Rho GTPases as Modulators of the Estrogen Receptor Transcriptional Response*

Laura F. Su‡, Roland Knoblauch§, and Michael J. Garabedian¶
From the Department of Microbiology and the Kaplan Comprehensive Cancer Center, New York University School of Medicine, New York, New York 10016

The estrogen receptor α (ER) is a ligand-dependent transcription factor that plays a critical role in the development and progression of breast cancer, in part, by regulating target genes involved in cellular proliferation. To identify novel components that affect the ER transcriptional response, we performed a genetic screen in yeast and identified RDI1, a Rho guanine nucleotide dissociation inhibitor (Rho GDI), as a positive regulator of ER transactivation. Overexpression of the human homologue of RDI1, Rho GDIα, increases ERα, ERβ, androgen receptor, and glucocorticoid receptor transcriptional activation in mammalian cells but not activation by the unrelated transcription factors serum response factor and Sp1. In contrast, expression of constitutively active forms of RhoA, Rac1, and Cdc42 decrease ER transcriptional activity, suggesting that Rho GDI increases ER transactivation by antagonizing Rho function. Inhibition of RhoA by expression of either the Clostridium botulinum C3 transferase or a dominant negative RhoA resulted in enhanced ER transcriptional activation, thus phenocopying the effect of Rho GDI expression on ER transactivation. Together, these findings establish the Rho GTPases as important modulators of ER transcriptional activation. Since Rho GTPases regulate actin polymerization, our findings suggest a link between the major regulators of cellular architecture and steroid receptor transcriptional response.

The estrogen receptor α (ER)1 is a ligand-dependent transcription factor that transduces the estrogen signal (1). Activation of ER is responsible for female sexual development and maintenance of bone density (2, 3). In addition, ER plays a critical role in the development and progression of breast cancer by regulating genes and signaling pathways involved in cellular proliferation (4). Regulation of gene expression by the ER requires the coordinate activity of ligand binding, phosphorylation, and cofactor interactions, with particular combinations probably resulting in the tissue-specific responses elicited by the receptor (5–7). However, the extracellular cues and intracellular signaling pathways modulating these components and regulating ER transcriptional activation are not fully understood.

To identify novel proteins that modulate ER transcriptional activation, we have carried out a genetic screen in the yeast Saccharomyces cerevisiae. The ability of the human ER to function within yeast allows a wide variety of genetic approaches to be taken toward further defining the mechanism of ER transcriptional activation given the ease of genetic manipulation and simplicity of gene identification in yeast. In addition, with the large number of orthologous proteins carrying out the same biological functions in both S. cerevisiae and metazoans (8–10), it is likely that the yeast factors affecting ER transactivation will have mammalian counterparts, which can be examined in vertebrate systems.

The genetic approach we have used to identify factors that affect ER transcriptional activation is dosage suppression analysis. In this technique, a mutant ER protein with a reduced ability to activate transcription is used as a substrate to isolate yeast gene product(s) that are capable of overcoming this mutant phenotype, thus restoring receptor transcriptional activity. The mutant ER derivative used in this screen is defective in transcriptional activation, thus phenocopying the effect of Rho GDI expression on ER transactivation. Together, these findings establish the Rho GTPases as important modulators of ER transcriptional activation. Since Rho GTPases regulate actin polymerization, our findings suggest a link between the major regulators of cellular architecture and steroid receptor transcriptional response.

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§ Supported by Army Breast Cancer Research Fund Predoctoral Grant DAMD17-98-8134 and NIH Grant T32 GM07308.
¶ To whom correspondence should be addressed: Dept. of Microbiology, NYU School of Medicine, 550 First Ave., New York, NY 10016. Tel.: 212-263-7662; Fax: 212-263-8276; E-mail: garabm01@med.nyu.edu.
1 The abbreviations used are: ER or ERα, estrogen receptor α; ERβ, estrogen receptor β; GDI, guanine nucleotide dissociation inhibitor; WT, wild type; GEF, guanine nucleotide exchange factor; GAP, GTPase-activating protein; GR, glucocorticoid receptor; AR, androgen receptor; SRF, serum response factor; ERE, estrogen response element; X-gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside; RLU, relative luminescence units.

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cellular transformation and metastasis (17–22). The Rho family members possess intrinsic GTPase activity and cycle between the inactive cytoplasmic GDP-bound and the active membrane-associated GTP-bound state. The exchange of GDP for GTP induces a conformational change in the G protein that allows effector molecules, such as protein kinases, to bind and initiate downstream signaling events (23). This GTP/GDP cycle is tightly regulated in response to extracellular signals by three different classes of proteins. Guanine nucleotide exchange factors (GEFs) catalyze the exchange of GDP for GTP, GTPase-activating proteins (GAPs) accelerate the intrinsic GTPase activity of the Rho GTPases, and GDIs antagonize their activity by blocking GEFs and GAPs (12, 24). However, since the cytoplasmic GDP-bound Rho GTPases predominate under physiological conditions, Rho GDI acts as a negative regulator of Rho GTPases mainly by blocking the dissociation of GDP. In addition, Rho GDI controls the subcellular localization of the GTPases, stimulating their release from the plasma membrane (12, 25). Interestingly, Rubino et al. (26) identified an ER-interacting protein, termed Brx, which contains a domain virtually identical to the Rho GEF Lbc and was first to demonstrate a link between ER signaling and the Rho GTPases.

In this report, we extend the findings of Rubino et al. (26) and examine the effects of human Rho GDIα as well as the Rho GTPases, RhoA, Rac1, Cdc42, on transcriptional activation by ER and other members of the steroid receptor family in mammalian cells. Our findings indicate that Rho GDIα specifically increases the transcriptional activity of ERα and ERβ as well as the glucocorticoid receptor (GR) and androgen receptor (AR), but not of the unrelated transcription factors serum response factor (SRF) and Sp1, and that this activation is mediated via repression of Rho GTPases. These results further establish the Rho-mediated signaling pathway as an important regulator of ER, GR, and AR transcriptional activity.

**EXPERIMENTAL PROCEDURES**

**Plasmids**

**Yeast—**The reporter plasmid ERE-CYC1-LacZ contains a single estrogen response element (ERE) upstream of a truncated CYC1 promoter linked to the β-galactosidase gene (27). The yeast high copy genomic library was described by Engebrecht et al. (28) and was generated by subcloning Saus3a partially digested yeast genomic DNA into the BamHI site of the YEP351 plasmid. WT ER and ERAAA were expressed from the Gal1–10 promoter in Trp1, 2 μm plasmid (p2T-GAL) (29). p2T-GAL-EREAAA was constructed by subcloning the BamHI fragment containing EREAAA sequence from pcDNA3 (30) plasmid into p2T-GAL (29).

**Mammalian Cells—**The reporter plasmid contains one ERE from the Xenopus vitellogenin A2 gene, upstream of the herpes simplex virus thymidine kinase promoter (–109) linked to the firefly luciferase coding sequence (XETL) (31). The GR reporter plasmid (XG46TL) is identical to XETL activity in the presence of ER. Since MCF-7 cells contain endogenous ER, transcriptional activity of the receptor is normalized to XETL activity in the presence of ER antagonist ICI 182,780. The data presented represent the average of two experimental values, with error bars representing the range of the data points.

**Immunoblotting**

To prepare protein extracts from transfected cells, whole cell extracts prepared for luciferase assay in 1× reporter lysis buffer were normalized for total protein and boiled for 3 min in SDS sample buffer. Protein extracts were fractionated by 12% SDS-polyacrylamide gel electrophoresis, transferred to Immobilon membrane (Millipore Corp.), and probed with anti-ERα polyclonal antibody (HC-20; Santa Cruz Biotechnology), anti-ERβ polyclonal antibody (A-20; Santa Cruz Biotechnology), or anti-c-Myc monoclonal antibody (9E10; Santa Cruz Biotechnology). The blots were developed using horseradish peroxidase-coupled sheep anti-mouse or goat anti-rabbit antibodies and the ECL substrate as per the manufacturer’s instructions (Amersham Pharmacia Biotech).

**RESULTS**

**A Genetic Screen for Activators of ERα Transcriptional Enhancement—**Concomitant serine to alanine mutations at N-terminal phosphorylation sites 104, 106, and 118 (ERAAAA) result in a ~50% reduction in ER transcriptional activity in mammalian cells (42, 43). To determine whether the transcriptional activity of ERAAAA is also reduced in yeast, strains were constructed containing a galactose-inducible expression vector encoding either WT ER or ERAAAA and an ER-responsive reporter plasmid. The transcriptional activities of WT ER and ERAAAA were measured as a function of hormone concentration. Compared with WT ER, ERAAAA exhibited ~40% reduction of...
that the approach was sound and that the library was probably together with the repeated isolation of certain genes indicates modulators of Rho GTPases. The recovery of known ER regulators is interesting to note that GAP and RDI1 are both negative regulators changing the ER AAA-expressing yeast from white to blue as a function of 17β-estradiol concentration. Yeast strains were transformed with either a galactose-inducible WT ER or ER AAA along with an ERE-containing β-galactosidase reporter plasmid. Transcriptional activation by the WT ER (dotted line) and ER AAA (solid line) in response to increasing 17β-estradiol concentration was determined by liquid β-galactosidase assay as described under “Experimental Procedures.” Note that the ER AAA in yeast exhibits ~40% of the WT ER transcriptional activity at each estradiol concentration tested. The dosage suppression screen was carried out in the presence of 1×10−10 M 17β-estradiol, conditions under which the ER AAA phenotype is the most pronounced. B, the relative activity of WT ER, ER AAA, and ER AAA with an ER activator. Three independent colonies on X-gal indicator plates in the presence of 1×10−10 M 17β-estradiol are shown and represent WT ER with an empty expression vector (WT ER), ER AAA plus an empty expression vector (ER (AAA)), and ER AAA plus the RDI1 suppressor plasmid (ER (AAA) + ER activator). Under these conditions, colonies expressing WT ER are blue, ER AAA-expressing colonies appear white, and ER AAA-expressing the ER activator RDI1 are blue.

transcriptional activity at all hormone concentrations tested, suggesting that the ER AAA is less efficient at engaging in the interactions necessary for transcriptional activation (Fig. 1A).

The ER AAA phenotype is most striking at 0.1 nM 17β-estradiol. Under these conditions, yeast colonies expressing WT ER are blue, while ER AAA-expressing colonies appear white (Fig. 1B). To screen for ER activators, yeasts expressing ER AAA, along with an estrogen-responsive reporter gene, were transformed with a high copy yeast genomic library and assayed for receptor transcriptional activation on X-gal indicator plates containing 0.1 nM 17β-estradiol. Candidate high copy suppressors changing the ER AAA-expressing yeast from white to blue were selected for further analysis (Fig. 1B). Of the 29,000 colonies screened, which represents approximately 3 times the size of the yeast genome, we identified six yeast open reading frames that enhance ER transcriptional activation (Table 1).

A search of the yeast genome data base revealed that two of the candidate suppressors were yeast homologues of mammalian proteins previously shown to affect ER transactivation. These include 1) CKA1 (44), a homologue of the mammalian α-subunit of casein kinase II that phosphorylates ER at serine 167 in vitro (45) and 2) CAD1 (46), a member of the Jun transcription factor family that synergizes with ER in mammalian cells (47). In addition to genes known to regulate ER activity, several genes not known to affect ER were identified. YAK1 (48), a serine/threonine kinase with homology to ANPK, a protein kinase that interacts with the zinc finger region of the AR and increases AR-dependent transcriptional activation, was isolated once (49). In addition, MCK1 (50), a protein kinase with homology to glycogen synthase kinase-3 (51), was identified once. We also isolated RDI1 (14), the yeast Rho guanine nucleotide dissociation inhibitor (Rho GDI), three times, and LRG1 (52), a yeast protein that contains a GTPase-activating protein (GAP) homology domain, once. Although LRG1 is presently linked to GAP merely through sequence homology, it is interesting to note that GAP and RDI1 are both negative regulators of Rho GTPases. The recovery of known ER regulators together with the repeated isolation of certain genes indicates that the approach was sound and that the library was probably screened to saturation. Since Rho GDI negatively regulates Rho GTPases, this result suggests that the Rho GTPases may modulate ER transcriptional activation and is the focus of this report.

Rho GDI Expression Increases ER Transactivation—Among the human Rho GDIs, RDI1 is most similar to human Rho GDIα, which negatively regulates the best studied Rho GTPases, RhoA, Rac1, and Cdc42. To examine whether the mammalian Rho GDI affects ER transcription in mammalian cells, we tested the ability of human Rho GDIα to enhance ER transcriptional activity in the human osteosarcoma cell line U2OS. ER-negative U2OS cells were transiently transfected with ERα, an ER-responsive reporter plasmid, along with increasing amounts of Rho GDIα. As shown in Fig. 2A, Rho GDIα stimulates ER transactivation in a dose-dependent manner. Enhancement of ER transcriptional activation by Rho GDIα was also observed for ER AAA mutant (not shown). To ensure that this enhanced transcriptional activity was not a result of increased ER protein production, we monitored protein expression in whole cell extracts using Western blot analysis. Fig. 2B illustrates that ER levels are not increased by Rho GDI expression. Indeed, the steady state concentration of ER decreased slightly with increasing Rho GDI expression, indicating that the effect of Rho GDIα on ER activity is greater than that observed. The effect of Rho GDI on ER transactivation is not restricted to single cell type, since Rho GDIα also enhanced ER transactivation in MCF-7 and Ishikawa cells (Fig. 2C). Thus, Rho GDIα can act as a positive regulator of ER-dependent transcriptional activation in a variety of mammalian cell lines.

Rho GDI Specifically Activates Steroid Hormone Receptors—We next tested the ability of Rho GDIα to affect transactivation by other members of the steroid receptor family, ERβ, GR, and AR, using transient transfection assays. Our results indicate that Rho GDIα also increased the transcriptional activity of ERβ, GR, and AR in a dose-dependent manner (Fig. 3, A–C). To determine whether Rho GDI-mediated activation is specific to steroid receptors, we tested the effect of Rho GDIα on Sp1- and SRF-dependent transactivation. Rho GTPase signaling has been previously shown to enhance transcriptional activation by SRF (18); thus, we would expect Rho GDIα, as a negative regulator of Rho GTPases, to decrease SRF transcriptional activity. Consistent with this idea, Rho GDIα expression decreased SRF activity from a reporter plasmid containing the c-Fos SRF element (Fig. 4A). Similarly, Sp1 transcriptional activity using an Sp1-responsive reporter also decreased in response to Rho GDI overexpression (Fig. 4B). Taken together, these results strongly suggest that Rho GDI specifically increases transactivation by steroid hormone receptors, perhaps through a mechanism involving suppression of Rho GTPase signaling.

Rho GTPases Inhibit ER Transactivation—The GTPases known to interact with Rho GDIα include RhoA, Rac1, and Cdc42. To determine whether Rho GDI increases ER transactivation by inhibiting the Rho GTPases, we expressed constitutively active forms of Rho GTPases (RhoA.V14, Rac1.L61, and Cdc42.L61) in U2OS cells and examined ER transcriptional activation. As shown in Fig. 5, expression of RhoA.V14, Rac1.L61, and Cdc42.L61 decreased ER transcriptional enhancement. Active forms of Rho GTPases also decreased ER transactivation in MCF-7 and Ishikawa cells (Fig. 6). In all three cell types, expression of the constitutively active forms of RhoA, Rac1, and Cdc42 resulted in an accumulation of filamentous actin, as determined by fluorescent phallolidin staining.3 These results are consistent with the model that Rho GDI

3 L. F. Su and M. J. Garabedian, unpublished observations.
activates ER transcriptional enhancement by antagonizing Rho GTPases.

As an independent means of examining the effect of RhoA inhibition on ER transcriptional activation, we ectopically expressed the Clostridium botulinum C3 transferase, a protein toxin that ADP-ribosylates and inhibits RhoA but not Rac1 or Cdc42 (37, 38). As with Rho GDI, expression of C3 transferase results in an enhancement of ER transcriptional activity but decreases SRF transcriptional activation in U2OS cells (Fig. 7A and data not shown). Inhibition of ER transcriptional activation by activated RhoA, but not Rac1 or Cdc42, was also relieved by C3 coexpression (not shown). Ectopic expression of C3

| Gene        | Function                        | Mammalian homologue          | Effect on ER                      | Times identified |
|-------------|---------------------------------|-------------------------------|-----------------------------------|------------------|
| CKA1        | α subunit of casein kinase II    | Casein kinase II              | Phosphorylates ER S167 in vitro   | 1                |
| CAD1        | Yeast Jun family                | c-Jun                         | Potentiates ER transactivation    | 1                |
| YAK1        | Ser/Thr kinase                  | ANPK                          | Enhances ER transactivation       | 1                |
| MCK1        | Ser/Thr/Tyr kinase              | GSK-3                         | Enhances ER transactivation       | 1                |
| RDI1        | Rho GDP dissociation inhibitor   | Rho GDIα                       | Enhances ER transactivation       | 3                |
| LRG1        | Contains LIM domains similar to Rho GTPase-activating proteins | Unknown                       | Enhances ER transactivation       | 1                |

Yeast genes that enhance ER transactivation in U2OS cells

At the top are shown yeast homologues of two previously known mammalian regulators that affect ER function. At the bottom are four factors that appear to link ER transcriptional activation to signal transduction pathways previously not known to affect ER function.

FIG. 2. Enhancement of ERα transcriptional activation by overexpression of Rho GDIα. A, ER-deficient U2OS cells (1.2 \times 10^5 cells/35-mm dish) were transiently transfected using LipofectAMINE Plus reagent with 0.1 μg of ERα expression construct or empty vector, 0.2 μg of the ERE-containing reporter gene XETL, and increasing amounts of Rho GDIα, as indicated. 12 h after the transfection, cells were treated with 100 nM 17β-estradiol (E2) (dark bars) or the ethanol vehicle (light bars) for 24 h, harvested, and assayed for luciferase activity. ERα transcriptional activity is normalized to XETL reporter activity in the absence of ER. The data represent the mean of an experiment done in duplicate, which was repeated three times. B, expression of ERα does not increase by Rho GDIα coexpression. Whole cell extracts were prepared from transfected cells as described under “Experimental Procedures,” and the expression of ERα and Rho GDIα was analyzed by Western blotting. C, MCF-7 and Ishikawa cells were transfected as above and assayed for luciferase activity. For Ishikawa cells, ERα transcriptional activity is normalized to XETL reporter activity in the absence of ER. The data represents the mean of experiments done in duplicate, which were repeated two times.

FIG. 3. Rho GDIα enhances the transcriptional activation by ERβ, GR, and AR. U2OS cells were transfected as described in Fig. 2 with paired expression and reporter plasmids for ERβ + XETL (A), GR + XG46TL (B), or AR + XG46TL (C) and, along with the indicated amount of Rho GDIα, were treated with 100 nM 17β-estradiol (E2), dexamethasone (Dex), and R1881, respectively, and harvested. In each case, receptor transcriptional activity shown is normalized to reporter activity in the absence of the receptor. The data shown represent experiments done in duplicate that have been repeated at least twice with similar results.

FIG. 4. Rho GDI inhibits transcriptional activation by SRF and Sp1. U2OS cells were transfected as in Fig. 2 with 0.4 μg of Rho GDIα together with 0.2 μg of SRF reporter (A) or Sp1 reporter (B), harvested after 24 h, and assayed for luciferase activity. Results shown represent an experiment done in duplicate and repeated twice.
We have demonstrated that Rho GDIα enhances the transcriptional activity of the ERα as well as ERβ, GR, and AR but not SRF or Sp1. We also showed that activated mutant forms of RhoA, Rac1, and Cdc42 decrease, whereas inhibition of endogenous RhoA by C3 transferase or dominant negative RhoA increases ER transcriptional activation. From these results, we conclude that the enhanced ER transactivation observed upon Rho GDIα overexpression is mediated by antagonism of Rho GTPases and implicates the Rho family proteins RhoA, Rac1, and Cdc42 in signaling to ER.

What is the mechanism underlying the modulation of ER transactivation by RhoA? Since Rho GTPases mediate actin cytoskeleton reorganization as well as the activation of multiple signaling pathways, such as c-Jun N-terminal kinase (JNK) and p38, Rho-mediated inhibition of ER may result from either of these events. The ability of the Rho GTPase family members to repress ER transcriptional activity suggests that RhoA-, Rac1-, and Cdc42-mediated signaling to ER may converge at some common point through a shared signaling molecule. An attractive candidate for such a common regulator is LIM kinase (53). The GTP-bound forms of RhoA and Rac1/Cdc42 activate LIM kinase via phosphorylation through effector kinases ROCK and Pak, respectively (54, 55). The activated LIM kinase phosphorylates cofolin, an actin-binding protein, thereby inhibiting its actin-depolymerizing activity and leading to the accumulation of actin filaments. Recently, it has been shown that changes in the actin cytoskeleton can affect transcriptional activation by SRF (56). In a model reminiscent of that proposed for regulation of SRF by actin (56), we speculate that suppression of ER transactivation could result either from releasing an ER corepressor that is associated with free G-actin or from binding a coactivator to actin filaments, thus preventing its interaction with the ER. One such putative actin-regulated factor is the SWI/SNF complex, which has previously been shown to be a coactivator for steroid receptors, including ER, in both yeast and mammalian cells (57–59) and contains β-actin as well as two actin-related protein subunits (60–62). We are currently testing whether SWI/SNF and/or LIM kinase mediate the modulatory effects of Rho GTPases on ER transactivation. While the effect of actin cytoskeletal changes on ER remains unknown, actin dynamics may provide a means of modulating ER transcriptional activity during normal development or in pathological settings, such as tumor progression, when cells undergo extensive actin reorganization.

Alternatively, changes in ER transcriptional regulatory properties may result from the activation of signal transduction pathways by Rho GTPases. For example, Rac1/Cdc42 activate JNK and p38 mitogen-activated protein kinase pathways, which may affect ER or its coregulatory factors via phosphorylation. Unlike Rac1/Cdc42, RhoA is not thought to activate the JNK and p38 pathways; therefore, it is unlikely that the activated Rho GTPases are effecting ER transactivation via these pathways. Nevertheless, since we have not excluded the possibility that JNK and p38 are mediating the effect of the Rho GTPases on ER transcriptional activation, we are currently testing whether SWI/SNF and/or LIM kinase mediate these pathways. We are currently testing the impact of activation and inhibition of JNK and p38 on receptor transactivation.

A cellular activity induced by activated RhoA, Rac1, and Cdc42 is NF-κB, which has been shown to inhibit steroid receptor transactivation by forming inhibitory heterocomplexes (63, 64). It is tempting to speculate that the inhibition of ER by the Rho GTPases is mediated by NF-κB. However, our preliminary findings suggest that inhibition of NF-κB by overexpressing 1xB does not relieve the repressive effects of Rho GTPases on ER transactivation (not shown), suggesting that Rho GTPases regulate ER independent of NF-κB.

Recently, an ER-interacting protein, termed Brx, was identified and shown to contain a domain virtually identical to the Rho GEF Lbc, although its enzymatic activity has not been demonstrated (26). Overexpression of Brx in Ishikawa cells increases ER transcriptional activation, and a dominant nega-
tive form of Cdc42, but not RhoA or Rac1, reduces its coactiva-
tor function (26). Our results differ from this report in assess-
ing the effect of Rho GTPases on ER transactivation and show-
ing that RhoA, Rac1, and Cdc42 negatively regulate ER trau-
scriptional activity. This apparent discrepancy between what
would be predicted from Rubino et al. (26), that Cdc42
increases ER transactivation, and our results showing that
Cdc42, RhoA, and Rac1 decreased ER transcriptional activa-
tion may be attributed to methodological or cell-specific dif-
ferences. Alternatively, since Brx coactivator function is prob-
able mediated by direct ER binding, the inhibition of ER activity by
the dominant negative form of Cdc42 may have resulted from
competition between ER and dominant negative Cdc42 for Brx
binding, rather than from blocking the signaling pathway
downstream of Brx. In contrast, the effect of Rho GDI and the
Rho GTPases on ER appears to be indirect. Localization studies
indicate that ER and Rho GDI are found in distinct subcellular
compartments, with Rho GDI residing in the cytoplasm, whereas
ER is confined to the nucleus. In addition, GST-Rho GDI
is unable to associate with estradiol-bound ER, although it
is capable of binding Rho A in vitro. While Brx and Rho
GTPases may modulate ER activity through distinct mecha-
nisms, the identification of different components in the Rho
signaling pathway as modulators of ER transactivation un-
derscores their importance in receptor regulation.

Our findings suggest that the Rho GTPases decrease transcrip-
tional activation by ERs, thus establishing a novel path-
way of cross-talk between cell surface receptors that regulate
Rho GTPase signaling and steroid receptor transcriptional ac-
tivity. Another example of cross-talk between the cell surface
and ER is the modulation of ER ligand-independent transcrip-
tional activation by the epidermal growth factor/Ras/mitogen-
activated protein kinase signaling pathway (65, 66). It has been
shown that treatment of cells with epidermal growth factor
results in ER ligand-independent activation and phosphoryla-
tion by the mitogen-activated protein kinase, Erk1 (31, 67). 
Although the mechanism of this increased ER transcriptional
activation remains to be elucidated, it probably involves phos-
phorylation-dependent cofactor recruitment (68). Thus, Ras
acts as a positive regulator of ER transcriptional enhancement
(67), whereas Rho GTPases suppress receptor transactivation.

We speculate that the opposing actions of Ras and Rho
GTPases on ER-mediated transcriptional activation provide a
means of fine tuning the ER transcriptional response to
changes in the extracellular environment.

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