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Promoter specificity of the two transcriptional activation functions of the human oestrogen receptor in yeast

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ABSTRACT

We have previously demonstrated that the human oestrogen receptor (hER) contains two transcriptional activation functions located in the N-terminal region (TAF-1) and in the hormone binding domain (TAF-2), which can act both independently and synergistically in a promoter- and cell-specific manner in animal cells. We have also demonstrated that hER can activate transcription from chimaeric oestrogen-responsive GAL1 promoters in yeast, and shown that transcriptional activation was due to TAF-1, whereas TAF-2 showed little, if any, transcriptional activity on these promoters. By using a more complex promoter derived from the URA3 gene, we now show that TAF-2 is also active in yeast, and that the activities of TAF-1 and TAF-2 are promoter-context-specific in yeast. We also confirm that the agonistic activity of 4-hydroxytamoxifen (OHT) can be ascribed to the activity of TAF-1.

INTRODUCTION

The oestrogen receptor (ER) is a ligand-inducible transcriptional enhancer factor. It stimulates transcription by interacting with cis-acting elements called oestrogen response elements (EREs) located in the vicinity of target genes. (for reviews see 1–8). Like other members of the nuclear receptor family, the ER contains functionally distinct domains. The most highly conserved region among all receptors is a 66–68 amino-acid-long sequence (core of region C) which contains two zinc fingers and is responsible for specific binding to EREs (9–16 and refs therein). The N-terminal A/B region is variable in length and amino acid composition for different receptors and is moderately conserved between oestrogen receptors from various species. This ER region contains a constitutive transcriptional activation function called TAF-1 (10, 17–20). Region E located in the C-terminal half of the receptors is also highly conserved and corresponds to the hormone binding domain (HBD). A hormone-dependent transcriptional activation function called TAF-2 is present within this region (10, 17, 18, 20–22).

In animal cells TAF-1 and TAF-2 are functionally different from one another and from acidic activating domains (AADs) by a number of criteria, including cell-type and promoter specificity of their activity (10, 17, 19, 23), their homo- and heterosynergizing properties (17), and their transcriptional interference/squelching properties (20). TAF-1 and TAF-2 appear to interact with limiting intermediary factors which mediate their action on the basal transcription machinery (20). Thus TAF-1, TAF-2 and AADs appear to activate transcription in mechanistically different ways.

We have previously shown that hER is able to stimulate transcription in yeast in a hormone-dependent manner as in animal cells (24, 25). The glucocorticoid, progesterone, vitamin D, thyroid hormone and androgen receptors (26–31) have also been shown to function in yeast, and the yeast activator GAL4 stimulates transcription in higher eukaryotes (32,33), indicating a conservation of the molecular mechanisms of transcriptional activation across eukaryotes. Using a truncated hER mutant (HE15) lacking the hormone binding domain, we have shown that TAF-1 was responsible for transcriptional activity of hER in yeast when tested on a chimaeric reporter gene containing an ERE upstream of the TATA box region of the yeast GAL1 gene (25), whereas a truncated hER mutant lacking the A/B region and containing TAF-2 (HEG19) was unable to activate transcription efficiently from this promoter in yeast (19).

To investigate whether TAF-2 is promoter-specific in yeast as it is in animal cells, we have now constructed more complex oestrogen-responsive promoters derived from the yeast URA3 gene, and have used them to test TAF-1 and TAF-2 activities in yeast. We show here that, when tested on chimaeric URA3 promoters, TAF-2 is able to activate transcription efficiently in yeast in a hormone-dependent manner, whereas TAF-1 is weakly active on this promoter, demonstrating that promoter context is important in yeast for the activities of both ER transcriptional activation functions. Furthermore, using the URA3-derived promoters, the non-steroidal antioestrogen 4-hydroxytamoxifen (OHT) exhibits on the whole receptor a low agonistic activity, correlated with the activity of TAF-1, which supports our previous conclusion that the agonistic activity of OHT is due to TAF-1 (19).

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MATERIALS AND METHODS

ER expression vectors and reporter genes

All recombinants were constructed using standard procedures (34). pYE45 is a multicopy yeast expression vector containing the TRP1 selectable marker and a unique EcoRI cloning site downstream of the phosphoglycerate kinase (PGK) gene promoter. It is derived from the previously described vector pTG848 (24) by filling in and religation of the EcoRI site to yield the plasmid pYE2, resulting in loss of the LEU2 gene and the EcoRI sites. The BglII site present downstream of the PGK promoter was converted into an EcoRI site by insertion of the adaptor oligonucleotide

5'-GATCCGAATTCG-3'
3'-GCTTAAGCCTAG-5'

giving pYE4. pYE45 was obtained by exchanging the URA3 marker with the TRP1 marker : the T4 polymerase-treated BglII fragment containing the TRP1 gene isolated from pFL45 (35) was cloned into pYE4 digested by HindIII and treated with T4 polymerase.

HE15F2 (see fig 1) was created by site-directed mutagenesis of HEG0 (36) using the oligonucleotide 5'-GGGCAGGGGTG-AAGTGGGTACCAGCCGTGGAGGGGCAT-3' resulting in deletion of aminoacids 282−552. pYE45 HEG0, pYE45 HEG19 and pYE45 HE15F2 were obtained by inserting the EcoRI fragment containing sequences encoding HEG0 , HEG19 (19) and HE15F2, respectively, into pYE45. pLRΔ20-G1ERE is the derivative vector of pLR1Δ20 described previously (24). The chimaeric URA3 promoters were constructed by replacing the HindIII-PstI fragment of pER7 (37), containing the URA3 promoter region, with a synthetic HindIII-PstI DNA fragment obtained with overlapping oligonucleotides corresponding to the wild type URA3 sequence, where nucleotides −216 to −139 with respect to the first nucleotide of the coding sequence were replaced by the sequence 5'-CCATGGTCACAGTGACC-3' or 5'-CCATGGTCACAGTGACCGGTCACAGTGACCGGTCA-CAGTGACC-3', resulting in pER7URA3-1ERE and pER7URA3-3ERE, respectively. The chimaeric constructs were confirmed to be correct by dideoxysequencing. pLRΔ21-U1ERE and pLRΔ21-U3ERE were constructed by cloning the T4 polymerase-treated SmaI-PstI fragment containing the modified URA3 promoter isolated from pER7URA3-1ERE or pER7URA3-3ERE respectively into the T4 polymerase-treated SmaI-BglII pLRΔ21 vector. pLRΔ21 is a derivative of pLR1Δ20 containing a BglII site between the TATA box region and start sites of the GAL1 promoter, obtained by site directed mutagenesis using the oligonucleotide 5'-TACTTTCAACATTTTAGAT-CTGGTTTGTATTACTT-3'.

Growth of yeast culture and β-galactosidase assay

Yeast strain FL100 ura3-373-251-328, trp1-4, ppr1-Δ1 (37) was transformed as described (24). Cells were grown in 0.67% yeast nitrogen base and 1% glucose. Ligands were added to mid-log cultures 2 hours before harvesting the cells. Cells were harvested, β-galactosidase assay performed and units of β-galactosidase activity calculated as previously described (24).

RESULTS

Construction of a chimaeric, highly inducible, oestrogen reporter gene with low background activity

In order to test if TAF-1 and TAF-2 activities could be promoter-dependent in yeast, we have constructed new lacZ reporter genes expressed from the extensively studied URA3 promoter (37, 38). This promoter normally supports a basal transcription, which is induced t five-fold under uracil starvation (39, 40). Basal URA3 transcription requires a poly(dA)-poly(dT) sequence located from

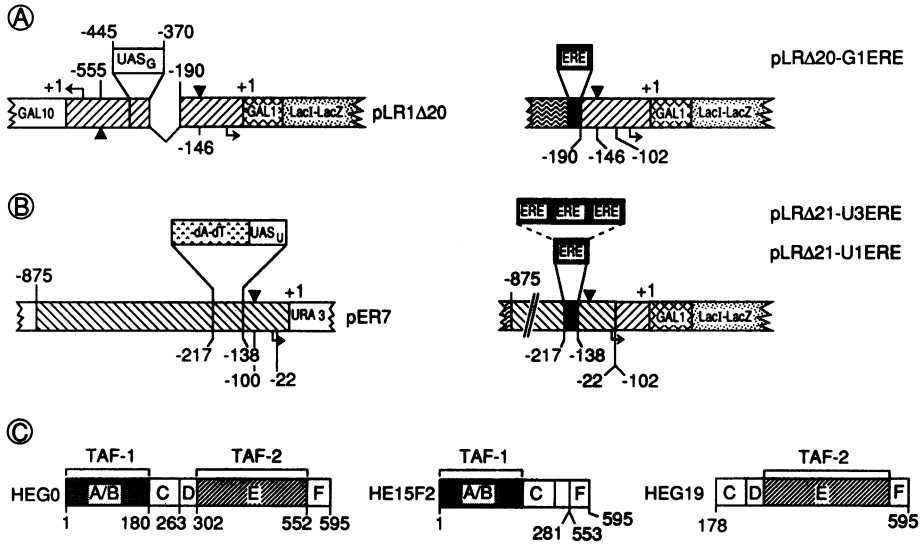

Figure 1. Schematic organisation of promoter regions, reporter vectors and activators. (A) Representation of GAL1-10 and the oestrogen-responsive GAL1 reporter gene promoter region. The GAL1-10 divergent promoter region from pLRΔ20 is shown hatched from right to left. The cross-hatched segment corresponds to the N-terminal GAL1 coding sequence fused to the LacI-LacZ sequence (dotted segment) coding for the β-galactosidase. The numbers refer to nucleotides (+1 corresponds to the first nucleotide of the relevant coding sequence), the arrow refers to the RNA startsite and the triangle to the TATA box. (B) Representation of the URA3 promoter region and oestrogen-responsive URA3 reporter gene promoter regions. The URA3 promoter region in pER7 is cross-hatched from left to right, the other symbols correspond to those in (A). (C) Representation of the hER activators. The regions containing TAF-1 and TAF-2 are indicated. The numbers refer to amino acid number taken from the amino acid sequence of hER (50; see also 36).

position −157 to −217 bp upstream of the coding sequence. Specific induction of URA3 requires an UAS_{URA} from position −138 to −155 bp upstream of the coding sequences (37, and Fig. 1B). URA3-derived reporter promoters were constructed by deleting the poly(dA)-poly(dT) sequence to reduce the constitutive transcription of the gene and replacing the UAS_{URA} with 1 or 3 ERE element(s) to make the reporter gene inducible by the oestrogen receptor. In order to compare these URA3 hybrid promoters with the previously used GAL1 hybrid promoters (17, 19, 24, 25), the chimaeric ERE-GAL1 promoter from pLRΔ20-G1ERE was replaced by the chimaeric ERE-URA3 promoters giving pLRΔ21-U1ERE and pLRΔ21-U3ERE reporter plasmids (Fig.1).

The above reporter genes were introduced into yeast by lithium acetate transformation. In the absence of ER, the β-galactosidase activity expressed from pLRΔ21-U1ERE or pLRΔ21-U3ERE was very low, and difficult to evaluate precisely, but clearly below five units and therefore, at least 24-fold lower than that obtained using pLRΔ20-G1ERE (Table 1). The level of background β-galactosidase expression was not affected by the presence of hER (HEG0) in the absence of hormone, whereas upon addition of oestradiol, both pLRΔ21-U1ERE and pLRΔ21-U3ERE promoters were strongly induced, demonstrating that these chimaeric reporters are oestradiol-responsive. Whereas the induction with pLRΔ20-G1ERE was at most 14 fold, it was more than 15 and 120 fold with pLRΔ21-U1ERE and pLRΔ21-U3ERE, respectively (Table 1; note that since the constitutive activity of pLRΔ21-U1ERE and pLRΔ21-3ERE could not be accurately determined but was certainly much lower than 5 units, the actual stimulations were certainly much higher than 15 and 120-fold). The level of induction on a chimaeric G-AL-1 reporter gene containing three EREs was not more than 4 fold, due to a high background activity with this reporter (data not shown). When compared with the Gal1-derived promoters, the URA3-derived promoters are less active in the presence of oestradiol, but the magnitude of β-galactosidase induction is higher.

Comparison of TAF-1 and TAF-2 activities on different oestrogen responsive reporter genes

TAF-1 activity was tested using the deletion mutant HE15F2 in which the hormone binding domain (region E) has been deleted (Fig. 1C). This mutant was constitutively active, being 50% as active as HEG0 in the presence of E2, on pLRΔ20-G1ERE, in agreement with our previous reports (19, 25). In contrast, the transcriptional activity of HE15F2 when tested on pLRΔ21-U1ERE or pLRΔ21-U3ERE was markedly lower: about 10 and 1.5% of HEG0, respectively (Table I). These results indicate that hER TAF-1 is very active on a GAL1-derived promoter, but much less active on an URA3-derived promoter, and suggest that TAF-2 is mostly responsible for HEG0 transcriptional activity on pLRΔ21-U1ERE and pLRΔ21-U3ERE.

To demonstrate that TAF-2 could function in yeast in the absence of TAF-1, we tested the hER receptor mutant HEG19, created by deleting the A/B region of HEG0 (see Fig. 1C). In the absence of hormone, HEG19 did not modify the basal transcriptional activity of either pLRΔ21-U1ERE or pLRΔ21-U3ERE, but the basal level transcriptional activity of pLRΔ20-G1ERE was decreased (30% reduction, Table 1). In the presence of oestradiol, HEG19 activated transcription from pLRΔ21-U1ERE and pLRΔ21-U3ERE (greater than 5-fold and 20-fold induction, respectively), whereas HEG19 did not activate transcription from pLRΔ20-G1ERE or pLRΔ20-G3ERE above the level observed in the absence of receptor (Table 1, and data not shown). However, the magnitude of HEG19 induction of transcription achieved with pLRΔ21-U1ERE and pLRΔ21-U3ERE was lower than that obtained with HEG0 (approximately 33% and 16%, respectively). All the activators used in this study were expressed at similar levels as determined by Western blot analysis (data not shown) indicating that the above differences in transactivation are not due to differences in protein levels. In addition, HEG19 was 20% as active as

Table 1. Transcriptional activity of HEG0, HE15F2 and HEG19 tested on chimaeric GAL1- and URA3- derived promoter reporters.

Receptor	E2	β-galactosidase activity)units pLRΔ20-G1ERE	pLRΔ21-U1ERE	pLRΔ21-U3ERE
—	−	115	<5	<5
	+	120	<5	<5
HEGO	−	120	<5	<5
	+	1700	75	600
HE15F2	−	750	7	8
	+	850	8	9
HEG19	−	70	<5	<5
	+	100	25	100

The β-galactosidase activity obtained from yeast cultures stably cotransfected with the reporter plasmids pLRΔ20-G1ERE, pLRΔ21-U1ERE or pLRΔ21-U3ERE, and the expression vectors pYE45, pYE45 HEG0, pYE45 HE15F2 or pYE45 HEG19 is given. The cultures were grown in the the absence (−) or presence (E2) of oestradiol (100nM). The results given are the average (+/−20%) of three independent experiments.

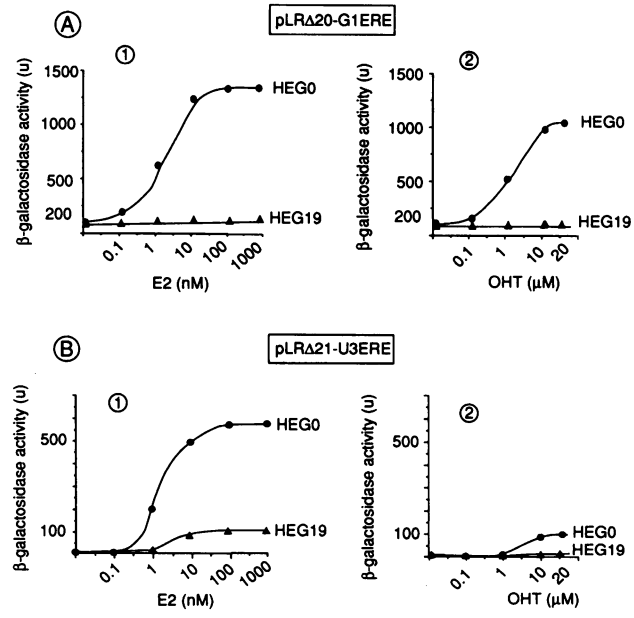

Figure 2. Effect of oestrogen and 4-hydroxytamoxifen on the transcriptional activity of HEG0 and HEG19 tested on different reporter genes. Yeast cultures expressing HEG0 or HEG19 were treated for 2 hours with different concentrations of oestradiol (E2) (A1 and B1) or 4-hydroxytamoxifen (OHT) (A2 and B2). The concentration of ligand is indicated on the X axis (logarithmic scale) and transcriptional activation from pLRΔ20-GIERE (A) or from pLRΔ21-U3ERE (B) is given on the Y axis in units (u) of β-galactosidase activity.

HEG0, and HE15F2 was only very weakly active when expressed from either low or high copy number plasmids and tested on an integrated chimaeric oestrogen responsive URA3 reporter gene (Pierrat et al., submitted). We therefore conclude that TAF-1 and TAF-2 activities are promoter-context-dependent and that the two transcription activation functions can act synergistically in yeast.

The agonistic activity of 4-hydroxytamoxifen on ER is promoter-context-dependent in yeast

We have previously concluded that the agonistic effect of OHT on hER in animal cells can be ascribed to the cell type- and promoter-context-dependent activity of TAF-1 (19). The present availability of a promoter on which TAF-2 was mostly responsible for transcriptional activation in yeast allowed us to further test this conclusion. The transcriptional activity of HEG0 and HEG19 on pLRΔ20-G1ERE and pLRΔ21-U3ERE, respectively, was determined in either the absence or presence of various concentrations of E2 or OHT (note that we used in these experiments the reporter genes which resulted in the highest induction). Maximum transcriptional activation of pLRΔ20-G1ERE and pLRΔ21-U3ERE by HEG0 was achieved with 100 nM E2 or 10 μM OHT. HEG0 was almost as active in the presence of optimal concentrations of OHT as in presence of 10 nM E2, when tested on pLRΔ20-G1ERE. However, when tested on pLRΔ21-U3ERE, HEG0 was less than 20% as active in the presence of OHT as in the presence of E2. As expected in the presence of OHT, HEG19 gave very little, if any, transcriptional activity on the reporter genes used in this study (see figure 2).

DISCUSSION

We have previously demonstrated that hER can stimulate transcription in yeast in a strictly oestrogen-dependent manner from a chimaeric promoter containing an ERE inserted upstream of the GAL1 TATA box region (24). Here we report the construction of other oestrogen-responsive promoters derived from the yeast URA3 promoter, and we show that the activities of the two hER transcriptional activation functions, TAF-1 and TAF-2, are promoter-context-dependent.

Reporter plasmids were constructed from a yeast multicopy plasmid, in which GAL1 and URA3 chimaeric promoters containing one or three EREs were fused to the E. coli LacZ coding sequence. Using yeast cells cotransformed with the expression vector encoding hER and the various reporter plasmids, we found that the addition of oestradiol in the growth medium resulted in a strong increase in the β-galactosidase activity expressed from either the GAL1 or URA3 chimaeric promoters. With the chimaeric URA3 reporter constructs containing three EREs and wild type HEG0 receptor as transactivator, we observe a greater than 135-fold increase in LacZ expression in the presence of 100 nM oestradiol, compared with uninduced conditions, whereas with the chimaeric GAL1 construct we observe at best a 14-fold increase. We have previously shown that using GAL1 chimaeric promoters, a greater induction of transcription activation was observed by RNase mapping, than with the β-galactosidase assay (24). The high β-galactosidase background activity observed with the GAL1 chimaeric construct can most probably be ascribed to transcriptional readthrough. A terminator function has previously been described in the URA3 promoter region (41). This element, which is present in the URA3 chimaeric reporter plasmids, is likely to be reducing readthrough transcription. However, the maximal transcriptional activity achieved with pLRΔ20-G1ERE was three-fold higher than from pLRΔ21-U3ERE. Thus when compared with GAL1, the URA3 constructs display both lower basal and lower maximal transcriptional activities, but the magnitude of oestradiol induction is higher.

The results obtained with yeast cells expressing truncated receptors containing either of the two independent hER transcriptional activation functions, TAF-1 or TAF-2, revealed marked differences depending on the nature of the reporter promoter which was used. The truncated ER containing TAF-1 (HE15F2) stimulated transcription constitutively and efficiently from the chimaeric GAL1 promoter (see also 19). On the other hand, it appeared very inefficient at activating transcription from the chimaeric URA3 promoters. A TAF-2 ER deletion mutant has also been shown to be transcriptionally inactive when tested on a chimaeric oestradiol responsive iso-1-cytochrome c promoter in yeast (42). In contrast to HE15F2, the N-terminally truncated ER (HEG19), containing TAF-2, exerted very little, if any stimulatory activity on the GAL1 promoter, whereas it efficiently induced the URA3 promoters in the presence of hormone. Thus the differential activities of TAF-1 and TAF-2 of hER in yeast can clearly be ascribed to the promoter regions used, since all other parameters were the same in these experiments.

The chimaeric GAL1 and URA3 promoters are different in several ways. The chimaeric oestrogen-responsive GAL1 promoter region corresponds to a 130-nucleotide-long region basically composed of an ERE and a TATA box separated by 45 nucleotides. The identified upstream regulatory elements, UAS_G interacting with the transcription factor GAL4, and URS_G responsible for glucose repression, have been deleted (43, 44 and figure 1A). The chimaeric oestrogen-responsive URA3 promoter region contains an ERE located 38 oligonucleotides upstream of the TATA box, and the poly dA-dT stretch responsible for constitutive transcription, as well as UAS_{URA} responsible for the uracil-controlled activation, have been deleted. The other promoter sequences and particularly the GA-BF binding sites surrounding the TATA box region (37) remain, whereas no such sequences are present in the GAL1 chimaeric construct. Therefore the URA3 chimaeric constructs are closer to the complex promoters, and the GAL1 chimaeric constructs to the minimal promoters, which have been used in animal cells (17, 19, 20). Thus TAF-2, which has very little, if any, transcriptional activity in yeast on a 'simple' promoter, but is active on a 'complex' promoter, may synergize with additional factors which interact with the URA3 promoter region, but not with the simpler GAL1 promoter region. Furthermore, since HEG19 and HE15F2 are only 33% and 10% as active as HEG0 respectively, on a URA3-derived promoter containing one ERE, it appears that TAF-1 and TAF-2 can synergize in yeast. These results are reminiscent of those obtained in animal cells, which showed that the activity of TAF-2 is strongly modulated by upstream promoter elements, and that TAF-1 and TAF-2 can synergize (17). However, whereas TAF-1 showed no strong promoter specificity in animal cells [HE15 was 50 to 100% as active as HEG0 when tested on different promoters in chicken embryo fibroblasts (17)], it was more active in yeast on the GAL1 than on the URA3 chimaeric constructs. This suggests that either TAF-1 synergizes with some uncharacterized factors interacting with the GAL1 promoter region, but not with the URA3 promoter region, or alternatively, that factors like GA-BF

bound to the chimaeric URA3 promoter region interfere with TAF-1 function.

The antioestrogen OHT allows hER to bind DNA, and its agonistic activity correlates with the cell-type and promoter-context-dependent activity of TAF-1 (19). As expected, since TAF-1 is highly active on chimaeric oestrogen-dependent GAL-1 promoters, and poorly active on chimaeric oestrogen-dependent URA3 promoters in yeast, OHT is an almost total agonist in the first case, and a very weak agonist in the second case. The apparently paradoxical higher transcriptional activity of hER in the presence of OHT, when compared to HE15F2 when tested on either pLRΔ20-G1ERE or pLRΔ21-U3ERE (see table 1 and Fig.2; note that as expected the presence of OHT did not affect the activity of HE15F2-data not shown) most probably results from the known higher affinity of HEG0 than HE15F2 for EREs, due to the presence of a strong dimerisation function in the ligand binding domain (45, 46). The residual activity of HEG0 on pLRΔ21-U3ERE in the presence of 4-hydroxytamoxifen (fig 2B) may indicate that TAF-1 has nevertheless some activity on URA3-derived reporters, or alternatively, but less likely, that TAF-2 activity is not fully blocked by 4-hydroxytamoxifen.

The fact that both TAF-1 and TAF-2 can work independently and synergistically in yeast as in animal cells, as well as the promoter specificity of the two transcriptional activation functions in yeast is further evidence that TAF-1 and TAF-2 are functionally different. We have previously proposed that different classes of activators may interact with the basic transcriptional machinery through transcriptional intermediary factors (TIFs; see 20). Evidence supporting the requirement of TIFs for transcriptional stimulation by the acidic activating activator GAL-VP16 in yeast and HeLa extracts has been provided (47–49). Our results suggest that TIFs for TAF-1 and TAF-2 have been conserved from yeast to man. The powerful genetic techniques available in yeast offer the opportunity to analyse further the mechanism of action of hER TAF-1 and TAF-2 in this simple eukaryotic organism.

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