Enhancement of B-MYB Transcriptional Activity by ZPR9, a Novel Zinc Finger Protein*

Received for publication, July 25, 2002, and in revised form, January 6, 2003
Published, JBC Papers in Press, January 6, 2003, DOI 10.1074/jbc.M207478200

Hyun-A Seong‡, Kyong-Tai Kim§, and Hyunjung Ha‡

From the ‡Department of Biochemistry, School of Life Sciences, Research Center for Bioresource and Health, Chungbuk National University, Cheongju 361-763 and the §Department of Life Science, Pohang University of Science and Technology, Pohang 790-784, Republic of Korea

B-MYB is a member of the MYB family of transcription factors, which is ubiquitously expressed and is involved in controlling cell proliferation and differentiation (1–5). B-MYB is phylogenetically the most divergent among the three MYB proteins, A-MYB, B-MYB, and c-MYB (6). Recent reports showed that the CDK2-cyclin A complex could induce phosphorylation of B-MYB and potentiate the B-MYB transactivating function and that this activation was also induced by truncation of the carboxyl terminus of B-MYB, suggesting that post-translational modifications are required for relieving the constitutive repression of B-MYB (7–10). In addition, a recent study (11) indicated that the B-MYB transactivation correlates with the binding of some cofactors to the carboxy-terminal conserved region, suggested as a protein binding domain and a putative phosphorylation site.

The MYB proteins are composed of three functional domains for transactivation, an amino-terminal DNA-binding domain, a central acidic region (transactivation domain), and a carboxy-terminal negative regulatory domain containing the leucine zipper motif (12). Recently, all these domains have been reported to be involved in interactions with several cellular proteins. The DNA-binding domain of c-MYB was found to bind with several proteins such as p100 coactivator (13, 14), c-Maf transcription factor (15), Cyp-40 peptidylprolyl isomerase (16), HSF3 (17), nucleolin (18), and retinoic acid receptor (19). In addition, recent reports have shown that the DNA-binding domain of A-MYB and B-MYB interacts with several nuclear proteins (18, 20) and poly(ADP-ribose) polymerase (PARP),1 which is associated with chromatin (21), respectively. On the other hand, the CAMP-response element-binding protein has been demonstrated to interact directly with the transactivation domain of both c-MYB and A-MYB and potentiate their transcriptional activity (22, 23). The leucine zipper motif of the carboxy-terminal domain was also found to associate with several proteins, including p26/28 (24), p67, and p160 (25, 26), and ATBF1 transcription factor (27), but these interactions except for ATBF1 have not been implicated in the regulation of MYB function so far. From these results, it is tempting to speculate that additional proteins may be involved in the regulation of transactivation by B-MYB, probably by association with the carboxy-terminal conserved region that shows significant homology with other members of the MYB gene family such as A-MYB and c-MYB.

ZPR9, a zinc finger protein, was originally identified as a novel cellular partner for the MPK38 serine/threonine kinase that may be involved in early T cell activation by concanavalin A (28) and embryonic development (29, 30). ZPR9 is a 52-kDa protein containing three zinc finger motifs and a physiological substrate of MPK38 kinase in vivo (31).

Here we show that ZPR9 binds to B-MYB in vivo and that the overexpression of ZPR9 induces apoptosis, instead of neural differentiation, in the neuroblastoma cells treated with retinoic acid. Binding of ZPR9 to B-MYB can stimulate the B-MYB transcriptional activity. In addition, we provide evidence that all three functional domains of B-MYB physically interact with ZPR9 in vivo. We also demonstrate that the coexpression of ZPR9 with all three MYB proteins causes the accumulation of both ZPR9 and B-MYB in the nucleus.

EXPERIMENTAL PROCEDURES

Reagents—The eukaryotic glutathione S-transferase (GST) expression vector (pEBG) and pFLAG-CMV-2 vector with a FLAG epitope were obtained as described previously (32). The anti-GST antibody was as described (32). The pT81luc 3xA reporter plasmid (33), containing part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Biochemistry, School of Life Sciences, Chungbuk National University, Cheongju 361-763, Republic of Korea. Tel.: 82-43-261-3233; Fax: 82-43-267-2306; E-mail: hyunjha@cbucc.chungbuk.ac.kr.

‡ To whom correspondence should be addressed: Dept. of Biochemistry, School of Life Sciences, Research Center for Bioresource and Health, Chungbuk National University, Cheongju 361-763, Republic of Korea. Tel.: 82-43-261-3233; Fax: 82-43-267-2306; E-mail: hyunjha@cbucc.chungbuk.ac.kr.

§ To whom correspondence should be addressed: Dept. of Biochemistry, School of Life Sciences, Research Center for Bioresource and Health, Chungbuk National University, Cheongju 361-763, Republic of Korea. Tel.: 82-43-261-3233; Fax: 82-43-267-2306; E-mail: hyunjha@cbucc.chungbuk.ac.kr.

1 The abbreviations used are: PARP, poly(ADP-ribose) polymerase; ZPR9, zinc finger-like protein 9; RA, retinoic acid; GST, glutathione S-transferase; GFP, green fluorescent protein; PBS, fetal bovine serum; DMEM, Dulbecco’s modified Eagle’s medium; TNF-α, tumor necrosis factor-α; DBD, DNA binding domain.

This paper is available on line at http://www.jbc.org

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 278, No. 11, Issue of March 14, pp. 9655–9662, 2003
Printed in U.S.A.
three copies of the “A box” Myb-binding sites from the 9m promoter, was a kind gift from Dr. Scott A. Ness (the University of New Mexico, Albuquerque, NM). The expression vector pCEV27 was kindly provided by Dr. D.-Y. Shin (Danguk University, Chonan, Korea). The anti-FLAG (M2) antibody, all-trans-retinoic acid (RA), BisBenzimide (H 33258), isopropyl-β-D-thiogalactopyranoside, dithiothreitol, aprotinin, and phenylmethylsulfonyl fluoride were purchased from Sigma. Polyvinylidene difluoride membrane was obtained from Millipore Corp. γ-32P-ATP was purchased from PerkinElmer Life Sciences. The human B-MYB antibody (C-20) raised against the carboxyl terminus was used for immunoprecipitation and Western analysis (Santa Cruz Biotechnology). Oligonucleotides were synthesized from Bioneer Corp. (Cheongwon, Chungbuk, Korea).

Coactivation of B-MYB by ZPR9

The human neuroblastoma cell line SK-N-BE (2C) and 293T cells, a derivative of human kidney embryonal fibroblast containing SV40 T antigen, were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS, 100 units/ml penicillin-streptomycin, and 1 μg/ml asparagine (34). For cell differentiation experiments, SK-N-BE (2C) cells grown in DMEM supplemented with 10% FBS were plated in 6-well flat-bottomed microplates at a concentration of 4 × 10^4 cells per well the day before retinoic acid (RA) treatment, and the medium was replaced with fresh medium without FBS, containing 5 μM all-trans-retinoic acid, every 3 days. The 293T cells were transfected by the calcium phosphate precipitation method as described previously (34).

Plasmids—The pEBG-B-MYB, an amino-terminally truncated version containing part of the acidic region and a complete conserved region, and pEBG-WT B-MYB, containing a full-length B-MYB cDNA, have been described previously (32). The deletion constructs, pEBG-B-MYB R1 and pEBG-B-MYB R2, were generated by PCR as described (32). To generate two deletion constructs, pFLAG-BM and pFLAG-IA, we performed a PCR using the full-length B-MYB cDNA as the template. The forward primers for DBD (5'-GCGGATCCCTCGAGCTCCAG-3') contain a BamHI restriction endonuclease recognition site (BamHI) and a complete DNA-binding domain, was constructed in pEBG and pFLAG-CMV-2 to generate the pEBG-B-MYB and pEBG-WT B-MYB constructs. The pEBG-TA was generated by subcloning of a carboxyl-terminally truncated version containing a DNA-binding domain, and a complete DNA-binding domain, was constructed in pEBG and pFLAG-CMV-2 to generate the pEBG-TA plasmid. For a confocal microscopy, the GFP-B-MYB antibody was used for immunoprecipitation and Western analysis (Santa Cruz Biotechnology). Oligonucleotides were synthesized from Bioneer Corp. (Cheongwon, Chungbuk, Korea).

The human neuroblastoma cell line SK-N-BE (2C) and 293T cells, a derivative of human kidney embryonal fibroblast containing SV40 T antigen, were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS, 100 units/ml penicillin-streptomycin, and 1 μg/ml asparagine (34). For cell differentiation experiments, SK-N-BE (2C) cells grown in DMEM supplemented with 10% FBS were plated in 6-well flat-bottomed microplates at a concentration of 4 × 10^4 cells per well the day before retinoic acid (RA) treatment, and the medium was replaced with fresh medium without FBS, containing 5 μM all-trans-retinoic acid, every 3 days. The 293T cells were transfected by the calcium phosphate precipitation method as described previously (34).

Plasmids—The pEBG-B-MYB, an amino-terminally truncated version containing part of the acidic region and a complete conserved region, and pEBG-WT B-MYB, containing a full-length B-MYB cDNA, have been described previously (32). The deletion constructs, pEBG-B-MYB R1 and pEBG-B-MYB R2, were generated by PCR as described (32). To generate two deletion constructs, pFLAG-BM and pFLAG-IA, we performed a PCR using the full-length B-MYB cDNA as the template. The forward primers for DBD (5'-GCGGATCCCTCGAGCTCCAG-3') contain a BamHI restriction endonuclease recognition site (BamHI) and a complete DNA-binding domain, was constructed in pEBG and pFLAG-CMV-2 to generate the pEBG-B-MYB and pEBG-WT B-MYB constructs. The pEBG-TA was generated by subcloning of a carboxyl-terminally truncated version containing a DNA-binding domain, and a complete DNA-binding domain, was constructed in pEBG and pFLAG-CMV-2 to generate the pEBG-B-MYB and pEBG-WT B-MYB constructs. The pEBG-TA was generated by subcloning of a carboxyl-terminally truncated version containing a DNA-binding domain, and a complete DNA-binding domain, was constructed in pEBG and pFLAG-CMV-2 to generate the pEBG-B-MYB and pEBG-WT B-MYB constructs. The pEBG-TA was generated by subcloning of a carboxyl-terminally truncated version containing a DNA-binding domain, and a complete DNA-binding domain, was constructed in pEBG and pFLAG-CMV-2 to generate the pEBG-B-MYB and pEBG-WT B-MYB constructs. The pEBG-TA was generated by subcloning of a carboxyl-terminally truncated version containing a DNA-binding domain, and a complete DNA-binding domain, was constructed in pEBG and pFLAG-CMV-2 to generate the pEBG-B-MYB and pEBG-WT B-MYB constructs. The pEBG-TA was generated by subcloning of a carboxyl-terminally truncated version containing a DNA-binding domain, and a complete DNA-binding domain, was constructed in pEBG and pFLAG-CMV-2 to generate the pEBG-B-MYB and pEBG-WT B-MYB constructs. The pEBG-TA was generated by subcloning of a carboxyl-terminally truncated version containing a DNA-binding domain, and a complete DNA-binding domain, was constructed in pEBG and pFLAG-CMV-2 to generate the pEBG-B-MYB and pEBG-WT B-MYB constructs. The pEBG-TA was generated by subcloning of a carboxyl-terminally truncated version containing a DNA-binding domain, and a complete DNA-binding domain, was constructed in pEBG and pFLAG-CMV-2 to generate the pEBG-B-MYB and pEBG-WT B-MYB constructs. The pEBG-TA was generated by subcloning of a carboxyl-terminally truncated version containing a DNA-binding domain, and a complete DNA-binding domain, was constructed in pEBG and pFLAG-CMV-2 to generate the pEBG-B-MYB and pEBG-WT B-MYB constructs.
interact with B-MYB, we used a two-hybrid specificity test technique that was usually employed to verify the interaction specificity between bait and the cDNA-encoded proteins and to eliminate quickly the majority of false positives detected in the yeast two-hybrid assay. Specific interacting proteins confer the galactose-dependent Leu"/LacZ" phenotype to yeast containing the related baits but not to yeast containing unrelated baits. To test this, the B-MYB library plasmid was rescued from the galactose-dependent Leu"/LacZ" yeast and re-introduced into the ZPR9 bait strain as well as the other strains containing approximately 20 different baits available in our laboratory. From this random screening, B-MYB cDNA was found to interact with the total seven baits tested (results not shown), including ZPR9 and B-MYB baits, suggesting that ZPR9, like B-MYB (32), can interact with B-MYB physically in mammalian cells.

To determine whether B-MYB and ZPR9 interact in vivo, we performed cotransfection experiments using GST- and FLAG-tagged eukaryotic expression vectors. In these experiments, the ZPR9 and wild-type B-MYB were coexpressed as a GST fusion protein and a FLAG-tagged protein in 293T cells, respectively. The interactions of FLAG-tagged B-MYB proteins to the GST-ZPR9 fusion proteins were analyzed by immunoblotting with an anti-FLAG antibody. As shown in Fig. 1A, the B-MYB was detected in the coprecipitate only when coexpressed with the GST-ZPR9 but not with the control GST alone, demonstrating that B-MYB physically interacts with ZPR9 in vivo. In order to verify further the interaction of B-MYB with ZPR9 in vivo, we performed communoprecipitation experiments using 293T cells transiently transfected with the vector alone or FLAG-tagged ZPR9 (Fig. 1B). Endogenous B-MYB was immunoprecipitated from cell lysates, and Western blot analysis shows that B-MYB was precipitated (Fig. 1B, lower panel). The binding of ZPR9 was subsequently analyzed using Western blotting with an anti-FLAG antibody, and as shown in Fig. 1B (upper left panel), ZPR9 was present in the B-MYB-immunoprecipitate. In conclusion, our results clearly demonstrate that B-MYB associates with ZPR9 in vivo.

Three Functional Domains of B-MYB Are Involved in ZPR9 Binding—Recently, together with other data (22, 23), it was reported that poly(ADP-ribose) polymerase binds to the B-MYB DNA-binding domain and enhances the transcriptional activity of B-MYB (21). Therefore, we speculated that ZPR9 might interact with the DNA-binding or transactivation domain of B-MYB, in addition to the carboxyl-terminal conserved region, and cause the modulation of B-MYB transactivation. To determine which regions of B-MYB were required for binding of ZPR9 in vivo, we generated nine deletion constructs fused to GST (Fig. 2, A and B). The GST-WT B-MYB, GST-CR, GST-B-MYB, GST-TA1, GST-B-MYB R1, GST-TA, and GST-B-MYB R2 constructs were expressed in 293T cells (Fig. 2, C and D, middle left panels) and used for the in vivo binding assay with ZPR9 and Two9, a partial clone of ZPR9 comprising amino acids 206–452 (31). The binding of the FLAG-tagged ZPR9 and Two9 with all six constructs tested, except for GST-B-MYB R2, was readily detectable (Fig. 2, C and D, top left panels). These results suggest that all three functional domains of B-MYB, a DNA-binding domain, a transactivation domain, and the carboxyl-terminal conserved region, are responsible for ZPR9 binding in vivo. To narrow down further the binding motif, we generated a DBD deletion construct (amino acids 1–206) and carried out a similar experiment. As a result, the FLAG-tagged DBD was coprecipitated with GST-tagged ZPR9 (or Two9) but not with GST alone (Fig. 2, C and D, top right panels). These findings, together with the binding of ZPR9 to GST-TA, clearly indicate that both DNA-binding and transactivation domains are required for ZPR9 binding. However, the GST-B-MYB R2 was not coprecipitated with FLAG-tagged ZPR9 or Two9, indicating that the conserved domain is only required for ZPR9 binding within the carboxyl-terminal domain of B-MYB. Taken together, these results suggest that each functional domain of B-MYB is sufficient for its association with ZPR9.

ZPR9 Enhances the Transcriptional Activity of B-MYB—Because ZPR9 is binding to the DNA-binding and transactivation domain of B-MYB (Fig. 2), it is likely that the interaction may affect the transactivation by B-MYB. To investigate the functional significance of binding of ZPR9 to B-MYB, we cotransfected the pT81luc 3xA reporter plasmid, containing three Myb-binding sites from the chicken mim-1 gene (33), with mammalian expression vectors encoding for ZPR9 and B-MYB. As shown in Fig. 3A, the addition of ZPR9 to B-MYB led to a significant enhancement of B-MYB transcriptional activity. To investigate further whether the expression of ZPR9 protein levels could influence the B-MYB transactivation, a dose dependence experiment by increasing the ZPR9 expression plas-
mid was performed. As shown in Fig. 3B, the stimulatory effect of ZPR9 in the B-MYB transactivation increased in a dose-dependent manner. However, the transfection of ZPR9 alone, as a control, did not influence a significant change in the basal transcription. These findings strongly suggest that ZPR9 is a potential coactivator of B-MYB.

**ZPR9 Stimulates the Nuclear Localization of B-MYB**—Because, in addition to the ZPR9 binding to the DNA-binding domain and transactivation domain of B-MYB, as shown in Fig. 3, coexpression of ZPR9 and B-MYB resulted in synergistic activation of the B-MYB-responsive promoter, pT81luc 3xA reporter, it is likely that ZPR9 could modify B-MYB movement. To address this point, 293T cells were transfected with FLAG-tagged ZPR9 alone or together with GFP-B-MYB. Cells expressing
Coactivation of B-MYB by ZPR9

Fig. 4. Subcellular localization of B-MYB and ZPR9. A, effect of ZPR9 on B-MYB subcellular localization. 293T cells were transfected with GFP-B-MYB alone (B-MYB), as a control, or GFP-B-MYB was coexpressed in cells together with FLAG-tagged ZPR9 (B-MYB/ZPR9). Forty-eight hours after transfection, cells were washed three times with ice-cold PBS and fixed with 4% paraformaldehyde for 10 min at room temperature prior to incubation with the anti-FLAG (M2) monoclonal antibody, which was followed by an incubation with a Texas Red-conjugated anti-mouse secondary antibody to label the ZPR9 construct. Slides were mounted and analyzed by confocal microscopy. B, effect of B-MYB on ZPR9 subcellular localization. 293T cells were transfected with FLAG-tagged ZPR9 alone (ZPR9), as a control, or GFP-B-MYB was coexpressed in cells together with FLAG-tagged ZPR9 (ZPR9/B-MYB). Cells were washed and fixed as described above. Cells were immunostained with the anti-FLAG (M2) monoclonal antibody, followed by Texas Red-conjugated anti-mouse secondary antibody, and analyzed by confocal microscopy. GFP is in green, and areas of colocalization appear as yellow (Merge).

Fig. 3. ZPR9 enhances B-MYB transactivating activity by direct interaction. A, 293T cells were transiently transfected with the pE3ECAT3 × 3A reporter (8 µg) together with empty pFLAG-CMV2 vector (10 µg) or pFLAG-CMV plasmid expressing wild-type B-MYB (6 µg) and pFLAG-ZPR9 (4 µg). Normalized luciferase expression from triplicate samples is presented relative to the LacZ expressions, and the standard deviations are less than 5%. Whole cell extracts from cells transfected with the indicated expression plasmids were analyzed by Western blotting (WB) using anti-FLAG antibody, and the cell extracts containing approximately equivalent amounts of B-MYB and ZPR9 were used for luciferase assay (lower panel). B, ZPR9 synergizes with B-MYB in the transactivating activity of B-MYB. 293T cells were transfected as described above with increasing amounts of ZPR9 (4, 8, and 16 µg) or B-MYB (2, 4, and 8 µg) as indicated in the presence or absence of B-MYB (4 µg). Luciferase assays were performed as described for A. The bottom panel shows the expression of the increasing amounts of ZPR9 and B-MYB in the cell extracts used for luciferase assay.

B-MYB and ZPR9 exhibit both cytosolic and nuclear staining, but the coexpression of ZPR9 with B-MYB resulted in nuclear localization of the B-MYB protein, as well as the ZPR9, with an average increase of 2.8- and 3.8-fold in the four experiments, respectively, when the percentage of the nuclear localization of B-MYB and ZPR9 was calculated as the number of GFP- (for B-MYB) and Texas Red-positive (for ZPR9) cells with nuclear staining divided by the total number of GFP- and Texas Red-positive cells (Fig. 4). These data show that ZPR9 is able to cooperate with B-MYB for the transactivation by B-MYB.

Constitutive Expression of ZPR9 Induces Apoptotic Neuroblastoma Cell Death by Retinoic Acid—To analyze the effect of ZPR9 gene expression on the differentiable or apoptotic potential of SK-N-BE (2/C), a human neuroblastoma cell line, we constructed an expression vector pCEV27-ZPR9, where a full-length human ZPR9 cDNA was placed under the control of Moloney murine leukemia virus long terminal repeat promoter. ZPR9-transfected SK-N-BE (2/C) cells were selected in medium containing G418 (800 µg/ml). Overexpression of the ZPR9 transcript in the selected transfectants was analyzed by Northern blot analysis. As shown in Fig. 5A, compared with parental SK-N-BE (2/C) cells and pCEV27 vector transfectants, the ZPR9 transcripts were identified at a high level in the selected ZPR9 transfected clones. In addition, similar results were obtained with all selected ZPR9 transfectant clones (results not shown). The growth rates under normal serum conditions were comparable in ZPR9 transfectants, pCEV27 vector transfectants, and parental SK-N-BE (2/C) cells, suggesting that the ectopic expression of ZPR9 did not affect the proliferative activity on neuroblastoma cells (results not shown). RA treatment resulted in a more rapid loss of viability in all ZPR9-expressing clones compared with the parental SK-N-BE (2/C) cells and the cell lines transfected with the pCEV27 vector (Fig. 5B). To confirm if the marked decrease in cellular viability of the RA-treated ZPR9 transfectants is due to apoptosis, we performed dual annexin V/propidium iodide staining as described under “Experimental Procedures” and obtained an experimental result similar to those in Fig. 5B. A significant increase in the number of apoptotic cells was observed in the ZPR9 transfectants after RA treatment, suggesting that the
overexpression of ZPR9 may induce apoptosis, instead of the neural differentiation, in the presence of RA (Fig. 5C). To investigate further the physiological roles of ZPR9 during apoptosis, 293T cells were transiently transfected with GFP alone, GFP and ZPR9, and GFP and B-MYB. In addition, cells were cotransfected with ZPR9 and B-MYB, together with GFP. After inducing apoptosis by TNF-α treatment, apoptotic cells were scored by a change in nuclear morphology among GFP-positive
cells. As shown in Fig. 5D, ~59% of 293T cells expressing ZPR9 were apoptotic following TNF-α treatment. In contrast, ~16% of cells transfected with B-MYB underwent TNF-α-induced apoptosis, similar to the percentage (about 15%) of control apoptotic cells expressing GFP alone. On the other hand, B-MYB coexpression markedly inhibited the apoptotic stimulation induced by ZPR9 (~55% inhibition).

To confirm further the involvement of ZPR9 in the enhancement of RA-induced apoptosis, we carried out a similar transient transfection experiment using SK-N-BE (2C) cells. As shown in Fig. 5E, the results obtained in this experiment were very similar to those in Fig. 5D. These findings suggest that the overexpression of ZPR9 is sufficient to stimulate apoptosis induced by various stimuli and raise the possibility that ZPR9 may be a potential pro-apoptotic protein.

**DISCUSSION**

In this report, we demonstrate that ZPR9 interacts with B-MYB in vivo and that each functional domain of B-MYB is necessary and sufficient to mediate direct protein-protein interactions with ZPR9. We found that ZPR9 enhanced the transactivating activity of B-MYB by direct interaction. Furthermore, we show that B-MYB moves to the nucleus following the coexpression of ZPR9, implying that ZPR9 may behave as an activator of the bound transcription factor, B-MYB.

The novel zinc finger protein, termed ZPR9 (zinc finger-like protein 9), was originally discovered as a protein partner for the MPK38 serine/threonine kinase (31). Recently, evidence has emerged that several zinc finger proteins such as ZPR1, tumor necrosis factor receptor-associated factor, CD40 receptor-associated factor, enigma, and LMP-associated protein act as modulators for receptor signaling, and that the formation of multiprotein complexes in many transcription factors results in an increased diversity and specificity in the regulation of gene expression (35–40). Zinc finger motifs of the Cys2-His2 type have been found in numerous transcription factors, including ZPR9. In this respect, the self-association of ZPR9 containing zinc finger motifs and the interaction of ZPR9 with the kinase catalytic domain of MPK38 provide an interesting aspect to the regulation of this factor (31). In addition, our recent study strongly suggests a possible role for phosphorylation of ZPR9 proteins in their translocation to the nucleus (31). Thus, these data open a new area of investigation on the potential interaction of ZPR9 with other cellular proteins.

Several lines of evidence indicate that the DNA-binding domain of MYB proteins has the potential of mediating contact with both DNA and proteins. It has been shown that PARP binds to the DNA-binding domain of B-MYB and enhances its transactivating activity and that the physical interaction between PARP and B-MYB is critical for the coactivating function (21), suggesting an important role for the direct interaction in the regulation of the B-MYB transcriptional activity. Recently, we have shown that B-MYB interacts in vivo with each other via the carboxyl-terminal conserved region (32). In addition, we have observed that the conserved region of B-MYB binds to several cellular proteins as well as ZPR9 in the yeast two-hybrid tests and in vivo binding assays (results not shown). This evidence led us to investigate whether ZPR9, a potential transcription factor containing zinc finger motifs, participates in the B-MYB-mediated transactivation. As shown in Fig. 3, a significant increase was observed in the transactivating activity of B-MYB by direct binding of ZPR9, suggesting that the in vivo association of B-MYB and ZPR9 plays a pivotal role in the modulation of B-MYB transcriptional activity. Based on this result, we imagine that rather than direct interaction with the carboxyl-terminal conserved region, the conformational change mediated through the DNA-binding or transactivation domain of B-MYB by direct binding of ZPR9 likely plays a role that is important in the regulation of B-MYB transcriptional activity. For activation of B-MYB transcriptional activity, our results, together with existing data (21–23), suggest a distinct mechanism in which, in addition to the truncation of the carboxyl terminus of B-MYB and the phosphorylation by cyclin A-CDK2 complex (7–10), cellular cofactors are bound to the B-MYB, for example through the binding of PARP and ZPR9, resulting in the enhancement of the B-MYB transcriptional activity (21, 31).

Recent studies (41) showed that all-trans-retinoic acid reduces human neuroblastoma growth by inducing either differentiation or apoptosis. To determine whether RA-treated ZPR9 stable transfectants can influence the differentiation or apoptosis in human neuroblastoma cells, the morphological and apoptotic analysis of ZPR9 transfectants was performed in addition to the examination of the number of viable cells (Fig. 5 and results not shown). The ZPR9 transfectants undergo apoptosis rather than differentiation after RA treatment, suggesting that ZPR9 may be one of the regulators controlling cellular growth arrest induced by RA in neuroblastoma cells. To test whether the observed apoptotic cell death after RA treatment in the ZPR9 stable transfectants is a consequence of direct interaction of B-MYB with ZPR9, we performed two separate transient transfection experiments using 293T and SK-N-BE (2C) cells in the presence of TNF-α and RA, respectively (Fig. 5, D and E). In these experiments, coexpression of B-MYB significantly inhibited rather than stimulated TNF-α and RA-induced apoptosis enhanced by ZPR9. In contrast, the percentage of apoptosis in cells transfected with B-MYB alone was similar to the percentage of control apoptotic cells expressing GFP alone in both 293T and SK-N-BE (2C) cells. Thus, it is tempting to suggest that the apoptotic cell death in RA-treated ZPR9 stable transfectants may be derived from the overexpression of ZPR9 itself, not through binding with B-MYB. On the other hand, one may raise the argument that ZPR9, like other zinc finger proteins, could be nuclear for its effect on target genes. Based on this, one possible explanation for ZPR9-induced apoptosis is that a subcellular location of ZPR9 may contribute to its apoptotic function in the presence of retinoic acid because a markedly increased nuclear accumulation of ZPR9, compared with the untreated control cells, was observed when the cells transfected with ZPR9 were treated with RA (results not shown). Additionally, it is not clear that the repression of ZPR9-induced apoptosis by B-MYB is dependent on the direct interaction of B-MYB with ZPR9 because B-MYB thought to mediate the anti-apoptotic functions is also involved in interactions with other cellular proteins that may compete with ZPR9 for binding (see Refs. 21 and 32 and results not shown). The biochemical and molecular mechanisms underlying the pro-apoptotic properties of ZPR9 are unknown at present. However, it seems that the most likely mechanism by which ZPR9 may accelerate apoptosis would be through the modulation of the potential cellular targets for ZPR9. In this context, future studies aimed at identifying cellular physiological targets for ZPR9 will be necessary to elucidate the exact mechanism through which ZPR9 can induce apoptotic cell death.

In addition, ZPR9 proteins, like PARP (21), are nuclear and enhance B-MYB transactivation. In this regard, it will be of further interest to determine the mechanistic interaction between B-MYB and ZPR9 to gain more insight into the role of ZPR9 in the B-MYB transactivation. Moreover, because B-MYB is thought to have a general role in cell growth control, differentiation, and cancer, the ZPR9-dependent modulation of this transcription factor may contribute to elucidate the mech-
anism by which B-MYB affects these processes as well as the mechanism of transcription activation by B-MYB.

Acknowledgments—We thank Dr. Scott A. Ness for providing the pT81luc 3xA reporter plasmid. We also thank Taenam Kim, Se-Yeon Kim, and Myeong-Suk Choi for technical assistance.

REFERENCES
1. Nomura, N., Takahashi, M., Matsui, M., Ishii, S., Date, T., Sasamoto, S., and Ishii, S. (1998) Nucleic Acids Res. 16, 11075–11089
2. Golay, J., Capucci, A., Arsura, M., Castellano, M., Rizzo, V., and Introna, M. (1991) Blood 77, 149–158
3. Sala, A., and Casella, I. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10415–10419
4. Rascilla, G., Negroni, A., Sala, A., Pisci, S., Romeo, A., and Calabretta, B. (1995) J. Biol. Chem. 270, 8540–8545
5. Sala, A., Kundu, M., Casella, I., Engelhard, A., Calabretta, B., Grasso, L., Paggi, G. M., Giordano, A., Watson, R. J., Khalili, K., and Peschle, C. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 532–536
6. Lane, S., Farlie, P., and Watson, R. (1997) Oncogene 14, 2445–2453
7. Ziebold, U., Bartoch, O., Marais, R., Ferrari, S., and Kempfauener, K. H. (1997) Curr. Biol. 7, 253–260
8. Nakauchi, H., Takemoto, Y., Ha, H., and Ishii, S. (1995) Oncogene 10, 1699–1707
9. Nakauchi, H., Takemoto, Y., and Ishii, S. (1993) J. Biol. Chem. 268, 14161–14167
10. Dash, A. B., Orrico, F. C., and Ness, S. A. (1996) Genes Dev. 10, 1858–1869
11. Tavner, F., Capucci, A., Tashiro, S., Favier, D., Jenkins, N. A., Gilbert, N. A., Copeland, N. G. E., Maltby, J., Keough, J., Ishii, R., and Gonda, T. J. (1998) Mol. Cell. Biol. 18, 989–1002
12. Hedges, J. D., and Ness, S. A. (1998) Mol. Cell. Biol. 18, 2729–2737
13. Levenson, J., and Ness, S. A. (1998) Mol. Cell. Biol. 18, 253–261
14. Kane-Ishii, C., Nakai, A., Morimoto, B. I., and Ishii, S. (1997) Science 277, 246–249
15. Ying, G.-G., Proost, P., Damme, J. V., Busch, M., Introna, M., and Golay, J. (2000) J. Biol. Chem. 275, 4152–4158
16. Pitzner, E., Kiefer, J., Becker, P., Rolke, A., and Schule, R. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5497–5504
17. Ying, G.-G., Arsura, M., Introna, M., and Golay, J. (1997) J. Biol. Chem. 272, 24921–24926
18. Oelgeschlager, M., Janneck, R., Krieg, J., Schreek, S., and Luscher, B. (1996) EMBO J. 15, 2771–2778
19. Dai, P., Nakai, A., Tanaka, Y., Hou, D. X., Yasukawa, T., Kane-Ishii, C., Takahashi, T., and Ishii, S. (1996) Genes Dev. 10, 528–540
20. Bartunek, P., Kralatka, V., Dvorakova, M., Zahorova, V., Mandikova, S., Zenke, M., and Dvorak, M. (1997) Oncogene 15, 2939–2949
21. Favier, D., and Gonda, T. J. (1994) Oncogene 9, 305–311
22. Gonda, T. J., Favier, D., Ferrao, P., MacMillan, E. M., Simpson, R., and Tavner, J. (1996) Curr. Top. Microbiol. Immunol. 211, 99–109
23. Kaspar, P., Dvorakova, M., Kralova, J., Pajer, P., Kozmik, Z., and Dvorak, M. (1996) J. Biol. Chem. 271, 14422–14428
24. Gil, M., Yang, Y., and Ha, H. (1996) Immunol. Lett. 64, 79–83
25. Heyer, B. S., Warsow, J., Solter, D., Knowles, B. B., and Ackerman, S. L. (1997) Mol. Reprod. Dev. 47, 148–156
26. Heyer, B. S., Kochanowski, H., and Solter, D. (1999) Dev. Dyn. 215, 344–351
27. Seung, H.-A., Gil, M., Kim, K.-T., Kim, S.-J., and Ha, H. (2002) Biochem. J. 361, 657–664
28. Kim, T., Jung, H., Min, S., Kim, K.-T., and Ha, H. (1999) FEBS Lett. 460, 363–368
29. Hess, S. A., Markwell, A., and Grof, T. (1989) Cell 59, 1115–1125
30. Jung, H., Kim, T., Chae, H. Z., Kim, K.-T., and Ha, H. (2001) J. Biol. Chem. 276, 15304–15310
31. Baleeva-Gargova, Z., Konstantinov, K. N., Wu, I. H., Klier, F. G., Barrett, T., and Davis, R. J. (1998) Science 272, 1797–1802
32. Rothe, C., Wang, S. C., Henzel, W. J., and Goeddel, D. V. (1994) Cell 78, 681–692
33. Hu, M. H., O’Flourke, K., Boguski, M. S., and Dixit, V. M. (1994) J. Biol. Chem. 269, 30069–30072
34. Cheng, G., Cleary, A. M., Ye, Z. H., Hong, D. I., Lederman, S., and Baltimore, D. (1995) Science 267, 1494–1498
35. Ishida, T., Tojo, T., Aoki, T., Kobayashi, N., Oishi, T., Watanabe, T., Yamamoto, T., and Inoue, J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9437–9442
36. Milaneso, G., Droual, B., Benincasa, L., Bernini, S., Piacentini, M., Reichert, U., and Cohen, P. (1997) Exp. Cell Res. 235, 55–61
Enhancement of B-MYB Transcriptional Activity by ZPR9, a Novel Zinc Finger Protein
Hyun-A Seong, Kyong-Tai Kim and Hyunjung Ha

J. Biol. Chem. 2003, 278:9655-9662.
doi: 10.1074/jbc.M207478200 originally published online January 6, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M207478200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 41 references, 22 of which can be accessed free at
http://www.jbc.org/content/278/11/9655.full.html#ref-list-1