Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 12265–12269, December 1995
Biochemistry

Functional antagonism between the retinoic acid receptor and the viral transactivator BZLF1 is mediated by protein–protein interactions

(transcriptional regulation/basic leucine zipper transcription factors/nuclear receptors/transrepression)

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Communicated by Elwood V. Jensen, University of Hamburg, Hamburg, Germany, September 1, 1995 (received for review June 21, 1995)

ABSTRACT **The Epstein–Barr virus-encoded protein BZLF1 is a member of the basic leucine zipper (bZip) family of transcription factors. Like several other members of the bZip family, transcriptional activity of BZLF1 is modulated by retinoic acid receptors (RARs). We present evidence that the RARα and BZLF1 can reciprocally repress each other's transcriptional activation by a newly discovered mechanism. Analysis of RARα mutants in transfection studies reveals that the DNA binding domain is sufficient for inhibition of BZLF1 activity. Analysis of BZLF1 mutants indicates that both the coiled-coil dimerization domain and a region containing the transcriptional activation domain of BZLF1 are required for transrepression. Coimmunoprecipitation experiments demonstrate physical interactions between RARα and BZLF1 *in vivo*. Furthermore, glutathione *S*-transferase-pulldown assays reveal that these protein–protein interactions are mediated by the coiled-coil dimerization domain of BZLF1 and the DNA binding domain of RARα. While RARα is unable to recognize BZLF1 binding sites, the RARα can be tethered to the DNA by forming a heteromeric complex with BZLF1 bound to DNA. Tethering RARs via protein–protein interactions onto promoter DNA suggest a mechanism through which RARs might gain additional levels of transcriptional regulation.**

Epstein–Barr virus is a human herpesvirus that infects B lymphocytes and epithelial cells of the nasopharynx (1). Infection of B cells typically results in a latent state, whereas infection of epithelial cells leads to viral replication and cytolysis (2). The switch from latency to the productive (replicative) cycle is induced by various agents such as calcium ionophores, sodium butyrate, and tumor promoters like phorbol 12-myristate 13-acetate, all of which activate expression of the Epstein–Barr virus immediate-early gene product BZLF1 (2). Overexpression of BZLF1 is sufficient to trigger the switch to the lytic cycle (2). Like the c-Jun/c-Fos proteins BZLF1 is a member of the basic leucine zipper (bZIP) transcription factor family (3, 4). BZLF1 binds as a homodimer to DNA (4) and transactivates several early viral promoters required in the cytolytic cycle of the virus. The BZLF1 response elements (ZREs) in these promoters are similar and sometimes identical to AP-1 binding sites (4).

Retinoids play an important role in development and differentiation and are well-known inhibitors of cell growth (for review, see ref. 5). Retinoic acid (RA) exerts biological effects by acting through at least two distinct classes of intracellular proteins including the RA receptors (RARs) and the retinoid X receptors (RXRs), both of which are members of the nuclear receptor superfamily (5). While both the RARs and RXRs are effective activators of some genes, RA is also known to repress expression of AP-1-dependent genes (6). RAR has been shown to inhibit transcriptional activity of the AP-1 complex by a mechanism that is not dependent on the receptor's ability to bind DNA and most likely reflects protein–protein interactions (6). This interaction of distinct regulatory pathways, termed "cross-talk," suggested a means by which nuclear hormone receptors could modulate the action of growth-inducing factors. Recent data suggested that RARα is able to repress the BZLF1-mediated transcriptional activation of the BMFR1 promoter via direct protein–protein interactions between RARα and BZLF1 (7).

To further extend our understanding of the cross-talk between RAR and members of the bZIP transcription factor family, we were interested in examining the mutual repression effects of RARα and BZLF1 in more detail. In this report, we show that RARα and BZLF1 repress each other's transcriptional activity. Transfection analysis of RARα and BZLF1 mutants reveals that the transactivation domain and the dimerization domain of the BZLF1 and the DNA binding domain (DBD) of RARα are required for repression. Glutathione *S*-transferase (GST)-pulldown assays and coimmunoprecipitation experiments demonstrate direct protein–protein interactions between BZLF1 and RARα both *in vitro* and *in vivo*. Furthermore, electrophoretic mobility shift assays (EMSAs) show that the RARα forms a specific heteromeric protein complex with BZLF1 bound to DNA. Our data suggest a strategy through which the RARs exert their transcriptional control. RARs can be tethered onto regulatory DNA sequences via protein–protein interactions with nonreceptor proteins, thereby gaining a new circuit of transcriptional regulation.

MATERIALS AND METHODS

Cell Culture and Transfection. NIH 3T3 and COS cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Transient transfection assays were carried out as described (6). Luciferase (LuC) activity was assayed as recommended by the manufacturer (Promega). All experiments were repeated at least three times.

Reporter Plasmids. The LuC reporter plasmids $(DR5)_2$-TKLuC and $(TREp)_2$-TKLuC have been described (8, 9). $(ZIIIA)_5$-TKLuC was generated by ligating 5 oligonucleotides containing the BZLF1 responsive element ZIIIA (10) in front of thymidine kinase (TK) LuC (8). To construct the $(ZIIIB)_4$-TATALuC reporter plasmid, two copies of an oligonucleotide containing two binding sites of the BZLF1-specific responsive element ZIIIB (10) were inserted into TATALuC.

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Abbreviations: RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor; GST, glutathione *S*-transferase; TK, thymidine kinase; LuC, luciferase; bZIP, basic leucine zipper; ZRE, BZLF1 response element; EMSA, electrophoretic mobility-shift assay; h, human; DBD, DNA binding domain.

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Expression Vectors. CMV-BZLF1 has been described (11). The expression vector CMX–NLS was created by inserting a Koszak consensus sequence and the nuclear localization signal of the simian virus 40 large T antigen (12) into CMX (9). To create the BZLF1 expression plasmids CMX–BZLF1 199*, CMX–BZLF1 ΔBR, CMX–BZLF1 178*, and CMX–BZLF1 CC the cDNAs coding for the respective domains were isolated from the plasmids BZLF1 N-*Pst* I (3), BZLF1 *Bsm* I/*Pst* I (4), and BZLF1 N-*Sty* (3) and recloned into CMX–NLS. The expression plasmids CMX–hRARα, CMX–hRXRα, GST–hRARα, and GST–RAR DBD have been described (13, 14). CMX–RAR DBD was generated by cloning the DBD of human (h) RARα (amino acids 82–167) into CMX–NLS. CMX–RAR ΔDBD was derived from RS–hRARα Δ81–153 (8). To create GST–RAR ΔDBD and GST–RAR C, the cDNA insert of CMX–RAR ΔDBD and a fragment coding for hRARα amino acids 187–351, was amplified by PCR and cloned into pGEX-1 (15).

***In Vitro* Binding Assays with GST Fusion Proteins.** The GST fusion proteins were expressed according to ref. 14. The *in vitro* interaction assays with [^{35}S]methionine-labeled, *in vitro* translated BZLF1 protein or mutated BZLF1 proteins were performed as recommended (16), except that all steps were performed at 37°C.

DNA Binding Studies. BZLF1 proteins were *in vitro* translated using the TNT-coupled wheat germ extract (Promega). BZLF1 primed lysate was preincubated with purified GST or GST–RAR fusion proteins (11 μg) in 20 mM Hepes, pH 7.6/100 mM KCl/10% (vol/vol) glycerol/0.2 mM EDTA/4 mM dithiothreitol (DTT)/1 μg of poly(dIdC) for 1 h at 37°C. ^{32}P-labeled ZIIIA ZRE probe (5′-AGCTTCATGAGCCA-GAG-3′) was added, incubated for 30 min at room temperature, and analyzed on a 4% polyacrylamide gel in 0.25× TBE at 4°C. To determine the composition of the various DNA–protein complexes, the following antibodies were included during incubation: anti-BZLF1 AZ 125 (17), anti-GST (Pharmacia), anti-Gal4 (Santa Cruz Biotechnology, Santa Cruz, CA), and anti-Flag (Kodak).

Coimmunoprecipitation and Immunoblotting. COS cells were transfected with 1 μg of CMV–BZLF1, CMX–hRARα, or both expression vectors. After 48 h, cells were harvested, resuspended in 300 μl of lysis buffer (50 mM Tris·HCl, pH 8.0/150 mM NaCl/1 mM DTT/0.5 mM EDTA/0.5% Nonidet P-40 supplemented with 200 μM Pefabloc, 2 mM leupeptin, and 1 mM pepstatin A) and lysed. Equal amounts of proteins were incubated with 2 μl of either a hRARα-specific monoclonal antibody (Rα10) (Dianova, Hamburg, Germany) or an unrelated anti-Gal4-specific antibody (Santa Cruz Biotechnology) for 4 h at 4°C. Protein complexes were precipitated with 10 μl of protein G-Sepharose (Pharmacia) for 1 h at 4°C, washed, and analyzed as described (18).

RESULTS

Both the Transactivation and the Coiled-Coil Dimerization Domains of BZLF1 Are Necessary for Repression of RA-Induced Gene Activation. Recent data suggested that BZLF1 can inhibit RA induction of the mouse RARβ promoter in the B-cell line Louckes (7). To determine whether this inhibitory effect could be extended to other cells and RA-responsive promoters, we cotransfected BZLF1 and RAR/RXR expression plasmids into NIH 3T3 cells and asked whether BZLF1 is able to inhibit RA-induced activation of a $(TREp)_2$-TKLuC reporter plasmid (9). As shown in Fig. 1, RAR/RXR strongly induced reporter activity in a hormone-dependent manner (Fig. 1, bar 1). Cotransfection of BZLF1 expression plasmids inhibited RA-induced reporter activity (compare bars 1 and 2). In contrast, basal level activity of the reporter constructs is not affected, demonstrating that the hormone-activated RARα is the target of BZLF1-mediated repression. Transfection of

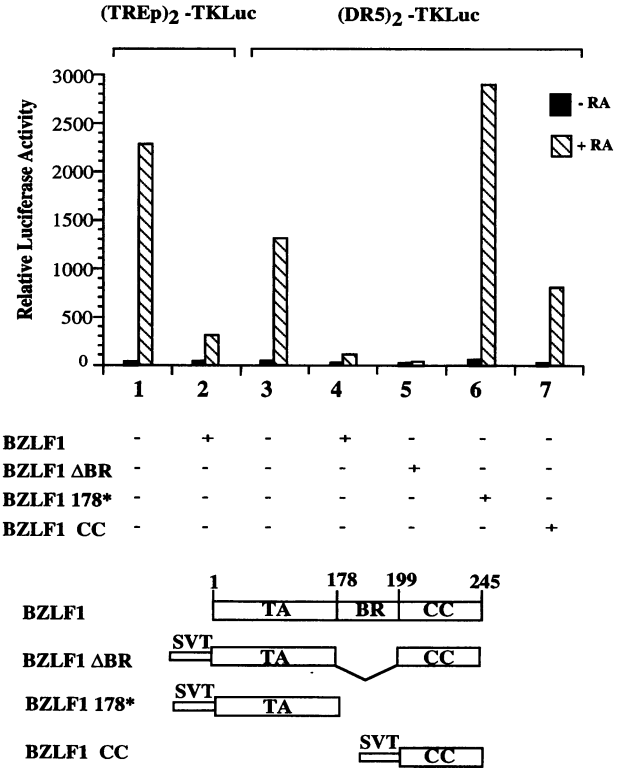

FIG. 1. Transactivation and coiled-coil dimerization domains of BZLF1 are required for inhibition of RA-mediated activation. $(TREp)_2$-TKLuC or $(DR5)_2$-TKLuC reporter plasmids (1.25 μg) were cotransfected in NIH 3T3 cells with 25 ng of each plasmid expressing hRARα and hRXRα and 250 ng of empty CMV expression plasmid or together with 250 ng of expression plasmids coding for full-length BZLF1 or the indicated mutants. Cells were either untreated (solid bars) or treated with 1 μM RA (hatched bars). BZLF1 protein is composed of the N-terminal transactivation domain TA, the DNA binding region BR, and the C-terminal coiled-coil dimerization domain CC.

parental plasmid CMV, lacking BZLF1 coding sequences, did not alter RA-induced activity of $(TREp)_2$-TKLuC (data not shown). To further demonstrate that BZLF1-mediated repression is not dependent on a particular RA response element, we transfected $(DR5)_2$-TKLuC reporter plasmids (9) into NIH 3T3 cells. RA-induced activity of this construct (bar 3) was efficiently repressed by increasing amounts of BZLF1 (compare bars 3 and 4).

To delineate the regions in the BZLF1 protein that are responsible for repression, we next tested a series of mutant BZLF1 proteins for their ability to repress RA-induced activity of the $(DR5)_2$-TKLuC reporter gene. To ensure proper translocation to the nucleus, we fused the simian virus 40 nuclear translocation signal to all BZLF1 mutants (12). All mutants were expressed in similar amounts in NIH 3T3 cells (data not shown). Deletion of the BZLF1 DBD resulted in a mutant that repressed even better than wild-type BZLF1 (Fig. 1, bar 5). In contrast, both the transactivation and the coiled-coil dimerization domains failed to repress (bars 6 and 7). In summary, these results indicate that the transactivation and the coiled-coil dimerization domains of BZLF1 act in concert to inhibit RA-mediated transactivation.

The RARα DBD Is Sufficient for Inhibition of BZLF1 Activity. To explore the possibility that RARα might be able to repress transcriptional activity of BZLF1, we transfected the BZLF1-inducible reporter construct $(ZIIIB)_4$-TATALuC into NIH 3T3 cells (Fig. 2, bar 1). This reporter contains four copies of a high-affinity BZLF1 binding site (ZIIIB) in front of a TATA minimal promoter (10). Cotransfection of BZLF1

expression plasmids resulted in a robust induction of reporter gene activity (bar 2). Expression of RARα inhibits BZLF1-mediated transactivation (bar 3). Interestingly, the RARα-mediated repression effect is hormone independent. In contrast, activity of the $(TREp)_2$-TKLuC reporter plasmid is induced by RARα in a RA-dependent manner (as shown in Fig. 1, bar 1).

To further reveal that RARα-mediated repression is independent of a particular BZLF1 binding site or promoter context, we asked whether RARα is able to repress the activity of the $(ZIIIA)_5$TKLuC reporter gene. This reporter contains five copies of a different BZLF1 binding site (ZIIIA) in front of the TK promoter (10). RARα alone does not influence basal level expression of the $(ZIIIA)_5$-TKLuC reporter in the absence of BZLF1 (Fig. 2, bar 5). Activity of this reporter is efficiently induced by BZLF1 (compare bars 4 and 6) and completely blocked by RARα (bar 7), demonstrating again that the BZLF1 protein is the target of RARα repression.

Next we analyzed several RARα mutants for their ability to inhibit the activity of the reporter plasmid $(ZIIIA)_5$-TKLuC. Deletion of the RARα DBD completely abolished the receptor's ability to repress (Fig. 2, bar 9). To demonstrate further that the RARα DBD is sufficient to inhibit BZLF1-mediated activation, we generated a mutant in which the simian virus 40 nuclear translocation signal was fused to the RARα DBD (12). This mutant repressed reporter gene activity even better than full-length RARα (bar 8). Together our results demonstrate that the RARα DBD alone is sufficient to block activity of BZLF1 inducible promoters. The obvious lack of a RARα DNA binding site in the $(ZIIIA)_5$-TKLuC reporter, in addition to the fact that RARα alone does not bind the ZRE (as shown in Fig. 4), suggests that the receptor's ability to bind DNA is not necessary for repression. Instead, the data suggest that the DBD facilitates repression, possibly through protein–protein interactions with BZLF1.

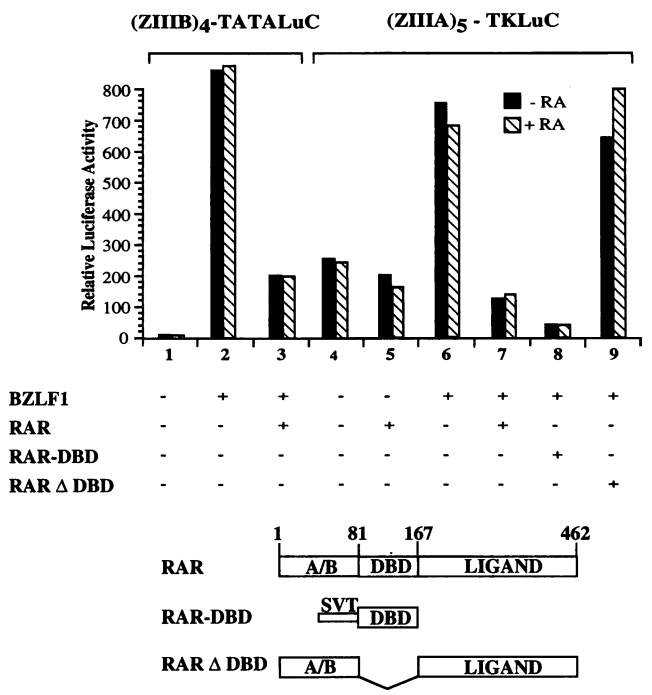

FIG. 2. hRARα DBD is sufficient to repress BZLF1-mediated transactivation. Transcriptional activity of 1.25 μg of reporter plasmids $(ZIIIB)_4$-TATALuC or $(ZIIIA)_5$-TKLuC in NIH 3T3 cells cotransfected as indicated (+) with 50 ng of expression plasmids coding for BZLF1 and 500 ng of empty CMV expression plasmid or together with 500 ng of expression plasmids coding for either full-length hRARα, the hRARα DBD, or a hRARα mutant lacking the DBD. Cells were either untreated (solid bars) or treated with 1 μM RA (hatched bars). Full-length hRARα protein is composed of the N-terminal A/B domain, the DBD, and the C-terminal ligand binding domain (Ligand).

RARα and BZLF1 Physically Interact *in Vitro*. To show direct protein–protein interactions between RARα and BZLF1, we performed GST-pulldown experiments. Recombinant full-length RARα (GST–RAR) interacted very strongly with *in vitro* translated [^{35}S]methionine-labeled full-length BZLF1 protein (Fig. 3*A*, lanes 2 and 7). This interaction was not dependent on RA (data not shown). In addition, the RARα DBD (GST–RAR DBD) bound to BZLF1 at levels similar to full-length RARα, demonstrating that the RARα DBD is necessary and sufficient to mediate direct protein–protein interactions with the BZLF1 protein (lane 3). Accordingly, the mutant GST–RAR C, which expresses RARα amino acids 187–351, and the mutant GST–RAR ΔDBD, which has the DBD deleted, failed to interact (lanes 4 and 8). Likewise, the GST control exhibits no specific binding (lane 5).

Next we asked which region in the BZLF1 protein might be responsible for the interaction with the RARα. *In vitro* translated [^{35}S]methionine-labeled full-length BZLF1 protein and several mutant proteins, respectively, were tested for interaction with GST–RAR. As shown above, BZLF1 and RARα interact very strongly (Fig. 3*B*, lane 2). The C-terminal deletion mutants BZLF1 178* and BZLF1 199* both failed to interact (compare lanes 5 with 6 and 8 with 9), whereas a mutant in which the BZLF1 DBD is deleted is still capable of interacting with RARα (lane 11). Importantly, a mutant that contains only the coiled-coil dimerization domain of BZLF1 interacts specifically with RARα (compare lanes 14 and 15). This result suggests that the coiled-coil dimerization domain of BZLF1 is necessary for protein–protein interaction *in vitro* and not the DBD.

RARα and BZLF1 Form a Heteromeric Complex Bound to DNA. To study the consequences of the physical interaction between RARα and BZLF1 on DNA binding we performed EMSAs with an oligonucleotide containing a single ZRE (ZIIIA). Recombinant RARα (GST–RAR) is unable to bind the ZRE, demonstrating that RARα alone does not recognize the BZLF1 binding site (Fig. 4*A*, lane 1). *In vitro* translated BZLF1 protein formed a retarded complex CI (lane 2). This complex is supershifted by a BZLF1-specific monoclonal antibody (lane 3), whereas an unrelated monoclonal antibody has no influence on the mobility of the DNA–protein complex (lane 4). However, performing the EMSA in the presence of both RARα and BZLF1 proteins resulted in generation of an additional complex, CII (lane 6). A mutant RARα protein lacking the DBD (GST–RAR ΔDBD) does not form the additional DNA–protein complex CII with BZLF1 (lane 7). As a further control, we tested GST protein, which is also unable to form complex CII (lane 5).

To establish the composition of complex CII, we challenged the EMSA with an antibody specific for the GST tag of GST–RAR. We used a GST antibody in order to exclude interference with possible protein–protein interactions between RARα and BZLF1. Complex CII is specifically supershifted by the anti-GST antibody, demonstrating that RARα fusion protein is part of this DNA–protein complex (Fig. 4*B*, lane 4). Accordingly, both complexes were supershifted by the addition of an anti-BZLF1-specific monoclonal antibody, indicating that BZLF1 is present in both complexes (lane 5). Incubation with two different, unrelated control monoclonal antibodies had no influence on the mobility of both complexes (lanes 6 and 7). Taken together, these data further support the results of the transient transfection experiments and GST-pulldown experiments in that the RARα DBD is sufficient to mediate both repression of BZLF1 activity *in vivo* and physical interaction *in vitro*. Our results also reveal that RARα, which alone is unable to bind to a ZRE, can be tethered onto DNA

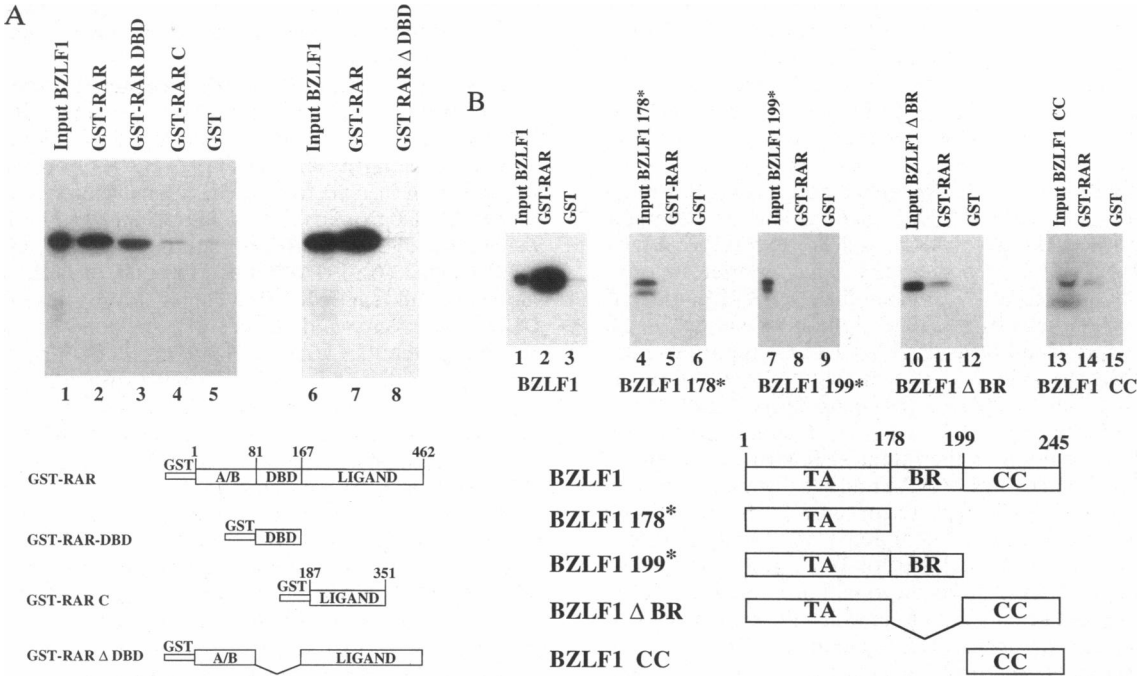

FIG. 3. BZLF1 and hRARα physically interact *in vitro*. (*A*) Ability of various GST–RAR fusion proteins to interact with [^{35}S]methionine-labeled *in vitro* translated full-length BZLF1 protein was evaluated in GST-pulldown assays. The same amounts of GST proteins, GST–RAR fusion proteins, or the GST–RAR mutant proteins were linked to glutathione Sepharose beads. Input control in lanes 1 and 6 reflects 10% of the total amount of [^{35}S]methionine-labeled BZLF1 proteins used for the pulldown experiments. To create the GST expression plasmids, the same hRARα cDNAs as those shown in Fig. 2 were used. (*B*) Coiled-coil dimerization domain of BZLF1 mediates physical interaction between BZLF1 and hRARα. Full-length BZLF1 or various mutants therefore were tested in GST-pulldown experiments for interaction with hRARα. Experimental setup is as described in *A*. BZLF1 cDNAs used for *in vitro* translation correspond to the constructs used in Fig. 1.

by forming a heteromeric protein–protein complex with BZLF1.

RARα and BZLF1 Directly Interact *in Vivo*. To demonstrate physical interaction *in vivo*, we performed coimmunoprecipitation experiments with cell extract from COS cells transiently transfected with BZLF1, RARα expression plasmids, or both expression plasmids. An anti-RARα-specific antibody (Rα10; Dianova) is able to coimmunoprecipitate BZLF1 proteins from COS cell extracts expressing both proteins (Fig. 5, lane 7). Even the low amounts of RARα protein, endogenously expressed in COS cells, were able to coimmunoprecipitate some BZLF1 protein from COS cell extracts transiently transfected with BZLF1 expression plasmids alone (lane 5). No BZLF1 could be immunoprecipitated from COS cell extracts transiently transfected with RARα expression plasmids alone (lane 6). Accordingly, an anti-Gal4 control antibody fails to immunoprecipitate BZLF1 from COS cell extracts transiently transfected with BZLF1 and RARα expression plasmids, demonstrating the specificity of the coimmunoprecipitation experiments (lane 8). Taken together, our results establish that RARα and BZLF1 are able to interact directly *in vivo*.

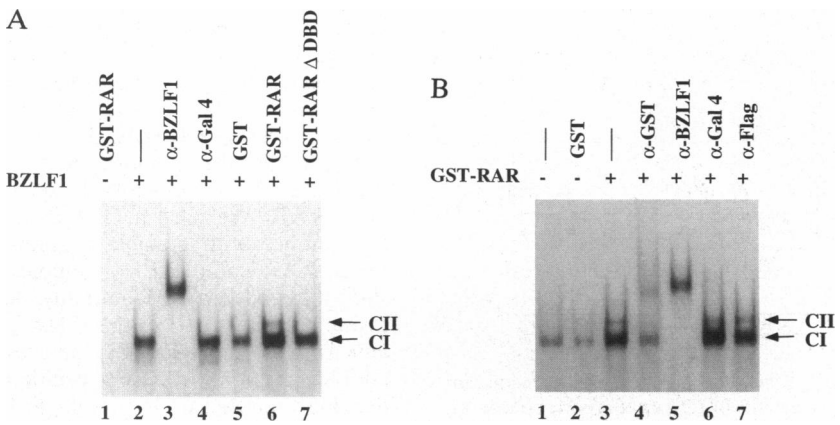

FIG. 4. hRARα and BZLF1 form a heteromeric complex on DNA. (*A*) EMSAs were performed with 0.25 μl of BZLF1 primed wheat germ extract (lanes 2–7) or 0.25 μl of unprimed lysate (lane 1). To determine the consequences of complex formation between BZLF1 and RARα on DNA binding of BZLF1, equal amounts of GST proteins (lane 5), GST–RAR fusion proteins (lane 6), or GST–RAR ΔDBD fusion proteins (lane 7) were incubated with *in vitro* translated BZLF1. Additional heteromeric RARα–BZLF1–DNA complex is denoted CII (lane 6). Where indicated, specific anti-BZLF1 antibodies (lane 3) or unrelated anti-Gal4 antibodies (lane 4) were added. (*B*) To establish the composition of the additional complex CII, *in vitro* translated BZLF1 proteins were incubated with GST–RAR fusion proteins (lanes 3–7) and challenged with specific antibodies directed against GST (lane 4), BZLF1 (lane 5), Gal4 (lane 6), and Flag (lane 7). The BZLF1–DNA complex is denoted CI, the heteromeric RARα–BZLF1–DNA complex is denoted CII.

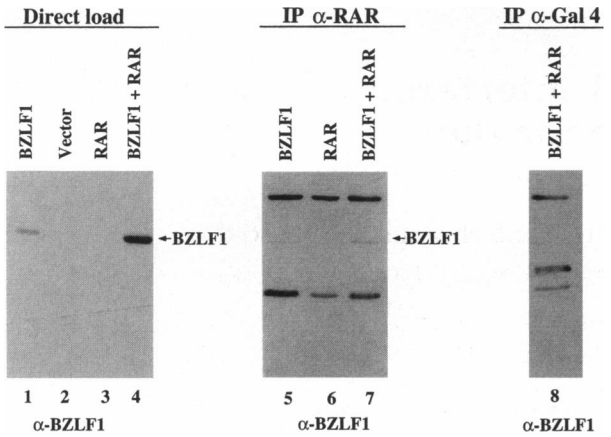

FIG. 5. RARα and BZLF1 directly interact *in vivo*. COS cells were transfected with expression plasmids coding for full-length BZLF1 (lanes 1 and 5), expression plasmids coding for full-length hRARα (lanes 3 and 6), or both (lanes 4, 7, and 8). As a control, COS cells were transfected with CMV expression plasmids (lane 2). Whole cell lysates of the transfected COS cells were divided and either loaded directly onto a SDS/polyacrylamide gel and then subjected to Western transfer (lanes 1–4) or first immunoprecipitated with an anti-hRARα antibody (lanes 5–7) or an unrelated anti-Gal4 antibody (lane 8) and then subjected to immunoblot analysis. The anti-BZLF1-specific antibody BZ 1 (Dako) was used to probe the immunoblot.

DISCUSSION

In this paper, we demonstrate that the Epstein–Barr virus transcription factor BZLF1 can function as a potent inhibitor of RA-induced gene activity. We provide evidence that BZLF1-mediated repression is not dependent on a particular RA response element or promoter context. Instead, repression depends on the presence of activated RARs. A BZLF1 mutant lacking the DBD represses as efficient as the wild-type BZLF1 protein, demonstrating that the ability of BZLF1 to bind DNA is not necessary for repression. *In vivo* both the coiled-coil dimerization domain and the transactivation domain are necessary for repression. This would be consistent with a model in which the coiled-coil dimerization domain is mediating a protein–protein contact with RARα, whereas the BZLF1 transactivation domain might possibly establish a nonproductive interaction with the basal transcription machinery (19). Interestingly, the coiled-coil dimerization domain of BZLF1 also facilitates interactions with the p53 and NFkB p65 proteins and the RXRα (16, 18, 20).

The mechanism of mutual repression between nuclear receptors and bZip transcription factors tempted us to explore whether RARα is able to block the activity of the bZip transcription factor BZLF1. Indeed, RARα severely inhibits activation of BZLF1-dependent promoters. In contrast to the data reported by Sista *et al.* (7), the repression effect seems to be RA independent. Addition of RA did not affect repression even when cells were cultured for a prolonged time in medium with delipidated serum or no serum at all.

We demonstrate that the RARα DBD is necessary and sufficient for transrepression. Because neither full-length RARα nor the RARα DBD alone bind to the ZREs, our results suggest that the receptor's ability to bind DNA is not important for transrepression. Instead, the DBD might facilitate direct protein–protein interactions between RARα and BZLF1. Accordingly, GST-pulldown experiments show that RARα and BZLF1 physically interact *in vitro* and that only the DBD of RARα is necessary for this interaction. Moreover, our EMSAs establish that RARα can be tethered onto DNA by forming a heteromeric protein–protein complex with BZLF1. In such a heteromeric complex, only BZLF1 would be involved in DNA contacts, whereas RARα would be tethered onto the promoter through protein–protein interactions with BZLF1.

Our findings add several new aspects to the previous definition of functional antagonism between nuclear receptors and bZip proteins. In contrast to the mutual repression of c-Jun/AP-1 by glucocorticoid receptor (GR) or RAR (6, 12), the transrepression between BZLF1 and RARα is accomplished by the receptor's DBD only. Furthermore, we provide evidence that the DBD of RARα alone mediates physical interaction with the BZLF1 protein. It is tempting to speculate that other members of the nuclear receptor superfamily might also be tethered onto DNA via protein–protein interactions with cellular transcription factors that do not belong to the receptor superfamily. Interestingly, similar types of interactions between the GR DBD and the cellular protein calreticulin have been proposed recently (21). In summary, our results suggest a mechanism of how members of the nuclear receptor superfamily might gain control of promoters that do not contain classical hormone response elements.

We thank Drs. Ronald M. Evans, Paul J. Farrell, Fritz Schwarzmann, and Wolfgang Hammerschmidt for plasmids and exchange of information prior to publication. We also thank the members of R.S.'s laboratory for discussion and critical reading of the manuscript. This work was supported in part by a grant of the Deutsche Forschungsgemeinschaft to R.S. (Schu 688/2-1).

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