Binding and Cooperative Interactions between Two B Cell–specific Transcriptional Coactivators

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Summary

The class II transactivator (CIITA) and B cell octamer-binding protein 1/octamer-binding factor 1/Oct coactivator from B cells (Bob1/OBF-1/OCA-B) represent two B cell–specific transcriptional coactivators. CIITA and Bob1 interact with proteins that bind to conserved upstream sequences in promoters of class II major histocompatibility genes and octamer-binding transcription factors Oct-1 and Oct-2, respectively. Both CIITA and Bob1 increase the expression from the DRA promoter, which is a prototypic class II promoter. Moreover, in the presence of CIITA, interactions between class II promoters and Bob1 are independent of the octamer-binding site. Using in vivo and in vitro binding assays, we confirm that Bob1 binds to CIITA. Thus, CIITA not only activates the expression of class II genes but recruits another B cell–specific coactivator to increase transcriptional activity of class II promoters in B cells.

Transcription of MHC class II genes (class II) is regulated by promoter elements, which consist of conserved upstream sequences (CUS) called the S, X, and Y boxes and downstream sequences, which in the DRA promoter, include an octamer-binding site (OBS)[1] (see Fig. 1) (for reviews see references 1–5). Whereas the B cell–specific and IFN-γ–inducible expression depend absolutely on the X box (6), these other promoter sequences are also required for optimal levels of class II transcription (7). Moreover, mutations in proteins, which interact with the X box, result in a severe combined immunodeficiency called the type II bare lymphocyte syndrome (BLSII) (1, 8). Genes mutated in two of the four complementation groups of BLSII have been isolated (9, 10). Of these, the regulatory factor X5 (RFX5) is a component of the RFX heterodimer, which binds to the X box (10). In contrast, the class II transactivator (CIITA) does not bind directly to DNA (9). CIITA is expressed at high levels in B cells and activated T cells but is also induced by IFN-γ and mediates effects of IFN-γ on the expression of class II in APCs (11, 12). Moreover, the absence of CIITA has been correlated with the extinction of class II on plasma cells (13).

Recently, the gene coding for the coactivator involved in the B cell–specific transcription of Ig genes was identified (10, 11). B cell octamer–binding protein 1/octamer-binding factor 1/Oct coactivator from B cells (Bob1/OBF-1/OCA-B; hereafter Bob1) binds to Oct-1 and Oct-2 and increases their ability to activate transcription in B cells (14–16). Since the DRA promoter contains an OBS, we wondered if Bob1 also regulates its expression. Indeed, Bob1 activated the DRA promoter and acted synergistically with CIITA. Using the DQβ promoter, which does not contain an OBS, and a DRA promoter bearing clustered point mutations in the OBS, we also demonstrated that this synergism was independent of the OBS. Furthermore, Bob1 bound to CIITA in vivo and in vitro, which explains their cooperative interactions in activating the transcription of class II genes.

Materials and Methods

Cell Culture, Transfection, and CAT Assay. Jurkat cells were grown in RPMI containing 10% FCS and antibiotics, under 5% CO_2. Transfections were performed by electroporation (960 mF, 250 V) using 10^7 cells in 0.4 ml RPMI. Unless otherwise stated, the total amount of DNA was kept constant at 40 μg. Specific amounts of DNA for each transfection are noted in the figure legends. 48 h after transfection, cells were harvested, and CAT enzymatic assays were performed as described (17).

In Vitro Protein Binding Assays. Glutathione-S-transferase (GST) CIITA fusion protein was produced using the baculovirus BaculoGold expression system from PharMingen (San Diego, CA). GST protein was produced in bacteria, from the plasmid pGEX2tk (Promega Corp., Madison, WI), as described (18). Equal amounts of GST or GSTCIITA were bound to glutathione–sepharose beads (Pharmacia, Uppsala, Sweden) in EBC-D buffer (50 mM Tris-HCl, pH 8.0, 120 mM NaCl, 0.5% NP-40, and 5 mM dithiothreitol), washed several times in EBC-D containing 0.075%

[1] *Abbreviations used in this paper:* Bob1, B cell octamer-binding protein 1; CIITA, Class II transactivator; OBS, octamer-binding site.

SDS, and resuspended in 100 ml EBC-D. To both samples, 5 μl of [^{35}S]–methionine labeled Bob1 which was transcribed/translated in vitro (TNT Coupled transcription/translation kit; Promega, Corp.) was added. The binding reaction was mixed for 1 h at 4°C, and washed four times in EBC-D, where the concentration of NaCl was increased to 250 mM. The Sepharose pellet was resuspended in 25 μl of SDS-loading buffer, boiled for 10 min, and loaded onto a 8% SDS-polyacrylamide gel. After electrophoresis, the gel was dried and autoradiographed overnight on ECL Hyperfilm (Amersham Corp., Arlington Heights, IL).

Results

Since the DRA promoter contains an OBS, we wanted to determine if Bob1 could regulate its levels of expression. Jurkat T cells were chosen because they express neither Bob1 nor CIITA (Fontes, J.D., and B.M. Peterlin, unpublished data). pDRASCAT, which contains the DRA promoter linked to the CAT reporter gene (Fig. 1 *A*) (7), was transfected alone and with increasing amounts of pCBOB1 which directs the expression of Bob1, into Jurkat cells. Expression from pDRASCAT increased with increasing amounts of pCBOB1 (Fig 1 *B*). At the maximum concentrations of pCBOB1, CAT levels were ~10-fold greater than those observed with pDRASCAT alone. This result indicates that Bob1 interacts with proteins that bind to the OBS, and activates transcription from the DRA promoter in the absence of CIITA.

We next examined the ability of Bob1 to activate transcription from the DRA promoter in the presence of CIITA. Cotransfections of pCCIITA (Fig. 1 *A*) with pDRASCAT (the ratio of the plasmid effector to target was 1:1) resulted in 12-fold higher CAT activities (Fig. 1 *C*). At the same ratio of the plasmid effector and target, the expression of Bob1 resulted in twofold higher CAT activities (Fig. 1, *B* and *C*). However, when both pCCIITA and pCBOB1 were cotransfected with pDRASCAT, CAT activities increased 40-fold (Fig. 1 *C*). This level of activation represents greater than additive effects between Bob1 and CIITA on the DRA promoter.

Of all class II promoters, only the DRA promoter contains an OBS. Thus, to determine if Bob1 could also activate other class II promoters alone or with CIITA, we used the DQβ promoter, which contains S, X, and Y boxes but lacks the OBS (19). pDQbAT (Fig. 2 *A*) was transfected alone, with pCBOB1 and with pCBOB1 and pCCIITA into Jurkat cells. CIITA activated the DQβ promoter sixfold (Fig. 2 *B*). As expected, Bob1 had no effect on the expression from pDQbCAT. However, when pCBOB1 was cotransfected with pCCIITA, CAT levels increased more than 15-fold (Fig. 2 *B*). Although these levels were slightly lower than those observed with the DRA promoter, they represent synergistic activation of the DQβ promoter by Bob1 and CIITA. That these synergistic activities between CIITA and Bob1 were indeed independent of the OBS was also revealed by another plasmid target, pDRASM11CAT, which contains clustered point mutations in the OBS of the DRA promoter (Fig. 2 *A*) (7). Fig. 2 *B* demonstrates that Bob1 alone could not increase expression from pDRASM11CAT.

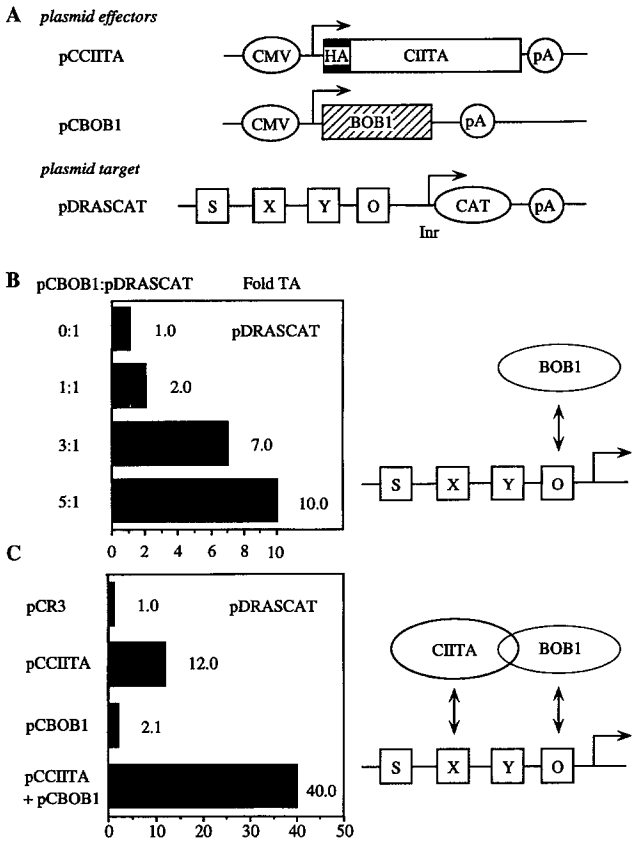

Figure 1. Bob1 activates the DRA promoter, alone and synergistically with CIITA in Jurkat cells. (*A*) Plasmid effectors pCCIITA and pCBOB1 direct the expression of their respective cDNAs from the CMV promoter. The plasmid target pDRASCAT was described previously (7). (*B*) Increasing amounts of pCBOB1 (0, 10, 30, and 50 μg) were co–transfected with a constant amount of pDRASCAT (10 μg) into Jurkat cells. In the absence of pCBOB1, the activity of the DRA promoter was set to 1. Bob1 led up to 10-fold–higher levels of expression from the DRA promoter. (*C*) pCCIITA (10 μg) and pCBOB1 (10 μg) alone increased expression of pDRASCAT (10 μg) 12- and 2.1-fold, respectively. Cotransfections of both pCCIITA and pCBOB1 (10 μg each) resulted in synergistically increased (40-fold) expression from the DRA promoter. (*HA*) Influenza hemagglutinin tag, (*S, X, Y,* and *O*) OBS, (*Inr*) initiator sequences from the DRA promoter, (*pCR3*) parental plasmid for pCCIITA and pCBOB1, and (*Fold TA*) fold transactivation. pCR3 was also added to keep total amounts of cotransfected plasmids constant. Plasmid effectors are given to the left of and plasmid targets inside the bar graphs. To the right of each bar graph are schematic representations of protein–protein interactions between coactivators and proteins, which bind to *cis*-acting sequences in class II promoters. Shown are representative experiments from three transfections done in duplicate. Standard errors of the mean were <10%.

However, CIITA increased the expression from this plasmid almost 9-fold, and together with Bob1, levels of expression from pDRASM11CAT increased more than 17-fold. Again, this increase represents greater than multiplicative effects of Bob1 and CIITA on the mutated DRA promoter that lacks the OBS.

That Bob1 and CIITA could also activate synergistically the expression from class II promoters lacking the OBS led to the possibility that the two coactivators were interacting directly. To test this hypothesis we used an in vivo binding

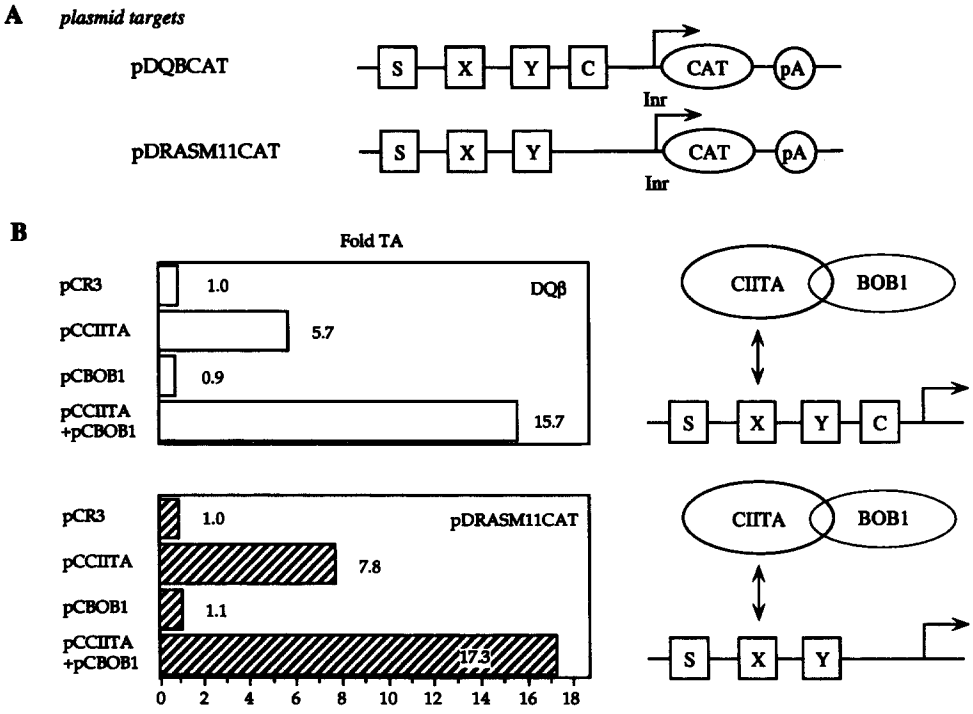

Figure 2. Bob1 and CIITA act synergistically on class II promoters even in the absence of the OBS. pDQbCAT contained the promoter of the DQβ gene, which is a class II promoter that lacks an OBS (Fontes and Peterlin, unpublished data). Instead of the OBS (O), DQβ contains a CCAA sequence (*C*). pDRASM11CAT contains clustered point mutations in the OBS of pDRASCAT (7). Alone and together, pCCIITA and pCBOB1 were cotransfected with the indicated plasmid targets. Amounts of DNA were as follows: 5 μg of pDQbCAT, 20 μg of pCCIITA, and 20 μg of pCBOB1; 5 μg of pDRASM11CAT, 10 μg of pCCIITA, and 20 μg of pCBOB1. pCR3 was added to keep total amounts of cotransfected plasmids constant. Plasmid effectors are given to the left of and plasmid targets inside the bar graphs. (*White and striped boxes*) Cotransfections with pDQbCAT and pDRASM11CAT, respectively. Shown are representative experiments from three transfections done in duplicate. Standard errors of the mean were <10%.

assay. The DNA-binding domain of Gal4 (DNABD) was linked to the full-length CIITA (pSGCIITA) (Fig. 3 *A*). Two plasmid targets were used. The first plasmid contains the binding site for Gal4 (UASg) upstream of the OBS, putative TATA sequence and initiator (Inr) from the DRA promoter (pDRASUASgCAT) (Fig. 3 *A*) (20). The second plasmid contains a single UASg upstream of the TATA box from the E1b promoter of adenovirus (pG1E1bCAT) (Fig.

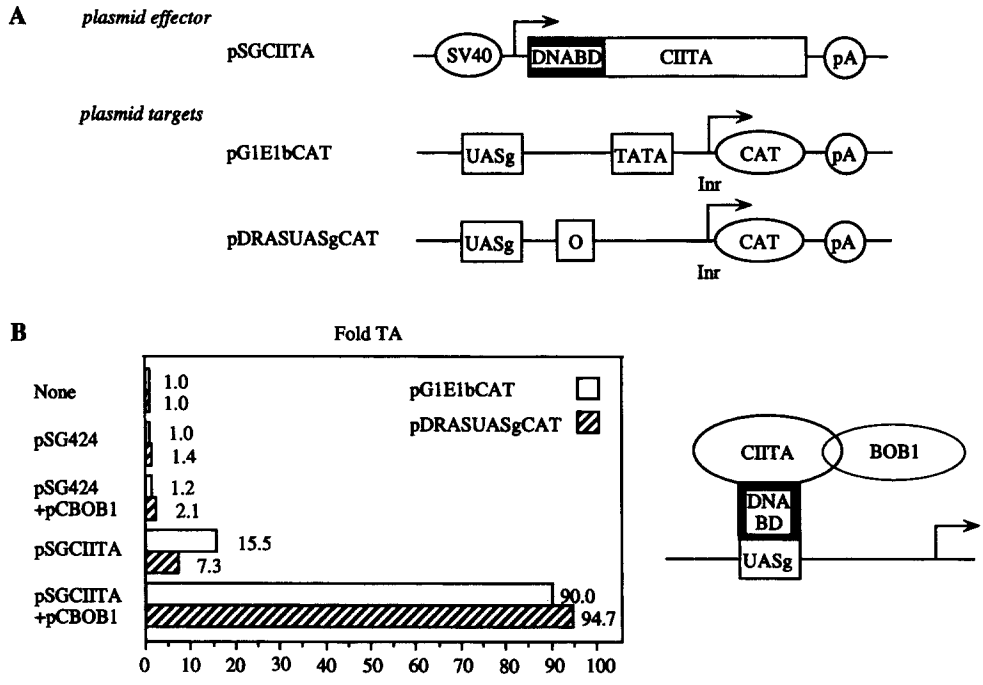

Figure 3. Bob1 interacts directly with CIITA to produce transcriptional synergy. (*A*) The plasmid effector (*pSGCIITA*) contains the Gal4 DNA-binding domain (DNABD) 5′ and linked to the full-length CIITA cDNA. These plasmid targets were used: pG1E1bCAT contains one Gal4 binding site 5′ to the TATA box from the E1b promoter of adenovirus (8) and pDRASUASgCAT contains a single Gal4 DNA binding site (*UASg*) 5′ to the OBS, putative TATA and Inr sequences from the DRA promoter (19). (*B*) Indicated plasmid effectors were cotransfected with and without pCBOB1 and pG1E1bCAT or pDRASUASgCAT into Jurkat cells. 5 μg of plasmid targets (*pG1E1bCAT* and *pDRASUASgCAT*), and 15 μg of plasmid effectors (*pSG424*, *pSGCIITA*, and *pCBOB1*) were used. (*None, left*) Only pCR3 was cotransfected with the plasmid targets. pSG424 is the parental plasmid of pSGCIITA. (*White and striped boxes*) Cotransfections with pG1E1bCAT and pDRASUASgCAT, respectively. Plasmid effectors are given to the left of and plasmid targets inside the bar graphs. Shown are representative experiments from three transfections done in duplicate. Standard errors of the mean were <10%.

3 *A*) (8). Using these plasmid targets, the interaction of CIITA and Bob1 was assessed in the presence and absence of the OBS. Both plasmids were activated to well above background levels by pSGCIITA. In fact, the expression from pG1E1bCAT and pDRAUASgCAT was increased more than 15- and 7-fold, respectively (Fig. 3 *B*).

pCBOB1 was also cotransfected with pSG424 (the parental vector of pSGCIITA) and these two plasmid targets. Bob1 had no effect on the expression from pG1E1bCAT, which does not contain the OBS. As expected, Bob1 increased twofold the expression pDRAUASgCAT, which contains the OBS (Fig. 3 *B*). These data were similar to those observed with pDRASCAT (Figs. 1 and 2). However, results obtained when both pSGCIITA and pCBOB1 were cotransfected with these plasmid targets were more significant. In cotransfections with pCBOB1 and pSGCIITA, the expression from pDRAUASgCAT increased 95-fold (Fig. 3 *B*). This finding was confirmed with pG1E1bCAT, where the same plasmid effectors increased the expression from pG1E1bCAT 90-fold. These degrees of activation were more than multiplicative and indicate that Bob1 and CIITA interacts with each other in cells. In control cotransfections, activation of pDRAUASgCAT and pG1E1bCAT by a fusion protein containing the Gal4 DNABD and the activation domain of VP16 from the HSV was not affected by pCBOB1, i.e., Bob1 did not interact with an irrelevant viral transactivator (19).

To confirm that Bob1 interacts with CIITA, a direct binding assay was performed with CIITA and Bob1 in vitro. A fusion protein between GSTCIITA fusion protein was incubated with Bob1 translated in the rabbit reticulocyte lysate. GSTCIITA was purified with glutathione–sepharose beads and bound proteins were analyzed by SDS-PAGE followed by autoradiography. Bob1 measured 40 kD (Fig. 4, lane *1*). When combined with Bob1, GSTCIITA also pulled down a protein of identical molecular weight (Fig. 4, lane *2*). However, when GST alone was incubated with Bob1, no Bob1 was detected (Fig. 4, lane *3*), indicating that interactions between Bob1 and CIITA were specific. Thus, GSTCIITA and Bob1 bind to each other directly. The degree of binding between Bob1 and CIITA in our gel was similar to that observed previously with Oct-1 and Bob1 (16, and Fontes and Peterlin, unpublished data).

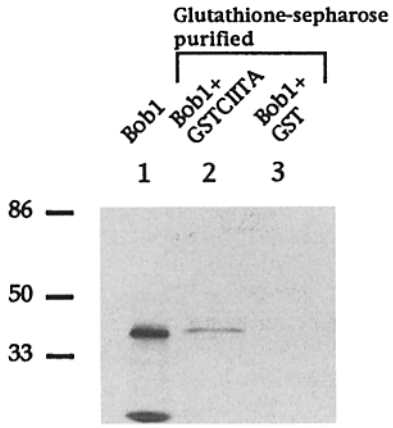

Figure 4. Bob1 and CIITA bind each other in vitro. Lane *1* contains the input Bob1, which was translated in the rabbit reticulocyte lysate. Lane *2* contains Bob1 which bound to GSTCIITA. Lane *3* contains Bob1 which was incubated with GST alone.

Discussion

This report represents the first demonstration of direct interactions between two transcriptional coactivators. CIITA interacts with proteins bound to class II promoters (20) and at least one human TBP-associated factor (TAF) (Fontes, J.D., and B.M. Peterlin, unpublished data). Since Bob1 contains an activation domain different than that of CIITA (21), the presence of Bob1 provides an additional surface for interactions with a second TAF. This scenario represents a variation on the theme of transcriptional synergy revealed by two Drosophila transactivators, bicoid and humpback, which bind to DNA and contact two distinct TAFs (22). Cooperative interactions with general transcription factors then result in more efficient binding of TFIID at the promoter, which leads to higher rates of initiation of transcription.

From this study, it is clear that CIITA is the primary regulator of class II transcription and that effects of Bob1 are dictated by CIITA. This observation divorces expression of class II from Ig genes in the ontogeny of B cells, where Igs are expressed before class II determinants appear and after their extinction in plasma cells (23). Moreover, the presence of both CIITA and Bob1 leads to the very high levels of class II in B cells, where they are required for optimal T cell help (24). In sharp contrast, IFN-γ induces the synthesis of only CIITA in APCs (25). Since these cells lack Bob1, levels of class II in APCs are lower than those in B cells and reflect only the requirement of class II for optimal antigen processing and presentation.

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