Regulation of GRIP1 and CBP Coactivator Activity by Rho GDI Modulates Estrogen Receptor Transcriptional Enhancement*

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Estrogen receptor α (ER) coordinates gene expression with cellular physiology in part by controlling receptor-cofactor interactions in response to extracellular signals. We have previously shown that the Rho signaling pathway modulates ER transcriptional activation. We now demonstrate that Rho GDI-dependent increase in ER transactivation is dependent on the ER AF-2 coactivator binding site, prompting us to examine regulation of receptor coactivators by Rho GDI. Indeed, Rho GDI cooperates with GRIP1 to increase ER ligand-independent and ligand-dependent transactivation and also enhances GRIP1 transcriptional activity when GRIP1 is tethered to DNA. The GRIP1 activation domain 1 (AD1), which binds CBP/p300, is necessary for Rho GDI to modulate GRIP1 activity. Using E1A to inhibit the endogenous CBP/p300 and a Gal4-CBP fusion protein to assay CBP activity, we find that the effect of Rho GDI on ER transactivation is CBP/p300-dependent. Importantly, the ability of CBP/p300 to transduce the Rho GDI signal to ER occurs through both GRIP1-dependent and independent pathways. These data suggest a complex interplay between ER transcriptional activation and the Rho signaling pathways through modulation of receptor cofactors, which may have evolved to coordinate receptor-dependent gene expression with Rho-regulated events, such as cell migration. We speculate that dysregulation of the Rho-ER axis may participate in cancer progression.

The estrogen receptor α (ER) is a ligand-dependent transcription factor that is an important regulator of cell growth, differentiation, and malignant transformation. Transcriptional activation by ER is accomplished through specific and general cofactor complexes that assemble with the receptor at target promoters to regulate transcription. Although many cofactors have been described that participate in ER transcriptional regulation, the cellular signals and physiological contexts that modulate the activity of these cofactors are not well understood.

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The abbreviations used are: ER, estrogen receptor α; AF, activation factor; CREB, cAMP response element-binding protein; CBP, CREB-binding protein; tk, thymidine kinase; DBD, DNA-binding domain; MAPK, mitogen-activated protein kinase; GDI, guanine nucleotide dissociation inhibitor.

ER contains at least two transcription activation functions (AFs); constitutively active AF-1 in the N terminus of the protein and ligand-dependent AF-2 at the ER C terminus. These AFs represent surfaces capable of interaction with general transcription factors (GTFs) and additional transcriptional regulatory factors termed coactivators. Estradiol binding to the ER promotes a conformational change in the receptor and the formation of a surface for protein-protein contacts between AF-2 and coactivators. Among the numerous coactivators identified to date, one of the best characterized is the p160 family of proteins comprised of SRC1 (NcoA1), GRIP1 (TIF2, NcoA2), and RAC3 (ACTR, AIB1, PCIP, TRAM). Although initial characterization of p160 proteins indicate that they participate in transcriptional activation by ER through AF-2 and interact with the receptor in a ligand-dependent manner (1–3), the p160s can also interact with ER N-terminal region and increase AF-1 transactivation independent of ligand (4). These interactions are mediated by two distinct regions on the p160s: the central nuclear receptor (NR) boxes bind ER AF-2 (5), whereas the p160 C terminus interacts with the ER N-terminal A/B domain (4). The p160 proteins are believed to enhance ER transactivation by recruiting other transcriptional regulatory factors through two activation domains, AD1 and AD2. AD1 interacts with CREB-binding protein (CBP) and p300 (6), whereas AD2 has been shown to associate with the coactivator-associated arginine methyltransferase 1 (CARM1) and the p68/72 family of proteins, which also bind to the ER A/B domain (7, 8). ER recruits CBP/p300 both indirectly, through an association with the p160 proteins, as well as directly, through the ER A/B domain, thereby increasing AF-1 activity and facilitating the interaction between ER AF-1 and AF-2 (9). CBP and p300 are multifunctional proteins that stimulate ER transactivation by interacting with components of the basal transcriptional machinery such as RNA Pol II, TBP, and TFII B, and facilitate an association with other transcription factors and coregulators such as p/CAF (10, 11). Additionally, CBP/p300 contains a histone acetyltransferase (HAT) activity that acetylates both histone and non-histone proteins, which, interestingly, include ER and the p160s (12, 13). Thus, a complex picture of signal transduction by ER is emerging that appears to rely on a collaboration of multiple factors for regulation of gene expression.

We have previously demonstrated that ER transcriptional activation is increased by overexpression of Rho guanine nucleotide dissociation inhibitor alpha (Rho GDI), and this effect is mediated through an inhibition of Rho GTPases (14). Rho GDI is a cytoplasmic protein that acts as a negative regulator of Rho GTPases, including RhoA, Rac1, and Cdc42, by blocking the dissociation of GDP (15). In addition, Rho GDI controls subcellular localization of the Rho GTPases through binding the C-terminal isoprenoid modification, thus preventing their insertion into the plasma membrane and modulating the ratio
of the active membrane-bound and inactive soluble forms of Rho proteins (16).

In this report we analyze the mechanism by which Rho GDI increases ER transcriptional activity. We determine the contribution of the ER transcriptional activation domains and examine the activity of p160 and CBP/p300 receptor cofactors by Rho GDI. Our results indicate that the Rho GDI signal is transduced to ER by CBP/p300 through GRIP1-dependent and -independent pathways.

EXPERIMENTAL PROCEDURES

Plasmids—The ER reporter plasmid XETL contains one GRE from the Xenopus vitellogenin A2 gene, upstream of the herpes simplex virus thymidine kinase (tk) promoter (165) linked to the firefly luciferase coding sequence. The Gal4 reporter plasmid, p5xGal4tk-kirase was a generous gift from Naoko Tanese. The human ER containing constructs pcDNA3-wtER and pcDNA3-ER1121, with amino acids 539–1121, and pM-GRIP1.

RESULTS

Rho GDI Increases ER Transcriptional Activity via AF-2-dependent and -independent Mechanisms—To understand how Rho GDI regulates ER transcriptional enhancement, we first examined whether the activities of AF-1 or AF-2 were modulated by Rho GDI expression. The contribution of each of these two transcriptional activation functions can be distinguished by using tamoxifen, which binds the ER ligand binding domain and alters the conformation of AF-2 such that coactivator association is disrupted. We transiently transfected U2OS cells with ER and Rho GDI expression vectors, and assessed ER transcriptional activation in the absence of ligand and in the presence of estradiol and tamoxifen on an ER-responsive reporter plasmid. ER activation by Rho GDI is roughly 7-fold upon estradiol stimulation and only 2-fold in the presence of tamoxifen (Fig. 1A; compare lanes 5 and 6 to lanes 9 and 10), suggesting that ER AF-2 mediates the majority, but not all of Rho GDI-dependent ER activation.

To further examine the role of AF-2 in mediating Rho GDI signaling, we used an ER derivative defective in p160 association. This mutant ER (ER121) contains twin leucine to alanine mutations at amino acids 539 and 540, which are located on helix 12 that forms a part of the p160 binding surface. It has been previously shown that these alterations decrease coactivator association with ER (18), without affecting ligand- or DNA-binding (19). In the absence of ligand treatment, we observed a 7-fold increase in wild type ER transcriptional activity by expression of Rho GDI, but only a 2-fold increase on ER121 (Fig. 1A; compare lanes 1 and 2 to lanes 3 and 4). Likewise, in the presence of estradiol, Rho GDI is less effective at increasing ER121 activity as compared with wild type ER (Fig. 1A, lanes 5–8). The ability of specific mutations in the coactivator binding site to disrupt Rho GDI-mediated increase in ER transcriptional activity supports the hypothesis that Rho GDI regulates ER largely by modulating ER AF-2 activity, implicating alterations in p160 binding or activity. Interestingly, Rho GDI does appear to regulate ER in an AF-2-independent manner, as neither tamoxifen treatment, AF-2 disruption by mutagenesis (ER2L), nor a combination of both completely abolish Rho GDI-dependent increase in ER transcriptional activity (Fig. 1A, lanes 9–12). Taken together, these data suggest that induction of ER transactivation by Rho GDI is mediated largely by an ER AF-2-dependent and to a lesser extent an AF-2-independent mechanism.

To further examine the effect of Rho GDI on ER activation domains, we tested whether Rho GDI can regulate AF-1 or AF-2 independently using ER fragments containing either AF-1 (ER1–269) or AF-2 (ER269–595) fused to the Gal4 DNA binding domain. As shown in Fig. 1P, Rho GDI increases the transcriptional activity of ER AF-2 in a ligand-dependent manner roughly 5-fold, providing further support for Rho GDI as an activator of ER AF-2. Additionally, Rho GDI increases the transcriptional activity of ER1–269 about 2-fold, consistent with AF-2-independent ER induction by Rho GDI in the presence of tamoxifen or mutations in the AF-2 coactivator binding site.

Rho GDI and GRIP1 Enhance ER Transcriptional Activation Cooperatively—Since a majority of Rho GDI-mediated increase in ER transcriptional activation is dependent on AF-2, which binds p160 coactivators, we examined the relationship between
Rho GDI and the p160 coactivator GRIP1. As shown in Fig. 2A, in the absence of hormone, ER transactivation is increased 3-fold by overexpressed Rho GDI, 3-fold by GRIP1, but is increased 22-fold when Rho GDI is coexpressed with GRIP1. Similarly, Rho GDI and GRIP1 together induce a 15-fold increase in ER transactivation upon estradiol treatment. This increase in ER transactivation is not the result of elevated ER protein expression (Fig. 2A, bottom panel) and ER nuclear localization is not affected by GDI or GRIP1 expression (not shown).

To determine if transcriptional synergy between GRIP1 and Rho GDI is a feature unique to GRIP1, we tested the effect of Rho GDI with the other members of the p160 family, SRC1 and RAC3. SRC1 also cooperates with Rho GDI to increase ER transactivation, although the fold induction of SRC1 with Rho GDI on ER transcriptional activation is less pronounced as compared with GRIP1, with only a 3-fold increase in the absence of ligand and a 6-fold enhancement upon estradiol treatment (Fig. 2B). In contrast, we were unable to demonstrate a significant increase in ER activation by RAC3 and Rho GDI.
over that of Rho GDI alone (Fig. 2C). These differences may reflect functional diversity between GRIP1, SRC1, and RAC3 or different levels of p160 expression. Since in our system Rho GDI cooperated most strongly with GRIP1, we have focused our efforts on characterizing the effect of Rho GDI on GRIP1.

**Rho GDI Increases GRIP1 Transcriptional Activity in an AD1-dependent Manner**—To determine the mechanism by which Rho GDI and GRIP1 increase ER transactivation, we first examined whether Rho GDI increases GRIP1 transcriptional activity. Although GRIP1 is not a sequence-specific transcription factor, it contains two activation domains whose activity can be monitored by tethering GRIP1 to DNA via a heterologous DNA binding domain. Rho GDI increased the transcriptional activity of GRIP1 fused to the Gal4-DBD (Gal4-GRIP1, Fig. 3A) roughly 3-fold (Fig. 3B), suggesting that Rho GDI is an upstream regulator of GRIP1, which amplifies its coactivation potential, thereby increasing ER transcriptional activation. These results also suggest that it is the activity of GRIP1, rather than GRIP1 binding to ER, that is stimulated by Rho GDI.

We next mapped the GRIP1 domain mediating the effect of Rho GDI on ER using GRIP1 derivatives lacking AD1 (Gal4-GRIP1.ΔAD1), AD2 (Gal4-GRIP1.ΔAD2), or AD1/2 (Gal4-GRIP1.ΔAD1/2) (Fig. 3A). Our results indicate that deletion of AD1 or AD1/2 abolishes the Rho GDI-dependent increase in GRIP1 activity, whereas deletion of AD2 does not (Fig. 3C). Although deletion of AD2 resulted in a large increase in GRIP1 activity, the activity of this derivative was further augmented by Rho GDI expression. This increase in GRIP1.ΔAD2 has not been explored. The expression of GRIP1 variants was confirmed by immunoblot analysis (Fig. 3C, bottom panel). Taken together, these results suggest that Rho GDI regulates GRIP1 transactivation, and that this requires the CBP/p300-interacting region, AD1.

**Rho GDI Regulates CBP Transcriptional Activity**—Since Rho GDI-responsive region of GRIP1 maps to the CBP/p300-binding domain, Rho GDI may increase GRIP1 activity by regulating CBP/p300 function. To determine if CBP/p300 is a downstream target of Rho GDI, we asked whether Rho GDI regulates the transcriptional activity of CBP. CBP-dependent transactivation was assayed by cotransfecting Gal4-CBP fusion protein with a Gal4 responsive reporter, with or without Rho GDI. Indeed, Rho GDI increases transcriptional activity of CBP in a dose-dependent manner (Fig. 4A).

The viral oncoprotein E1A has been shown to associate with the HAT domain and the neighboring CH3 region of CBP and p300, thereby inhibiting their HAT-dependent and -independent activities (10, 20, 21). We cotransfected a Gal4 fusion of full-length GRIP1 with or without Rho GDI and in the presence or absence of E1A-12S. As shown in Fig. 4B, E1A decreases GRIP1 activity and completely blocks stimulation of GRIP1 transactivation by Rho GDI, suggesting that CBP/p300 are required for Rho GDI to increase GRIP1 activity. The activity of a Gal4-GRIP1.ΔAD1 derivative, which lacks the CBP/p300-interacting region, is not blocked by ectopic E1A expression, suggesting that the inhibitory effect of E1A on GRIP1-dependent transcriptional activation is specific for CBP/p300 (Fig. 4C). These findings are consistent with our results that GRIP1’s CBP/p300-interacting AD1 domain mediates the effect of Rho GDI on GRIP1. Furthermore, these results suggest that CBP/p300, in addition to GRIP1, may be largely responsible for mediating the increase in ER transactivation induced by Rho GDI.

**CBP/p300 Are Required for Rho GDI to Increase ER Transcriptional Activation**—We next examined whether CBP is required for Rho GDI to increase ER transactivation, by cotransferring ER and Rho GDI with E1A. Fig. 5A shows that inhibition of CBP/p300 by E1A inhibits ER transcriptional activity, consistent with the role of CBP/p300 as being important ER regulators in U2OS cells. Importantly, E1A also abolishes Rho GDI-mediated increase in ER transactivation without decreasing the level of ER or Rho GDI protein expression (Fig. 5A, bottom panels). If CBP/p300 are required to transduce Rho GDI signaling to ER via GRIP1, we anticipate that E1A will decrease the cooperativity with respect to ER transactivation. Indeed, the addition of E1A reduced ER transcriptional activation (Fig. 5B, compare lanes 1 and 5) and blocked the synergistic increase in ER activity induced by coexpression of Rho GDI and GRIP1 (Fig. 5B, compare lanes 4 and 8). Since CBP and p300 are inhibited by E1A, these results indicate that Rho GDI modulates ER activity through an E1A-sensitive step that most likely requires the activity of CBP/p300.

**Increased ER Transcriptional Activation Is Partially Dependent on Recruitment of CBP/p300 by GRIP1**—Although in-
Inhibition of Rho GDI/GRIP1 synergy by E1A supports a model whereby Rho GDI activates ER through a CBP/p300-dependent step, it does not address whether recruitment of CBP/p300 to GRIP1 is important in this process. As CBP can also interact with ER directly, independent of p160 proteins, it is conceivable that the synergistic enhancement of ER transcriptional activation by Rho GDI and GRIP1 results from CBP/p300 both increasing ER transcriptional activity directly, as well as indirectly through an increase in GRIP1 activity. We examined the ability of GRIP1ΔAD1, a known target of Rho GDI, to increase ER transcriptional activity in the presence of Rho GDI (Fig. 6A). Thus, recruitment of CBP/p300 by GRIP1 only partly accounts for the ER activation by Rho GDI in combination with GRIP1. It is likely that CBP/p300 transduces Rho GDI signaling by binding and coactivating ER through parallel pathways involving both GRIP1 AD1-dependent and -independent mechanisms.

**FIG. 4. CBP is a target of Rho GDI.** A, Rho GDI increases CBP transcriptional activity. U2OS cells were transfected as in Fig. 1 with 0.5 μg of Gal4-CBPc, 0.2 μg of p5xGal4tk-luciferase reporter, and the indicated amount of Rho GDI. B, E1A blocks the Rho GDI-dependent increase in GRIP1 transcriptional activity. U2OS cells were transfected as above with 1.0 μg of Gal4-GRIP1 FL, 0.2 μg of p5xGal4tk-luciferase reporter, and the indicated amount of Rho GDI in the absence (−) or presence (+) of 80 ng of pCI-HA-E1A-12S. C, inhibition of GRIP1 transcriptional activation by E1A is dependent on the CBP/p300 interaction region AD1. U2OS cells were transfected with either 1.0 μg of Gal4-GRIP1 (white bar) or Gal4-GRIP1ΔAD1 (light gray bar), along with 0.2 μg of p5xGal4tk-luciferase reporter and the indicated amount of Rho GDI in the absence (−) or presence (+) of 80 ng of pCI-HA-E1A-12S. Cells were harvested and luciferase activity was measured 36 h post-transfection. Shown are representatives of three independent experiments. Error bars represent the range of the mean.

**FIG. 5. Rho GDI-mediated increase in ER transcriptional activation is E1A-sensitive.** A, U2OS cells were transfected with 0.2 μg of XETL reporter, 0.5 μg of ER, along with the indicated amount of Rho GDI and E1A-12S. Cells were treated with 100 nM 17β-estradiol and luciferase activity was measured as in Fig. 1. Whole cell extracts were prepared from transfected cells, and the expression of ER and Rho GDI was analyzed by immunoblotting using anti-ERα and anti-Rho GDIα antibodies (bottom panels). B, E1A blocks the increase in ER transcriptional activity by Rho GDI and GRIP1. U2OS cells were transfected as in Fig. 1 with 0.2 μg of XETL reporter, 0.1 μg of ER, along with the either 0.6 μg of empty expression vector only (lanes 1 and 5), Rho GDI (lanes 2 and 6), GRIP1 (lanes 3 and 7), or Rho GDI and GRIP1 (lanes 4 and 8) in the absence or presence of E1A-12S (80 ng/dish; lanes 5 and 6 or 400 ng/dish; lanes 7 and 8). Cells were treated as described in the legend to Fig. 1 and ER transcriptional activation was measured. Data shown are the mean of a representative experiment performed in duplicate and repeated three times with similar results. Error bars represent the range of the mean.
induced interaction between these two activation domains (9). Since Rho GDI activates CBP/p300, and CBP/p300 also appear to be essential for cooperativity between Rho GDI and GRIP1, we speculated that ER activation by Rho GDI and GRIP1 may reflect a collaboration between ER AF-1 and AF-2. This hypothesis was tested using truncated receptors containing either the DNA binding domain and AF-2, but not AF-1, extending from amino acid 179 to 545 (ERADP-2) or the reciprocal receptor derivative containing the DNA binding domain and AF-1, but not AF-2, from amino acids 1 to 269 (ERAF-1) (Fig. 7A). As shown in Fig. 7B, no synergy between Rho GDI and GRIP1 is observed on either the isolated ERADP-2 or ERADP-1, suggesting that both domains play an essential role in mediating cooperative ER activation by Rho GDI and GRIP1. These data suggest that induction of ER transcriptional activation by Rho GDI and GRIP1 relies on the cooperative actions of ER AF-1 and AF-2.

Since AF-1 phosphorylation sites at Ser-104, Ser-106, and Ser-118 are important for AF-1 activity, we next addressed the role of AF-1 phosphorylation in mediating cooperativity between Rho GDI and GRIP1. Using full-length ER with a serine to alanine mutation at three phosphorylation sites in AF-1 (serines 104, 106, and 118) that are phosphorylated by MAPK and cell cycle-regulated kinases. ERADP-2 encompasses the DNA binding domain and AF-2, but lacks the A/B domain containing AF-1, whereas ERAF-1 contains the N terminus and the DNA binding domain, but lacks AF-2. As shown in Fig. 7B, no synergy between Rho GDI and GRIP1 is observed on either the isolated ERADP-2 or ERAF-1, suggesting that both domains play an essential role in mediating cooperative ER activation by Rho GDI and GRIP1. These data suggest that induction of ER transcriptional activation by Rho GDI and GRIP1 relies on the cooperative actions of ER AF-1 and AF-2.

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Rho GDI action on ER is not mediated by the AF-1 phospho-domain is important for Rho GDI to induce ER transactivation, Rho GDI action on ER is not mediated by the AF-1 phosphorylation sites examined.

**DISCUSSION**

We provide evidence that Rho GDI increases ER transactivation by regulating the transcriptional activity of both AF-1 and AF-2 through the coactivators GRIP1 and CBP/p300. Several lines of evidence suggest the cooperative induction of ER by Rho GDI and GRIP1 is mediated by CBP/p300. First, this cooperation is dependent in part on AD1, the CBP/p300 interacting domain of GRIP1. Second, Rho GDI stimulates the transcriptional activity of CBP tethered to DNA. Third, inhibition of endogenous CBP/p300 by the viral oncoprotein E1A blocks the increase in ER transcriptional activity by Rho GDI, as well as cooperativity between Rho GDI and GRIP1 on ER transactivation. While E1A also binds a component of the mammalian Mediator complex, hSur2 (23, 24), which is a subunit of the GRIP/TRAP complex that binds nuclear receptors and stimulates ER transactivation (25), it is unlikely that inhibition of this complex results in the E1A-mediated inhibition of ER transcriptional activation, since the E1A-12S isoform used is the alternative splice product that does not interact with hSur2. Rather, we suggest that the Rho GDI-dependent increase in ER transactivation is through an E1A-sensitive step that most likely involves CBP/p300.

How might Rho GDI increase ER transactivation cooperatively with GRIP1? We speculate that Rho GDI increases GRIP1 transcriptional activity through enhancement of CBP/p300 binding or activity. However, because a GRIP1 derivative that no longer binds CBP/p300 (GRIP1ΔAD1) retains some activity, Rho GDI may also cooperate with GRIP1 via an AD1-independent mechanism. This mechanism, nevertheless, appears to involve CBP/p300 since cooperative ER activation by Rho GDI and GRIP1ΔAD1 is E1A-sensitive. Taken together, our data suggest that Rho GDI enhances ER transcriptional activation by stimulating CBP/p300 action, which, in turn increases ER transactivation via parallel GRIP1 AD1-dependent and-independent mechanisms (Fig. 8). This result is consistent with recent findings from the Kraus laboratory that demonstrate CBP/p300 interactions with GRIP1 are required for ER transcription initiation in vitro (26).

Our initial studies suggested that Rho GDI increases ER transactivation largely by regulating ER AF-2, as inhibition of AF-2 activity either pharmacologically using tamoxifen or with ER mutations, greatly diminishes the effect of Rho GDI on ER (Fig. 1). However, in light of the observation that AF-1 deletion abolishes synergistic increase in ER transactivation by Rho GDI and GRIP1 (Fig. 7), it appears that ER AF-1 plays an essential role in permitting Rho GDI and GRIP1 to cooperatively enhance ER transcriptional activity. This requirement for ER AF-1 is also consistent with ligand-independent induction of ER by Rho GDI and GRIP (Fig. 2A). Thus, both ER AF-1 and AF-2 are necessary, but individually not sufficient for the cooperative effect of Rho GDI and GRIP1 on ER transcription activation. We suggest that the synergistic increase in ER activity most likely reflects a collaboration between ER AF-1 and AF-2, with both contributing to overall transcriptional enhancement. Our data are consistent with a model whereby Rho GDI overexpression increases the number of CBP/p300 molecules recruited to ER, either directly or indirectly through GRIP1 binding, thereby enhancing the functional interaction between AF-1 and AF-2 (Fig. 8).

While the AF-1 domain is required for Rho GDI to induce ER transactivation, Rho GDI action on ER does not require Ser-104, Ser-106, and Ser-118 phosphorylationsites (Fig. 7B). Thus, direct phosphorylation of Ser-104 and Ser-106 by cyclinA/Cdk2 (17, 27) and of Ser-118 by MAPK (28) or cyclinH/Cdk7 (29) is unlikely to mediate Rho GDI-dependent increase in ER transcriptional activity. However, MAPK activation and cell cycle regulation by the Rho signaling pathway may still contribute to changes in ER transactivation via phosphorylation and modulation of coactivator function. Indeed, recent studies have shown that the p160 coactivators are modified by MAPK signaling (30–32). CBP/p300 are also targets for MAPK phosphorylation, which appears to stimulate HAT activity (33–36). As MAPKs regulate p160 and p300/CBP activity, a link between the MAPK pathway and Rho GDI-dependent increase in ER transactivation appears plausible. Alternatively, GRIP1 and CBP/p300 may be regulated by other common effectors of the Rho signaling pathway, such as the PAK family of serine/threonine kinases.

Rho signaling has been implicated in transcriptional regulation of a handful of transcription factors, including SRF and NF-κB (37, 38). We report here that Rho GDI targets CBP/p300 and increases CBP transcriptional activity. Since CBP and p300 modulate the activity of a large number of transcription factors, induction of CBP/p300 activity by Rho GDI could result in widespread changes in gene expression. Thus, it is possible that the role of Rho signaling in regulating gene transcription is currently underappreciated, with many more transcription factors responsive to Rho GDI still to be identified. With respect to ER transactivation, modulation of CBP/p300 and GRIP1 activity by Rho signaling pathways provide an additional regulatory input to modulate ER transcriptional activity in response to extracellular signals.

Although the overall consequences of ER activation by Rho GDI is currently unknown, the interplay between Rho signaling and ER function may prove particularly important during normal development when regulation of cellular proliferation by ER may need to be coordinated with Rho-regulated events, such as cellular migration. Dysregulation of the Rho-ER axis may uncouple this regulation, thereby contributing to cancer progression. Activation of ER is an early mitogenic event in breast cancer; however, it has also been suggested that the receptor may restrict tumor progression by inhibiting cell invasion and metastasis. For example, introduction of ER into an ER-negative metastatic breast cancer cell line results in reduced invasiveness in vitro and metastatic tumor formation in...
in vivo (39). Similarly, MCF-7 cells with a high ER content display decreased motility in vitro (40). ER-expressing breast tumors are less assertive and invasive with a more favorable disease outcome, whereas ER-negative tumors are typically more aggressive and metastatic, and are associated with a worse prognosis. Thus, although ER promotes cellular proliferation, it also appears to inhibit cell migration and loss of ER results in a more aggressive tumor phenotype.

Interestingly, while ER expression is decreased in advanced breast tumors, the level of Rho GTPases increases with the degree of tumor progression (41). Indeed, overexpression of RhoC is sufficient to stimulate invasion of melanoma cells and is overexpressed in a particularly aggressive type of breast cancer prone to early metastasis (42), whereas the dominant negative RhoA represses the invasiveness of melanoma cells (43).

The opposing effects of Rho GTPases and ER on cell invasion is consistent with a model where Rho GTPases inhibit ER transcriptional activity, thereby enhancing cell motility by blocking ER target genes that suppress cell migration. In contrast, Rho GDI overexpression would promote expression of ER target genes that restrain cell invasion by inhibiting Rho GTPases. It would be interesting to examine the effect of the Rho signaling pathway on endogenous genes regulated by ER, and determine whether Rho GDI specifically regulates genes involved in cell migration and invasion that correlate with breast tumor progression.

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