that the association occurs with both DREAM and MMB and that co-immunoprecipitation is detected in cells lacking pocket proteins suggest that binding occurs through a core MuvB protein. Interestingly, a recent extensive proteomic screen performed in HEK293 cells to identify SIN3A/B interaction partners found RBAP48-containing complexes, but not MuvB, suggesting that the SIN3B-MuvB interaction could be cell type-specific [104].

Other DREAM-associated repressive mechanisms at E2F4 genes in the context of differentiation have been explored. It was found that the histone demethylase KDM5A, which removes activating histone marks, is recruited to E2F4 promoters [105]. While KDM5A can be detected at many cell-cycle gene promoters, it appears that it does not require E2F4/DREAM for recruitment to those promoters and that the repressive effects of KDM5A and DREAM on gene expression are additive [105]. It has also been shown that DREAM also co-localizes in the fly genome with insulator proteins, especially at divergently transcribed genes, further connecting DREAM to mechanisms that repress gene expression through manipulating chromatin architecture [106].

A growing body of evidence suggests that MuvB has intrinsic repressive activity independent of the pocket proteins. Depletion and knockout experiments suggest that the pocket protein-E2F complex and MuvB synergize in repression in *C. elegans* and *Drosophila* [51, 60]. Based on several observations, we have proposed that the association of MuvB with nucleosomes is a critical mechanism underlying repressive function. Direct binding of nucleosomes is mediated by components of MuvBN, including LIN37 and RBAP48, which when deleted lead to specific defects in gene repression [10, 13, 25, 62, 63]. In addition, MuvB-dependent repression of cell-cycle genes in quiescence is correlated with the

presence of a strongly positioned +1 nucleosome directly downstream of the transcription start site [25, 35]. MuvB binds this nucleosome, and other evidence with purified reagents demonstrates that MuvB stabilizes nucleosome incorporation into a reconstituted CHR promoter [25]. Interestingly, an enhancement of nucleosomes downstream of the TSS was also shown for genes binding E2F4 and SIN3B in differentiated myotubes as compared to cycling myoblasts [107]. We envision that this nucleosome stabilization inhibits any remodeling that is required for transcription and that association of MuvB with histone tails may also inhibit modifications by co-activator proteins.

Interactions of MuvB with histones likely play an important part in the mechanisms for how it regulates transcription, even if evidence from the mammalian system is still limited. Histone binding was first detected between nonacetylated H4 peptides and the DREAM complex biochemically purified from fly cells [50]. At some promoters, the association of the fly complex with histones may be mediated by the subunit L3MBT, which colocalizes to promoters with fly MuvB proteins [108]. L3MBT has not been detected in mammalian MuvB complexes [28]. However, the MuvB core complex has histone H3 and nucleosome binding activity through the MuvBN structural unit. Other studies have demonstrated in flies and worms that orthologs of the histone variant H2A.Z are essential for DREAM repression and that H2A.Z is increased in the gene body of DREAM-repressed genes [109, 110]. However, the role of H2A.Z in the function of mammalian DREAM is unclear, particularly considering that this variant is thought to decrease nucleosome stability [111].

The mechanism by which the MMB complex activates transcription remains uncertain (Fig. 3). Like the transactivation domains of other transcription factors such as E2F or FOXM1, the B-MYB TAD binds co-activator histone acetyltransferases and drives gene expression when B-MYB is overexpressed [112, 113]. However, the role of the B-MYB TAD in the context of MMB complex activity remains uncharacterized. Much of the initial genetic evidence in model organisms suggests that B-MYB antagonizes MuvB repressor function to relieve gene repression in addition to recruiting other transcription factors like FOXM1 and YAP. In rescue experiments performed in flies, the MuvB-binding domain is necessary and, remarkably, sufficient to rescue viability and gene expression defects observed upon MYB deletion [49]. Deletion of MuvB subunits in flies rescues a MYB deletion phenotype [114, 115], and expression of fly MYB in *C. elegans*, which lacks a known MYB ortholog, results in a synMuvB phenotype similar to MuvB subunit deletion [48]. Together these results support a model in which the core MuvB complex acts as a repressor, and at least part of MYB activity is to function as a MuvB inhibitor that relieves MuvB-dependent gene repression. The evidence described above suggests that the physical association of B-MYB is sufficient to inhibit MuvB repressive activity. One intriguing possibility is that B-MYB association induces a conformational change that results in nucleosome release and loss of co-repressor recruitment (Fig. 3). Such a mechanism would likely entail structural coupling of the LIN9 and LIN52 MYB-binding domains with the MuvBN subcomplex that associates with nucleosomes.

Concluding remarks and important questions

Despite the identification of the DREAM and MMB complexes nearly twenty years ago, we still do not understand definitively the mechanisms for how they regulate transcription of cell-cycle genes. As discussed, our current models for biochemical activity invoke a role of MuvB in influencing chromatin structure at promoters, either directly through nucleosome association or indirectly through recruitment of proteins that are themselves implicated in influencing chromatin architecture for regulation of transcription (Fig. 3). Therefore, a more detailed characterization of the impact of MuvB on the structure of cell-cycle gene promoters and how that promoter structure changes when a gene transitions from a state of repression to activation is needed. A related question is what role histone marks play in the function of MuvB and how its activity may be controlled. The presence of RBAP48 and the LIN9 Tudor domain suggests MuvB acts as a histone reader, and the recruitment of complexes such as SIN3B and transcriptional co-activators via B-MYB and FOXM1 suggests MuvB can indirectly function as a histone writer. However, there have not been studies demonstrating these activities or even correlating the presence of MuvB with specific marks. More broadly, there have been remarkably few studies that correlate cell cycle-dependent gene expression with specific histone marks.

One of the most intriguing unanswered questions surrounding MuvB is what regulatory elements and structural mechanisms change its structure from that of a repressor to an activator upon cell-cycle entry. The fact that MuvB is sufficient for nucleosome binding *in vitro* but that nucleosome binding is detected only under conditions of cell-cycle arrest raises the question of whether MuvB structure changes for gene activation in a manner that nucleosome binding is lost. It may be that B-MYB and/or FOXM1 binding or MuvB phosphorylation induces such a change that leads to loss of MuvB repressor function.

Further understanding the structure, function, and regulation of MuvB ultimately may inform cancer therapeutic discovery. Molecules that arrest the cell cycle, including inhibitors of CDKs and activators of p53, are in the clinic or trials and motivate the discovery of additional cell-cycle regulatory proteins as drug targets [116, 117]. Considering the central role of DREAM and activating MuvB complexes in regulating cell-cycle gene expression, it is tempting to speculate that manipulation of these complexes could be used to control cancer cell proliferation. One likely approach is to identify molecules that disrupt complex formation. Considering that B-MYB activity is essential for cancer cell proliferation and that B-MYB relies on MuvB for proper recruitment to mitotic genes [70], inhibition of B-MYB-MuvB and FOXM1-MuvB formation may be a viable therapeutic approach in cancers that depend on B-MYB overexpression. The interface between B-MYB and the MuvBC complex informs potential design of such inhibitors, and mutagenesis studies in particular point to the interaction of M677 in B-MYB with a hydrophobic pocket formed at the LIN9-LIN52 heterodimerization interface as a hotspot. The interface between the LIN52 N-terminus and p130 may also have potential as a therapeutic target. It has been demonstrated, for example, that inhibition of DREAM formation deters tumor cell dormancy and stimulates apoptosis by activation of cell-cycle genes [118, 119]. While LxCxE-site inhibitors have been reported [120], more work is needed to develop these and identify other compounds. Additional insights into MuvB function and regulation and a better structural

understanding of how MuvB binds protein partners are likely to have significant impact on developing other inhibitor approaches.

Acknowledgments

Research in the authors' laboratory is funded by grants from the National Institutes of Health (R01GM124148, R01GM127707, and R01CA228413 to S.M.R.). Figs. 1 and 3 were created with BioRender.com.

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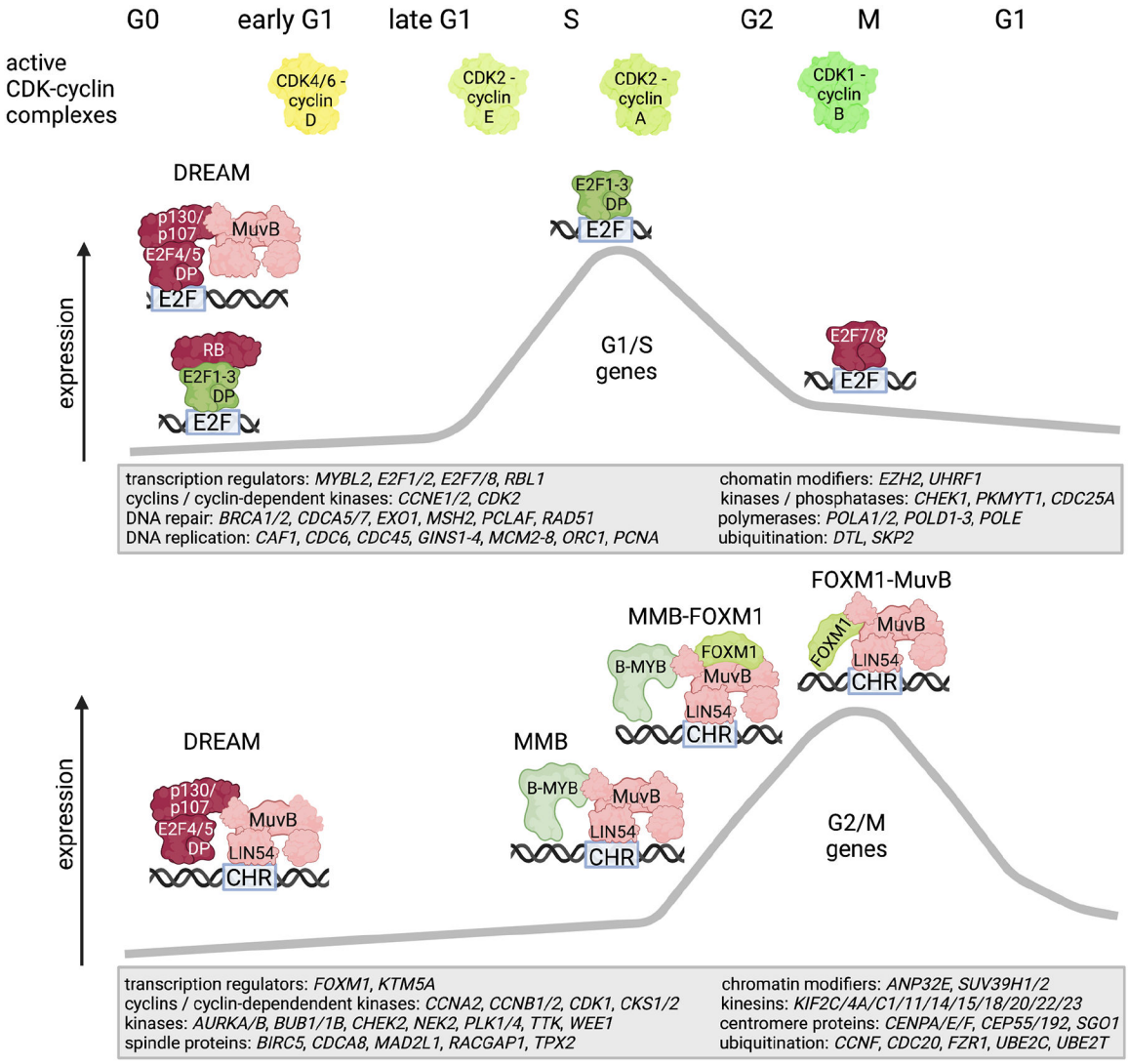

Fig. 1: Regulation of cell-cycle gene expression by MuvB and RB-E2F complexes.

Two sets of genes are maximally expressed in either G1 and S phases (G1/S genes), or in G2 and mitosis (G2/M genes). Central features that discriminate both gene sets are E2F promoter elements in G1/S genes and CHR promoter sites in G2/M genes. The MuvB complex (pink) regulates expression of these genes with other proteins that act as repressors (red) and activators (green) of transcription. The DREAM complex can bind and repress both sets in G0 and early G1 through the interaction of E2F4/5-DP with E2F promoter elements, and of the MuvB component LIN54 with CHR sites, respectively. RB together with the activator E2Fs (E2F1–3a) also bind and repress G1/S genes by contacting E2F promoter sites. When cells enter the cell cycle from G0, CDK-cyclin complexes are activated and phosphorylate RB, p107, and p130, resulting in the disruption of the repressive DREAM and RB-E2F complexes. Expression of G1/S genes is stimulated in late G1 by activator E2Fs that remain bound to E2F sites, with peak expression occurring in S phase. At the end of S phase, activator E2Fs are degraded, and G1/S genes are repressed by the non-canonical E2F7/8. One cell cycle-regulator encoded by a G1/S gene is the transcription

factor B-MYB. B-MYB binds to MuvB to form the MMB complex. This interaction is essential to recruit FOXM1 (MMB-FOXM1). In G2/M, B-MYB is degraded, and maximum expression of genes coincides with binding of the FOXM1-MuvB complex to CHR elements in promoters. Examples of G1/S or G2/M genes that encode for prominent cell-cycle regulatory proteins are given in the grey boxes.

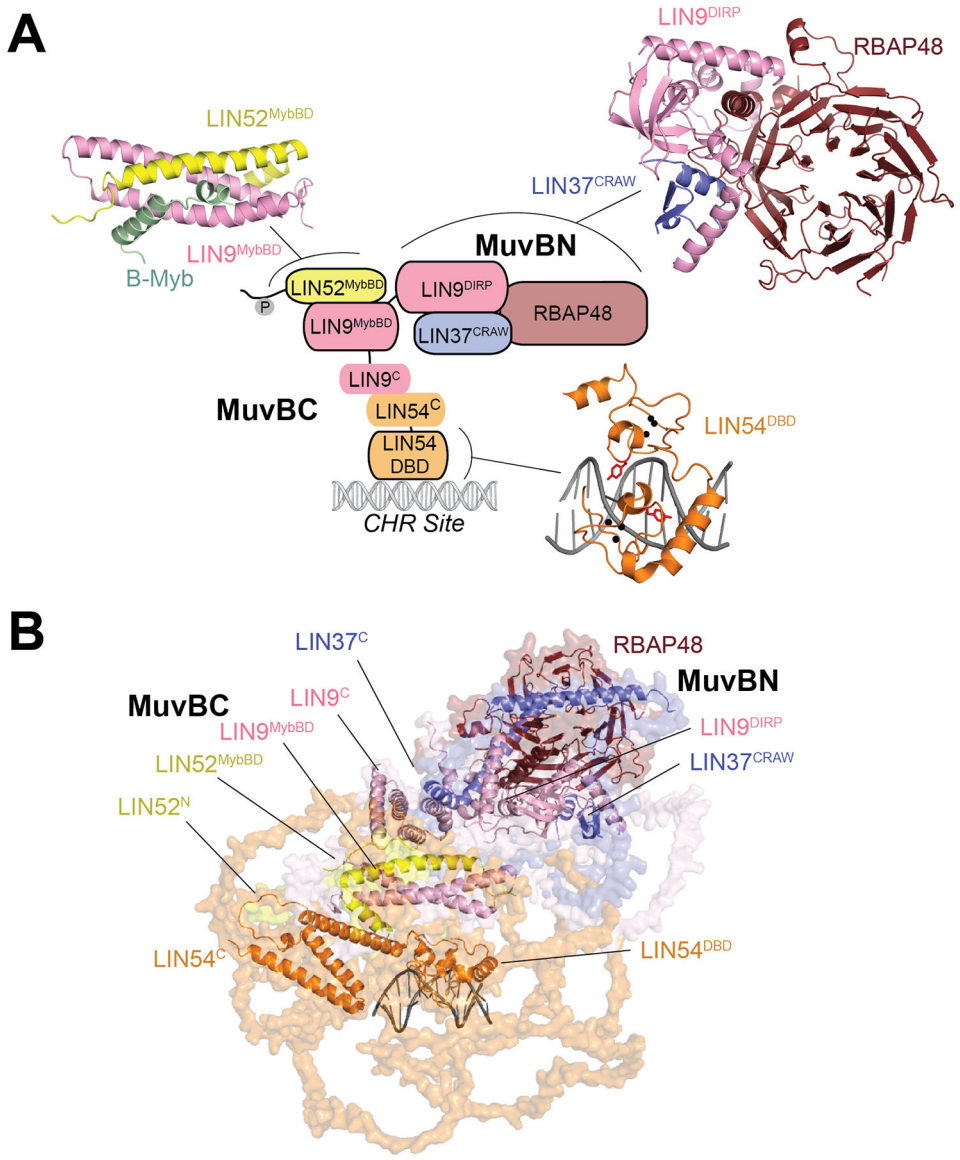

Fig. 2: MuvB structure.

(A) MuvB domain architecture and known domain structures. The PDB codes are 6C48 ($LIN9^{MybBD}$-$LIN9^{MybBD}$-B-Myb), 7N40 ($MuvB^{N}$), and 5FD3 ($LIN54^{DBD}$). (B) Overall model for atomic resolution structure generated by aligning known domain structures with structural predictions from AlphaFold. Regions of predicted disorder are shown with a surface representation only.

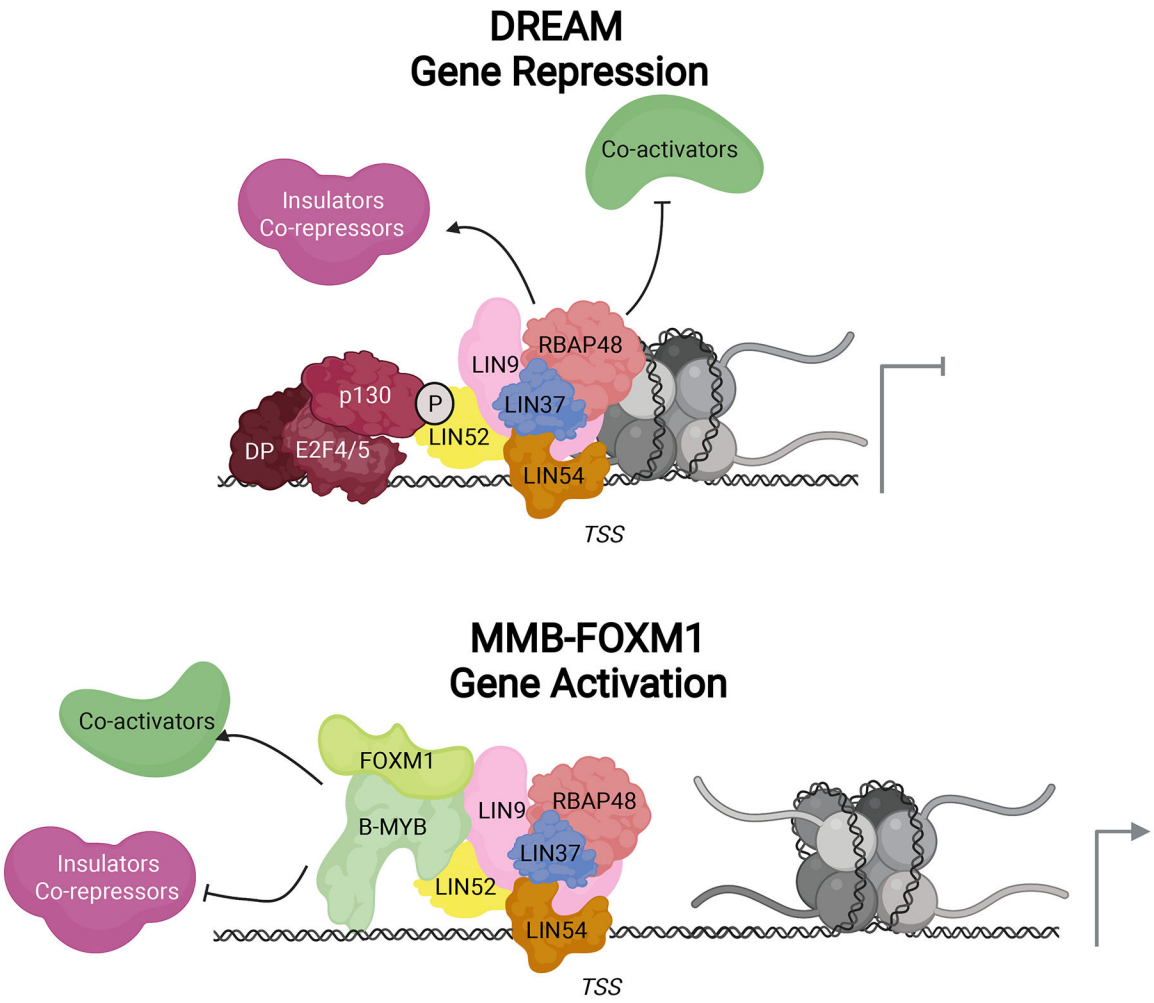

Fig. 3: Model for MuvB function in repression and activation of cell-cycle genes.

DREAM-mediated gene repression occurs in G0 and early G1. MuvB forms a repressive complex near the transcription start site (TSS) with p130/p107, E2F4/5, and DP proteins facilitated by LIN52 phosphorylation by DYRK1A. Through MuvBN, MuvB binds and stabilizes the +1 nucleosome of target gene promoters and may recruit co-repressors and inhibit the activity of co-activators required for gene transcription. In G2/M the sequential binding of B-MYB and later FOXM1 to the MuvB core forms activator MuvB complexes which may prevent binding to the +1 nucleosome, inhibit the activity of co-repressors, and recruit factors that promote gene transcription.

Table 1:

Protein names and functions

Core MuvB Components	
LIN9	Scaffold protein critical for MuvB assembly Required for both activating and repressive functions of MuvB
LIN37	Binds LIN9 and RBAP48 through CRAW domain Repressive function
LIN52	Recruits p107/p130 through phosphorylated N-terminus Recruits B-MYB together with LIN9
LIN54	Binds CHR promoter elements of G2/M genes Assembles with MuvB through C-terminus
RBAP48	Ubiquitous component of chromatin complexes Binds histones
DREAM proteins and other repressors	
p107/p130	Retinoblastoma (RB) protein homologs that bind LIN52 and E2F4/E2F5 Potentially recruit other co-repressors
E2F4/E2F5	Repressor members of the E2F family Bind E2F promoter sites of G1/S genes
DP	Obligate heterodimerization partner of E2F family members (except E2F7/E2F8) Two human paralogs (DP1 and DP2) that promote E2F activity
SIN3B	Recruits histone deacetylase (HDAC) proteins Unclear how assembles with MuvB complexes
Activator Proteins	
B-MYB	Transcription factor that binds MuvB to form MMB complex Activates expression of G2/M genes, likely in part through relieving MuvB repression Interaction with MuvB is essential for recruiting FOXM1
FOXM1	Transcription factor that binds MuvB during S/G2/M phase Promotes maximum expression of G2/M genes
YAP	Transcriptional co-activator that binds MMB Recruited to enhancers by TEAD and activates MMB target genes via DNA looping
Proteins Regulating MuvB Complexes	
CDKs	Ser/Thr kinases that broadly control cell-cycle progression Phosphorylate MuvB components, p107/p130, E2Fs, B-MYB, and FOXM1
PLK1	Ser/Thr kinase that plays crucial roles in mitosis Phosphorylates and activates B-MYB and FOXM1
DYRK1A	Kinase that phosphorylates LIN52 to promote DREAM assembly
PP2A	Ser/Thr phosphatase that dephosphorylates p107/p130 to promote DREAM formation
PAF	Binds RBAP48 and disrupts DREAM