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Activation of human B-MYB by cyclins

(cell cycle/cyclin A/cyclin E/phosphorylation/transactivation)

ARTURO SALA*†‡, MONDIRA KUNDU†§, IDA CASELLA*, ANDREW ENGELHARD*, BRUNO CALABRETTA*, LUIGI GRASSO*, MARCO G. PAGGI¶, ANTONIO GIORDANO*, ROGER J. WATSON‖, KAMEL KHALILI§, AND CESARE PESCHLE*,**

*Thomas Jefferson University, Kimmel Cancer Institute, Department of Microbiology–Immunology and §Molecular Neurovirology Section, Jefferson Institute of Molecular Medicine, Department of Biochemistry and Molecular Biology, Philadelphia, PA 19107; **Laboratorio di Ematologia Oncologia Istituto Superiore di Sanita', Rome, Italy; ¶Istituto Regina Elena Centro Ricerca Sperimentale, Rome, Italy; and ‖Ludwig Institute for Cancer Research, St. Mary's Hospital Medical School, London W21PG, United Kingdom

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ABSTRACT **B-MYB expression is associated with cell proliferation and recent studies have suggested that it promotes the S phase of mammalian cells. Based on its homology to the transcription factors c-MYB and A-MYB, B-MYB is thought to be involved in transcriptional regulation; however, its activity is not detectable in several cell lines. It was postulated that B-MYB function may depend on the presence of a cofactor, and recent studies suggested that B-MYB is phosphorylated specifically during S phase in murine fibroblasts. In this report we provide evidence that the product of the human *B-myb* gene can be activated *in vivo* by coexpression with cyclin A or cyclin E. Transfection studies showed that B-MYB was a weak transcriptional activator in SAOS-2 cells and was unable to promote their proliferation. In contrast, overexpression of both B-MYB and cyclin A or cyclin E caused a drastic increase in the number of SAOS-2 cells in S phase. Also, overexpression of cyclin A and cyclin E in SAOS-2 cells enhanced the ability of B-MYB, but not c-MYB, to transactivate various promoters, including the *cdc2* promoter, the HIV-1-LTR, and the simian virus 40 minimal promoter. A direct role for cyclin-dependent activation of B-MYB was demonstrated using an *in vitro* transcription assay. These observations suggest that one mechanism by which cyclin A and E may promote the S phase is through modification and activation of B-MYB.**

B-MYB is a transcriptional regulator whose activity appears to be associated with cellular proliferation and differentiation (1–5). B-MYB is highly homologous to c-MYB within the DNA-binding domain region, and this correlates with a similar affinity displayed by both proteins for the *myb*-binding sequence (C/T)AAC(G/T)G (reviewed in ref. 6). A large body of evidence implicates B-MYB as a player in cell-cycle progression. First, although *B-myb* expression is ubiquitous, it is strictly regulated in cycling cells: *B-myb* transcription is downregulated in quiescent cells and expression is detected, upon reentry into the cell cycle, in late G_1 (7, 8). Repression of *B-myb* transcription in G_0/early G_1 is dependent upon an E2F binding site within the promoter and appears to involve negative regulation by the retinoblastoma-related proteins p107 and p130 (9, 10). B-MYB is a downstream target of growth suppressors such as p107 and p53 (5, 11) and its transcription is induced by E2F-1 (12), whose activity is associated with cell-cycle progression and, possibly, transformation. Overall, these data support the hypothesis that B-MYB may play a role in G_1/S transition or during S phase itself. B-MYB can affect the growth rate of certain, but not all, cell lines, and it can activate or repress transcription depending on the promoter, cell type, and species. (13–15). The parameters regulating the specificity of B-MYB transcriptional activity have not been elucidated; however, there is evidence to suggest that the conserved region of B-MYB protein binds to a set of cellular factors that may be involved in cell-type-dependent transcriptional activity (16). Recent studies suggested that B-MYB is phosphorylated in a cell-cycle-dependent manner in NIH 3T3 murine fibroblasts, and it also was shown that the cyclin A-cdk2 complex can induce phosphorylation of murine B-MYB when expressed in insect cells (17). To investigate the possibility that cyclin-induced phosphorylation of human B-MYB protein could be associated with a change in its activity, we have performed several experiments that revealed that cyclin A and cyclin E positively regulate B-MYB in terms of control of cell proliferation and transactivation of cellular and viral promoters.

MATERIALS AND METHODS

Cell Lines. The SAOS-2 and T98G cell lines were purchased from the American Type Culture Collection and were passaged and maintained as described (5, 11).

Plasmids. CMV-CD20, CMV-B-MYB, CMV-cyclinA, CMV-cyclinE, CMV-cyclinB1, CMV-CDK2, CMV-CDK2DN, CD34-CAT, HIV-1-LTR-CAT, CDC2-7N-CAT, CDC2-7N-MUT, and pMbM1(containing the *c-myb* cDNA driven by the simian virus 40 (SV40) promoter) were all described (18, 11, 19–24). The plasmid pGL2-promoter, containing the SV40 early promoter linked to the luciferase gene, was purchased from Promega.

Cell-Cycle Analysis. Briefly, 2 μg of the marker plasmid pCMV-CD20 was cotransfected with 5 μg of pCMV-cyclinA or pCMV-cyclinE and 10 μg of pCMV-B-MYB constructs into SAOS-2 cells with the calcium phosphate method. Forty-eight hours after transfections, cells were collected and stained with fluorescein isothiocyanate-labeled anti-CD20 antibody followed by propidium iodide staining, as described (18). Cell-cycle data were analyzed with the program MULTICYCLE (Phoenix).

Transient Transfections and Chloramphenical Acetyltransferase (CAT) and Luciferase Assays. Transient transfections and CAT assays were performed according to the calcium

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Abbreviations: SV40, simian virus 40; CMV, cytomegalovirus; FITC, fluorescein isothiocyanate; CAT, chloramphenicol acetyltransferase; LTR, long terminal repeat.

†A.S. and M.K. contributed equally to this work.
‡To whom reprint requests should be addressed at: Kimmel Cancer Institute, Thomas Jefferson University, 233 South 10th Street, BLSB Building, Philadelphia, PA 19107. e-mail: A_Sala@lac.jci.tju.edu.

phosphate precipitation method, and luciferase assay was performed with the aid of the Luciferase Assay Kit (Promega), following the manufacturer's instructions. Transfections were normalized by cotransfecting the pSVBGAL (Promega) or CMV-CD20 plasmids with reporter and effector constructs. Transfection efficiencies ranged between 10% and 30%.

Western Blots and Immunoprecipitation Studies. For Western blot analysis, SAOS-2 cells were collected 24–36 h after transfections. Cells were directly lysed in SDS-sample buffer. The equivalent of 10^5 cells was loaded onto a 7.0% acrylamide gel and run for 5–6 h until the 83-kDa marker was located in the middle of the gel. After transfer onto nitrocellulose and blocking with 5% dry milk in PBS, the filter was incubated with serum of a rabbit immunized with recombinant human B-MYB protein (see ref. 4 for details on the B-MYB antibody, a gift from R. E. Lewis, University of Nebraska) diluted 1:1000 in PBS and 5% dry milk. After washings and incubation with secondary antibody coupled with peroxidase, the filter was incubated with enhanced chemiluminescence (Amersham) reagent and exposed to x-ray film.

Gel Shift. Double-stranded oligonucleotide probes representative of the *cdc2*, HIV-LTR, and MIM-1 promoters and containing putative *myb*-binding sites (23, 25, 26) were incubated with 5 μg of SAOS-2 nuclear extracts mixed with 10^5 cpm of probe, loaded onto a 5% polyacrylamide nondenaturing gel as described (4).

***In vitro* Transcription Assay.** *In vitro* transcription assay was performed with an *in-vitro* transcription kit according to manufacturer's protocol (Promega). Templates were prepared by restriction digestion of −219/+80-CAT and −117/+80-CAT with *Eco*RI for 1 h at 37°C. B-MYB eluates were prepared by immunoprecipitating B-MYB from SAOS-2 cells, transiently transfected with CMV-B-MYB plasmid, lysed in RIPA buffer (1% Nonidet P-40/0.1% SDS in PBS), washing immune pellets five times in RIPA buffer and three times in a nondenaturing low-salt buffer (20 mM Hepes, pH 7.9/50 mM KCl/0.2 mM EDTA/0.5 mM DTT, 20% (vol/vol) glycerol) and eluting proteins in a high-salt buffer (20 mM Hepes, pH 7.9/800 mM KCl/0.2 mM EDTA/0.5 mM DTT/20% glycerol). Salt concentration was adjusted to 100 mM KCl by diluting the eluted proteins in 1× transcription buffer containing no salt (20 mM Hepes, pH 7.9/0.2 mM EDTA/0.5 mM DTT/20% glycerol). Presence of equal amounts of B-MYB protein in its phosphorylated or native form in the eluted extracts was verified by Western blot analysis, and 1/7 of the total eluate was added to the transcription reaction.

RESULTS

B-MYB and Cyclins Cooperate in Promoting Cell Proliferation. The finding that cyclin A/cdk2 kinase complex is able to phosphorylate B-MYB protein in insect cells (17) prompted us to investigate whether this might result in a change of B-MYB activity. We previously showed that overexpression of B-MYB did not increase DNA synthesis in SAOS-2 cells (5); thus, we asked whether concomitant overexpression of cyclin A, cyclin E, or cyclin B1 would result in synergistic activation of DNA synthesis of those cells as a result of B-MYB modification induced by the cyclins. Fluorescence-activated cell sorter analysis of SAOS-2 cells transiently transfected with 10 μg of B-MYB or 5 μg of cyclin A, both driven by the cytomegalovirus (CMV) promoter, revealed a cell-cycle distribution similar to that obtained by transfection of the control plasmid. A striking increase of the percentage of cells in the S phase was instead observed when B-MYB and cyclin A were concurrently overexpressed (Table 1, experiments 1 and 2). Transfection of 5 μg of cyclin E induced a significant increase in the number of cells in S- and G_2/M phases, and the addition of B-MYB augmented the fraction of cells residing in S phase, although with some fluctuations in separate experiments (Table 1, experiments 3 and 4). In contrast, cotransfection of cyclin B1 and *B-myb* cDNAs did not significantly alter SAOS-2 cell-cycle profiles (Table 1, experiment 5).

Table 1. Cell-cycle phase of transfected SAOS-2 cells

Transfection	% cells in phase		
	G_1	S	G_2/M
Exp. 1			
CMV	43.8	32.9	23.3
B-MYB	48.3	30.8	20.9
Cyclin A	43.9	35.3	20.8
B-MYB + cyclin A	29.9	52.5	17.6
B-MYB + cyclin A	29.9	59.5	10.6
Exp. 2			
CMV	39.0	27.7	33.3
B-MYB	28.1	34.9	37.0
B-MYB	35.5	28.2	36.3
Cyclin A	33.9	29.0	37.0
Cyclin A	30.9	27.9	41.1
B-MYB + cyclin A	19.2	44.4	36.5
B-MYB + cyclin A	16.7	46.1	37.2
Exp. 3			
CMV	30.2	37.5	32.3
B-MYB	31.5	40.5	28.0
Cyclin E	22.0	37.9	40.2
B-MYB + cyclin E	24.5	41.6	34.0
B-MYB + cyclin E	21.3	45.6	33.1
Exp. 4			
CMV	40.8	39.9	19.3
B-MYB	41.2	32.7	26.1
B-MYB	34.5	34.0	31.5
Cyclin E	18.2	48.4	33.4
Cyclin E	19.9	47.8	32.3
B-MYB + cyclin E	19.4	57.6	23.0
B-MYB + cyclin E	16.3	59.0	24.7
Exp. 5			
CMV	30.7	35.6	33.7
B-MYB	30.4	35.3	34.3
Cyclin B1	31.4	36.0	32.5
Cyclin B1	34.1	31.3	34.6
B-MYB + cyclin B1	26.0	36.0	37.4
B-MYB + cyclin B1	26.0	35.6	38.4

Data are expressed as the percentage of the FITC-anti-CD20-stained cells in the various phases of the cell cycle. Various independent transfection experiments were performed on separate days. We preferred to present the entire set of data, rather than the averages with standard deviations, because variations in the efficiency of transfection and staining, in separate experiments, may cause large fluctuations, so that a statistical analysis would not add significance to the raw data.

Cyclin A and Cyclin E Overexpression Results in Activation of B-MYB Transactivating Activity, Independently of *myb* Sites in the Target Promoters. The hypothesis that kinase complexes containing cyclin A or cyclin E may modulate B-MYB transactivating potential was tested by transfecting several promoters, including the HIV-1, *cdc2*, CD34, and SV40 promoters, in cells transiently overexpressing combinations of B-MYB and either cyclin A or cyclin E. The HIV-1, *cdc2*, and CD34 promoters were used because they all contain *myb*-binding sequences whose integrity is required for c-MYB-induced transactivation and therefore have the potential to be regulated by B-MYB (21, 23, 25). The SV40 early promoter previously was shown to be susceptible to transactivation by B-MYB, also in the absence of *myb*-binding sites (27). SAOS-2 cells were chosen for these studies because they display low levels of endogenous B-MYB protein and almost undetectable levels of cyclin A (see below). Transfection of B-MYB, cyclin A or cyclin E cDNAs alone had no significant effect on transcriptional activity of the CD34, *cdc2*, or HIV-1 promot-

ers; however, we observed a 2- to 3-fold transactivation of the SV40 early promoter by B-MYB alone. Interestingly, the activity of *cdc2*, HIV-1 and the SV40 early promoter constructs was markedly increased upon coexpression of B-MYB and cyclins (Fig. 1 *A* and *B*). By contrast, cyclin A did not significantly activate B-MYB-mediated transactivation of the CD34 promoter, suggesting that the effect is specific to certain promoters. Overexpression of cyclin A did not potentiate, but rather decreased, c-MYB-dependent transactivation of the CD34 promoter, supporting the hypothesis that B-MYB is specifically activated by the cyclin A/cdk2 kinase complex (Fig. 1*C*).

To investigate whether activation of B-MYB was a consequence of its increased affinity for DNA, we performed gel-shift assays with nuclear extracts prepared from SAOS-2 cells transfected with B-MYB and cyclins in different combinations. An oligonucleotide containing the prototype c-*myb*- and *B-myb*-binding site derived from the MIM-1 promoter was used as a probe in all assays (26). The bands induced by B-MYB transfection were blocked by competition with 200 ng of unlabeled wild-type probe, but not by the probe bearing a mutated *myb*-binding sequence, demonstrating that they contained B-MYB protein (data not shown). Surprisingly, double-stranded oligonucleotides representative of the *cdc2* and the HIV-1 promoters or restriction fragments encompassing the *myb* sites located in the *cdc2* promoter and the responsive region of the HIV-1-LTR, containing a putative low-affinity *myb* site (see below), did not compete with the MIM-1 probe (data not shown). These findings were substantiated by the

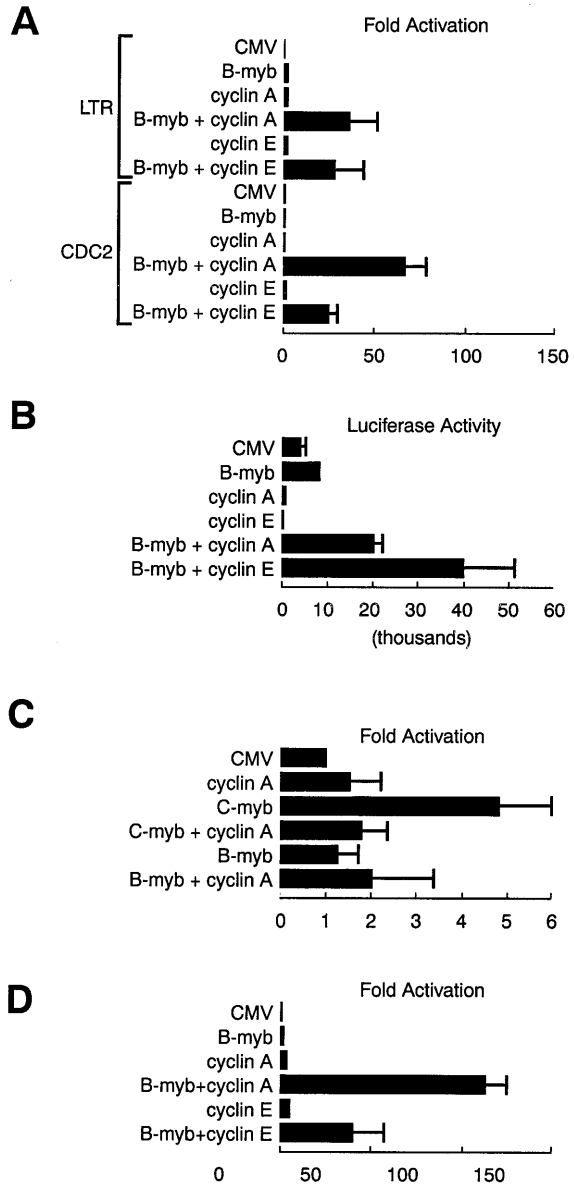

FIG. 1. Synergistic activation of human and viral promoters upon cotransfection of B-MYB with cyclins. (*A*) Five micrograms of HIV-1-LTR (long terminal repeat) and *cdc2* promoter constructs was cotransfected with 10 μg of B-MYB expression vector with or without 5 μg of CMV-cyclin A or CMV-cyclin E. CAT assays were performed as described in the text. (*B*) One microgram of pGL2-promoter was cotransfected with 1 μg of CMV-B-MYB with or without 1 μg of CMV-cyclin A or CMV-cyclin E in 35-mm dishes and each experiment measurement was performed in triplicate. Luciferase assay was carried out 36 h after transfections, and luciferase activity was assayed with the aid of a scintillation counter and expressed as thousands of cpm. (*C*) The CD34 promoter linked to CAT was cotransfected with either CMV-B-MYB or SV40-c-MYB, with or without cyclin A, as described for *A*. (*D*) The *cdc2* promoter, bearing mutations in the *myb*-binding sites, linked to CAT was cotransfected with CMV-B-MYB, with or without CMV-cyclin A or E, as described for *A*. Standard deviations are indicated by the error bars.

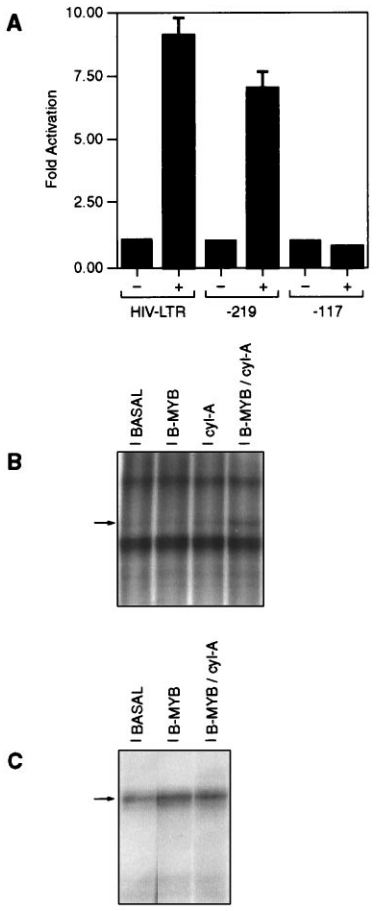

FIG. 2. Cyclin A-activated B-MYB induces *in vitro* transcription of the responsive region mapped within the HIV-1-LTR. (*A*) SAOS-2 cells were transfected with the full-length HIV-1 promoter or two progressive deletions linked to the CAT gene with (+) or without (−) B-MYB plus cyclin A. (*B*) *In vitro* transcription assays were performed by adding B-MYB protein immunoprecipitated from cells transfected with B-MYB and cyclin A, alone or in combination, as described in the text. The template used was the +80/−219 section of the HIV-1 promoter that was also activated *in vivo* by B-MYB and cyclin A (see *A*). This experiment was repeated three times with similar results. A typical experiment is shown. (*C*) Same as *B* except that the template used was the +80/−117 segment of the HIV-1 promoter that was not responsive *in vivo* to activation by B-MYB and cyclin A (see *A*). Arrows indicate the relevant transcripts.

observation that B-MYB, in combination with cyclin A or cyclin E, transactivated a *cdc2* promoter construct with mutated *myb* binding sites (Fig. 1*D*). Thus, it appears that B-MYB-induced transactivation, following activation by cyclins, is independent from binding of B-MYB protein to *myb* sites located in the target promoters.

The activity of various HIV-1 promoter deletion constructs was assayed in the context of B-MYB and cyclin A overexpression to demonstrate that the responsive region is located between nucleotides −219 and −117 relative to the transcription initiation site (+1) in the HIV-1-LTR (Fig. 2*A*). It is interesting to note that the responsive region does not contain previously mapped high-affinity, but only one low-affinity *myb*-binding site (25). Although this assay provided further evidence that cyclin A is able to potentiate the transcriptional activity of B-MYB, it did not provide the means to determine whether this is a direct result of cyclin A's ability to induce B-MYB modification. Toward this end, we used a cell-free assay to evaluate the transcriptional activity of the minimal responsive HIV-1 promoter construct in the presence of B-MYB immunoprecipitated from plain or cyclin A-transfected SAOS-2 cells. B-MYB protein was prepared using a polyclonal antibody prepared against recombinant B-MYB (4). The integrity of the eluted B-MYB was verified by Western blot analysis (data not shown). Consistent with the transactivation studies performed *in vivo*, we observed that the B-MYB protein immunoprecipitated from cells overexpressing cyclin A was a better transcriptional activator *in vitro* than that immunoprecipitated from cells transfected with B-MYB alone (Fig. 2*B*). Furthermore, B-MYB was unable to transactivate the truncated HIV-1 promoter that lacked the responsive region (Fig. 2*C*). These data suggest that a modification of B-MYB induced by cyclin A is directly involved in the activation of B-MYB transactivating potential.

Coexpression with Cyclin A and Cyclin E, but Not Cyclin B1, Alters the Electrophoretic Mobility of B-MYB Protein. Two forms of B-MYB protein were immunoprecipitated from SAOS-2 cells transiently transfected with B-MYB and cyclin A expression vectors, whereas only a single form was detected in immunoprecipitates from cells transfected with B-MYB and CMV empty vector (Fig. 3*A*). To confirm and extend this result, we ran an acrylamide gel low-percentage SDS and performed Western blot analysis of cell extracts after transfection with B-MYB and different cyclins. The electrophoretic mobility of B-MYB was altered in SAOS-2 cells overexpressing cyclins, with the appearance of a slower migrating form in cells transfected with cyclin A and two or more forms in cells

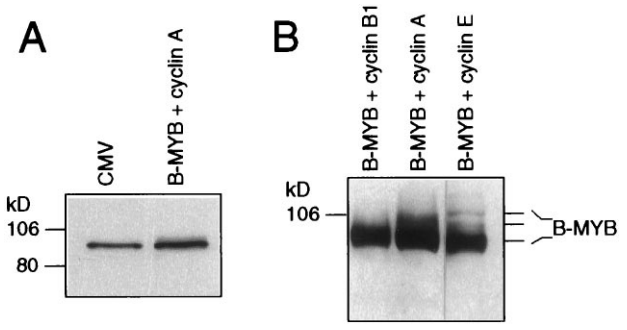

FIG. 3. B-MYB protein is modified following transfection with cyclin A and cyclin E. (*A*) SAOS-2 cells were transiently transfected with 5 μg of CMV-B-MYB and 5 μg of CMV-cyclin A or CMV empty vector. After 36 h the cells were lysed and immunoprecipitates were loaded onto a 10% acrylamide minigel (Bio-Rad) and electrophoresed. (*B*) SAOS-2 cells were transiently transfected with B-MYB in combination with various cyclins, and after 36 h cells were directly lysed in SDS sample buffer, boiled and loaded onto a low-percentage (7%) acrylamide/SDS gel. Western blotting was carried out as described in the text.

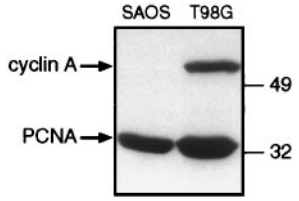

FIG. 4. Cyclin A protein levels in SAOS-2 and T98G cell lines. Nuclear extracts were prepared and 50 μg of protein were loaded onto a 10% acrylamide/SDS minigel. After blotting onto nitrocellulose the membrane was incubated at the same time with an antibody against cyclin A (Oncogene Science) and one against proliferating cell nuclear antigen (PCNA) (Santa Cruz).

transfected with cyclin E, reminiscent of the phosphorylated forms observed in murine B-MYB protein (17). In contrast, cyclin B1 expression was not accompanied by modification of B-MYB protein (Fig. 3*B*).

B-MYB Promotion of Proliferation of a Human Cell Line Correlates with the Presence of Cyclin A. A Western blot performed with nuclear proteins extracted from SAOS-2 cells revealed that cyclin A levels were almost undetectable in this cell line, whereas T98G human glioblastoma cell extracts displayed high levels of cyclin A expression (Fig. 4). We analyzed cell-cycle profiles from the two cell lines transiently transfected with B-MYB and, in agreement with previous studies (5), we observed that B-MYB alone can stimulate DNA synthesis of T98G but not of SAOS-2 cells, further indicating that B-MYB activity may require the presence of cyclin A (Table 2).

DISCUSSION

Although several studies have established a link between B-MYB expression and control of mammalian cell cycle, the molecular mechanisms remain elusive due to the variable effects of B-MYB on cellular growth and promoter activation, depending on the cell type and promoter. It was therefore suggested that additional factors are involved in determining the specificity of B-MYB activity. Recent studies have demonstrated that murine B-MYB protein is phosphorylated in a cell-cycle-specific manner during S phase and that the cyclin A/cdk2 kinase complex is able to phosphorylate murine B-MYB in insect cells, but no information was provided regarding the functional consequences of this event (17).

Phosphorylation of murine B-MYB protein starts in late G_1 and is maintained during S phase, a time in which cyclin E, followed by cyclin A, reaches its peak. Thus, we were interested in examining the possibility that cyclin-directed phosphorylation of human B-MYB might be associated with modulation of B-MYB activity. Coexpression of B-MYB with cyclin A and cyclin E resulted in promotion of S phase in cells refractory to B-MYB effects; in contrast, B-MYB was unable to cooperate with cyclin B1, whose role is required during G_2 and mitosis (Table 1). We also observed that overexpression of B-MYB with

Table 2. Comparison of transfected SAOS-2 and T98G cells

	% cells in phase		
Transfection	G_1	S	G_2/M
SAOS-2			
CMV	41.0	34.5	24.5
B-MYB	39.2	33.0	27.8
B-MYB	37.1	39.6	23.3
T98G			
CMV	34.9	47.4	17.7
B-MYB	**21.5** (35.7)	**57.4** (46.3)	21.1 (18.0)
B-MYB	**24.3** (36.5)	**58.9** (46.7)	16.8 (16.8)

Parentheses indicate the data from untransfected cells in the same tube. Boldface type indicates a significant difference from control.

cyclin A or cyclin E resulted in transactivation of promoters, such as the *cdc2* promoter and the HIV-1 LTR, which are regulated in a cell-cycle-dependent fashion (Fig. 1*A* and *B*). In contrast, cotransfection of B-MYB and/or cyclins did not transactivate the promoter of genes such as CD34 and MIM-1 that are involved in hematopoietic differentiation (Fig. 1*C* and data not shown). It also should be noted that cyclin A overexpression decreased c-MYB-dependent transactivation of the CD34 promoter, suggesting that cyclins might modulate the activity of other members of the MYB family in a negative way (Fig. 1*C*).

An *in vitro* transcription assay was used to demonstrate that the synergistic activation of responsive promoters by B-MYB and cyclin A in transient transfection experiments was associated with cyclin A-induced modification of B-MYB (Figs. 2 and 3). These data together suggest that following activation by cyclin E and A during late G_1 and S phase, B-MYB might induce expression of genes, such as *cdc2*, that are needed for completion of S phase and mitosis (20). It seems reasonable to speculate that the modification of human B-MYB protein observed after transient overexpression of cyclin A or E is related to phosphorylation (Fig. 3). Preliminary experiments with phosphatase support this hypothesis (not shown), although only mutations of the relevant sites in the B-MYB protein will conclusively demonstrate the relationship between cyclin activity, B-MYB phosphorylation, and its functional activation.

Because most of the promoters used in the transient transfection assays contain high-affinity *myb*-binding sites, the observation that B-MYB/cyclin A- or E-induced transactivation occurs independently from those sites was interesting and led to the hypothesis that this activity is regulated by other sequences. This hypothesis is corroborated by the observation that mutation of the *myb* sites in the *cdc2* promoter does not affect B-MYB/cyclin A-mediated transactivation (Fig. 1*D*). It was not the purpose of our investigation to assess in detail the mechanisms underlying B-MYB transactivating activity, nevertheless, our results suggest that it will be worthwhile to determine whether B-MYB activates promoter sequences through interaction with other transcription factors. The observation that B-MYB transactivation is independent from binding to DNA is not entirely without precedent. For example, chicken B-MYB is able to transactivate the heat shock promoter through a DNA-binding-independent mechanism mediated by the TATA box (28,) and human B-MYB has been shown to activate the SV40 early promoter through a not-yet-identified indirect mechanism (27).

Recent studies showed that cyclin A induces phosphorylation of E2F1 (29, 30). Although both cyclin A and E2F1 are required for S phase progression (31–33), E2F1 phosphorylation results in the inhibition of E2F1's ability to bind to and transactivate promoter sequences with loss of its ability to stimulate DNA synthesis (29, 30). This paradox was resolved by hypothesizing that this event is required for turning off E2F-activated genes as cells exit from S phase. Here we propose that cyclin A-dependent activation of B-MYB protein is associated with initiation and progression of DNA replication, based on our results that show the synergistic effects resulting from coexpression of B-MYB with cyclin A (Fig. 1 *A*, *B*, and *D* and Table 1). Thus, we can hypothesize that cyclin A directs the temporal events required during DNA synthesis by modification of several factors, either in a positive or in a negative fashion, depending on the function and distribution of the various players within the cell-cycle clock. This is consistent with a direct role of cyclin A in the replication of DNA that was postulated based on its localization at nuclear replication foci (34). It is our next goal to reveal the molecular targets of B-MYB activity, probably related to its capacity to transactivate genes upon activation by cyclins and associated kinases.

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