Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 10762–10767, September 1997
Genetics

Corepressor SMRT binds the BTB/POZ repressing domain of the LAZ3/BCL6 oncoprotein

(transcriptional repression/diffuse large cell lymphomas/yeast two-hybrid screen)

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ABSTRACT **The *LAZ3/BCL6* (lymphoma-associated zinc finger 3/B cell lymphomas 6) gene frequently is altered in non-Hodgkin lymphomas. It encodes a sequence-specific DNA binding transcriptional repressor that contains a conserved N-terminal domain, termed BTB/POZ (bric-à-brac tramtrack broad complex/pox viruses and zinc fingers). Using a yeast two-hybrid screen, we show here that the LAZ3/BCL6 BTB/POZ domain interacts with the SMRT (silencing mediator of retinoid and thyroid receptor) protein. SMRT originally was identified as a corepressor of unliganded retinoic acid and thyroid receptors and forms a repressive complex with a mammalian homolog of the yeast transcriptional repressor SIN3 and the HDAC-1 histone deacetylase. Protein binding assays demonstrate that the LAZ3/BCL6 BTB/POZ domain directly interacts with SMRT *in vitro*. Furthermore, DNA-bound LAZ3/BCL6 recruits SMRT *in vivo*, and both overexpressed proteins completely colocalize in nuclear dots. Finally, overexpression of SMRT enhances the LAZ3/BCL6-mediated repression. These results define SMRT as a corepressor of LAZ3/BCL6 and suggest that LAZ3/BCL6 and nuclear hormone receptors repress transcription through shared mechanisms involving SMRT recruitment and histone deacetylation.**

The *LAZ3/BCL6* (lymphoma-associated zinc finger 3/B cell lymphomas 6) gene has been cloned by virtue of its frequent structural alteration in both diffuse large cell and follicular lymphomas (1–3). These alterations include translocations, small deletions, and point mutations. Most of them have been found in a genomic region, called the major translocation cluster, containing the first noncoding exon and the first downstream intron of the *LAZ3/BCL6* locus (4–8). It usually is proposed that such structural alterations lead to the deregulation of LAZ3/BCL6 expression and, hence, contribute to lymphomagenesis (4, 7). The normal *LAZ3/BCL6* expression pattern suggests its implication in B cell differentiation and in the control of T cell-dependent immune response (9). Recent genetic experiments in mouse abrogating LAZ3/BCL6 expression or leading to the expression of an inactive deleted version of this protein substantiate this hypothesis. Indeed, mice deficient for LAZ3/BCL6 activity are devoid of germinal centers, present a Th2-type inflammatory disease and a defect in T cell-dependent antibody response (10, 11). Taken together, these results suggest that LAZ3/BCL6-associated lymphomas may occur as a consequence of a deregulated *LAZ3/BCL6* expression.

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The *LAZ3/BCL6* gene encodes a sequence-specific transcriptional repressor that harbors six C-terminal C2H2 krüppel-like zinc fingers. These zinc fingers are responsible for the sequence-specific DNA binding of the protein. At its N-terminal part, LAZ3/BCL6 also contains an ≈130-aa conserved domain termed the BTB/POZ (bric-à-brac tramtrack broad complex/pox viruses and zinc fingers) domain (12, 13). This domain has been identified in ≈40 proteins found in Metazoans and poxviruses (13). In LAZ3/BCL6, the BTB/POZ domain mediates self-interaction and targets the protein into nuclear dots (9, 14). Moreover, it is required for full LAZ3/BCL6-mediated repression, and holds an autonomous transcriptional repressing activity when tethered to DNA by a heterologous DNA binding domain (15–18).

To further examine the function of the LAZ3/BCL6 BTB/POZ domain, we performed a yeast two-hybrid screen (19) using this domain as a bait. Here we show that one of the isolated cofactors is the SMRT (silencing mediator of retinoid and thyroid receptor) protein. SMRT previously was identified as one of the related corepressors collectively referred to as TRACs (thyroid and retinoid receptors associated corepressors) (20–26). We demonstrate that the BTB/POZ domain of LAZ3/BCL6 is necessary and sufficient for its interaction with SMRT. Moreover, both proteins colocalize in nuclear dots when expressed in mammalian cells. Finally, SMRT expression enhances LAZ3/BCL6-dependent transcriptional repression. Collectively, these results define SMRT as a LAZ3/BCL6 corepressor and suggest that the nuclear receptors and LAZ3/BCL6 (possibly as well as other BTB/POZ transcriptional repressors) could repress transcription through a shared mechanism.

MATERIALS AND METHODS

Yeast Methods. The Y190 yeast strain (CLONTECH) was transformed using the LiAc/polyethylene glycol method (27) with the pGBT9-LAZ(1–181) construct and a cDNA library from human Epstein–Barr virus-transformed lymphocytes cloned in the pACT vector (CLONTECH) and then incubated in a selective medium without leucine and tryptophane at 30°C for 4 days. Two of 6.10^5 colonies were positive for β-galactosidase (β-gal) activity using a 5-bromo-4-chlor-3-indoly β-D-galactoside (Sigma) filter assay. For quantitative β-gal

Abbreviations: BTB/POZ, bric-à-brac tramtrack, broad complex/pox viruses and zinc fingers; LAZ3/BCL6, lymphoma associated zinc finger 3/B cell lymphomas 6; SMRT, silencing mediator of retinoid and thyroid receptor; β-gal, β-galactosidase; RAR, retinoic acid receptor; TR, thyroid hormone receptor; VP16, viral protein 16; GAL4dbd, GAL4 DNA binding domain; GAL4act, GAL4 activation domain; GST, glutathione *S*-transferase; PLZF, promyelocytic leukemia zinc fingers.

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activity measure, Y190 yeast cells were transformed using the same method, and three growing colonies were used to inoculate 5 ml of yeast extract/peptone/dextrose medium. Aliquots of the resulting overnight culture at 30°C were used to perform liquid β-gal assays using ortho-nitrophenyl-β-D-galactopyranoside (Sigma) as a reporter. The β-Gal activities are expressed according to ref. 28. Experiments were repeated three times for each clone, and three clones were used for each interaction tested.

Plasmids. *Yeast expression vectors.* The chimeras between the GAL4 DNA binding domain (GAL4dbd) (pGBT9) or GAL4 activation domain (GAL4act) (pGAD424) with the LAZ3/BCL6 derivatives were generated either by using PCR [LAZ(1–140) and LAZ(1–181)] or a PCR-produced adaptor

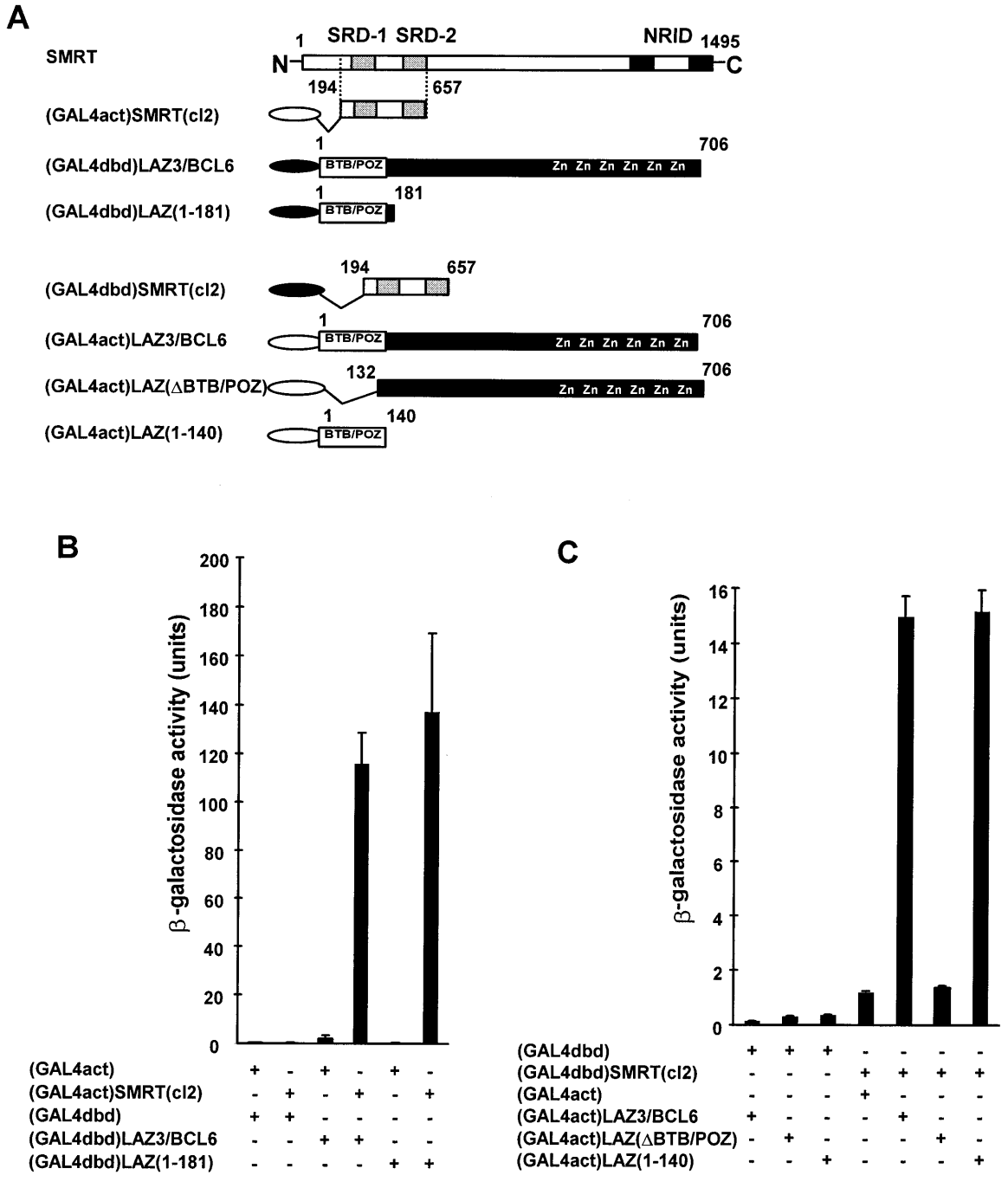

FIG. 1. Interaction between the LAZ3/BCL6 BTB/POZ domain and SMRT in the yeast two-hybrid assay. (*A*) Schematic drawing of the LAZ3/BCL6 and SMRT chimeras used in the two-hybrid experiments in *B* (*Upper*) and *C* (*Lower*). The SMRT protein is shown, with its nuclear receptors interaction domains (NRID) (black boxes) and its two SMRT repressive domains (SRD-1 and SRD-2) (gray boxes) (22–26) to point out the SMRT(cl2) cloned in the two-hybrid screen. In the LAZ3/BCL6 protein, the N-terminal BTB/POZ domain and the six C-terminal zinc fingers are shown. GAL4dbd, black ovals; GAL4act, open ovals. (*B*) Both full-length LAZ3/BCL6 and LAZ(1–181) proteins interact with SMRT(cl2). The proteins expressed (+) or not expressed (−) in the corresponding yeast two-hybrid test are mentioned. β-Gal activity was determined by liquid assay and expressed as a unit defined in ref. 28. The average of three experiments in triplicate are plotted. SD are shown. (*C*) The BTB/POZ domain mediates the interaction between LAZ3/BCL6 and SMRT(cl2) in the yeast two-hybrid system. The proteins expressed (+) or not expressed (−) in the corresponding test are mentioned. The average of three experiments in triplicate are plotted. Results are expressed as in *B*. All GAL4act chimeras are expressed in yeast at similar level as indicated by Western blot analyses using an anti-GAL4act antibody (data not shown).

(LAZ3/BCL6, ΔBTB/POZ). The constructs were checked by DNA sequencing. The pGBT9 derivative encoding the (GAL4dbd)SMRT(cl2) chimera was obtained by cloning the *Bgl*II 1.4-kb fragment of the pACT-SMRT(cl2) into a *Bam*HI-opened pGBT9.

Bacterial and mammalian expression vectors. The pGEX-KG-LAZ(5–121) was generated using a PCR-amplified LAZ3/BCL6 fragment encoding amino acids 5–121. The following vectors previously have been described: pGEX-RARα (retinoic acid receptor α), pCMX-SMRT (22), pCMX-VP16 (viral protein 16) (25), pTL-LAZ3BCL6-flg, pTL-LAZ3/BCL6-GFP (green fluorescent protein) (14), pSG424-LAZ(BTB/POZ), pSG424-LAZ(ΔBTB/POZ), and pTL-LAZ3/BCL6 (15, 16). The pCMX-VP16-SMRT(cl2) was obtained by cloning the 1.4-kb *Eco*RI fragment of the pSG5424-SMRT(cl2) into an *Eco*RI-opened pCMX-VP16. The pSG-FNV-(flag-nuclear localization signal-VP16)-SMRT(cl2) was generated by cloning the 1.4-kb *Xho*I fragment of the pACT-SMRT(cl2) into a *Xho*I-opened pSG-FNV (29). Further detailed information on the vectors used in this study are available upon request. All of the plasmids used in this study were prepared using Qiagen columns.

Glutathione *S*-Transferase (GST) Pulldown Assay. The GST-fusion proteins were induced, purified, and stored in protein storage buffer (50 mM Tris, pH 7.5/10% glycerol/5 mM DTT). Before the binding assay, approximately equal amounts of the proteins were loaded on glutathione Sepharose 4B beads (Pharmacia LKB) and blocked in binding buffer (20 mM Hepes, pH 7.9/100 mM NaCl/1 mM EDTA/4 mM $MgCl_2$/1 mM DTT/0.02% Nonidet P-40/10% glycerol with fresh 0.5 mM phenylmethysulfonyl fluoride and protease inhibitors) supplied with 1 mg/ml BSA for 15 min at 4°C with gentle rocking. The beads then were spun down and resuspended as a 40% suspension in binding buffer. *In vitro* transcription-translation mixtures (TNT kit, Promega) containing [^{35}S]methionine were programmed with the relevant plasmids. Ten microliters of programmed lysates were incubated with 50 μl of preblocked GST proteins at 4°C with rotation for 30 min. The beads then were washed three times with binding buffer. Bound proteins were eluted in SDS sample buffer, resolved by SDS/PAGE, and visualized by autoradiography.

Cell Culture and Transfections. C2 mouse muscle cells were maintained in MCDB202/DMEM (1/1) supplemented with 20% fetal calf serum (30–32). One day before transfection, cells were plated at 30–40% confluency in 35-mm plates or 6-well plates (Falcon). For transfection, cells were incubated with 2.1 μg of plasmid DNA and 12 μl of Lipofectamine (GIBCO/BRL) for 5 hr in 1 ml of optiMEM (GIBCO/BRL) and then in fresh MCDB202/DMEM (1:1) and 20% fetal calf serum culture medium. For reporter assays, detailed plasmids transfection conditions are described in figure legends for each experiment presented. The pSG5-β-gal vector (0.1 μg) was cotransfected in each assay to correct for variation in transfection efficiency. Cells were washed 36 hr after transfection in PBS 1× and lysed in universal lysis buffer (Promega). Luciferase activity was measured using beetle luciferin (Promega) (15, 16). β-Gal activity was measured using the galacto-light kit (Tropix). Both luciferase and β-gal activities were measured with a Berthold chimioluminometer. Results are the means (±SD) of at least three transfections. For immunofluorescence analyses, 1 μg of each expression vector was transfected.

Immunofluorescence Experiments. Transfected C2 cells were fixed 24 hr after transfection using neutralized formalin (Sigma), permeabilized by 1× PBS, 0.25% Triton X-100, and neutralized with 1× PBS containing 50 mM NH_4CL_2. Each step was separated by three washes with 1× PBS. Incubation with the antibodies was performed in 1× PBS, 0.2% gelatin for 1 hr at room temperature. The primary monoclonal anti-Flag-M2 (Kodak) and the polyclonal anti-SMRT antibody [raised against the bacterially purified SMRT(cl2)] were used at the 1/500th and 1/200th dilution, respectively. The secondary fluorescein isothiocyanate-conjugated anti-mouse antibody (Dako) and the biotinylated anti-rabbit antibody (Amersham) were used at the 1/40th and the 1/400th dilution, respectively, for 30′ at room temperature. Then, tetramethylrhodamine isothiocyanate-conjugated streptavidin (Amersham) was used at the 1/200th dilution for 20 min at room temperature. Each incubation was separated by three washes in 1× PBS, 0.2% gelatin. Finally, plates were mounted and observed on a optical microscope (Leica). Pictures were taken using 400ASA Ektachrome Kodak film.

RESULTS

We used the first 181 amino acids of LAZ3/BCL6, encompassing the BTB/POZ domain, as a bait in a yeast two-hybrid screen to isolate cofactors interacting with LAZ3/BCL6 (19). We isolated a cDNA encoding amino acids 194–657 [thereafter referred to as SMRT(cl2)] of the SMRT protein (Fig. 1 *A* and *B*).

Next, we set out to identify the region of LAZ3/BCL6 that is sufficient to interact with SMRT(cl2). When fused to GAL4act, a LAZ3/BCL6 derivative lacking the BTB/POZ domain [(GAL4act)LAZ(ΔBTB/POZ)] failed to interact with SMRT(cl2) in the yeast two-hybrid system (Fig. 1*C*), whereas the isolated BTB/POZ domain [(GALact)LAZ(1–140)] shows strong association (Fig. 1*C*). We conclude that the BTB/POZ domain is both necessary and sufficient for LAZ3/BCL6 to interact with SMRT(cl2). Interestingly, this region of SMRT includes the N-terminal repressing domains SRD-1 and SRD-2 (Fig. 1*A*), but not the C-terminal nuclear receptors interaction domains (22–26).

We next attempted to confirm the interaction between the BTB/POZ domain and SMRT *in vitro* (Fig. 2). In GST pulldown experiments, the BTB/POZ domain is able to retain the *in vitro* translated and ^{35}S-labeled full-length SMRT (Fig. 2, lane 4) about as efficiently as RARα does (Fig. 2, lane 3). As a control, GST alone is unable to pull down SMRT (Fig. 2 lane 2). We conclude that the LAZ3/BCL6 directly binds the SMRT protein *in vitro* through its BTB/POZ domain.

We next addressed in mammalian cells the interactions observed in yeast and *in vitro* between SMRT and the LAZ3/BCL6

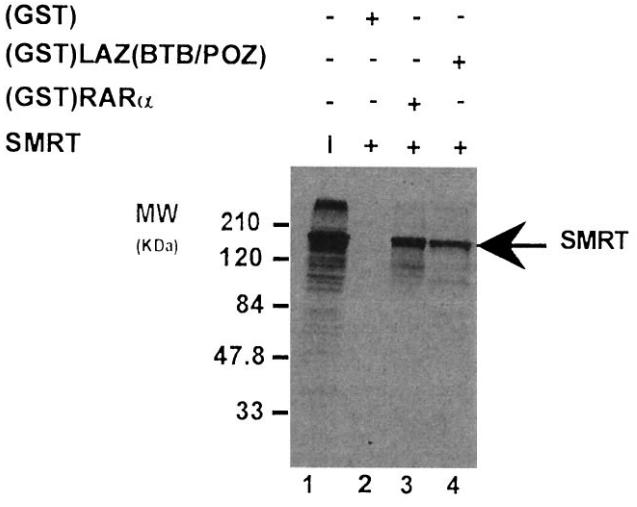

FIG. 2. The BTB/POZ domain of LAZ3/BCL6 directly interacts with SMRT in GST pulldown assays. *In vitro* translated and ^{35}S-methionine-labeled SMRT (lane 1, I represents 40% of the SMRT amount used in the pulldown experiments) was incubated with either GST alone (lane 2), or GST-LAZ(BTB/POZ) (lane 4). Interaction between SMRT and GST-RARα is shown as a positive control (lane 3). Molecular masses are indicated on the left.

BTB/POZ domain. To this end, we performed two-hybrid experiments in mouse C2 cells, using GAL4dbd-LAZ3/BCL6 and VP16 transactivation domain (VP16)-SMRT(cl2) chimeras. As expected, we observed a ≈300-fold increase in the GAL4 responsive reporter activity when a (VP16)SMRT(cl2) chimera is coexpressed with (GAL4dbd)LAZ(BTB/POZ) in these cells (33) (Fig. 3*B*). In contrast, the cotransfection of (VP16)SMRT(cl2) with either the isolated GAL4dbd or a chimera fusing the GAL4dbd to a LAZ3/BCL6 derivative lacking only the BTB/POZ domain [(GAL4dbd)LAZ(ΔBTB/POZ)] completely failed to activate the same reporter (Fig. 3*B*). Thus, in complete agreement with our results both in yeast and *in vitro*, the BTB/POZ domain of LAZ3/BCL6 interacts with SMRT in mammalian cells. We next addressed the possibility for DNA-bound LAZ3/BCL6 to recruit SMRT in mammalian cells (Fig. 3*C*). Concomitant expression of LAZ3/BCL6 and VP16-SMRT(cl2) leads to a ≈10-fold increase of the B6BStkluc reporter activity (18), which contains a LAZ3/BCL6 target sequence upstream of the minimal tk promoter (Fig. 3*C*). We conclude that DNA-bound LAZ3/BCL6 is indeed able to recruit SMRT in mammalian cells.

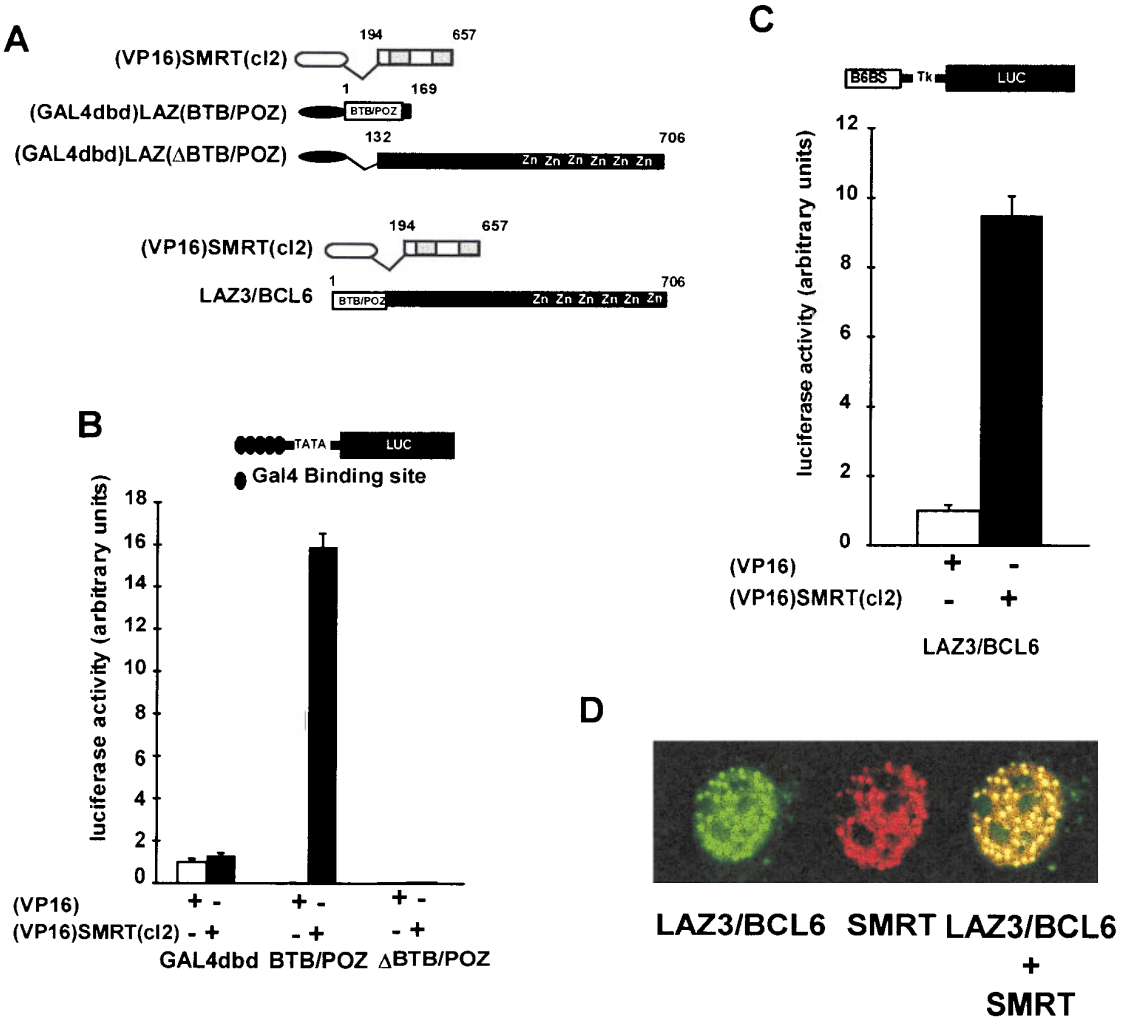

FIG. 3. The BTB/POZ domain mediates the interaction between LAZ3/BCL6 and SMRT(cl2) in mammalian cells. (*A*) Schematic representation of the mutants used in *B* (*Upper*) and *C* (*Lower*). VP16 activating domain (open boxes) and GAL4dbd (black ovals) are shown (see Fig. 1 *A* for the other legend conventions). (*B*) DNA-tethered BTB/POZ recruits SMRT(cl2) in mammalian cells. GAL4dbd, (GAL4dbd)LAZ(BTB/POZ), or (GAL4dbd)LAZ(ΔBTB/POZ) were coexpressed with either the isolated VP16 activating domain (VP16) (empty bars) or the VP16 activating domain fused to SMRT(cl2) [(VP16)SMRT(cl2)] (filled bars). The G5-TATA-Luc reporter construct (33) is shown above the panel. Only the coexpression of (GAL4dbd)LAZ(BTB/POZ) with the (VP16)SMRT(cl2) construct elicits a dramatic increase in the activity of the G5-TATA-Luc reporter (33). Either pSG424, pSG424-LAZ(BTB/POZ), or pSG424-LAZ(ΔBTB/POZ) (34) (0.2 μg) was transfected together with either VP16 or (VP16)SMRT(cl2) expressing pSG-FNV vectors (0.2 μg) (36) and with the G5-TATA-Luc (1.6 μg) reporter (33). Normalized luciferase activity are plotted (arbitrary units). The results obtained with the coexpression of the isolated GAL4dbd with the VP16 activating domain are arbitrarily taken as 1. Note that we observed a repressive effect as previously described when the VP16 alone was coexpressed with the GAL4dbd LAZ3/BCL6 chimeras (13–18). (*C*) DNA-bound LAZ3/BCL6 recruits SMRT(cl2) in mammalian cells. Full-length LAZ3/BCL6 was coexpressed with either the isolated VP16 activating domain (empty bar) or with the (VP16)SMRT(cl2) chimera (filled bar). The B6BS-tk-LUC reporter (*Upper*) contains one LAZ3/BCL6 binding site upstream of the minimal tk promoter (18). The pTL1-LAZ3/BCL6 expression vector (0.2 μg) was cotransfected along with the B6BStkLuc reporter vector (1.6 μg) (18) and either pCMX-VP16 or pCMX-VP16-SMRT(cl2) expression vector (0.2 μg). Results are expressed as normalized luciferase activity. The activity obtained for the coexpression of LAZ3/BCL6 and VP16 was arbitrarily taken as 1. (*D*) LAZ3/BCL6 and SMRT colocalize in nuclear dots. A SMRT and an epitope-tagged LAZ3/BCL6 (LAZ3/BCL6-flag) encoding vectors were cotransfected in C2 cells. The nucleus of a cotransfected C2 cell is shown for the LAZ3/BCL6-flag pattern in green (*Left*) and for the SMRT pattern in red (*Middle*). Both patterns are completely identical as shown by the resulting yellow image after superimposition of both staining (*Right*). Note that the same results were obtained with two different anti-SMRT polyclonal antibodies, or when SMRT was cotransfected with the "self-detectable" LAZ3/BCL6-GFP (green fluorescent protein) chimera ruling out that the observed colocalization results from antibody crossreactions (data not shown).

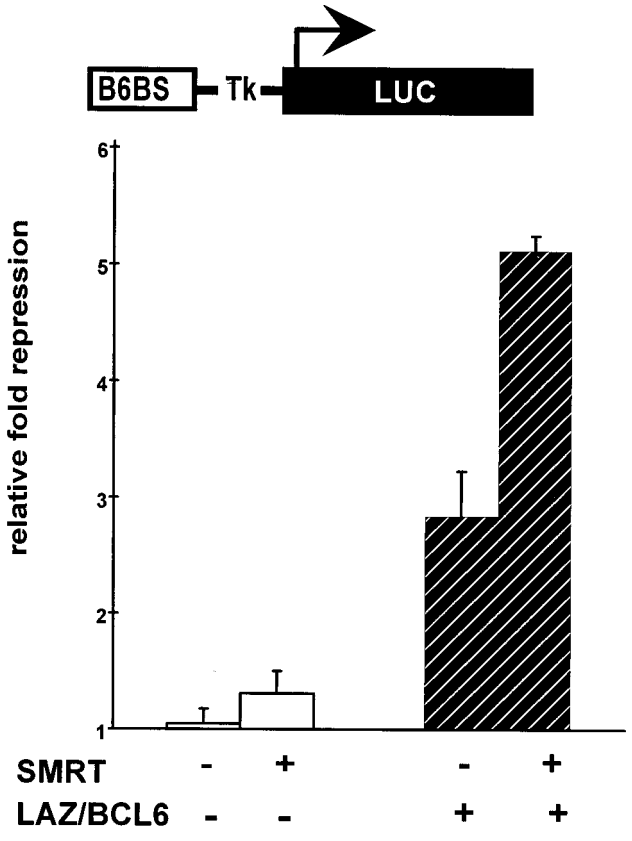

FIG. 4. Overexpression of SMRT potentiates the LAZ3/BCL6-mediated repression. The LAZ3/BCL6 encoding vector (pTL1-LAZ3/BCL6) was transfected without (−) or with (+) the SMRT encoding vector (pCMX-SMRT) (hatched bars). The coexpression of LAZ3/BCL6 and SMRT elicits a ≈ 2-fold increase in the LAZ3/BCL6-mediated repression of the B6BS reporter activity (18). Cotransfection of pCMX-SMRT with the empty pTL1 vector results in a slight decrease of the B6BS reporter activity, as compared with the mock transfected cells (empty bars). This is presumably due to the weak expression of the endogenous LAZ3/BCL6 protein in C2 cells (ref. 15, data not shown). Either empty pTL or pTL-LAZ3/BCL6 (17) expression vector was transfected together with B6BS-tkLuc (1.5 μg) (18), and with either empty pCMX or pCMX-SMRT expression vector (0.2 μg). pSG5-β-gal (0.1 μg) was cotransfected in each assay to correct for variation in transfection efficiency. Results are expressed as normalized luciferase activity.

To further substantiate the interaction between SMRT and LAZ3/BCL6 in mammalian cells, we took advantage of the observation that overexpressed LAZ3/BCL6 forms readily detectable nuclear dots (9, 14). By immunofluorescence analyses, we show in Fig. 3*D* that LAZ3/BCL6 and SMRT completely colocalize in such nuclear dots when simultaneously overexpressed in C2 cells. These results again support the conclusion that LAZ3/BCL6 and SMRT form complexes in mammalian cell nuclei.

We next explored the functional consequences of the physical interaction between LAZ3/BCL6 and SMRT. We and others have shown that the LAZ3/BCL6 protein acts as a transcriptional repressor on its cognate DNA-binding sequence (15–18). SMRT interacts with unliganded RARs and thyroid hormone receptors (TRs) and represses transcription when tethered to DNA, thereby fulfilling the criteria of a corepressor. If SMRT is also a limiting corepressor of LAZ3/BCL6, then its overexpression should potentiate the efficiency of LAZ3/BCL6-mediated repression. Results shown in Fig. 4 fully substantiate this hypothesis. Overexpression of SMRT enhances the silencing effect mediated by LAZ3/BCL6 on its own binding site (Fig. 4). Furthermore, the effect of SMRT on LAZ3/BCL6-mediated repression is dose-dependent (data not shown). Thus, SMRT both interacts with LAZ3/BCL6 and potentiates its repressing activity.

DISCUSSION

The present study defines SMRT as a corepressor of the LAZ3/BCL6 oncoprotein. This conclusion arises from the following data: (*i*) yeast and mammalian two-hybrid systems show that SMRT interacts with the repressing BTB/POZ domain of LAZ3/BCL6; (*ii*) GST pulldown assay further demonstrates that SMRT and BTB/POZ directly associate *in vitro*; (*iii*) DNA-bound LAZ3/BCL6 is able to recruit SMRT *in vivo*; (*iv*) LAZ3/BCL6 and SMRT fully colocalize in nuclear dots; and (*v*) SMRT potentiates the LAZ3/BCL6-mediated transcriptional repression. In addition, our results point out that SMRT function is not restricted to nuclear receptor-mediated repressing activity. Rather, SMRT is a shared corepressor recruited to DNA by unliganded nuclear receptors, in particular RAR and TR, the BTB/POZ domain/zinc finger transcription factors, and Mad/Max heterodimeric complex (26).

The understanding of the LAZ3/BCL6-mediated transcriptional repression could shed light on the mechanisms underlying its implication in the appearance of diffuse large cell lymphomas. Indeed, *LAZ3/BCL6* is a specific regulator of germinal center formation (10, 11) and is structurally altered in most cases of diffuse large cell lymphomas, a germinal center-derived tumor. Importantly, these structural alterations seem to be restricted to the 5′ noncoding region, and hence are thought to alter *LAZ3/BCL6* expression while leaving intact its product (4–8). Thus, upon *LAZ3/BCL6* structural alteration and deregulation, the ectopic repression of normal LAZ3/BCL6 target genes in late stages of B cell differentiation, especially after the exit of germinal center (9, 11), may contribute to diffuse large-cell lymphoma pathogenesis. In addition, as SMRT is a shared corepressor, misregulated LAZ3/BCL6 exerts an indirect effect by interfering with other SMRT-dependent regulatory pathways. In this regard, it should be noted that a shift from Myc/Max to Mad/Max complexes occur during cellular differentiation, and some RAR ligands appear to play an important role in the control of B cell proliferation (35, 36).

Several *Drosophila* and vertebrate BTB/POZ domain/zinc fingers proteins can, like LAZ3/BCL6, repress transcription through sequence-specific DNA binding (37–43). Interestingly, the BTB/POZ of promyelocytic leukemia zinc fingers (PLZF), a relative of LAZ3/BCL6 (13, 42), both autonomously represses transcription (43) and interacts with SMRT (R.J.L. and R.M.E., unpublished work), suggesting that SMRT may be a common corepressor for BTB/POZ-containing transcriptional repressors. PLZF originally was cloned because of its fusion to RARα in a subset of acute promyelocytic leukemia (42), and its BTB/POZ domain is chiefly responsible for the dominant negative properties of the PLZF-RARα protein on the ligand-dependent RARα transcriptional activity (44, 45). Together with our data, these findings suggest that the interaction between SMRT and the BTB/POZ domain of PLZF may interfere with the transcriptional activity of the RARα moiety within the PLZF-RARα chimera and thus directly contribute to its oncogenic activity.

It has been postulated that several nuclear BTB/POZ proteins could act by remodeling chromatin structures (13). In this respect, it is worth noting that SMRT recently was shown to form a ternary complex with mSin3A, a corepressor of the Mad and Mxi bHLH-ZIP proteins, and HDAC-1, a histone deacetylase homologous to the yeast *rpd-3* gene product (26, 46–51). Interestingly, LAZ3/BCL6 interacts with the region of SMRT that has strong repression activity (Fig. 1*A*), raising the possibility that it also may associate with mSin3A/HDAC-1

and form a multimeric repression complex involving histone deacetylation activity.

We thank Christelle Desbois and Pierre Jalinot for the gift of the pSG-FNV plasmid, Riccardo Dalla-Favera for the gift of the B6BS-tk-Luc reporter construct, Danièle Lantoine for her expertise in cell culture, and Hélène Pelczar for advice in transfection experiments. P.D. is a Fellow of the Secrétariat d'Etat à la Recherche. O.A. is a Fellow of the Institut de Recherches sur le Cancer de Lille. R.J.L. is a Predoctoral Fellow of the Lucille P. Markey Charitable Trust. R.M.E. is an Investigator of the Howard Hughes Medical Institute at the Salk Institute for Biological Studies. This work was supported by the Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Association pour la Recherche contre le Cancer, Ligue Nationale contre le Cancer, and Fondation pour la Recherche Médicale.

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