Hierarchical Requirement of SWI/SNF in Retinoblastoma Tumor Suppressor-mediated Repression of Plk1* 

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Plk1 (Polo-like kinase 1) is a critical regulator of cell cycle progression that harbors oncogenic activity and exhibits aberrant expression in multiple tumors. However, the mechanism through which Plk1 expression is regulated has not been extensively studied. Here we demonstrate that Plk1 is a target of the retinoblastoma tumor suppressor (RB) pathway. Activation of RB and related pocket proteins p107/p130 mediate attenuation of Plk1 expression. Conversely, RB loss deregulates the control of Plk1 expression. RB pathway activation resulted in the repression of Plk1 promoter activity, and this action was dependent on the SWI/SNF chromatin remodeling complex. Although SWI/SNF subunits are lost during tumorigenesis and cooperate with RB for transcriptional repression, the mechanism through which SWI/SNF impinges on RB action is unresolved. Therefore, we delineated the requirement of SWI/SNF for three critical facets of Plk1 promoter regulation: transcription factor binding, corepressor binding, and histone modification. We find that E2F4 and pocket protein association with the Plk1 promoter is independent of SWI/SNF. However, these analyses revealed that SWI/SNF is required for histone deacetylation of the Plk1 promoter. The importance of SWI/SNF-dependent histone deacetylation of the Plk1 promoter was evident, because blockade of this event restored Plk1 expression in the presence of active RB. In summary, these data demonstrate that Plk1 is a target of the RB pathway. Moreover, these findings demonstrate a hierarchical role for SWI/SNF in the control of Plk1 promoter activity through histone modification.

Progression through the cell cycle is a carefully choreographed process that is often deregulated in cancer cells (1–3). It is believed that deregulation of proliferation control serves to fuel tumor development and progression. Interplay between cell cycle regulatory proteins is increasingly relevant for understanding the underlying basis of appropriate cell cycle control and the development of aberrant proliferation in cancer. Here we demonstrate a novel regulation of the mitotic Plk1 (Polo-like kinase 1) by the retinoblastoma tumor suppressor (RB) pathway.

Initially identified through homology with the Drosophila polo, Plk1 governs multiple events associated with G1/M progression (4–7). For example, Plk1 is a determinant of mitosis promoting factor, CDK1/cyclin B, which stimulates entry into mitosis (6, 8). Additionally, centrosome duplication and assembly of the mitotic spindle apparatus are regulated through the action of Plk1 (6, 9). Recently, a number of studies have implicated a role for Plk1 in cancer. For example, numerous tumor types (e.g., colorectal cancer, squamous cell carcinomas of the head and neck, and melanoma) aberrantly express Plk1 (10–13). Consistent with a causative role in tumorigenesis, ectopic expression of Plk1 can transform cells in culture (14). Conversely, Plk1 ablation in tumor cells results in mitotic failure and cell death (9, 15–17).

Plk1 expression is repressed in resting cells and induced only as cells progress through G1/S (18, 19). The induction of Plk1 protein levels is largely dependent on the regulation Plk1 promoter activity, and cis-acting elements that modulate cell cycle dependence have been defined (18, 20). Particularly, the Plk1 promoter is subject to transcriptional repression through cell cycle-dependent element/cell cycle-gene homology region elements that are required for the cell cycle dependence of Plk1 promoter activity (20, 21). However, the mechanism through which silencing of Plk1 expression occurs as cells exit the cell cycle and the source of the deregulated expression in tumor cells is unknown.

A critical regulator of G1/S-dependent gene expression is the RB pathway. RB is functionally inactivated in the majority of tumors via a number of discrete mechanisms (1–3, 22). It is believed that RB, in concert with the related pocket proteins p107 and p130, functions as a tumor suppressor through its capacity to repress the transcription of critical targets in a cell cycle-dependent manner and thus prevent proliferation (22, 23). In G0 or early G1 cells, RB and related proteins are hypophosphorylated and form complexes with the E2F family of transcription factors (24–26). The E2F family of transcription factors are involved in the regulation of numerous genes required for cell cycle progression, RB, p107, and p130 mediate transcriptional repression and subsequent attenuation of E2F-regulated genes by recruiting additional corepressors (e.g., histone deacetylases) that modify chromatin structure (25–27). Repression is alleviated when RB family members are phos-
phorylated by CDK complexes in mid G1, thus enabling progression through the cell cycle (28). Transcriptional repression is viewed as requisite for RB function in tumor suppression based on genetic and biochemical data (29, 30). Additionally, we and others have found that the loss of SWI/SNF chromatin remodeling factors compromises RB-mediated transcriptional repression (31–34).

SWI/SNF is a heterogeneous multisubunit chromatin remodeling complex (35, 36). This complex utilizes the energy of ATP to remodel chromatin structure and contains either BRG1 or BRM as the central ATPase (36). The activity of the core ATPase subunit is required by the SWI/SNF complex to regulate gene transcription (35, 37, 38). Prior studies have demonstrated that the combined losses of BRG1 and BRM result in resistance to the activation of the RB pathway and aberrant cell cycle progression (32, 33). Additionally, the loss of SWI/SNF activity is associated with a failure of RB to elicit transcriptional repression of specific targets (e.g. cyclin A). Based on studies in yeast, the loss of SWI/SNF could disrupt virtually any step associated with transcriptional repression (35, 38). For example, SWI/SNF could be required for the assembly of E2F proteins at promoter, for the retention of RB or related proteins at the promoter, or for subsequent modifications of the promoter. However, the mechanism through which SWI/SNF cooperates with RB for transcriptional repression is not understood.

In this study, we specifically focused on elucidating the regulation of Plk1 expression. We show that Plk1 is repressed via activation of the RB pathway and that Plk1 expression is deregulated through targeted RB loss. Moreover, we demonstrate that the repression of Plk1 by RB is dependent on SWI/SNF activity. Analysis of SWI/SNF function demonstrates that chromatin remodeling is not required for the association of E2F or RB family members at the Plk1 promoter. In contrast, histone deacetylation of the Plk1 promoter was dependent on SWI/SNF and critical for the observed transcriptional repression. Thus, this study provides critical insight into the mechanism through which Plk1 transcription is regulated and demonstrates the intricate relationship between SWI/SNF and histone deacetylases during RB-mediated transcriptional repression.

**MATERIALS AND METHODS**

**Cell Culture, Plasmids, Infections, and Transfections—**SW13, TSUPR-1, U2OS, and A5-1 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 units of penicillin-streptomycin, 2 mM L-glutamine at 37 °C in 5% CO2. In addition, A5-1 cells were maintained in G418 (200 μg/ml), hygromycin B (200 μg/ml), and doxycycline (Dox, 1 μg/ml). Primary RbloxP/loxP mouse adult fibroblasts (MAFs) were isolated from RbloxP/loxP mice (39). The cells were propagated by routine subculturing in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum supplemented with 100 units/ml penicillin-streptomycin and 2 mM L-glutamine. Primary cultures were between passage 2 and 6. Plasmids encoding β-gal, p16ink4a, PSM-RB, and BRG1 have been previously described (20, 31, 40). Adenovirus encoding GFP and p16ink4a, PSM-RB, and BRG1 have been previously described (20, 31, 44). However, the mechanisms through which Plk1 expression is controlled or deregulated in humans cancers are poorly understood. In a microarray screen we identified Plk1 as a target for RB-mediated repression (45). Therefore, we initially determined the activity of the RB pathway on Plk1 protein levels (Fig. 1). To activate endogenous RB (and the related proteins p107 and p130), U2OS cells were infected with adenoviruses encoding either GFP (control) or p16ink4a, which prevents their phosphorylation by inhibiting CDK4 activity. As expected, the expression of p16ink4a resulted in RB dephosphorylation (Fig. 1A), indicating endogenous RB pathway activation. Plk1 protein levels were significantly attenuated in those cells infected with p16ink4a encoding adenovirus, as compared with cells infected with the GFP encoding virus (Fig. 1A).

To determine whether targeted RB loss, such as occurs in cancer, influences Plk1 expression, conditional knockout of the Rb gene was employed. MAFs of the RbloxP/loxP genotype were infected with recombinant adenoviruses encoding either GFP or Cre-recombinase. In this system, the endogenous Rb locus is subject to recombination through the expression of the Cre-recombinase, and RB protein expression is ablated (not shown). Under this condition, we find that Plk1 protein levels were elevated following RB loss (Fig. 1B, compare lanes 1 and 2). Similarly, by microarray analysis we observe a 2.4-fold elevation in Plk1 RNA levels with RB loss. Thus, endogenous RB serves to maintain the appropriate levels of Plk1. To determine the subsequent action of endogenous RB on attenuating Plk1 protein levels under physiological stress, we examined the expression of Plk1 following exposure to camptothecin (CPT). CPT induces an RB-dependent checkpoint response, wherein specifically those cells deficient in RB (Cre-infected) continue cell cycle progression in the presence of CPT (Fig. 1C). Consistent with protein analyses, MAFs deficient in RB showed upregulation of Plk1 RNA levels compared with MAFs harboring RB (Fig. 1D, compare lanes 1 and 2). Treatment with CPT resulted in the repression of Plk1 RNA levels in RB-proficient MAFs (Fig. 1D, compare lanes 1 and 2). However, Cre-mediated ablation of RB largely relieved the repression of Plk1 transcription following treatment with CPT (Fig. 1D, compare lanes 2 and 4). These observations were further supported by analysis of Plk1 protein levels (Fig. 1E, compare lanes 2 and 4). Collectively, these results demonstrate that Plk1 is a target of the RB pathway, and specific RB loss results in deregulation of this critical target.

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RB-mediated Repression of Plk1 Is Compromised in SWI/SNF-deficient Cells—Having established that Plk1 protein levels are modulated by the RB pathway, we next sought to elucidate the mechanism of this regulation. Because SWI/SNF activity is compromised in specific cancers (33, 46) and known to be required for repression of selected RB target genes (e.g. cyclin A) (31, 34), the action of SWI/SNF in Plk1 regulation was investigated. The SW13 cell line does not express the BRG1 and BRM ATPases requisite for SWI/SNF activity, whereas TSUPr-1 cells express BRM and are sensitive to RB-mediated signaling (32). To activate the endogenous RB pathway, adenoviral transduction of p16ink4a that maintains RB in its hypophosphorylated/active state was utilized. As shown, expression of p16ink4a led to the dephosphorylation of RB and related proteins p107 and p130 in both cell types (Fig. 2A). To determine the coordinate action of the RB pathway and SWI/SNF upon Plk1 expression, we initially analyzed Plk1 promoter activity. SW13 cells and TSUPr-1 cells were cotransfected with Plk1 reporter plasmid and either vector control or p16ink4a-encoding plasmids (Fig. 2B). p16ink4a expression potently repressed Plk1 promoter activity in TSUPr-1 cells. In contrast, p16ink4a failed to repress Plk1 in the SW13 cell line. Consistent with the reporter assays, we observed attenuation of endogenous RNA of Plk1 in TSUPr-1 cells infected with p16ink4a (Fig. 2C, compare lanes 3 and 4). As expected, expression of GAPDH did not change in either of these cell lines even when infected with p16ink4a. In contrast, p16ink4a failed to attenuate endogenous RNA levels of Plk1 in SW13 cells (Fig. 2C, compare lanes 1 and 2). The SWI/SNF-dependent reduction in promoter activity and RNA levels were reflected in the specific attenuation of Plk1 protein levels by p16ink4a infection in TSUPr-1 cells (Fig. 2D). Taken together, these results indicate that SWI/SNF activity is critical for RB pathway-mediated repression of Plk1. To confirm this observation, BRG1 expression was restored in SW13 cells in combination with p16ink4a, and Plk1 promoter activity was analyzed through reporter analysis (Fig. 2E). However, cotransfection of BRG1 and p16ink4a significantly repressed Plk1 promoter activity in the BRG1/BRM-deficient SW13 cells. Collectively, these data demonstrate that SWI/SNF activity is required for RB-mediated repression of the Plk1 promoter and attenuation of its RNA and protein levels.

SWI/SNF Is Dispensable for the Assembly of E2F or Pocket Proteins at the Plk1 Promoter—Failure of the RB pathway to repress Plk1 in the absence of SWI/SNF activity could be attributed to multiple functions of this complex. In yeast, it has been shown that SWI/SNF activity is required for the assembly...
of transcription factor complexes on chromatin (38, 47). Therefore, SWI/SNF loss could bypass RB-mediated repression through two basic mechanisms. First, SWI/SNF could be required for E2F action. Specifically, SWI/SNF could be required for the expression of E2F family members or for E2F-chromatin interactions that are required for RB pathway-mediated repression (48). Second, SWI/SNF could be required for pocket proteins to associate with E2F factors on target promoters.

Initially, we probed the action of SWI/SNF directly on E2F proteins. The E2F family is broadly defined as activating (E2F1–E2F3) or repressive (E2F4 and E2F5) E2Fs based on their predominant transcripational role (49). Immunoblot analyses demonstrated that both SW13 and TSUPr-1 cells expressed similar levels of E2F1 (“activating E2F”) and E2F4 proteins (“repressive E2F”) (Fig. 3A). This result is consistent with microarray analyses that did not identify E2F family members as targets of SWI/SNF (35) and suggests that limitation of E2F expression does not underlie the resistance to RB pathway activation in SW13 cells. Therefore, several approaches were utilized to subsequently delineate the role of SWI/SNF on the functional interaction of E2F with chromatin. Initially, we determined whether E2F proteins were compromised for transcriptional activation, because the RB pathway is compromised for transcriptional repression. To perform these analyses we used a synthetic promoter composed of multimerized E2F sites (3xE2F-Luc), wherein promoter activity is dependent on E2F binding for activity. Endogenous E2F activity was readily detected with this reporter (Fig. 3B). Additionally, ectopic expression of E2F2 clearly activated the reporter (3-fold) in the absence of SWI/SNF (Fig. 3B). These results suggest that E2F binding to a simple promoter element and stimulation of transcription is independent of SWI/SNF action.

Because transcription factors interact with chromatin in a dynamic fashion, we assessed the action of SWI/SNF on E2F2 retention in living cells. In the case of E2F proteins, we have previously shown that the diffusion rate of these proteins in living cells is dependent on chromatin association (50). Here we constructed an expression vector encoding enhanced GFP fused to the N terminus of human E2F2 (GFP-E2F2). Expression of the GFP-E2F2 fusion protein was verified by immunoblotting (Fig. 3C). Additionally, the GFP-E2F2 construct efficiently stimulated transcription from the 3xE2F-Luc reporter (not shown). Having validated the functional activity of GFP-E2F2, it was utilized in fluorescence recovery after photobleaching (FRAP) analysis to determine the influence of SWI/SNF on the nuclear retention of E2F2. SW13 and TSUPr-1 cells were transfected with GFP-E2F2 expression plasmids, and fluorescence recovery after photobleaching analysis was performed 18 h post-transfection. Under these conditions, we did not observe a significant difference in the mobility of GFP-E2F2 between the two cell lines (data not shown). To specifically address whether SWI/SNF influenced the mobility of E2F2 in
SW13 cells, we cotransfected these cells with GFP-E2F2 and either vector or BRG1. We failed to observe any difference in fluorescence recovery in the presence or absence of BRG1 (Fig. 3D). As expected, freely diffusible GFP rapidly recovered fluorescence after photobleaching, whereas GFP-histone H2B failed to recover because of its tight association with chromatin. Taken together, these findings demonstrate that SWI/SNF does not globally affect E2F2 chromatin retention as assessed by live cell imaging.

To specifically determine whether SWI/SNF is required for the association of an E2F family member with the Plk1 promoter, ChIP assays were performed. SW13 and TSUPr-1 cells were infected with p16ink4a encoding adenovirus, and protein-DNA complexes were cross-linked with formaldehyde and immunoprecipitated with antibodies to E2F4 and Dbf4 (nonspecific). Input and immunoprecipitated DNA was amplified by PCR with primers specific for the Plk1 promoter. Amplification of the DNA was well within the linear range of PCR (data not shown). E2F4 occupancy was observed in both TSUPr-1 and SW13 cells (Fig. 3E, compare lanes 1 and 2). Together, these results demonstrate that SWI/SNF is not required for the assembly of E2F4 on promoters.

In addition to regulating transcription factor association, SWI/SNF could regulate the ability of pocket proteins to assemble at promoters. As shown in Fig. 2A, RB and the related proteins p107 and p130 are expressed in TSUPr-1 and SW13 cells, and these proteins were dephosphorylated following the expression of p16ink4a. Next, we determined whether RB had the capacity to functionally interact with E2F proteins in the absence of SWI/SNF activity. As such, the 3xE2F-Luc reporter construct was utilized to determine whether RB has the capacity to physically interact with E2F family members on a promoter. In SW13 cells the expression of a constitutively active allele of RB (PSM-RB) inhibited virtually all activation of the reporter (Fig. 4A). This result indicates that RB retains the capacity to efficiently interact with E2F in the absence of SWI/SNF activity.

To determine the requirement for SWI/SNF in the assembly of pocket proteins on the Plk1 promoter, ChIP analysis was performed (51, 52). We observed approximately equal recruitment of p130 to promoters in the presence or absence of SWI/SNF (Fig. 4B, lanes 1 and 2, respectively). In contrast, there was enhanced p107 recruitment in the SWI/SNF-deficient cells (Fig. 4B, lanes 1 and 2). Thus, SWI/SNF is not required for these pocket proteins to assemble on promoters. Similarly, we detected RB at the Plk1 promoter in the absence of SWI/SNF activity.
Requirement of SWI/SNF in RB-mediated Repression of Plk1

PLK1 promoter.

amplified by PCR using primers specific for the PLK1 promoter.

encoding adenoviruses. The ChIP assays were performed with

iciency.

Fig. 5A). As shown, infection with P16INK4A resulted in signif-

(47). Similarly, the recruitment of GCN5-containing complexes

mediated repression in this system. Removal of Dox from the

media results in the coordinate induction of both PSM-RB and

mutant BRG1 as we have previously reported (42). Under these

conditions RB-mediated attenuation of Plk1 RNA levels were

compromised (Fig. 5C, lanes 3 and 4). Therefore, RB-mediated

repression of Plk1 is dependent on SWI/SNF activity consistent

with what was observed in SW13 cells. To address whether

inhibition of histone deacetylase activity specifically has the po-

tential to block RB-mediated attenuation of Plk1, a pharmaco-

logical inhibitor of deacetylase enzymes, trichostatin A (TSA),

was utilized. As shown, TSA significantly reversed the RB-medi-

ated attenuation of Plk1 RNA levels (Fig. 5C, lanes 5 and 6). To
directly investigate Plk1 promoter activity, the Plk1 reporter

construct was integrated into A6-1 cells. In this system, there

was a 10-fold reduction in Plk1 promoter activity in presence of

active RB (Fig. 5D). Treatment with the histone deacetylase

inhibitor TSA partially alleviated RB-mediated repression of

Plk1 (Fig. 5D). Collectively, these data demonstrate that SWI/

SNF is required for the deacetylation of the Plk1 promoter and

that this event is critical for repression mediated by the RB

pathway. Our findings support a model wherein both SWI/SNF

and histone deacetylation are required for RB-mediated repres-

sion of Plk1 (Fig. 6). Moreover, histone deacetylation of Plk1

promoter requires SWI/SNF activity, thus placing SWI/SNF and

histone deacetylation in a hierarchical order for the repression

of Plk1 expression.

DISCUSSION

Plk1 plays critical roles in progression through the cell cycle.

Specifically, Plk1 is implicated in a variety of processes asso-

ciated with mitotic progression. These activities range from

roles in centrosome duplication (which occurs at the G1/S tran-

sition) to spindle pole maturation required for a productive

nuclear division (6, 7). As such, the regulation of Plk1 expres-

sion is tightly controlled, and modification of Plk1 levels is

associated with diverse effects on cell biology. For example, loss

of Plk1 is inconsistent with cellular viability and induces cat-

astrophic events in mitosis (15). In contrast, ectopic expression

of Plk1 is associated with cellular transformation and is dereg-

ulated in human cancers (14, 19, 43). It has been previously

demonstrated that Plk1 expression is stimulated as cells pro-

gress toward the G1/S transition (20, 44). Here we show that

this gene regulation is manifested through the activity of the

RB pathway. Because the RB pathway is compromised in the

majority of tumors, these analyses provide a likely mechanism

for the deregulation of Plk1 expression observed in tumors.

The mechanism through which RB regulates critical down-

stream target gene expression has been hypothesized to involve

the recruitment of corepressors (25, 26). Prior studies have

demonstrated that RB can recruit a myriad of corepressors to

chromatin but also subsequent histone modifications at the

promoter (25). Specifically, RB and related proteins recruit

histone deacetylase activities that result in promoter hypo-

cacylation. We have recently shown that such modifications

represent a critical means through which RB functions to

repress transcription (42). Therefore, we determined whether

SWI/SNF influences promoter histone acetylation. ChIP assays

were performed using antibodies for p107 and p130. Input and

immunoprecipitated DNA was amplified by PCR using primers

specific for the Plk1 promoter.

(data not shown). However, in our hands the occupancy of RB

at the Plk1 promoter (as well as other promoters) is not con-

sistently detectable. Therefore, similar to the situation with

E2F factors, BRG1/BRM was not required for promoter associ-

ation of p107/p130.

SWI/SNF Is Required for Plk1 Promoter Histone Deacetyla-

tion—It is believed that one of the key components of RB-

mediated transcriptional repression is not only its recruitment

to chromatin but also subsequent histone modifications at the

promoter (25). Specifically, RB and related proteins recruit

histone deacetylase activities that result in promoter hypo-

cacylation. We have recently shown that such modifications

represent a critical means through which RB functions to

repress transcription (42). Therefore, we determined whether

SWI/SNF influences promoter histone acetylation. ChIP assays

were performed using antibodies for histone H4 antibody to deter-

mine the acetylation status of histone H4 at the Plk1 promoter

(Fig. 5A). As shown, infection with P16INK4A resulted in signif-

icant histone deacetylation of the Plk1 promoter in TSUPr-1

cells (Fig. 5A, top left panel, compare lanes 3 and 4), which was

evident from quantification of independent experiments (Fig.

5A, right panel). In contrast, P16INK4A infection failed to induce

any histone deacetylation of Plk1 promoter in SW13 cells,

indicating that SWI/SNF is required for histone deacetylation

of the Plk1 promoter.

To determine the effect of histone deacetylation on Plk1 ex-

pression, we utilized an immortalized rat fibroblast cell line that

expresses a dominant negative mutant of BRG1 was utilized to

specifically determine the requirement for SWI/SNF during RB-

mediated repression in this system.

FIG. 4. SWI/SNF is not required for pocket protein association

with the Plk1 promoter. A, SW13 cells were transfected with cytom-

egalovirus p-gal and 3XE2F-Luc reporter plasmids and either vector, PSM-

RB, or PSM-RB and E2P2 expression plasmids as indicated. The relative

luciferase activity was normalized to p-gal and 3XE2F-Luc reporter plas-

mids and either vector, PSM-RB, or PSM-RB and E2P2 expression plas-

mids as indicated. The relative luciferase activity was normalized to

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mids and either vector, PSM-RB, or PSM-RB and E2P2 expression plas-

mids as indicated. The relative luciferase activity was normalized to
to specific promoters is dependent on SWI/SNF activity (38). In the case of the RB pathway this transcription factor would be the E2F family of transcription factors, and it is well known that disruption of E2F-chromatin association represents one means to bypass RB-mediated arrest (30, 55). However, we show here that SWI/SNF is not required for the basic retention of E2F proteins on chromatin, using both reporter assays and a live cell imaging approach. Additionally, we can readily detect E2F4 at the Plk1 promoter in the absence of SWI/SNF activity.

Second, RB and related proteins could fail to stably interact with E2F at the promoter in the absence of SWI/SNF. We addressed this possibility by delineating the binding of RB to E2F in a simple functional interaction study, which revealed that RB does retain the capacity to interact with E2F proteins to inhibit transactivation. Additionally, we could clearly detect the RB-related proteins p107 and p130 at the Plk1 promoters by ChIP. Thus, assembly of potential repressor complexes at target promoters occurs independently of SWI/SNF. Last, SWI/SNF activity could in fact be required for chromatin modifications leading to repression. It has been previously established that RB-repressor complexes can utilize histone deacetylation as a means to facilitate transcriptional repression (34, 42). Analyses of the Plk1 promoter clearly demonstrated histone deacetylation during repression. However, this deacetylation was dependent on SWI/SNF activity. In the case of the RB family of proteins, histone deacetylation plays an important role in transcriptional repression of specific genes. For example, the cyclin E, TopoIIα/β, TS, and Cdc2 genes are repressed via the RB pathway in a histone deacetylase-dependent manner (34, 42). Our results suggest that the requisite action of SWI/SNF in the repression of these genes could be solely through the control of the histone deacetylation of these promoters. Such a possibility is demonstrated in the case of Plk1 where the
inhibitor of histone deacetylation, TSA, significantly reversed RB-mediated repression.

In conclusion, these studies delineate a critical mechanism through which Plk1 is transcriptionally regulated. Specifically, the RB pathway is responsible for repression of the Plk1 promoter. This repression is dependent on SWI/SNF functioning in a hierarchical manner to control histone deacetylation of the Plk1 promoter.

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