Structure-Function Analysis of the Estrogen Receptor α Corepressor Scaffold Attachment Factor-B1

IDENTIFICATION OF A POTENT TRANSCRIPTIONAL REPRESSION DOMAIN

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Scaffold attachment factor-B1 (SAFB1) is a nuclear matrix protein that has been proposed to couple chromatin structure, transcription, and RNA processing. We have previously shown that SAFB1 can repress estrogen receptor (ERα)-mediated transactivation. Here we present a structure-function study showing that transactivation is mediated via an intrinsic and transferable C-terminal repression domain (RD). A similar C-terminal RD was found in the family member SAFB2. Removal of the RD from SAFB1 resulted in a dominant-negative SAFB1 protein that increased ligand-dependent and -independent ERα activity. SAFB1RD-mediated repression was partly blocked by histone deacetylase inhibitors; however, no histone deacetylase inhibitors were identified in a yeast two-hybrid screen using the RD as bait. Instead, SAFB1RD was found to interact with TAFII68, a member of the basal transcription machinery. We propose a model in which SAFB1 represses ERα activity via indirect association with histone deacetylation and interaction with the basal transcription machinery.

SAFB1 belongs to a family of nuclear proteins that are localized in the nuclear matrix. The matrix is a proteinaceous structure consisting of a network of ribonucleoproteins and non-histone proteins, such as transcription factors, that serve as a scaffold for the higher organization of chromatin into loop structures (1). A characteristic of SAFB family members is the presence of an N-terminal SAF box (2–4) that binds to DNA regulatory regions termed scaffold/matrix attachment regions (S/MARs). S/MARs are bound to the nuclear matrix, define the loop structure of higher order chromatin (5–8), divide the genome into structural and functional domains, and are implicated in the regulation of gene expression (1).

SAFB proteins contain a central RNA recognition motive (RRM), which suggests a role in mRNA processing. Because SAFB1 also has been shown to interact with members of the RNA processing machinery and with RNA polymerase II, it has been suggested that this protein is part of a “transcriptosome” complex, coupling chromatin structure to transcription and RNA processing (9–12).

We have previously reported that SAFB1 plays an important role in breast cancer because its overexpression results in growth inhibition (13). SAFB1 maps to a chromosomal locus that displays unusually high rates of loss of heterozygosity (14), and mutations have been identified in breast tumors (14). We have also shown that SAFB1 can bind to and repress transcriptional activity of the estrogen receptor α (ERα), thereby functioning as an ERα corepressor (15).

ERα is a steroid receptor that regulates transcription of genes involved in proliferation, apoptosis, migration, and other cellular processes (16). ERα corepressors and coactivators are components of large protein complexes that tightly control the activity of ERα (17, 18). Although the role of coactivators in the activity of ERα is well established, the role of ERα corepressors is less clear. Based on recent studies, it is believed that ERα corepressors play a role in: (i) modulating the estrogen response and thereby provide tissue specificity; (ii) conferring anti-estrogen-mediated repression of ERα; (iii) mediating estrogen-induced repression of genes; and (iv) controlling the activity of ERα bound to DNA in the absence of ligand (for a recent review see Ref. 19). Emerging studies show that the repression of ERα activity is essential in preventing the cell from responding inappropriately to estrogen, and it is hypothesized that the failure of these control mechanisms is a characteristic of estrogen-responsive breast cancer (14, 20, 21).

A number of ERα corepressors contain domains that can repress the transcriptional activity of a reporter construct when they are transferred to heterologous proteins. These independent repression domains recruit proteins that modulate activity by remodeling local chromatin or by interacting directly with members of the basal transcription machinery. For example, SMRT, NCoR (22), and metastasis-associated protein 1 (MTA1) (23, 24) recruit histone deacetylases (HDAC) or HDAC-containing protein complexes to target promoters. Additionally, NCoR can also function by locking the central components of the transcription initiation machinery (TFIIIB and TATA-binding protein-associated factors) into a nonfunctional complex or confirmation that is not favorable for transcription (25). In contrast to repressors containing independent and transferable repression domains, the repressor of estrogen receptor activity functions by competing with coactivators for ERα binding sites (26), and the testicular orphan nuclear re-
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ceptor 2 prevents ERα homodimerization and its subsequent interaction with DNA (27). Other proteins such as repressor of tamofoxifen transcriptional activity may modulate ERα by regulating facets of mRNA processing or stability (28).

Here we report that the ERα corepressor SAFB1 functions similarly to SMRT and NCoR in that it has an independent repression domain that maps to the C terminus, a characteristic also found in SAFB2, a protein that is very similar to SAFB1. Repression through this domain can be partially released with HDAC inhibitors. Detailed structure-function studies of SAFB1 showed that the repression domain is separable from the interaction domain used by Cre recombinase for transgene repression and other chromatin-occupied regions. Yeast two-hybrid studies revealed an interaction with TAFI168, a member of the basal transcription machinery. Based on these and other previously published data, we suggest that SAFB1 provides a scaffold for recruiting members of both the chromatin remodeling and the basal transcription machinery to estrogen-regulated promoters.

EXPERIMENTAL PROCEDURES

Cell Culture and Chemicals—Cell lines CV-1 (African green monkey kidney cells), NIH-3T3 (mouse fibroblast), HeLa (cervical adenocarcinoma), 293 (primary human embryonal kidney), Saos-2 Endo cells (osteosarcoma), SAFB1 and SAFB2 negative (see Ref. 4), and the human breast cancer cell line, MCF-7, were maintained in improved Minimum essential medium (IMEM) (Invitrogen) supplemented with 200 units/ml penicillin, 200 μg/ml streptomycin, 6 ng/ml insulin, and 5–10% fetal bovine serum (HyClone, Logan, UT). For estrogen induction experiments, cells were switched to serum-free medium, which consisted of phenol red-free IMEM, 10 mM HEPES, pH 7.4, 1 μg/ml fibronectin (Invitrogen), trace elements (Biofluids, Camarillo CA), and 1% bovine serum albumin (BSA) or 100 nM 17β-estradiol. After 8–10 h of transfection, the medium was replaced with HDAC inhibitors (100 nM trichostatin A, 500 nM sodium butyrate). In estradiol-containing medium, luciferase activity was measured at 20 min 37 °C and were then inactivated by heat at 65 °C for 5 min. 2–5 μl were transferred into BW2374 (30) or PirI (Invitrogen) cells. Restriction digests and their ability to generate GST fusion proteins of the appropriate size confirmed all constructs.

Gal4-DBD fusion constructs were generated using the pCMX-Gal4N expression vector. To clone full-length SAFB1 into pCMX-Gal4N, SAFB1 was cloned into the EcoRI site of pCDNA1.1 which was digested with BamHI and EcoRI and ligated in-frame into the pCMX-Gal4N vector. Other Gal4 fusion constructs in pCMX-Gal4N were generated by PCR with appropriate primers and 5′ restriction sites or by restriction digests of full-length SAFB1 to produce SAFB1 fragments that were then cloned in-frame to pCMX-Gal4N.

Analysis of Protein-Protein Interactions—Proteins translated in vitro were produced using the TnT rapid in vitro translation kit (Promega) according to the manufacturer’s instructions and as described previously by us (4). For the yeast two-hybrid interactions, cDNA of SAFB1 was subcloned into the pCDNA1.1 vector were digested with BamHI and EcoRI (pGEX2T) or BamHI and EcoRI (pBluescript KS(+)) (Stratagene). The DNA for the mutant protein was then cloned into pUNI10. GST fusion constructs of the repression domain mutants, SAFB1 fragments that were then cloned in-frame to the pCMX-Gal4N vector. MCF-7 cells were lysed in a high stringency lysis buffer, and 500 μg of protein were precluded with protein G-agarose and incubated with 4 μg of the appropriate antibody, as described previously by us (4). For the yeast two-hybrid interactions, the terminal SAFB1 fragment (aa 599–915) was cloned into pGBK-T7 vector (BD Biosciences). Yeast two-hybrid assays using a normal breast tissue cDNA library were undertaken using the Matchmaker3 system (BD Biosciences) according to the manufacturer’s instructions and as described previously (29).

In Vitro RNA-binding Assay—A modified version of an in vitro RNA-binding assay (31) was used to identify RNA molecules bound by the RRM of SAFB1. SAFB1 fragments containing the RRM (GST-RRM, amino acids 354–538) and ΔRRM (GST-ΔRRM, amino acids 354–428 and 483–538) were cloned into the host vector, pUNI10, using PCR. A Cre recombinase vector with the polyadenylation signal pBl22-GST produced the GST fusion protein. All steps were performed at 4 °C, unless otherwise stated. Total RNA was isolated from MCF-7 cells using the RNAasy mini prep kit from Qiagen. Bacterially expressed fusion proteins were produced in BL21-Gold cells as described above and bound to 200 μl of GST-agarose beads. The beads were washed in 10 ml volumes of phosphate-buffered saline. The GST-Sepharose beads with bound GST fusion proteins were resuspended in 500 μl of phosphate-buffered saline with 1 unit/ml RNasin (Promega) and 100 μg of Escherichia coli 16S rRNA. The beads were boiled with the RNA at 100 °C for 10 min followed by the addition of 60 μg of MCF-7 RNA. The RNA was bound for 30 min at room temperature, and the beads were washed by centrifugation at 1000 × g for 1 min. The beads were then resuspended in 10 ml of phosphate-buffered saline and washed twice more with vortexing. The RNA was extracted using TRI reagent from Sigma. Extracted RNA was precipitated using 1 ml of 75% ethanol and inactivated by heat for 10 min. The reverse transcription reaction, which was performed using 2–4 μl of purified RNA and the SMART RACE cDNA amplification kit from Clontech. A modified primer, 5′-TAA GTA GCC GCG GGT GAA GTG TTG (T(20) AGC AGC CTG)3′, was used for the reverse transcriptase reaction. 

PfX DNA polymerase (Invitrogen). Annealing temperatures and extension times for each primer pair were determined for use on either an MJ Research PTC 200 thermal cycler (MJ Research Inc., Waltham, MA) or a PerkinElmer 9600 (PerkinElmer Life Sciences). PCR products were digested with BamHI and EcoRI (pGEX2T) or BamHI and NdeI (pUNI10) and gel-purified using the Qiagen Gel Extraction Kit (Qiagen Inc., Valencia, CA). The resulting reverse transcriptase PCR products were cloned into the...
SAFB1 Contains Two Domains That Interact with Estrogen Receptor α—The SAFB1 protein contains a number of conserved domains (indicated in Fig. 1, top panel) that may function as protein-protein interaction domains, and we asked, first, if any of these motifs were important for the SAFB1-ERα interaction. A series of GST-SAFB1 fusion proteins containing a deletion in one or another of these motifs were constructed and then used to assay in vitro protein-protein interactions with in vitro translated ERα. All of the SAFB1 mutants bound ERα (Fig. 1, bottom panel), suggesting that none of these domains were required for ERα binding.

Next, we generated a number of SAFB1 GST deletion constructs as depicted in Fig. 2A, left panels, and confirmed the correct protein expression (Fig. 2A, right panels). Performing in vitro protein-protein interactions with the N-terminal (aa 1–260), central (aa 260–600), and C-terminal (aa 600–915) regions, we identified the central region of SAFB1 as the major ERα interaction domain (Fig. 2B, EID). We repeatedly observed weak binding of ERα to the SAFB1 C terminus (aa 600–915) (Fig. 2B, EID2) that could not be conclusively defined using smaller GST fusion proteins because of the relatively weak binding. To further refine EID1, a series of iterations using consecutively smaller fusion proteins from aa 426–600 were generated (Fig. 2A). The results from the GST pull-down assays (Fig. 2C) suggest that the SAFB1 region encompassing aa 426–600 contains at least three interaction domains, which may bind in a cooperative manner. The existence of multiple interaction domains may explain how a mutant missing the central RRM is still able to bind ERα (Fig. 1). The EIDs in SAFB1 are illustrated in Fig. 2D.

SAFB1 Binds RNA but This Function Is Not Required for Corepressor Activity—A number of nuclear receptor cofactors contain RNA recognition motifs, and in some cases (e.g. repressor of tamoxifen transcriptional activity (see Ref. 28)) these domains have been shown to be essential for corepressor activity. SAFB1 also has a central RRM, and we asked, first, if this RRM could in fact bind RNA. Based on secondary structure predictions and molecular modeling of the RRM in SAFB1, we chose to remove amino acids 429–483, which constitute β2β3α2β4 of the canonical RRM β1α1β2β3α2β4 motif, to produce a ΔRRM-SAFB1 construct. GST-SAFB1 (aa 354–538) and GST-SAFB1-ΔRRM (aa 354–428 and 483–538) were expressed in bacteria, bound to GST-agarose beads, and combined with total RNA isolated from MCF-7 cells. The beads were extensively washed, and the RNA was purified to make cDNA, which was then amplified by PCR and separated on agarose gels. The presence of PCR product in the GST-SAFB1-RRM lane indicated that this domain is able to bind RNA in vitro, whereas the GST-SAFB1-ΔRRM could not (Fig. 3A).

Second, we asked whether RNA binding and, therefore, the RRM domain was essential for SAFB1 corepressor activity as measured in the transient transfections assays. Full-length SAFB1 (aa 1–915) or SAFB1-ΔRRM (aa 1–428 and 483–915) were transiently transfected into MCF-7 cells along with an ERα-responsive reporter construct, ERE-TK-Luc. Both SAFB1 and SAFB1-ΔRRM repressed ERα activity, indicating that RNA binding is not an essential component for the repression of ERα activity (Fig. 3B), at least as measured in these assays. Further experiments included a series of ERE reporter assays in which we cotransfected expression plasmids that encoded SAFB1-interacting proteins, which function in mRNA splicing and stability, such as heterogeneous nuclear ribonucleoprotein A1, tra2, and AUF1/heterogeneous nuclear ribonucleoprotein D, along with SAFB1. These experiments, however, failed to show any synergestic effects (data not shown), suggesting that there might be a delineation between SAFB1 activities in RNA processing and transcriptional regulation.

SAFB1 Has an ERα-independent Transferrable Repression Function When Tethered to DNA—Next, we tested whether SAFB1 has an intrinsic repression activity that is independent of an interaction with ERα as demonstrated for other corepressors such as SMRT (32). Conventional repression assays using Gal4-DBD fusion proteins and a Gal4-responsive reporter construct were performed with CV-1 cells, and the N-terminal repression domain (RD) of SMRT (SMRTRD) (aa 1–1230) was used as a positive control (22). The Gal4-DBD-SMRTRD and -SAFB1 fusion proteins were expressed at comparable levels (Fig. 4, inset). Like SMRTRD, full-length SAFB1 repressed the reporter activity in a dose-dependent manner (Fig. 4). Transfection of SAFB1 that was not fused to Gal4-DBD (pcDNA1-SAFB1) failed to reduce the basal reporter activity (data not shown) verifying that SAFB1 must be bound to DNA to repress transcription. Additionally, we tested an SAFB1ΔRRM-Gal4-DBD mutant using this assay, and, as expected, we did not see a loss of repression (data not shown). The Gal4-DBD-SAFB1 repression assay was also performed with NIH3T3, MCF-7, HEK 293, Saos-2E, and HeLa cells, and, although we detected minor differences in the level of repression, SAFB1 was able to repress transcription in those cell lines (data not shown).

The Repression Domain Maps to the C-terminal Region in SAFB1—Having shown that SAFB1 harbors intrinsic repression activity, we next mapped the region in SAFB1 responsible for this activity. We generated a series of truncated Gal4-DBD-SAFB1 expression constructs (Fig 5A, top panel) that, when expressed in CV-1 cells, led to the expression of peptides of the expected sizes (Fig. 5A, bottom panel) and were expressed at levels comparable with SMRTRD. Transient transfections using the Gal4-DBD-SAFB1 deletions showed that the repression domain resides in the C terminus (aa 599–915) (Fig. 5B). The repressive activity of this C-terminal region is comparable with that of SMRT (Fig. 5B, compare SMRTRD and aa 599–915).

To investigate whether the SAFB1 C terminus contained more than one intrinsic repression domain and whether we could finely map the repression domain(s), a series of overlapping Gal4-DBD fusion proteins from the C-terminal region were constructed (Fig. 5C, top panel), and correct protein expression was confirmed (Fig. 5C, bottom panel). The data in Fig. 5D indicate that all deletion fragments were able to repress, at least to some extent, and that there may be multiple elements in this region that are able to direct repression.
We previously demonstrated that HDAC inhibitors can reduce SAFB1 corepressor activity on ERα/H9251 (33), and here we investigated whether HDAC inhibitors would affect SAFB1-mediated repression in an ERα-independent assay. As shown in Fig. 6, the addition of TSA led to a release of repression of both SAFB1RD and SMRTRD; however, TSA did not fully reverse SAFB1-mediated repression of the reporter. Similar results were obtained with sodium butyrate, the HDAC inhibitor (data not shown), suggesting that SAFB1, like other ERα corepressors (34, 35), might have multiple mechanisms of repression, including HDAC-
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SAFB1 has an independent and transferable repression domain. SAFB1 represses TK promoter activity when tethered to DNA. CV-1 cells were transiently transfected with 1 domain.

In the absence of ligand. We repeated these experiments using an expression construct that contained the main ERα-interacting domain. As shown in Fig. 7, the SAFB1 C-terminal mutant, which lacks the RD, functions as a dominant negative in both the presence and absence of ligand. The RD interacts with TAFII68, which is a member of the basal transcription machinery.

To further characterize SAFB1 as an ERα copressor, we first analyzed the EID. We determined that the major EID in SAFB1 maps to aa 426–600, and this region does not contain any previously described ER interaction motifs such as the “CoRNR box.” The CoRNR box, which contains the (L/I)XX[I/V] motif, can be found in NCoR and SMRT (36). It is related to the LXXLL motif (“NR box”) commonly found in the EID in ERα coactivators (37, 38) as well as in a few corepressors, including LCoR (34) and RIP140 (39). Other corepressors such as repressor of estrogen receptor activity (26) harbor novel ERα interaction domains. Likewise, SAFB1 mediates ERα interaction via a novel domain, and therefore our results confirm the existence of additional binding motifs, other than NR and CoRNR boxes, used by corepressors.

SAFB1 is a large protein containing a number of functional domains. Like many other proteins, such as poly pyrimidine tract-binding protein-associated splicing factor (PSF/p100) (40) and heterogeneous nuclear ribonucleoprotein units/SAF-A (41–45), SAFB1 is involved in transcription, mRNA stability and/or processing, and chromatin regulation. SAFB1 has been shown previously to bind DNA (2), and here we show that it is also able to bind RNA. Although there is evidence that RNA binding can be important for ERα copressor activity (28), a SAFB1 mutant deficient in RNA binding was able to repress ERα activity, comparable with wild-type SAFB1. Although additional relevant assays are needed to draw a final, solid conclusion, these data suggest that the role of SAFB1 in transcriptional repression might be separate from its role in mRNA processing and/or stability. A similar separation of functions has been demonstrated previously for Sam68 (46). This separate role in mRNA processing and other undiscovered activities of previously known SAFB1-interacting proteins, including SF2/ASF (SFRS1), SF3b (SFRS9), and Sam68, confirming the validity of our screen. To our surprise, we did not identify any HDACs, suggesting that there might be an indirect interaction between SAFB1 and HDAC proteins.

One of the intriguing interacting proteins was TAFII68 (also known as TAF15, RF56, and TAF2N), a protein that has been shown to be associated with both TFIID and RNA polymerase II. Using directed yeast two-hybrid assays, we were able to show that this interaction was specific for the C-terminal end of the repression domain (Fig. 8A, aa 720–915). The interaction between SAFB1 and TAFII68 was confirmed in GST pull-down assays using in vitro translated TAFII68 and the SAFB1 C terminus fused to GST (Fig. 8B). We also were able to detect an interaction of endogenous SAFB and TAFII68 proteins in co-immunoprecipitation as shown in Fig. 8C. Finally, we generated a Gal4-DDB-SAFB1 construct in which the interaction domain was deleted (Fig. 8D, aa Δ720–915), which was used in repression assays. As shown in Fig. 8D, deletion of the TAFII68 interaction domain resulted in the loss of repression. This result suggests that, within the context of the entire protein, the interaction with TAFII68 is necessary for the repressive activity of SAFB1.

**DISCUSSION**

The transcriptional activator ERα is regulated by a number of coactivators and corepressors. The studies presented here deal with the further characterization of SAFB1, a recently identified ERα corepressor. Here we have shown that SAFB1 harbors an intrinsic RD that is indispensable for its corepressor activity and is separate from its main ERα-interacting domain. An SAFB1 mutant, which lacks the RD, functions as a dominant negative in both the presence and absence of ligand.

To further characterize SAFB1 as an ERα corepressor, we first analyzed the EID. We determined that the major EID in SAFB1 maps to aa 426–600, and this domain can be further divided into smaller but weaker interaction domains. This region does not contain any previously described ER interaction motifs such as the “CoRNR box.” The CoRNR box, which contains the (L/I)XX[I/V] motif, can be found in NCoR and SMRT (36). It is related to the LXXLL motif (“NR box”) commonly found in the EID in ERα coactivators (37, 38) as well as in a few corepressors, including LCoR (34) and RIP140 (39). Other corepressors such as repressor of estrogen receptor activity (26) harbor novel ERα interaction domains. Likewise, SAFB1 mediates ERα interaction via a novel domain, and therefore our results confirm the existence of additional binding motifs, other than NR and CoRNR boxes, used by corepressors.

SAFB1 is a large protein containing a number of functional domains. Like many other proteins, such as poly pyrimidine tract-binding protein-associated splicing factor (PSF/p100) (40) and heterogeneous nuclear ribonucleoprotein units/SAF-A (41–45), SAFB1 is involved in transcription, mRNA stability and/or processing, and chromatin regulation. SAFB1 has been shown previously to bind DNA (2), and here we show that it is also able to bind RNA. Although there is evidence that RNA binding can be important for ERα copressor activity (28), a SAFB1 mutant deficient in RNA binding was able to repress ERα activity, comparable with wild-type SAFB1. Although additional relevant assays are needed to draw a final, solid conclusion, these data suggest that the role of SAFB1 in transcriptional repression might be separate from its role in mRNA processing and/or stability. A similar separation of functions has been demonstrated previously for Sam68 (46). This separate role in mRNA processing and other undiscovered activities...
of SAFB1 may be important in the effect of SAFB1 in ERα negative breast cancer cell lines and tissues.

We have shown previously that SAFB1 interacts with the DBD/Hinge region in ERα but that SAFB1-mediated repression is not a result of inhibition of the DNA binding of ERα (15). We have also performed a number of experiments that exclude simple competition with coactivators.² Consistent with these studies, we show here that SAFB1 has an independent and transferable repression domain similar to that described for the ERα corepressors NCoR and SMRT (47, 48). Removal of this repression domain resulted in a dominant-negative SAFB1 protein that enhanced ERα-driven transcription. Intriguingly, SAFB1 RD functioned as a dominant negative not only in the presence but also in the absence of ligand, suggesting a role for SAFB1 in repression of ligand-independent ERα. Similarly, mouse embryo fibroblasts that are deficient in BRCA1 (BRCA1−/−), which functions as an ERα corepressor (49), showed increased ligand-independent ERα activity when compared with wild-type mouse embryo fibroblasts (50).

The repression domains in NCoR and SMRT recruit different HDAC-containing complexes (22). Because the SAFB1 RD-mediated repression was partially released by treatment with HDAC inhibitors, we expected that a yeast two-hybrid assay using SAFB1 RD as bait would reveal an interaction with known HDACs or members of the HDAC complexes. Because we failed to detect such interaction, we concluded that there is

² S. M. Townson, unpublished data.
an indirect interaction between SAFB1 and HDACs. Supporting this conclusion is a recent finding by Tai et al. (51) who discovered an interaction between SAFB and the chromodomain protein CHD1. CHD1 has been proposed to play a role in chromatin architecture and transcriptional regulation through its interactions with HDACs and NCoR. Like SAFB proteins, CHD1 binds to stretches of AT-rich DNA in so-called S/MARs. We therefore suggest that SAFB1-mediated repression is in part mediated through an indirect interaction with HDACs via binding to CHD1. Ongoing experiments in our laboratory will decipher the role of S/MARs in this repression.

The yeast two-hybrid screen identified TAFII68 as a SAFB1RD-interacting protein. TAFII68 belongs to the TET (TLS/FUS, EWSR1, and TAFII68/TAF15/RBP56) family of proteins that are best known through their role in transformation (52). Following chromosomal translocation, the N termini of TET proteins are fused to a variety of C-terminal DNA binding domains of transcription factors (29), resulting in dominant onco genes, presumably by functioning as transactivators (reviewed in Ref. 54). The TET family member TAFII68 was shown to be associated with both TFIID and RNA polymerase II (29, 55), and we therefore suggest that the interaction between SAFB1 and TAFII68 bridges the SAFB1-ER complex to the basal transcription machinery. Previous studies have shown that SAFB1 can also directly interact with RNA polymerase II (10). Deletion of the TAFII68 interaction domain in SAFB1 resulted in the loss of SAFB1RD-mediated repression, suggesting that this interaction is necessary for the effect of SAFB1. Interestingly, the GST pull-down experiments indicated that the C-terminal domain has multiple repression domains that, when used independently, can result in some degree of repression (see Fig. 5D). It is feasible, however, that within the context of the entire protein, the small fragments can not recruit other repressors efficiently enough to transfer repression, and thus deletion of the TAFII68 interaction domain results in a loss of repression.

In summary, we propose a model whereby SAFB1 serves as a scaffold to provide a structural framework for chromatin and the transcriptional machinery that allows for regulation of ERα at several steps during the transcriptional process. In this model, the SAFB1 complex defines a repressed chromatin structure through the indirect recruitment of HDACs, possibly via CHD1, and/or the inhibition of RNA polymerase II, either directly or through interaction with TAFII68. Ongoing studies in our laboratory that test this model will enable us to further understand how SAFB1 modifies the structure of chromatin for the regulation of ERα activity and will show how these mechanisms are disrupted in the development of breast cancer.

**FIG. 7.** SAFB1 C-terminal RD is required for corepression of ERα activity. MCF-7 cells were transfected with 1 μg of ERE-TK-Luc, 50 ng of ERα, 25 ng of β-galactosidase, and 250 ng of expression constructs for SAFB1 and SAFB1ΔRD (aa 1–600). Cells were treated with (+) or without (−) estradiol (E2, 10−8 M; 24 h) ligand as indicated. The inset shows the result from a GST pull-down assay confirming that SAFB1ΔRD can indeed bind to ERα. RLU, relative luciferase units.

**FIG. 8.** SAFB1 C-terminal RD interacts with TAFII68. **A,** TAFII68 interacts with the SAFB1 C-terminal amino acids 720–915. Yeast expression plasmids for TAFII68 and SAFB1 deletion mutants were transformed into yeast cells that were then plated onto selection media containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). Interaction between p53 and large T antigen (Tag) and lack thereof between TAFII68 and empty pGBKT7 vector served as positive and negative controls, respectively. **B,** TAFII68 interacts with SAFB1 in GST pull-down assays. In vitro translated TAFII68 was tested for interaction with GST-SAFB1 (aa 600–915) or GST only. IP, immunoprecipitate; WB, Western blot. **C,** MCF-7 cell lysate was precipitated with antibodies as indicated, and the membrane was immunoblotted with anti-SAFB antibodies. **D,** an SAFB1 protein missing the TAFII68 binding domain failed to function as a repressor. CV-1 cells were transiently transfected with 1 μg of 17x4-UAS-TK-Luc, 25 ng of β-galactosidase, and 250 ng of empty vector Gal4-DBD-SAFB1 or Gal4-DBD-SAFB1Δ720–915. RLU, relative luciferase units.
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