A Novel Zinc Finger Transcription Factor with Two Isoforms That Are Differentially Repressed by Estrogen Receptor-α*

Andrew T. Conroy‡, Manju Sharma‡, Ann E. Holtz‡, Chengbiao Wu‡, Zijie Sun‡, and Ronald J. Weigel¶†

From the ★Department of Surgery, Stanford University School of Medicine, Stanford, California 94305 and §Clontech Laboratories, Palo Alto, California 94303

Estrogen receptor-α (ERα) can induce the expression of genes in response to estrogen by binding to estrogen response elements in the promoters of target genes. There is growing evidence that ERα can alter patterns of gene expression in response to ligand by regulating the activity of other factors through a direct protein-protein interaction. To identify other factors that are regulated by ERα, a yeast two-hybrid screen was performed that identified a novel Cys2His2 zinc finger protein named ZER6. The ZER6 protein contains a Kruppel-associated box domain and six Cys2His2 zinc fingers. Transcripts from the ZER6 gene can have alternate 5′ exons and encode either a p71 or p52 isoform. The p52-ZER6 protein interacts strongly with ERα exons and encode either a p71 or p52 isoform. The p52-ZER6 protein interacts strongly with ERα in the presence of 17β-estradiol, whereas the p71-ZER6 isoform has a HUB-1 amino-terminal domain that inhibits the interaction with ERα. A consensus ZER6 binding element was defined using PCR-assisted binding site selection. In COS-1 cells, both the p52 and p71 isoforms can activate transcription through the ZER6 binding element; however, in the presence of ERα, transactivation by the p52 isoform is specifically repressed. Overexpression of the p52 isoform was able to abrogate activation by p71-ZER6. Expression of ZER6 was largely restricted to the mammary gland with a lower level of expression in the kidney. We conclude that ZER6 is a novel zinc finger transcription factor in which regulation of transcription in hormone-responsive cells can be controlled by the relative level of expression of two distinct isoforms.

Two human estrogen receptors (ERs)† have been identified: ERα and ERβ (1–4). These nuclear receptors are members of the steroid-thyroid-retinoic acid superfamily of transcription factors (5). In the classic model of transactivation by the recep-

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† Supported by a George H. A. Clowes, M.D., F.A.C.S., memorial research career development award through the American College of Surgeons. To whom correspondence should be addressed: Dept. of Surgery, MSLBS, Rm. P214, 1201 Welch Rd., Stanford University School of Medicine, Stanford, CA 94305-5494. Tel.: 650-723-9799; Fax: 650-724-3229; E-mail: ronald.weigel@stanford.edu.

‡ The abbreviations used are: ER, estrogen receptor; ZBE, ZER6 binding element; GST, glutathione S-transferase; contig, group of overlapping clones; KRAB, Kruppel-associated box; BD, binding domain; AD, activation domain; HT, His tag; PBS, phosphate-buffered saline; BSA, bovine serum albumin; IGF, insulin-like growth factor.
forms: p71-ZER6 and p52-ZER6. The p71 isoform contains a HUB-1 domain, which was previously identified to be important for repression of long terminal repeat-mediated transactivation (16). The p52 isoform of ZER6 interacts strongly with ERα, with 5′-GGGGAATTCGCCGCCACCATGCATCATCATC-3′ and 3′-GAGACTCGTAGTTGCCCTTCATG-3′) and the 5′-I and 3′-I site. The PCR product was cloned into TA cloning kit (Invitrogen, Carlsbad, CA). The resulting plasmid and the ZER6 cDNA clone I572-1 were restricted with EcoRI and the appropriate fragments were excised from agarose gels and ligated together creating pcDNA3.1-HT-p52-ZER6. To create an expression plasmid for p71-ZER6, the vector pcDNA3.1-HT was digested with S1 nuclease, and ligated to destroy the ApaI site. pcDNA3.1-HT-p52-ZER6 was digested with XhoI and the insert moved into the pcDNA3.1 vector lacking the ApaI site. The 5′ end of the p71-ZER6 cDNA was amplified by RT-PCR using MCF7 mRNA as template with the primers ZER6alt5 (5′-GGGGATT-TGCGGCCACCATGCATCATCATCTGTCAGTGAGGGCC-CCGGGCCGACATCAGTGA-3′) and ZER6alt3 (5′-GAATGAAACTGTGGCTTACTAAGT3′). The PCR product was cloned using TA cloning kit, sequenced, and excised using EcoRI and ApaI. This fragment was used to replace the 5′ end of the HT-p52-ZER6 clone, and the resulting plasmid was called pcDNA3.1-HT-p71-ZER6.

**GST Pull-down Assay**—The GST fusion protein with the ligand domain of ERα was expressed as described in Section 2.1 (17).

**Identification of ZBE**—PCR-aided binding site selection was performed as described previously (17). A GST-HT-p52-ZER6 fusion protein was created by cloning the HT-p52-ZER6 cDNA as an EcoRI/HindIII fragment into the vector pGEX-KG-1 (Amersham Biosciences, Inc., Piscataway, NJ). The resulting plasmid was transformed into *E. coli* and the plasmid was recovered from the bound complex on the dried gel-shift blot after autoradiography. After four rounds of binding selection, the PCR amplification products were cloned into the TA cloning vector pCR2.1 (Invitrogen Inc., Carlsbad, CA) and 100 cloned inserts were sequenced. Two rounds were performed using the oligonucleotides 5′-TCGGAGGGTTGGGGGTGGCCC-3′ and 5′-TGAGGAGGCT-CCCACCCCAT-3′. A nonbinding probe, which had G nucleotides changed to A and T nucleotides to G was created by annealing the two oligonucleotides 5′-AAAAAAAGAGAAAAAGAAT-3′ and 5′-AAAAATAATTTC- TTTCTTT-3′. Gel shift was performed with the bacterially produced protein as described previously. The anti-ER antibody used to super-shift the ZER6 complex was SC-8036X (Santa Cruz Biotechnology).
**Transactivation Assays**—The IL2-LUC minimal reporter has been described previously (17). ZBE-IL2-LUC was created by inserting a double-stranded ZBE oligonucleotide into the XhoI site of IL2-LUC. DNA was introduced into either MCF-7 or COS-1 cells by lipid-mediated transfection using FuGENE (Roche Molecular Biochemicals) according to the manufacturer's instructions. For COS-1 cells, the cells were plated at 2.5 × 10^5 cells/well in six-well plates 24 h prior to transfection and were 80% confluent at the time of transfection. Transfection medium contained zinc chloride to 100 μM. Each transfection contained 200 ng of luciferase reporter plasmid, 50 ng of pβGal-Control vector, and 750 ng of a ZER6 expression vector or plasmid DNA control as indicated. All transfections were performed in triplicate, and cells were harvested 48 h after transfection. Cell extracts for luciferase or β-galactosidase assays were prepared using a luciferase assay system (Promega, Madison, WI). Luciferase assays were performed with 10 μl of cell extract and 100 μl of luciferase assay buffer. The enzyme activity was measured for 10 s in a luminometer (Analytical Luminiscence Laboratory, San Diego, CA). β-Galactosidase activity in cell extracts was assayed using a Galacto-Light system (Applied Biosystems, Bedford, MA).

For MCF7 cell transfections, cells were seeded at 5 × 10^5/well in six-well plates in growth medium the day before transfection. On the day of transfection, the medium was replaced with fresh growth medium. Generally, 250 ng each of reporter plasmid, 1.5 μg of ZER6 expression plasmid, and 250 ng of pβGal-control plasmid were used per sample. After 24 h of incubation in the presence of the DNA-lipid complexes, the transfection mixture was removed, the cells were washed twice with phosphate-buffered saline, and the transfection mixture was replaced with minimal essential medium with 100 μM zinc chloride, 10 μM 17β-estradiol, and 1% FBS. The cells were replaced at 37°C, 5% CO₂ until harvest. Luciferase and β-galactosidase activity was assayed as described above. For each sample the luciferase relative light units were normalized with the β-galactosidase relative light units.

**Tissue-specific Expression of ZER6**—The cDNA for the ZER6 gene was hybridized to a Human RNA Master Blot (CLONTECH, Palo Alto, CA) according to the instructions of the manufacturer.

**RESULTS**

**Ligand-dependent Interaction of ERα with a Novel Zinc Fingerm Protein**—The yeast two-hybrid system was used to identify proteins with ligand-dependent interaction with ERα. Full-length ERα was cloned as a fusion protein with the DNA binding domain of GAL4. A normal human mammary epithelial cell cDNA library cloned to create a fusion protein with the GAL4 activation domain used was identified in yeast co-transformation. Yeast transformants were screened on SD Leu–/Trp–/His–/Ade– medium to select for double transformants. To identify proteins that interact with ERα in a ligand-specific fashion, yeast colonies were selected on SD Leu–/Trp–/His–/Ade– medium supplemented with 17β-estradiol, tamoxifen, or no ERα ligand. Approximately 300 yeast colonies underwent a secondary screening procedure. Twenty-five colonies were identified that demonstrated a ligand-dependent growth phenotype, and four colonies were isolated that demonstrated growth only with 17β-estradiol (data not shown). The plasmids encoding the activation domain fusion proteins were recovered from these four yeast transformants, and the inserts were sequenced. One of these inserts (clone 6) was found to contain an insert of 993 bp that encoded an in-frame protein fragment of 293 amino acids that was terminated by a stop codon. Protein analysis of the predicted amino acid sequence indicated that part of a cDNA encoding a novel zinc finger protein had been cloned. This protein has been tentatively called ZER6 (zinc finger–estrogen receptor interaction, clone 6).

The 5' and 3' regions of the ZER6 protein fragment were subcloned into pGADT7. The 5' region contained the first 143 amino acids, and the 3' region contained the carboxyl-terminal 151 amino acids. These two plasmids were transformed into AH109 yeast with the full-length clone in parallel and assayed for growth on selected media with various ERα ligands. As seen in Fig. 1, all transformants grew on SD Leu–/Trp– medium; however, on SD Leu–/Trp–/His–/Ade– medium, the full-length ZER6 insert and the 5' region grew only in the presence of 17β-estradiol. No growth was observed on selective media with the plasmid encoding an activation domain fusion protein with the carboxyl-terminal 151 amino acids of ZER6. Yeast transformed with expression plasmids encoding a GAL4 DNA binding domain/p53 and GAL4 activation domain/T antigen (AD/T) demonstrated growth on selected media independent of ERα ligand.

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**Cloning the Full-length ZER6 cDNA**—The cDNA insert for ZER6 obtained from the yeast two-hybrid screen was used as a probe to screen a cDNA library prepared from MCF7 cells (an ERα-positive human breast carcinoma cell line). Four cDNAs were obtained and were entirely sequenced. As seen in Fig. 2, the sequence of the four cDNA clones matched the genomic cosmid DJ0800G07 and allowed an identification of exon/intron borders. ZER6 was determined to be encoded by five exons spanning a genomic region of ~28,000 bp. All introns began with a GT and ended with AG. The first ATG in-frame occurred in the second exon, which was 127 bp long. The open reading frame extended beyond the 5' end of the longest cDNA, which suggested the possibility that the gene encoded a longer protein not completely contained in the cDNA clones.

To obtain longer cDNAs encompassing the full open reading frame, 5'-RACE was performed using primers at the most proximal ATG in the second exon. Fourteen RACE clones were obtained and are diagrammed in Fig. 3. As shown in Fig. 3, variable splicing results in a number of possible 5' ends for ZER6 transcripts. Five clones (I811-2, I815-1, I815-6, I815-11, and I815-7) utilized upstream exons which were not included in the genomic contig. Two of these (I811-2 and I815-1) encoded cDNAs that had a stop codon in-frame and indicated an amino-terminal boundary for the coding region. In mRNAs with this structure, the ATG in the 127-bp exon would be the 5' terminal ATG and would encode a protein with a predicted size of 52 kDa. Two of the 5'-RACE clones (I815-6 and I815-11) had identical sequence and demonstrated exon skipping. This
cDNA structure extended the open reading frame; however, no
in-frame ATG was encountered. Similarly, I815-13 utilized a
unique exon sequence that also terminated the open reading
frame and would utilize the same ATG as the aforementioned
transcripts. Seven of the 5'/H11032 RACE clones (I811-1, I815-5, I815-8,
I 815-4, I815-9, I815-3, and I815-14) appear to be premature
termination of larger cDNAs and do not provide any additional
structural information.

Clone I815-10 was unique in that it utilized 41 bp of an exon
that extends the open reading frame and contained an in-frame
ATG. An mRNA with this structure would encode a predicted
protein of /H11011 71 kDa. The full-length cDNA encoding p71-ZER6
is shown in Fig. 4. The ATG beginning the translation of
p52-ZER6 is also noted. There are six complete Cys2His2 zinc
fingers, which are aligned with the consensus sequence as
shown in Fig. 5. Each of the six fingers utilizes a leucine as the
hydrophobic residue before the His2 domain. In addition, each
of the linkers between the Cys 2 residues comprises two amino
acids. A BLASTp search of the protein sequence unique to
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Fig. 5. Amino acid sequence of the six consensus Cys2His2 zinc fingers of ZER6. The six Cys2His2 zinc fingers of the ZER6 protein are shown aligned to highlight the consensus amino acid structure shown at the top. Letters X indicate any possible residue, and ψ denotes a hydrophobic residue, which in each case in ZER6 is a leucine. Numbers to the left indicate the amino acid residue in p71-ZER6, which denotes the location of the first amino acid of the finger shown on that line. In the numbering scheme used in the text, position −1 is the residue immediately preceding the α helix, which corresponds to the second residue in the Xψ block before the ψ residue. For the six zinc fingers of ZER6, residue −1 of the α helices is D, L, G, R, R, and Y, respectively.

Fig. 6. GST pull-down demonstrates ligand-dependent interaction between ERα and p52-ZER6. The p71-ZER6 and p52-ZER6 proteins were synthesized using in vitro transcription/translation and incubated with a GST fusion protein containing the ligand binding domain of ERα. As shown, p52-ZER6 binds to ERα in the presence of 17β-estradiol. The p71 isoform does not interact with ERα. Similarly, in an experiment where the p52 and p71 isoforms of ZER6 were synthesized together (MIX), only the p52-ZER6 protein demonstrates ligand-dependent interaction with ERα.

ERα-ZER6 interaction in intact cells. COS-1 cells were co-transfected with expression vectors for ERα and either p52-ZER6 or p71-ZER6. The ZER6 proteins were expressed with an HT epitope. A representative cell staining pattern is shown in Fig. 7. Expression of both proteins was nuclear with faint cytoplasmic staining seen with the anti-HT antibody. Confocal microscopy demonstrates co-localization of both proteins with slight cytoplasmic staining with the anti-HT antibody. Confocal microscopy demonstrates co-localization (yellow) of p52-ZER6 and ERα. Co-transfection of p71-ZER6 and ERα (bottom panel) demonstrates predominantly nuclear staining. However, confocal microscopy to examine both proteins demonstrates separate red (p71-ZER6) and green (ERα) nuclear immunofluorescence.

Fig. 7. Indirect immunofluorescence demonstrates co-localization of ERα and p52-ZER6. Indirect immunofluorescence was used to examine localization of ZER6 (red) and ERα (green) in COS-1 cells transfected with expression vectors for the proteins indicated. Co-transfection of p52-ZER6 and ERα (top panels) demonstrate nuclear staining for both proteins with slight cytoplasmic staining with the anti-HT antibody. confocal microscopy demonstrates co-localization (yellow) of p52-ZER6 and ERα. Co-transfection of p71-ZER6 and ERα (bottom panels) demonstrates predominantly nuclear staining. However, confocal microscopy to examine both proteins demonstrates separate red (p71-ZER6) and green (ERα) nuclear immunofluorescence.

The repressing effect of ERα on ZER6 was evident by a yellow staining color, and would translate a protein with predicted size of 52 kDa. The location of splice sites is indicated by arrowheads connected by a line. The six consensus zinc finger domains are indicated in bold type. The stop codon is indicated by a star, and the polyadenylation signal AATAAA is underlined. The GenBank accession number for this cDNA is AY049744. The sequence of the cDNA from MCF7 cells matched the genomic clone DJ080007, which is shown with the exception that the nucleotide G201 was A201, nucleotide T438 was C438, and the nucleotides 2068 to 2072 (CTAGA) in the untranslated 3' region were deleted. None of these polymorphisms alter the protein structure. Recent BLASTp searches identified homology with partial cDNAs submitted as expressed sequence tags and listed under accession numbers BAA92577 (KIAA1339 protein), AAD45824 (similar to zinc finger proteins), and XP_035724 (KIAA1339 protein).
possible that overexpression of the p52 isoform might interfere with transactivation by p71-ZER6. To test this hypothesis, MCF7 cells were transfected with p71-ZER6 expression vector with increasing amounts of expression vector for p52-ZER6. As seen in Fig. 9D, overexpression of p52-ZER6 abrogated transactivation by p71-ZER6. There was a statistically significant difference between transfections in which the ratio of p71 to p52 expression vector was 2:1 versus 1:2 (99.3 ± 7.8 versus 69.1 ± 6.7, p = 0.007). These data suggest that the interaction between ERα and p52-ZER6 repressed ZER6-mediated transactivation. We conclude that because the p71 isoform does not interact with ERα, p71-ZER6 is able to activate transcription independent of ERα expression.

ZER6 Expression Is Restricted to the Mammary Gland—The physiologic relevance of an interaction between ERα and ZER6 requires that the two proteins are co-expressed in cells. To determine the pattern of ZER6 expression, a RNA dot blot with RNA from 50 different human tissues was hybridized with a ZER6 cDNA probe. These results are shown in Fig. 10. The ZER6 gene was most prominently expressed in the mammary gland. This finding agrees with the fact that the ZER6 gene was cloned from cDNA libraries derived from normal mammary gland and MCF7 breast carcinoma cells. The gene is also expressed in the kidney, although the level of expression is significantly less than in the breast. Negligible expression was detected in 41 other adult human tissues and 7 human fetal tissues. Weak hybridization signals were also detected with total human and bacterial DNA. We conclude that the expression pattern of the ZER6 gene is largely restricted to the mammary gland with relatively lower levels of expression in the kidney.

DISCUSSION

In the presence of estrogen, ERα forms a homodimer that is able to activate transcription by binding to estrogen response elements in the promoters of target genes (6). The specific transcriptional response to ERα ligands varies with the cell line and is dependent upon the repertoire of ERα cofactors or corepressors expressed in the cell (21–23). Over the last several years, it has become clear that ERα can have effects on gene expression independent of the DNA binding function of the receptor. This alternate pathway of gene regulation is dependent upon the ability of ERα to affect the function of other nuclear factors through direct protein-protein interactions. In this report, we described a novel Cys2His2 zinc finger protein, ZER6, with two isoforms (p71-ZER6 and p52-ZER6), which demonstrate differential ligand-dependent interaction with

![Figure 8](http://www.jbc.org/)

**Fig. 8.** ZER6 is a sequence-specific DNA-binding protein. Gel shift was performed with a DNA probe containing the ZBE as defined by PCR-assisted binding site selection. Protein extract was from bacterially expressed p52-ZER6 protein synthesized as a GST fusion protein with an HT. The ZER6 protein forms a stable complex, which can be competed with excess cold probe sequence (ZBE) but not a nonbinding probe (NBP). Specific complexes can be supershifted with an anti-HT antibody.

![Figure 9](http://www.jbc.org/)

**Fig. 9.** ZER6 can activate transcription but the p52 isoform is repressed by ERα. Transcriptional activation of the ZER6 proteins was examined using luciferase reporter constructs that utilize the interleukin-2 minimal promoter (IL2-LUC) or the identical construct containing a ZBE (ZBE-IL2-LUC). Luciferase reporter constructs were co-transfected with expression vectors pcDNA3.1-HT-p71-ZER6 or pcDNA3.1-HT-p52-ZER6 as shown. Results presented are representative for three or four independent experiments; all transfections were performed in triplicate, and β-galactosidase expression was used to normalize relative light units (RLU). A, COS-1 cells were transfected as indicated. B, COS-1 cells were transfected with expression plasmids for p71-ZER6 and/or p52-ZER6 as indicated. The numbers indicate the nanograms of each expression plasmid transfected. Plasmid DNA was added to adjust for amount of DNA so that all transfections were performed with 2 μg of DNA. The cells were assayed 48 h after transfection.
ERα. Although both isoforms were able to activate transcription through a ZBE, transactivation by p52-ZER6 was repressed in the presence of ERα. Hence, in hormone-responsive cells, regulation of transcription by ZER6 can be controlled by the relative level of expression of the two isoforms.

The Cys2His2 zinc finger is one of the most common motifs found in eukaryotic DNA-binding proteins (24). Zinc finger proteins containing four or more finger domains are usually able to form stable, sequence-specific DNA interactions (25, 26). ZER6 contains six complete Cys2His2 zinc finger domains, and it would be expected that the protein would be able to bind DNA with a core motif of 15–18 nucleotides (27). The amino acids at positions −1, 2, 3, and 6 of the α-helix in each finger are most critical in determining the sequence specificity of the DNA binding motif (25). The residues at −1, 3, and 6 are positioned to make contact with the primary DNA strand, whereas the residue at position 2 contacts the complimentary strand. Neighboring finger domains coordinately interact with the DNA sequence with each finger making contact with 3 nucleotides of the primary DNA strand. This arrangement offers the possibility of designing zinc finger proteins with defined DNA sequence specificity (27–29). Although the data are not yet available to make precise predictions, some amino acid residues have been defined to have preferences for specific nucleotides. In the case of ZER6, the α-helix of fingers F3 through F5 will create a binding pocket with the residues RREHNR at positions 6, −1, 2, 3, 6, and −1. This arrangement of residues at these key locations is very similar to the α-helix structure created by fingers 1–3 of Zif268 with the sequence RRDHTR (25). The glutamic acid residue at position 2 in ZER6 is very similar to aspartic acid residue in Zif268. Based on the similarity in this region, one might expect this region of ZER6 to have DNA specificity similar to that of the analogous region of Zif268. This region of Zif268 binds the core motif 5′-GTGGG, and this sequence motif is indeed found in the ZBE identified by PCR-assisted binding site selection. Although the α-helix structure of the other ZER6 fingers suggests a GC-rich binding site, data to allow a prediction of DNA interactions for the other fingers of ZER6 are incomplete. Combining the structural information of this novel zinc finger protein with the DNA binding specificity will provide additional information to help develop a robust model of zinc finger-DNA interactions.

A search for the ZER6 binding element was performed in the eukaryotic promoter database (www.epd.isb-sib.ch/), which contains promoter sequence information on 1390 eukaryotic promoters from −499 to +100 relative to the gene cap site. The promoter region of three genes (serum albumin, histocompatibility antigen γ, and an early embryonic gene called H19) contained an exact match for the sequence 5′-GGGGTGGGGTGGG. Because there is likely to be some degeneracy in sequence specificity, a search was performed with less stringency with a particular interest in genes known to be expressed in the breast or kidney. The insulin-like growth factor II (IGF-II) gene from human and rat contains multiple copies of sequences closely related to the ZBE, with the closest match being 5′-GGGGTGGGGTGGG-3′. This finding is interesting because tamoxifen has been shown to stimulate expression from the IGF-II promoter (30). If the IGF-II promoter was targeted by ZER-6, it is conceivable that the ERα-ZER6 interaction might be disrupted by tamoxifen thereby releasing active p52-ZER6. The mouse renin-1 gene promoter was found to contain the sequence 5′-CTGGGGTGGGAG-3′. Renin is synthesized in the renal juxtaglomerular apparatus, and these data are consistent with the possibility that renin expression is regulated by ZER6. It is not known if the expression of these other potential ZER6 target genes is altered by estrogen.

A BLASTp analysis of the ZER6 proteins identified a domain in the amino-terminal region, which was partially contained in p52-ZER6, with homology to the KRAB domains (31). The KRAB domains are common features of many zinc finger proteins and are associated with transcriptional regulation. The region of p71-ZER6 that is not contained in the p52 isoform demonstrated a region of striking homology with HUB-1(16). Over a region of 139 amino acids (from amino acid 33 to 171), there is 74% identity and 84% homology with the HUB-1 repressor domain. The HUB-1 gene was cloned based on the ability for the protein to bind to the U5RE of human T-cell lymphotrophic virus type I. HUB-1 is a zinc finger protein with five finger domains that was shown to repress long terminal repeat-mediated transactivation. We hypothesize that the HUB-1 domain is involved in orchestrating protein-protein interactions involved in generating transcriptional complexes. In the case of ZER6, this domain inhibited the ability of ZER6 to interact with ERα, which allowed the p71 isoform to escape repression in hormone responsive cells. To date, HUB-1 and ZER6 are the only two proteins with this motif and as more data are accumulated, this domain may define a subset of KRAB-related zinc finger proteins.

Alternate splicing of the 5′ end of ZER6 transcripts is one mechanism that can regulate expression of the p52 and p71 isoforms. The structure of the majority of transcripts obtained by 5′-RACE using RNA from MCF7 cells would encode the p52 isoform. This conclusion is supported by the fact that several of the clones contained the entire open reading frame and the most amino-terminal methionine residue in those clones correspond to initiation of p52-ZER6. Only 1 of the 14 5′-RACE clones encoded the p71-ZER6 isoform, which indicates that mRNAs with this structure are in the minority. Although transcripts encoding the p71 isoform may arise from alternate splicing, initiation by an alternative promoter is a distinct possibility. An alternate ZER6 promoter transcribing an mRNA that encodes p71-ZER6 would provide an additional mechanism to control the ratio of the p71 and p52 isoforms. Clearly, the two isoforms have different mechanisms controlling ZER6-mediated transactivation. The implications of the transcriptional data derived from reporter assays indicate that the p52 isoform is repressed by ERα. In addition, overexpres-
version of p52 can have a dominant-negative effect that can repress transactivation by the p71 isoform (see Fig. 9D). One potential mechanism for the dominant-negative effect might be competitive binding of the ZBE in target genes. Alternatively, the two isoforms may compete for a limiting co-activator. Identification of ZER6 target genes and mechanisms of regulating activation and repression by the two isoforms will be important areas for further investigation.

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