Relationships of the Antiproliferative Proteins BTG1 and BTG2 with CAF1, the Human Homolog of a Component of the Yeast CCR4 Transcriptional Complex

INVOLVEMENT IN ESTROGEN RECEPTOR α SIGNALING PATHWAY*

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We have reported previously the physical interaction of B-cell translocation gene proteins (BTG1) and BTG2 with the mouse protein CAF1 (CCR4-associated factor 1) and suggested that these proteins may participate, through their association with CAF1, in transcription regulation. Here we describe the in vitro and in vivo association of these proteins with hPOP2, the human paralog of hCAF1. The physical and functional relationships between the BTG proteins and their partners hCAF1 and hPOP2 were investigated to find out how these interactions affect cellular processes, and in particular transcription regulation. We defined their interaction regions and examined their expression in various human tissues. We also show functional data indicating their involvement in estrogen receptor α (ERα)-mediated transcription regulation. We found that BTG1 and BTG2, probably through their interaction with CAF1 via a CCR4-like complex, can play both positive or negative roles in regulating the ERα function. In addition, our results indicate that two LXLL motifs, referred to as nuclear receptor boxes, present in both BTG1 and BTG2, are involved in the regulation of ERα-mediated activation.

The pathways that inhibit cell proliferation allow normal cycling cells to exit from the cell cycle in response to changes in environmental conditions (e.g. nutrient deprivation, growth-inhibiting factors, or high cell density). The BTG1 family, whose founding member is BTG1 (B-cell translocation gene 1) (1, 2), is a family of functionally related genes involved in the negative control of the cell cycle. In vertebrate, this family comprises at least nine distinct members: BTG1, BTG2/TIS21/P3, BTG3/ANA, TOB, TOB2, B9.10, PC3K, PC3B, and B9.15. Two short conserved domains (Box A and Box B) define the signature of this family (3). BTG family proteins have been reported to be involved in some aspects of cell growth, differentiation, and survival (4-9). For example BTG1, BTG2/PC3, TOB, TOB2, and ANA were reported to display antiproliferative properties (2, 10-14). Furthermore, BTG2 expression is regulated by p53 and has been found to be involved in DNA damage-induced G2/M cell cycle arrest (8). Rat BTG2, known as PC3 (for pheochromocytoma cell-3) was recently shown to inhibit S-phase entry in an Rb-dependent fashion, correlated with the inhibition of cyclin D1 expression (15). Therefore the authors suggest that PC3 may act as a transcriptional regulator of cyclin D1. These results support our previous work, indicating that BTG1 and BTG2 may play a role in transcription regulation. We have shown that these proteins associate physically and functionally with the homeoprotein HOXB9 and enhance HOXB9-mediated transcription (16). In addition, we have demonstrated that both BTG1 and BTG2 interact with mCAF1 (17), whose yeast homolog is a component of the CCR4-NOT transcriptional complex, which can affect transcription either positively or negatively (18).

The association of BTG1 with hCAF1, the human homolog of mCAF1, has been confirmed by a study (19) that showed that hCAF1 overexpression in different cell lines leads to a proliferation block, demonstrating its involvement in growth suppression. Furthermore, a search in GenBank with the hCAF1 cDNA sequence revealed sequence identity with a human cosmid (DDBJ/EMBL/GenBank accession number AB020860), localized in the short arm of chromosome 8 at 8p12.3-p22, a region frequently deleted in numerous human tumors (20-22). CAF1 was subsequently shown to interact with two other members of the BTG family, TOB and TOB2 (13). Recently, the paralog of the hCAF1 gene, hPOP2, has been identified (23) (EMBL/GenBank accession number AF053318). The authors mapped this gene on chromosome 5q31-q33 and suggested that hPOP2 might be the tumor suppressor gene associated with the development of the myelodysplastic (5q-) syndrome. The human POP2 protein was later described (under the name of CALIF) as interacting with hNOT2 and hNOT3, the human homologs of the yeast proteins involved in the formation of the CCR4-NOT complex (24).

In other words, CAF1 and POP2 seem to be involved in transcriptional regulation, and both are localized on chromosome regions frequently deleted in human tumors. The structural and functional characterization of these genes should help to establish their role in transcription regulation and in tumorogenesis. In the present study, we demonstrate that POP2 protein, like CAF1, interacts with both BTG1 and BTG2,
and we define their interaction regions. We also examine the expression of CAF1 and POP2, together with BTG1 and BTG2, in different human tissues. Finally, we present functional results indicating the involvement of these proteins in estrogen receptor α (ERα)-mediated transcription regulation.

**EXPERIMENTAL PROCEDURES**

Cloning of hPO2 Open Reading Frame and Preparation of a Fusion Probe—The cDNA encompassing the entire hPO2 open reading frame, along with the 3′-nontranslated regions (NT) of hCAFI and hPO2 mRNA, were cloned by reverse transcriptase-PCR. 1 μg of total RNA from a human lymphoblastoid cell line was reverse-transcribed using 100 ng of oligo(dT) as primer, following the Superscript 2 (Life Technologies, Inc.) protocol. One-tenth of this mixture was used as a template for PCR. 50 pmol of specific oligonucleotides were added, and the reaction was carried out following Promega’s instructions, at an annealing temperature of 50 °C. The expected cDNAs were recovered, cloned, and sequenced. Sequences of the synthetic oligonucleotides used were as follows:

- **hPO2** 3′: 5′-TAAGCACCACCTCTGGGATCA-3′;
- **hPO2** 5′: 5′-ACTTCTCAGGTTTCTTCAGG-3′;
- **hCAFI** 3′-NT 3′: 5′-AGTTATTTAATAATTTCAATGATG-3′;
- **hCAFI** 3′-NT 5′: 5′-TTGTAGCCCTTGGCTTGG-3′;
- **hPO2** 3′-NT: 5′-ATGGAGTAGAGAAGTGGGAG-3′;
- **hPO2** 5′-NT: 5′-TGATCCAGGATGTGGTACCTA-3′.

**Bacterial Expression Constructs**—To generate the bacterial expression vectors for BTG1, BTG2, and CAF1, their full-length coding sequences were inserted into the pGEX-ET expression vector (Amersham Pharmacia Biotech) in-frame with the glutathione S-transferase (GST) coding sequence.

**Mammalian Reporter Plasmid**—The pCI-1 CAT reporter plasmid contains the P1 promoter (nucleotides −229–247 and −70–132) and 70–132 (corresponding to Box B. pSG5FlagBTG1ML2, M3L2, M3L1 were obtained from pSG5FlagBTG1 by directed mutagenesis (USE mutagenesis kit), deleting, respectively, bases 724–780 and 392–351, corresponding to Box B. pSG5FlagBTG1ML2, M3L2, M3L1, and M3L were obtained from pSG5FlagBTG1 by the same technique, replacing, at one or both LXXNL sites of the ERα consensus elements (ERE) upstream from the TATA box fused to the luciferase gene, was provided by V. Lauvet (ENSIL, Lyon, France). The p4–TG-CK-TK reporter plasmid contains six GAL4 consensus elements upstream from the thymidine kinase (TK) promoter region, fused to the CAT gene.

**Mammalian Expression Constructs**—All mammalian expression constructs used are derivatives of the SV40 promoter-driven expression vector pSG5 (Stratagene). The plasmid pSG5Flag was derived from pSG5 by insertion between the EcoRI and BamHI sites of an oligonucleotide containing the Flag peptide sequence (IBI Flag system; Eastman Kodak Co.) and a polyclinase. The full-length BTG1 DNA, and the fragments coding for the regions containing amino acids 1–96, 1–117, 1–126, and 38–171, obtained by PCR, were cloned in-frame with the Flag epitope to generate pSG5FlagBTG1, pSG5FlagBTG1/1–96, pSG5FlagBTG1/1–117, pSG5FlagBTG1/1–126, and pSG5FlagBTG1/38–171. pSG5FlagBTG1/1–126, and pSG5FlagBTG1/38–171 were obtained from pSG5FlagBTG1 by directed mutagenesis (USE mutagenesis kit, Amersham Pharmacia Biotech), respectively, replacing the AGC codon (coding for serine 159) by a GCG (coding for alanine) and deleting bases 292–351, corresponding to Box B. pSG5FlagBTG1ML2, M3L2, M3L1, and M3L were obtained from pSG5FlagBTG1 by the same technique, replacing, at one or both LXXNL sites of the ERα consensus elements (ERE) upstream from the TATA box fused to the luciferase gene, was provided by V. Lauvet (ENSIL, Lyon, France). The p4–TG-CK-TK reporter plasmid contains six GAL4 consensus elements upstream from the thymidine kinase (TK) promoter region, fused to the CAT gene.

**Immunoblot Analysis**—For protein expression assays, 50 μg of transfected Hela cell lysates was subjected to electrophoresis on a 10% polyacrylamide-SDS gel. The separated proteins were transferred to a polyvinylidene difluoride membrane (Millipore) by electoblotting. Equal amounts of protein were loaded onto each lane, as measured by Bradford assay and confirmed by Red Ponceau staining of the transferred membranes. ERα was detected with an anti-human monoclonal antibody (HC-20; Santa Cruz Biotechnology, Inc.). The membranes were incubated with horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulins. The protein was visualized using the enhanced chemiluminescence kit (Amersham Pharmacia Biotech), following the manufacturer’s instructions.

**RESULTS**

**The Two Members of the CAF1/POP Family Are Widely Expressed in Human Tissues:** Relationship with BTG1 and
BTG2—hCAF1 and hPOP2 proteins exhibit 76% sequence identity (see Fig. 1A), but their corresponding cDNA are divergent in the 5'- and 3'-nontranslated regions. To determine the tissue-specific patterns of CAF1 and POP2 gene expression, Northern blots containing human RNA from various tissues were performed using specific probes corresponding to the 3'-nontranslated regions of each transcript.

Transcripts of hCAF1 and hPOP2 were observed in a wide variety of tissues, with the highest levels for hCAF1, in the skeletal muscle, heart, and pancreas and for hPOP2, the heart and pancreas. A 2.4-kb mRNA species was detected with both the CAF1 and POP2 probes (see Fig. 1B). Besides the 2.4-kb transcript, the hCAF1 probe also detected significant amount of a 4.3-kb transcript in the skeletal muscle and a 1.35-kb transcript, which was the most abundant in the testis and was not detected in the lung or brain (Fig. 1B). Both forms were absent in the stomach, small intestine, and thymus. The nature and function of these variant transcripts are still unknown. At the same time we monitored the expression of BTG1 and BTG2 (see Fig. 1B): BTG1 transcript was found, though at barely detectable levels, in most tissues assayed other than the pancreas, heart, and lung, unlike BTG2 expression, which was abundant in the majority of the tissues analyzed, less so in the brain, and absent from the liver and testis.

Interaction of hPOP2 with BTG1 and BTG2—Given that hPO2 and hCAF1 exhibit 76% amino acid sequence identity, we next investigated whether hPOP2 interacted with BTG1 and BTG2. We also tested the possibility that hPOP2 could interact with CAF1. The hCAF1 and mCAF1 proteins have only one amino acid sequence difference (Fig. 1A), so we used CAF1 to indicate both human and mouse proteins. The cDNA encompassing the entire hPOP2 open reading frame was cloned by reverse transcriptase-PCR, as described under “Experimental Procedures.” The interaction assay was performed in the mammalian two-hybrid system, as already described (17). As shown in Fig. 2A both BTG1 and BTG2 interacted with hPOP2 in mammalian cells, but hPOP2 did not associate with CAF1. To verify that hPOP2 can interact directly with BTG1 and BTG2, we performed in vitro association assays with purified recombinant GST fusion proteins. GST-BTG1, GST-BTG2, and, as a control, GST alone, were coupled to glutathione-Sepharose beads and incubated with [35S]methionine-labeled hPOP2. As shown in Fig. 2B, the specific retention of hPOP2 was observed with the GST-BTG1 and GST-BTG2 beads, but not with the control GST beads. The incubation of GST-BTG1 and GST-BTG2 with [35S]methionine-labeled luciferase, used as a control, failed to show any specific interaction (Fig. 2B). These results point to a direct physical interaction of hPOP2 with both BTG1 and BTG2 and indicate that POP2 does not interact with CAF1 and that it does not homodimerize (data not shown).

Mapping of the Protein Domains Required for BTG-CAF1 Interaction—Our previous studies indicated that BTG1 and BTG2 were able to interact with mCAF1 in yeast and in mammalian cells and that Box B was necessary to this interaction (17). More recent work has confirmed that BTG1 protein interacts with hCAF1 (19), but the authors indicate that this inter-
action is mediated by the phosphorylation of BTG1 Ser-159. To define further the BTG1 regions involved in the interaction with CAF1, we used the mammalian two-hybrid system, given that proteins are more likely to be appropriately modified post-transcriptionally and that the results are therefore more likely to represent biologically significant interactions. Several deletion mutants of BTG1, described in Fig. 3A, were fused to the DNA binding domain of the GAL4 protein and assayed for possible interaction with CAF1 fused to the VP16 transactivation domain (VP16CAF1) in HeLa cells (see Fig. 3B). As expected, no interaction was found with the GALBTG1ΔBoxB chimera, lacking Box B (amino acids 98–117), whereas GALBTG1S159A, in which the BTG1 Ser-159 is mutated into Ala, did not prevent BTG1 interaction with CAF1. The results of this assay indicate that the phosphorylation of serine 159, unlike Box B, is not indispensable for BTG1-CAF1 interaction. In contrast to the results obtained with yeast (17), the deletion mutant GALBTG1/1–117 was unable to interact with CAF1, showing that Box B is necessary but not sufficient for this interaction in HeLa cells. As the deletion mutant GALBTG1/1–126 was still able to interact with CAF1, we conclude that the interaction in question does not require the C terminus of BTG1 but is strictly dependent on the short sequence flanking Box B (amino acids 118–126), which is highly conserved between BTG1 and BTG2. The deletion mutant GALBTG1/38–171 did not interact with CAF1, showing that the N terminus of BTG1 is required for the interaction. This region (amino acids 1–38) is also involved in the interaction of both BTG1 and BTG2 with HOXB9 protein in yeast (16) and contains a short motif (EIAAAV) that is conserved in all the members of the BTG family. It is possible that this motif has some functional significance, at least in protein-protein interactions. All of the results obtained with the GALBTG1L chimeric mutants were confirmed in vitro, using a GST pull-down method with purified GST-CAF1 and 35S-radiolabeled mutant BTG1 proteins (Fig. 3D). We conclude that the interactions observed were direct and not modified by the presence of the GAL4 domain in the fusion proteins. Analysis of the interactions of the VP16POP2 chimeric protein with the GALBTG1 fusion mutants gave similar results (data not shown). Taken together, these results demonstrate that the direct interaction of CAF1 and POP2 with BTG1 involve two regions of BTG1 (amino acids 1–38 and 98–126). As these two regions are highly conserved between BTG1 and BTG2, it is probable that they are also necessary for the interactions of CAF1 and POP2 with BTG2.

Seeking then to delineate the regions of CAF1 which are important for its association with BTG1 and BTG2, we made a series of deletion constructs of CAF1 (Fig. 4A). Using the mammalian two-hybrid assay (Fig. 4B), we found that two regions, corresponding to residues 11–31 and 229–247, are important for the interaction between CAF1 and both BTG2 (Fig. 4B) and BTG1 (data not shown). None of the hybrid proteins activated expression of CAT reporter gene on its own. In fact, as described in our previous studies (17), CAF1 and BTG proteins do not seem to be capable of stimulating transcription when tethered to multimerized DNA sites through a GAL4 binding domain in HeLa cells. We confirmed all of these interactions by in vitro interaction using a GST pull-down assay, showing that the interactions observed in HeLa cells are direct (Fig. 4D). As these regions are conserved between CAF1 and POP2, they are probably also involved in BTG-POP2 interactions.
BTG Proteins—The fact that BTG1 and BTG2 interact with CAF1 and POP2, which are homologs of the yeast transcription factor yCAF1/POP2 and act as cofactors for HOXB9-mediated transcription (16), supports the hypothesis that these proteins play a role in transcription regulation. In addition, BTG1 and BTG2 contain two copies of an LXXLL motif known as the NR (nuclear receptor) box (Fig. 6A), which was identified as being essential for the interaction of a number of coactivators with nuclear receptors (29). One motif (referred to below as L1) is located in the N-terminal part of the two proteins; the other motif (referred to below as L2) is located within the middle part, at the beginning of Box B, one of the two conserved domains that constitute the BTG family signature. These observations incited us to study the possible role of the BTG proteins in the transcriptional regulation of the nuclear receptors. We first focused on ER\(\alpha\) because both BTG and ER\(\alpha\) are involved in the regulation of cell proliferation: hormone binding to ER\(\alpha\) induces conformational changes leading to the recruit-
The cells were washed and treated for 24 h with a medium containing 10 nM 17β-estradiol. HeLa cells were transfected with the pSG5HEO vector, expressing through three half-EREs dispersed in the promoter (25). HeLa Reporter activity, which is expressed as pg of CAT protein/ml of lysate, was normalized by activity in the absence of ERα proteins, was transiently cotransfected into HeLa cells with 0.5 μg of pP1-CAT reporter plasmid, as described under “Experimental Procedures.”

Report activity, which is expressed as pg of CAT protein/ml of lysate, was normalized by β-galactosidase activity. Bars indicate S.D. from the mean of at least three independent transfections. The pERE-Luc and pP1-CAT reporter vectors are represented at the bottom of the figure.

Fig. 5. Promoter-selective coactivator or repressor effect of BTG1 and BTG2 for ERα. Panel A, coactivator effect of BTG1 and BTG2 on ERα function. HeLa cells were transiently transfected with 100 ng of pERE-Luc plasmid in the presence of 40 ng of pSG5HEO and increasing amounts (white bar, 20 ng; gray bar, 100 ng; black bar, 200 ng) of BTG1- and BTG2-expressing vectors or 200 ng of control plasmids. After 24 h, the cells were washed and treated for 24 h with a medium containing 10 nM 17β-estradiol. The transfected cells were washed and collected 48 h after transfection, then assayed for luciferase and β-galactosidase activity. Normalized values are expressed as in Fig. 3B. Panel B, inhibition of ERα-mediated activation of transcription on the P1 promoter by BTG1 and BTG2. 200 ng of expression plasmids, corresponding to the indicated proteins, was transiently cotransfected into HeLa cells with 0.5 μg of pP1-CAT reporter plasmid, as described under “Experimental Procedures.”

Taken together, the results of these transfection studies suggest that BTG1 and BTG2 can function either as coactivator or corepressor of ERα (Fig. 5, A and B), depending on the promoter context, which in turn suggest that the BTG proteins can act as effectors of the ERα signaling pathway.

Role of NR Motifs in Transcriptional Regulation by BTG1—To determine the relative importance of the two LXXLL motifs, which are common to both BTG1 and BTG2, we constructed a series of full-length BTG1 derivatives bearing individual leucine to alanine substitutions as illustrated in Fig. 6B; XXAL in both NR boxes (see Fig. 6B; M1L2) were fully responsive to ERα-mediated transcriptional activation. The results of four independent experiments (Fig. 6, B and C) demonstrated that the mutation of LXXLL to LXXAL in both NR boxes (see Fig. 6B; M1L2) were fully responsive to ERα. In contrast, mutations that converted the three hydrophobic leucine to alanine (M3L1 and M3L2) in the L1 or in the L2 motif, prevented BTG1 for having an effect on ERα transcriptional activity, suggesting that both motifs participate in the observed regulation. As expected the mutant BTG1/1–96, lacking Box B and L2, had no effect on ERα-mediated transcriptional activation. Thus, the activity of BTG1 and BTG2 on ERα appears to depend on the presence of two functional NR motifs.

Mechanisms for BTG Regulation of ERα-mediated Activation—To investigate whether the ERα-BTG functional interaction takes place directly, we carried out GST pull-down experiments. In vitro translated ERα did not appear to interact directly with either GST-BTG1 or GST-BTG2 used as baits, either in the presence or the absence of the ligand (data not shown). But although the BTG proteins seem to not interact directly with ERα, they possibly interact with other components of regulatory complexes involved in ERα-dependent transcriptional activation.
BTG and CAF1 Modulate ERα Transcription

Involvement of BTG1 NR box motifs in the modulation of ERα-mediated transcription. Panel A, schematic representation of the LXXLL sites of BTG1 and the corresponding mutants. L1 and L2 refer to the mutated site, and M and M3 refer to the number of leucine(s) replaced by alanine(s). Panels B and C, HeLa cells were transiently transfected with the ERα and the corresponding mutants expression vectors and either the pERE-Luc or the NR BTG1 mutants expression vectors and either the pERE-Luc (panel B) as indicated in Fig. 5A or pP1-CAT (panel C), as indicated in Fig. 5B. Data are presented as described for Fig. 5. Panel D, expression of FlagBTG1 and the indicated mutants in lysates from transfected HeLa cells analyzed by Western blotting with the anti-Flag (M2) antibody.

FIG. 6.

DISCUSSION

In this study we compared structural and functional features of CAF1 and POP2 gene products and their relations with the BTG proteins. The POP2 coding region has a high degree of homology with mouse and human CAF1, resulting in a protein that has 76% amino acid identity with mouse and human CAF1. A noticeable difference between POP2 and CAF1 is that has 76% amino acid identity with mouse and human CAF1, resulting in a protein that has 76% amino acid identity with mouse and human CAF1. A noticeable difference between POP2 and CAF1 is that has 76% amino acid identity with mouse and human CAF1. A noticeable difference between POP2 and CAF1 is that has 76% amino acid identity with mouse and human CAF1. A noticeable difference between POP2 and CAF1 is that has 76% amino acid identity with mouse and human CAF1. A noticeable difference between POP2 and CAF1 is that has 76% amino acid identity with mouse and human CAF1.
interactions with different partners. The fact that hPOP2/CALIF but not hCAF1, is able to interact with hNOT2 and hNOT3 (24) suggests that the two proteins are functionally distinct and that they could participate in the formation of different complexes in mammals. This observation is particularly interesting because in yeast the CCR4 \(\text{z}\) NOT complex appears to be composed of at least two groups of proteins which are physiologically and functionally distinct (34).

Different findings indicated the possibility that BTG1 and BTG2 play a role in transcription regulation: (a) both proteins interact with HOXB9 and modulate its transcription activity (16); (b) both of them interact with CAF1 (17) and POP2 (this report), which are homologs of a yeast transcription factor; (c) BTG2 acts as a transcriptional regulator of cyclin D1 (15); (d) BTG1 and BTG2 contain two copies of an LXXLL motif (see Fig. 6A), which has been identified as being essential for the interaction of a number of coactivators with nuclear receptors (29); (e) they also interact with the protein-arginine N-methyltransferase (PRMT1) (35), and a relationship between the methylation of proteins and the transcription regulation of nuclear receptors has recently been described (36, 37). We therefore investigated the possible involvement of the BTG proteins in the transcriptional

**Fig. 7.** Effect of CAF1 on ER\(\alpha\)-dependent transcription. Panels A and B, HeLa cells were transiently transfected with the ER\(\alpha\), CAF1, or FOLL expression vectors, and either the pERE-Luc (panel A) as described in Fig. 5A or pP1-CAT (panel B), as indicated in Fig. 5B. Data are presented as described in Fig. 5. Panel C, expression of ER\(\alpha\) in lysates from nontransfected HeLa cells (HeLa), or from HeLa cells transfected with pSG5HEO (200 ng), either alone (ER\(\alpha\)) or in the presence of BTG1-, BTG2-, or CAF1-expressing vectors (200 ng) (ER+BTG1, ER+BTG2, ER+CAF1). The cell lysates were subjected to Western blot analysis after SDS-PAGE and immunoblotted with the anti-ER\(\alpha\) antibody. Panel D, ER\(\alpha\) interacts directly with CAF1 in the presence of 17\(\beta\)-estradiol. Purified GST, GST-CAF1, and the control protein GST-FLRG, shown as Coomassie staining, were subjected to SDS-PAGE, transferred from the gel to membrane, and probed with \(^{35}\)S\textit{-labeled ER\(\alpha\) proteins suspended in binding buffer in the presence or absence of 100 nm 17\(\beta\)-estradiol. Specific hybridization was observed with GST-CAF1, but not with control GST or GST-FLRG, after incubation with labeled ER\(\alpha\) in the presence of 17\(\beta\)-estradiol. No interaction was observed in the absence of hormone. Molecular size markers are shown in kDa.

**Fig. 8.** Dependence of the physical interaction of BTG1 with CAF1 on the integrity of its LXXLL sites. Shown is a GST pull-down assay with interactions between \(^{35}\)S-labeled \textit{in vitro} translated BTG1 proteins mutated in LXXLL boxes, as described in Fig. 6A, and GST-CAF1, as described in Fig. 2B.
regulation of the estrogen nuclear receptor ERα. Our results indicate that BTG1 and BTG2 can function as coactivators and corepressors of ERα (Fig. 5) and that the LXXL sequences are involved in the effect of BTG proteins on ERα-dependent transcription (Fig. 6). This result along with the fact that (a) the BTG proteins need the LXXL motifs to modulate ERα-mediated transcription and to interact with CAF1 and (b) that CAF1 also acted as a modulator in this assay and can bind directly to ERα in vitro strongly suggests that BTG proteins modulate ERα-mediated transcription through their interaction with CAF1 via a CCR4-like complex or complexes. As regards yeast, it is thought that a complex of this type may affect transcription either positively or negatively (18). This hypothesis is supported by our recent results showing that in mammalian cells CAF1 and BTG1 bind together in a large multiprotein complex.2 Alternatively, it may be that the LXXL sites are important for the correct conformation of BTG proteins and for their biological activity. In fact this motif normally takes on a helical conformation and facilitates transcription and to interact with CAF1 and (38). Although the mechanistic basis of the inhibitor 11. Matsuda, S., Kawamura-Tsuzuku, J., Ohsugi, M., Yoshida, M., Emi, M. (1999) Oncogene 12, 705–713 12. Yoshida, Y., Matsuda, S., Ikematsu, N., Kawamura-Tsuzuku, J., Inazawa, J., Usui, T., Nakamura, H., and Yamamoto, T. (1999) Oncogene 18, 2687–2693 13. Ikematsu, N., Yoshida, Y., Kawamura-Tsuzuku, J., Ohsugi, M., Onda, M., Hirai, M., Fujimoto, J., and Yamamoto, T. (1999) Oncogene 18, 7432–7441 14. Malatesta, P., Gotz, M., Barsacchi, G., Price, J., Zoncu, R., and Cremisi, F. (1999) Mol. Cell. 4, 17–22 15. Guardavaccaro, D., Corrente, G., Covone, F., Micheli, L., D’Agnano, I., Starace, G., Carruo, M., and Tironi, F. (2000) Mol. Cell. Biol. 20, 1797–1815 16. Bogdan, J. A., Adams-Burton, C., Pedicord, D. L., Sukovich, D. A., Benfield, P. A., Corjay, M. H., Stoltenberg, J. K., and Dicer, I. B. (1999) Biochem. J. 346, 471–481 17. Verma, R. S., Manikal, M., Conte, R. A., and Godec, J. C. (1999) Cancer Invest. 17, 441–447 18. Levy, A., Dang, U. C., and Bookstein, R. (1999) Genes Chromosomes Cancer 24, 42–47 19. Pineda, P., Nagai, H., Frigeri, S., Wei Y., Gyapay, G., Weissbenbach, J., Traut, P., Ruesu, M., and Dejazet, A. (1999) Oncogene 18, 3147–3154 20. Fidler, C., Wainscoat, J. S., and Bouloul, J. (1999) Genomics 56, 134–136 21. Albert, T. K., Lemaire, M., van Berkum, N. L., Gentz, R., Collart, M. A., and Tirone, F. (2000) J. Biol. Chem. 275, 473–736 22. Rappold, B., Kalkhoven, E., and Tirone, F. (2000) J. Biol. Chem. 275, 137–138 23. Horvitz, K. B., Jackson, T. A., Bain, D. L., Richer, J. K., Takimoto, G. S., and Tung, L. (1996) Mol. Endocrinol. 10, 1139–1311 24. Tora, L., Mullik, A., Metzger, D., Ponglikitmongkol, M., Park, I., and Chambon, P. (1989) EMBO J. 8, 181–186 25. Green, S., Inseman, I., and Sheer, E. (1988) Nucleic Acids Res. 16, 369 26. Planas-Silva, D. M., and Weinberg, R. A. (1997) Mol. Cell. Biol. 17, 4059–4069 27. Heery, D. M., Kalkhoven, E., Heare, S., and Parker, M. G. (1997) Nature 387, 733–736 28. Beato, M., Herrlich, P., and Schutz, G. (1995) Cell 83, 851–857 29. Görnemann, H., and Ladet, V. (1995) Protein Protocols 2, 173–1308 30. Horwitz, K. B., Jackson, T. A., Bain, D. L., Richer, J. K., Takimoto, G. S., and Tung, L. (1996) Mol. Endocrinol. 10, 1167–1177 31. Manglesdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Rastelli, P., Mark, M., Chambon, P., and Evans, R. H. (1995) Cell 83, 853–859 32. Bai, Y., Salvadore, C., Chiang, Y. C., Collart, M. A., Liu, H. Y., and Den, C. L. (1996) Mol. Cell. Biol. 16, 6642–6651 33. Lin, W. J., Gray, D. J., Yang, M. C., Clarke, S., and Herschman, H. R. (1996) J. Biol. Chem. 271, 15034–15044 34. Chen, D., Ma, H., Hong, H., Koh, S. S., Huang, S. M., Scherter, B. T., Aswad, D. W., and Stallcup, M. R. (1994) Science 264, 2174–2177 35. Koh, S. S., Chen, D., Lee, Y. H., and Stallcup, M. R. J. Biol. Chem. 276, 1089–1098 36. Kalkhoven, E., Valentine, J. E., Heery, D. M., and Parker, M. G. (1998) EMBO J. 17, 223–222 37. Zwijsen, R. M., Luijendijk, E., Klompaker, R., van der Sman, J., Bernards, R., and Michalides, R. J. (1997) Cell 88, 405–415
Relationships of the Antiproliferative Proteins BTG1 and BTG2 with CAF1, the Human Homolog of a Component of the Yeast CCR4 Transcriptional Complex: INVOLVEMENT IN ESTROGEN RECEPTOR α SIGNALING PATHWAY
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