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Regulation of human estrogen receptor α-mediated gene transactivation in *Saccharomyces cerevisiae* by human coactivator and corepressor proteins

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Abstract

Human estrogen receptor α (ERα)-mediated transcription activation was evaluated in the yeast *Saccharomyces cerevisiae* using both the native ERα and a G400V variant. A previous study [1] demonstrated that coexpression of human SRC-1, a potent stimulator of ERα function in mammalian cells, potentiated ERα-mediated gene expression in yeast over five-fold in an E_2-dependent manner. In the present study, two additional human coactivator proteins were shown to potentiate ERα-mediated gene expression in yeast. SRC2 potentiated transactivation two- to three-fold while SRC3 potentiated transactivation five- to eight-fold. Both human coactivators potentiated both the native ERα and the G400V variant in an E_2-dependent manner. The effect of a human corepressor protein was also evaluated in yeast. Repressor of estrogen receptor activity (REA) did not affect E_2-induced transactivation by ERα (either isoform). However, in a strain that coexpressed human SRC1, REA reduced E_2-induced transactivation to that observed with ERα alone. Furthermore, repression of SRC1 potentiation was specific for the native ERα since REA had no effect on SRC1 potentiation of the G400V variant. Additionally, REA repression was specific for SRC1 since potentiation of ERα (either isoform) transactivation by SRC2 and SRC3 was unaffected by coexpression of REA. These results support previous observations in mammalian cells that REA does not prevent ERα from binding to DNA but does inhibit potentiation of ERα-mediated transactivation by SRC1. The results in the present study further characterize REA-mediated repression, and demonstrate the utility of this yeast system for dissecting molecular mechanisms involved in regulating gene transactivation by human ERα.

Keywords: Estrogen receptor α; Coactivator; Corepressor; Yeast surrogate host

1. Introduction

The yeast *Saccharomyces cerevisiae* has proven to be a useful surrogate host for the analysis of human protein function. Many molecular mechanisms have been conserved between yeast and humans (reviewed in [2]), but this simple eukaryote has a much smaller genome (less than 1% that of humans) and the number of expressed proteins is less than 20% that of humans. It was originally demonstrated that human gluccocorticoid [3] and estrogen [4] receptors function in *S. cerevisiae* in a hormone-dependent manner. Subsequently, a number of other human steroid hormone receptors (SHR) have been expressed in yeast and shown to transactivate reporter genes containing the cognate hormone response DNA element in a ligand-dependent manner (reviewed in [5]). Studying human SHR function in *S. cerevisiae* has the advantage that yeast is a null host for nuclear receptors. That is, the yeast genome does not encode homologues of mammalian steroid receptors or other nuclear receptors. Furthermore, yeast do not have genes that encode homologues of the mammalian p160 family of nuclear receptor coactivators. Studies of SHR function in mammalian cells are complicated by the presence of endogenous cofactors, both coactivators and corepressors, which may vary between mammalian cell types. In contrast, by utilizing *S. cerevisiae* as a surrogate host, steroid receptor function can be combinatorially analyzed in a defined, minimal cell system reconstructed with a specific composition of human pro-

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teins. In addition to yeast RNA polymerase II and the basal transcription factors, other yeast proteins that participate in human steroid receptor transactivation include HSP90 [6], SWI1, SWI2, SWI3 [7], SUG1 [8], PSU1 [9] ADA complex and GCN5 [10,11]. Native yeast proteins that may potentiate human steroid receptor coactivation have been reported based on genetic screens. These include SPT6 [12] and RSP5 [13].

Estrogen receptor α (ERα) plays an important role in normal development, organ and bone homeostasis, and the neoplastic progression of some cancers. ERα-mediated gene transactivation involves the binding of estradiol (E_2) to the receptor, receptor dimerization and subsequent localization and binding to the estrogen response element (ERE) in the promoter of estrogen-responsive genes (reviewed in [14]). Upon binding to DNA, gene transcription is stimulated by activation domains (AF-1 and AF-2) on the surface of the ERα polypeptide. The level of gene expression may be further regulated by interactions with additional regulatory proteins. Maximal ERα-mediated gene expression depends on coactivators, which associate with E_2-bound ERα and enhance transcription by interacting with basal transcription factors and/or altering chromatin structure around estrogen-responsive genes (reviewed in [15–17]).

Steroid receptor coactivator-1 (SRC-1) [18,19] was the first identified member of the p160 family of coactivators, which includes SRC-2 (originally named TIF2 [20]; the human homologue of mouse GRIP1 [21]), and SRC-3 (originally named AIB1 [22]; the human homologue of mouse p/CIP [23]). These structurally related coactivators contain multiple leucine-rich motifs (LXXLL) that mediate the interaction between coactivator and nuclear receptor [24]. The p160 family of coactivators potentiate transactivation of multiple types of nuclear receptors, including ERα. Although SRC-1 expression does not appear to be critical for normal development and fertility [25], ERα:SRC-1 interactions have been implicated in mediating tissue-specific responses to certain physical stimuli and synthetic ERα ligands [25,26].

In a previous study from this laboratory, hybrid promoters that contain an ERE were constructed and demonstrated to be activated by human ERα in a ligand-dependent manner in yeast [1]. Those studies demonstrated the following: (1) the level of E_2-induced transactivation by ERα is dependent on both the ERE sequence and its position relative to the transcription start site; (2) the yeast system is able to distinguish isoforms of human ERα that have different E_2 responsiveness; (3) transactivation by ERα in yeast is potentiated by the human coactivator protein, SRC1; (4) variants of human SRC1 have similarly altered activities in yeast as in mammalian cells; (5) hormone responsiveness and coactivator dependence in yeast are qualitatively similar to that observed in mammalian cells.

In the present study, the response of human ERα to additional human coregulator proteins expressed in yeast was evaluated. Both SRC2 and SRC3 potentiate E_2-induced ERα transactivation. It is also demonstrated, for the first time, that human corepressor proteins function in yeast. The results obtained in these analyses demonstrate the utility of *S. cerevisiae* as a surrogate host for dissecting the molecular mechanisms of human ERα transactivation and regulation.

2. Materials and methods

2.1. Expression vectors and genes

The reporter plasmid pGP381(ERE3)/Z was described previously [1]. Vectors for expressing native human ERα (ER-G; pBT26/ER-G), the ERα G400V variant (ER-V; pBT26/ER-V) and the human coactivator protein SRC1 (pMETc/SRC1a) were also described previously [1].

cDNAs for human SRC2 (GenBank accession # NM_006540) and SRC3 (GenBank accession # NM_006534) were obtained from OriGene Technologies (Catologue #'s FB2095_C12, PR1338_H04). The entire SRC2 coding sequence (55 bp 5′ to ATG intiation codon to 61 bp 3′ of termination codon) was isolated from FB2095_C12 as a *BglII* fragment and subcloned into pMETc (p416MET25; [27]) to generate pMETc/SRC2. PR1338_H04 was digested with *BamH1* and *SacI* to release codon 9 of SRC3 through 114 bp 3′ of the termination codon and subcloned into pMETc. The 5′ end of the SRC3 coding sequence was reconstructed by cloning the synthetic oligonucleotide

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5'-GATCCAAGATGAGTGGATTAGGAGAAAACTTA
    GTTCTACTCACCTAATCCTCTTTTGAATCTAG-5'
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into the *BamH1* site of the intermediate vector and screening for a clone with the synthetic duplex in the correct orientation to generate pMETc/SRC3. These expression vectors were transformed into strain YPH500 containing pGP381(ERE3)/Z and expressing either ER-V or ER-G.

A full length cDNA of REA [28] (repressor of estrogen receptor activity, GenBank accession #NM_007273) was purchased from Stratagene (Cat. # C50835). The entire coding sequence was PCR amplified with the primers.

5′-CGCACTAGTGCCGGCCTCAAGATCAG-3′ and 5′-GCGCCTCGAGGGTGGAGTTCTTGGTGACTAG-3′. After digestion with *SpeI* and *XhoI*, the REA cDNA was subcloned into pBT6 [29] to generate pBT6/REA. Both pBT6/REA and pBT6 were introduced into strain YPH500 harboring pGP(ERE3)/Z and various combinations of other expression vectors (Section 3), selecting for uracil protrophs (*URA3* gene on pBT6).

All expression vectors used in this study contain the centromeric sequences CEN3/ARS1 (pGP381, pGP171 and pBT26) or CEN6/ARS4 (pMETc) and are therefore stably maintained at 1–2 copies per cell.