Estrogen receptor corepressors – a role in human breast cancer?

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Abstract

Estrogen receptor α (ERα) has an established role in promoting breast cancer. Transcriptional activation by ERα is a complex and multistep process, and it is influenced by coactivator and corepressor proteins that can either positively or negatively modulate ERα-mediated transcriptional activity. Corepressors are proposed to provide a counterbalance to the estrogen-induced transactivation, and represent a potential mechanism employed by the cell to regulate hormonal responses. In this review, we present evidence from tissue culture, animal and clinical studies, supporting the hypothesis that corepressors are crucial regulators of ERα-mediated action, and that their loss could promote breast cancer development and resistance to endocrine therapy. We propose that ERα corepressors play an important biological role by controlling the magnitude of the estrogen response, mediating antiestrogen inhibition of ERα, repressing DNA-bound ERα in the absence of the ligand, and conferring active repression of ERα-downregulated genes. Different ERα corepressors regulate steroid receptor activity through a variety of mechanisms, including formation of multiprotein complexes that are able to affect chromatin remodeling, histone deacetylation, or basal transcription. Other mechanisms include competition with coactivators, interference with DNA binding and ERα homodimerization, alteration of ERα stability, sequestration of ERα in the cytoplasm, and effects on DNA repair. Most ERα corepressors can control the receptor’s activity through more than one mechanism, and it is possible that the synergy between different pathways cooperates to fully inhibit ERα transcriptional activity, and create an integrated response to a variety of different cellular signaling pathways. We will discuss the role of corepressors in tumor suppression and the link they might present between ERα regulation and DNA repair. Finally, we will discuss major challenges in the field and speculate on the exciting findings that await us in the next few years.

Introduction

The estrogen receptor (ERα) is a transcription factor that regulates genes involved in development, reproduction, differentiation and transformation (Osborne et al. 2001a). ERα modulates gene expression by binding to short sequences of DNA termed estrogen response elements (ERE) that are usually found in the promoters of estrogen-responsive genes (Klinge 2001). The consensus ERE is a 13-bp palindromic sequence containing two inverted repeats of 5′-GGATC-3′ separated by three base pairs. Although perfect EREs have only been discovered in two human estrogen-regulated genes (cytochrome c oxidase subunit VIIa-related protein (COX7RP) (Watanabe et al. 1998) and estrogen responsive finger protein (Efp) (Inoue et al. 1993)), ERα can bind to non-perfect or half ERE sequences, particularly in the context of appropriate flanking sequences. ERα can also affect transcription without directly binding to DNA, for example through interaction with SP-1 and AP-1 transcription factors (for recent reviews see Kushner et al. 2000, Safe 2001).

ERα has a modular structure, containing an N-terminal, ligand-independent transcriptional activation domain (AF-1), a conserved DNA binding domain (DBD) consisting of two zinc fingers, a flexible hinge domain, a C-terminal activation domain (AF-2) located within the ligand binding domain (LBD), and finally an F domain of an as yet to be determined function. The AF-2 function requires ligand binding for transcriptional activity, and the contribution of AF-1 and AF-2 to ERα activity is both cell type- and promoter-specific.

Recently, a second ER has been discovered, termed ERβ (Kuiper et al. 1996). ERα and ERβ are encoded by different genes and clearly have both overlapping and different functions (Paech et al. 1997). Our review will focus on ERα, since ERβ was discovered more recently, and thus few
studies have addressed ERβ-corepressor interactions. As suggested by recent studies there will be some corepressors shared between ERα and ERβ (Montano et al. 1999), and there will be others which have different effects on ERα and ERβ (Seol et al. 1998). Interestingly, in vitro and in vivo data show that ERα is negatively regulated by ERβ (Hall & McDonnell 1999, Weihua et al. 2000). It is clear that additional studies need to be conducted to understand the role of ERβ in human breast cancer (Palmieri et al. 2002).

Transcriptional regulation of target genes by nuclear receptors (NRs) is a complex, multistep and tightly regulated process. One of the major breakthroughs in understanding NRs was the discovery of the interacting coregulator proteins that can either positively (coactivators, CoA) or negatively (corepressors, CoR) modulate NR activity (McDonnell & Norris 2002). For detailed description of many of these cofactors, we point the reader to recently published reviews (Glass et al. 1997, McKenna & O’Malley 2002, Tremblay & Giguere 2002).

While the role of coactivators for ERα is well established, the importance of corepressors is still somewhat controversial. This controversy mainly arises from the dogma that ERα’s main mechanism is completely different from that of many NRs, such as the thyroid hormone (TR), retinoic acid receptor (RAR), and retinoid X receptor (RXR). TR/RAR/RXR bind to DNA in the absence of ligand, and actively repress transcription through transferable repression domains (Banaihamad et al. 1992). A search for factors that would confer active gene repression led to the identification of two closely related proteins, NCoR (nuclear receptor corepressor) (Horlein et al. 1995) and SMRT (silencing mediator of RAR and TR) (Chen & Evans 1995). In the presence of ligand, corepressors are released from TR/RAR, coactivators are recruited, and transcription is initiated. In contrast, it is generally believed that ERα only binds to DNA in the presence of ligand, eliminating the perceived need for corepressors. However, an increasing number of ERα corepressors has been reported in the literature in the last few years (Klinge 2000), and in this review, we will present evidence originating from a number of laboratories to support the hypotheses that (a) corepressors are important for ERα-mediated actions, and (b) their loss could be involved in breast cancer development and resistance to endocrine breast cancer treatment.

**ERα corepressors**

**Definition of ERα corepressors**

NR corepressors have been defined as factors that ‘interact with nuclear receptors and lower the transcriptional rate at their target genes’ (McKenna et al. 1999). They are rate limiting for NR repression, and do not significantly repress basal transcription. This broad definition has resulted in a large and diverse set of proteins being incorporated into this expanding field. There are ‘classical’ corepressors, proteins that contain an intrinsic and transferable transcriptional repression domain. However, a larger set of ‘non-classical’ corepressors have been found to interact with ERα and repress its action. For example, this includes proteins that cannot affect transcription themselves, but can repress ERα via competition with coactivators or with DNA binding. As our understanding of ERα action has developed, so must our vocabulary for describing this divergent set of proteins. For instance, it is predicted that a new set of proteins will be responsible for controlling the ever expanding novel mechanisms of ERα action such as the rapid cytoplasmic/membrane signaling, and the coupling of transcription and RNA processing. In this review we will describe the identification, functional characterization, and role of corepressors in breast cancer.

**Identification of ERα corepressors**

Given the broad definition of ERα corepressors (as stated above), at least 23 of them have been identified over the last 6 years (Table 1). The best characterized corepressors, NCoR and SMRT, were initially identified as factors binding to TR/RXR family members (Chen & Evans 1995, Horlein et al. 1995, Kurokawa et al. 1995, Zamir et al. 1997, Ordentlich et al. 1999). Subsequently, it was shown that ERα can also interact with these corepressors in the presence of antagonist (Xu et al. 1996, Jackson et al. 1997, Smith et al. 1997, Lavinisky et al. 1998).

Yeast two-hybrid screens have often been applied to identify ERα corepressors, such as the repressor of ERα activity (REA) (bait: AF-2 with point mutation L540Q; library source: MCF-7) (Montano et al. 1999), the repressor of tamoxifen transcriptional activity (RTA) (bait: N-terminus amino acids 51–149; library source: HeLa) (Norris et al. 2002), the ligand-dependent corepressor (LCoR) (bait: LBD in the presence of estradiol; library source: fetal kidney and prostate) (Fernandes et al. 2003), the DEAD box RNA helicase (DP79) (bait: LBD complexed with the antigestrog tamoxifen; library source: MCF-7) (Rajendran et al. 2003), and the SMRT/HDAC1-associated repressor protein SHARP (bait: SMRT, library source: mouse embryo E17) (Shi et al. 2001). The orphan nuclear receptor SHP (short heterodimer partner) was originally isolated in a yeast two-hybrid screen using several conventional and orphan members of the receptor superfamily, including RAR and TR (Seol et al. 1996), and was subsequently shown to interact with and repress ERα (Seol et al. 1998, Johansson et al. 2000). SHP is not the only orphan receptor implicated as a corepressor – TR2 (testicular receptor 2) (Hu et al. 2002), DAX-1 (DSS-AHC critical region on the X, gene 1) (Zhang et al. 2000) and COUP-TF (chicken ovalbumin upstream promoter-transcription factor) (Klinge et al. 1997) also modulate ERα actions.
### Table 1: Estrogen receptor corepressors

The binding sites of corepressors in ERα are listed as reported in the original publications. The inclusion of a single domain does not preclude the possibility of binding in other domains that were not studied. Also, in some studies the LBD and AF-2 were not defined in detail. The mechanisms of repression represent those that are proven and some that are more speculative. Other cellular functions are listed but are not exhaustive.

<table>
<thead>
<tr>
<th>Name</th>
<th>ER binding site</th>
<th>Mechanisms of repression</th>
<th>Other cellular functions</th>
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<tr>
<td>NCoR</td>
<td>LBD</td>
<td>HDACs</td>
<td>Repression of other of transcription factors</td>
<td>Lavinsky et al. (1998)</td>
</tr>
<tr>
<td>SMRT</td>
<td>LBD</td>
<td>HDACs</td>
<td>Repression of other of transcription factors</td>
<td>Smith et al. (1997)</td>
</tr>
<tr>
<td>SHARP</td>
<td>SMRT</td>
<td>HDACs, competition with SRA</td>
<td>RNA and S/MAR binding, inhibits cell growth</td>
<td>Shi et al. (2001)</td>
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<tr>
<td>SAFB1</td>
<td>DBD/hinge</td>
<td>HDAC-dependent and independent</td>
<td></td>
<td>Oesterreich et al. (2000)</td>
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<tr>
<td>SAFB2</td>
<td>ND</td>
<td>ND</td>
<td>Inhibition of cell growth</td>
<td>Townson et al. (2003)</td>
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<td>RIP140</td>
<td>LBD</td>
<td>HDACs, CtBP, competition with coactivators</td>
<td>Interaction with other NRs</td>
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</tr>
<tr>
<td>LCoR</td>
<td>LBD</td>
<td>HDAC-dependent and independent</td>
<td></td>
<td></td>
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<tr>
<td>SHP</td>
<td>AF-2</td>
<td>Competition with coactivators, interference with DNA binding</td>
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<td>DAX-1</td>
<td>AF-2</td>
<td>Competition with coactivators, inhibition of ERα dimerization, competition for ERE</td>
<td>Cofactor for SF-1</td>
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<td>COUP-TF</td>
<td>ND</td>
<td>Inhibition of ERα DNA binding</td>
<td>Negative regulation of a range of NRs</td>
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<td>DP97</td>
<td>LBD/AF-2</td>
<td>ND</td>
<td>ATP-dependent RNA helicase</td>
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<tr>
<td>NSD1</td>
<td>LBD</td>
<td>ND</td>
<td>HMTase activity</td>
<td>Huang et al. (1998)</td>
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<tr>
<td>BRCA1</td>
<td>LBD/AF-2</td>
<td>CTIP interaction, p300 down regulation</td>
<td>DNA repair, recombination transcription</td>
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</tr>
<tr>
<td>MTA1</td>
<td>AF-2</td>
<td>HDACs</td>
<td>Increase in metastasis, member of NURD complex</td>
<td>Mazumdar et al. (2001)</td>
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<tr>
<td>MTA1s</td>
<td>AF-1, DBD, AF-2</td>
<td>Sequestration of ERα in cytoplasm</td>
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<td>RTA</td>
<td>AF-1</td>
<td>HDAC-independent</td>
<td>RNA binding</td>
<td>Norris et al. (2002)</td>
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<td>REA</td>
<td>LBD</td>
<td>Competition with coactivators</td>
<td>Transcription factor that regulates apoptosis and cell cycle</td>
<td>Montano et al. (1999)</td>
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<td>FKHR</td>
<td>LBD</td>
<td>ND</td>
<td>Transcription factor, interaction with HDACs</td>
<td>Zhao et al. (2001)</td>
</tr>
<tr>
<td>TR2</td>
<td>DBD/hinge</td>
<td>Inhibition of ERα dimerization</td>
<td>Transcription factor, interaction with HDACs</td>
<td>Hu et al. (2002)</td>
</tr>
<tr>
<td>NEDD8</td>
<td>ND</td>
<td>Proteolysis of ERα</td>
<td>Modification of cullins</td>
<td>Fan et al. (2003)</td>
</tr>
<tr>
<td>TAF-1β</td>
<td>DBD/hinge</td>
<td>Decrease of ERα acetylation</td>
<td>Decrease of histone acetylation and modulation chromatin structure</td>
<td>Loven et al. (2003b)</td>
</tr>
<tr>
<td>Smad4</td>
<td>AF-1</td>
<td>ND</td>
<td>Transcriptional regulator</td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td>ND</td>
<td>Inhibition of ERα-ERE binding</td>
<td>DNA repair, inhibition of apoptosis and cell growth</td>
<td>Wu et al. (2003) Yu et al. (1997)</td>
</tr>
</tbody>
</table>

ND, not determined; S/MAR, scaffold/matrix attachment region.

RIP140 (or nuclear receptor interacting protein 1, Nrip1) was originally identified as an ERα coactivator by expression cloning using the ERα AF-2 in the presence of estrogen (Cavailles et al. 1995). Subsequent studies, however, showed that RIP140 appears to repress receptor activity rather than activating it, and hence it is now widely accepted as an ERα co-repressor (Lee et al. 1998, Treuter et al. 1998, Lee & Wei 1999). There are a number of ERα cofactors which have been proposed to function as both coactivators and corepressors, including the FKHR (forkhead homolog in rhabdomyosarcoma) (Schuur et al. 2001, Zhao et al. 2001), ERRα (estrogen-related receptor α) (Vanacker et al. 1998, 1999, Kraus et al. 2002), and the NR-binding SET-domain-containing protein 1 (NSD1) (Huang et al. 1998). The bifunctional activity has been attributed to the presence of separate activation and repression domains. Such proteins...
could be important intermediary factors whose regulatory activity is strictly dependent upon the tissue-, cell-, and promoter-specific context.

Some of the most critical players in breast tumorigenesis also repress ERα. The tumor suppressor gene, BRCA1, was originally identified by linkage analysis from breast cancer families (Hall et al. 1990), subsequently cloned (Miki et al. 1994), and has now emerged as a crucial regulator of transcription, DNA repair, recombination, and cell cycle checkpoint control (Venkitaraman 2002). Intriguingly, BRCA1 not only regulates estrogen-dependent but also ligand-independent activity of ERα. More recently, the tumor suppressor gene, p53, which plays critical roles in cell cycle regulation and apoptosis (Levine 1997, Yang et al. 2002), was reported to interact with ERα by glutathione S-transferase (GST)-pulldown and in mammalian two-hybrid assays, and to repress ERα’s activity (Yu et al. 1997, Liu et al. 1999). Finally, MTA1 (metastasis-associated protein 1), which was originally identified by differential expression screening in rat mammary adenocarcinoma metastatic cells (Toh et al. 1994), and which has later been shown to be associated with metastasis in both breast cancer cell lines (Nicolson et al. 2003) and human breast cancer specimens (Martin et al. 2001), also functions as an ERα corepressor.

Our laboratory is studying the ERα corepressor function of the scaffold attachment factors SAFB1 and SAFB2. Scaffold attachment factor B1 (SAFB1) was identified as a nuclear matrix protein binding to the matrix attachment regions (Renz & Fackelmayer 1996) and as a protein repressing heat shock protein, hsp27 (Oesterreich et al. 1997). Since hsp27 is an estrogen-regulated gene, and its promoter contains ERα-like elements, we began to consider a potential role of SAFB1 in ERα’s activity (Oesterreich et al. 2000). To date, we know that not only SAFB1, but also its other family member SAFB2 (Townson et al. 2003), can bind to ERα and repress its activity.

As with coactivators, the list of ERα corepressors is growing, and other recently described ERα corepressors include TERP-1 (truncated estrogen receptor product-1) (Rensnick et al. 2000), the POU transcription factor Brn-3a (Budhram-Terpend-1 (truncated estrogen receptor product-1) (Rensnick et al. 2000), the POU transcription factor Brn-3a (Budhram et al. 2000), and SMRT contain a NR box-related conserved bipartite NR domain (SM Townson, K Kang, AV Lee & S Oesterreich; unpublished observations), confirming the existence of additional binding motifs, other than NR and CoRNR boxes, to specific CoRNRs, as well as flanking sequences (Hu et al. 2001). FKHR also contains an LXXLL sequence, but this motif does not seem to be required for interaction with ERα (Qin & Schiff, unpublished observations). Other corepressors such as REA (Delage-Mourroux et al. 2000), and DP97 (Rajendran et al. 2003) harbor novel ERα interaction domains. Likewise, SAFB1 mediates ERα interaction via a novel domain (SM Townson, K Kang, AV Lee & S Oesterreich; unpublished observations), confirming the existence of additional binding motifs, other than NR and CoRNR boxes, utilized by corepressors. The versatility of the interaction domains is likely to be a reflection of the different mechanisms of repression (see below).

### Structural basis for interaction between ERα and corepressors

The interaction of NR boxes with the ERα LBD is now understood in considerable detail (Brzozowski et al. 1997). Ligand activation is associated with structural rearrangements within the LBD/AF-2 domain, permitting the recruitment of coactivators. In the presence of antiestrogens, the AF-2 helix 12 translocates to a position that overlaps with the site of coactivator interaction, which prevents coactivator binding and facilitates corepressor recruitment. These fundamental crystallographic studies provide a useful paradigm for the structural basis of ERα agonism and antagonism. The big questions, however, are these: if only the antiestrogen-occupied receptor conformation allows corepressor recruitment, are corepressors

### Corepressor-interaction domains in ERα

To date, corepressors have been identified that interact with ERα in AF-1, DBD/hinge, and LBD/AF-2 regions (see also Table 1). The majority of reported ERα cofactors bind to AF-2, a finding that most likely results from investigators concentrating on the ligand-dependent activation function. However, it has become clear that AF-1 and DBD/hinge domains are equally important, and there is no doubt that they will receive more attention in various screens.

### ERα-interaction domains in corepressors

ERα coactivators and corepressors differ in their interaction with ERα. In contrast to ERα coactivators, where detailed analysis has revealed the existence of multiple highly conserved amphipathic ‘LXXLL’ helical motifs (NR box), there does not seem to be a commonly shared ERα interaction domain for corepressors. To date, only LCoR (Fernandes et al. 2003), MTA1s (short form of MTA1) (Kumar et al. 2002), SHP (Johansson et al. 2000), and RIP140 (Heery et al. 2001) were shown to contain a functional NR box. NCoR and SMRT contain a NR box-related conserved bipartite NR interaction domain (NRID), which is predicted to form a different helical structure compared with the coactivator NR box (Perissi et al. 1999). As with coactivators, the specificity of CoRNR’s interactions has been shown to be dependent upon the preferential binding of different NRs to specific CoRNRs, as well as flanking sequences (Hu et al. 2001). FKHR also contains an LXXLL sequence, but this motif does not seem to be required for interaction with ERα (Qin & Schiff, unpublished observations). Other corepressors such as REA (Delage-Mourroux et al. 2000), and DP97 (Rajendran et al. 2003) harbor novel ERα interaction domains. Likewise, SAFB1 mediates ERα interaction via a novel domain (SM Townson, K Kang, AV Lee & S Oesterreich; unpublished observations), confirming the existence of additional binding motifs, other than NR and CoRNR boxes, utilized by corepressors. The versatility of the interaction domains is likely to be a reflection of the different mechanisms of repression (see below).
a pharmacological anomaly? Or are we going to find novel natural ligands with structural similarity to antagonists? Or do ERα corepressors play other important roles in the regulation of ERα, independent of antiestrogen binding? While we will provide evidence for the latter possibility in the following section, it is clear that to fully understand ERα–corepressor interactions, we need to know the structure of full-length ERα (or at least larger parts than AF-2 only) in the presence of corepressor peptides. To complicate matters further, the DNA sequence of the ERE also affects the conformation of ERα and thus affects interaction with cofactors (Hall et al. 2002), suggesting that co-crystals of ERα complexed with its binding site have to be obtained and analyzed. Vigorous attempts are being made to further our understanding of the interaction between NRs and corepressors (Xu et al. 2002), and we can certainly look forward to exciting revelations in this important area over the next years.

Expression of ERα corepressors in normal tissues

The expression pattern of some corepressors is very high in hormone-responsive tissues. For example, LCoR (Fernandes et al. 2003), RIP140 (Parker et al. 2003), SAFB1/2 (Townson et al. 2003), and FKHR (Zhao et al. 2001) are abundant principally in brain and various parts of the reproductive system. Perhaps surprisingly, however, most ERα corepressors are not restricted to estrogen-responsive tissues, but rather they are widely found in human and mouse tissues. This could be explained by the fact that the majority of corepressors do not exclusively interact with ERα, but function for a variety of unrelated transcription factors which regulate completely diverse cellular functions. One of the very few exceptions is REA (Montano et al. 1999) whose function is ERα-specific.

Considering the promiscuity of the corepressors, and their subsequent potential to influence a broad spectrum of cellular processes, multiple levels of control of their action would be expected. Indeed, corepressors undergo posttranslational modifications, such as phosphorylation, acetylation, and proteolysis. Additionally, they can shuttle between nucleus and cytoplasm, and perhaps there is an important spatio-temporal regulation even within the nucleus. These modifications (Hermanson et al. 2002, McKenna & O’Malley 2002) would allow corepressors to control a broad range of developmental, physiologic, and metabolic processes.

Mechanisms of ERα corepressor action

Corepressors function through a number of mechanisms (illustrated in Fig. 1), as briefly discussed below. While the corepressors appear to act most prominently through modifications of chromatin, they also seem to be able to regulate transcription at additional levels. Common to all is the recruitment of dynamic multiprotein complexes which have been more readily identified through advancements in biochemical and protein technologies. Interestingly, a number of corepressors may function through more than one mechanism, and it is likely that the mechanism in play depends upon the promoter and cellular context.

Chromatin remodeling

Most fully characterized are NCOR and SMRT, which function by recruiting different HDAC protein complexes. We would like to point the interested reader to a recent review by Jepsen and Rosenfeld (2002) in which details are elegantly described. Briefly, NCOR has been found to interact with components of both the SAP (Sin-associated protein) and the NURD (nucleosome remodeling and histone deacetylation) complexes (Alland et al. 1997, Heinzel et al. 1997, Nagy et al. 1997, Li et al. 2002a). More recently, both Lazar’s and Evan’s laboratories have discovered that NCOR can also function through mSin3/HDAC1-independent mechanisms which involve recruitment of class II HDACs (Huang et al. 2000, Kao et al. 2000). A further distinct complex contains HDAC3, NCOR/SMRT, and transducin (beta)-like protein 1 (TBL1) (Guenther et al. 2000, Li et al. 2000). Interestingly, NCOR has also been shown to bind the methyl-CpG-binding protein MeCP2 (Kokura et al. 2001), and thereby to play a role in the Smad4-mediated repression of ERα via the recruitment of a Ski-MeCP2 repressor complex (Kokura et al. 2001, Ueki & Hayman 2003, Wu et al. 2003). Taken together, these data not only show that NCOR/SMRT can utilize various mechanisms, but also suggest that previously distinct methods of repression such as chromatin remodeling, histone deacetylation, silencing, and DNA methylation are closely connected cellular processes.

The ERα corepressor BRCA1 interacts with CtBP, a protein originally identified on the basis of its association with the C-terminal binding protein CBP (Wong et al. 1998, Yu et al. 1998b). CtBP is known to mediate repression through recruitment of HDAC and the polycomb group genes (PcG) (Chinnadurai 2002).

A number of other ERα corepressors use HDAC-dependent mechanisms, at least in part, for repression. These include RIP140 (Wei et al. 2000), LCoR (Fernandes et al. 2003), MTA1 (Mazumdar et al. 2001), and TR2 (Franco et al. 2001). While dramatic progress has been made in the biochemical characterization of the HDAC-containing complexes, many questions are still open, including the very basics of histone deacetylation and repression. For instance, transcriptional activation is not necessarily connected with increased acetylation (Deckert & Struhl 2001), and conversely hyperacetylated histones can be found in transcriptionally inactive regions (Martens et al. 2002).

Therefore, this fast moving field might be open for some surprising findings.
Basal transcription

One of the mechanisms that can influence NR activity is the effect that corepressors exert on the basal transcription apparatus. For example, NCoR interacts with the basal transcription factors TFII B, TAF1, and TAF70 (Muscat et al. 1998). Also, BRCA1 is present in the RNA pol II holoenzyme complex (Monteiro 2000), and SAFB1 binds to the C-terminal domain of RNA pol II (Nayler et al. 1998). The direct interaction with central components of the transcriptional process suggests that corepressors could lock them into a non-functional complex or into a conformation that is not conducive to transcription.

Competition

Another mechanism of corepression is the competition for NR binding sites between coactivators and corepressors. For example, REA and SHP compete with SRC-1 and TIF-2 respectively, for ERα binding sites, and can reverse coactivator-mediated enhancement of ERα activity (Johansson et al. 1999, Delage-Mourroux et al. 2000). In addition, RIP140 and GRIP1 have been shown to compete for binding to c-Jun and ERα to modulate estrogen-mediated AP-1-dependent transcriptional activation (Teyssier et al. 2003). While this competition is direct and involved binding to the same domain, repression can also occur as a result of indirect competition, i.e. through sequestration. SHARP, for example, can bind to the steroid receptor RNA coactivator SRA (Lanz et al. 1999), leading to decreased SRA-induced steroid receptor activity in the presence of SRC-1 (Shi et al. 2001). Notably, it has been proposed that estrogen-mediated repression of erbB2 is a result of sequestering the ERα coactivator SRC-1 away from an enhancer which drives erbB2 expression in the absence of estrogen, or in the presence of antiestrogens (Newman et al. 2000).
RNA processing

More than 20 years ago, Chong and Lippman (1982) provided data which suggested that ‘steroid-receptor complexes may play a role in posttranscriptional control’ and that there is an ‘interaction between steroid hormone-receptor-complexes, RNA, and ribonucleotides’. Today we know that this indeed is the case. While a number of studies indicated a role of NR cofactors in coupling transcription and RNA processing, most notably the elegant analysis of PGC-1 (Monsalve et al. 2000), direct evidence came recently from the O’Malley laboratory which showed that steroid hormones can affect RNA processing, and that cofactors are intimately involved in this process (Auboeuf et al. 2002).

In contrast to ERα coactivators, less is known about ERα corepressors and their role in RNA processing. A subset of corepressors (SHARP, RTA, SAFB1/2) contain an RNA recognition motif (RRM) (Weighardt et al. 1999, Shi et al. 2001, Norris et al. 2002). To date, the RRM has only been shown to be important for ERα corepression in the case of RTA (Norris et al. 2002). For SAFB1 (SM Townsend, K Kang, AV Lee & S Oesterreich; unpublished observations) and DP97 (Rajendran et al. 2003), for example, repressor activity and RRM- and DEAD box-motif-containing regions, respectively, are physically and functionally separable.

This is clearly an evolving field which might also benefit from reconsideration of dogmas. RRMIs are involved not only in RNA binding but also in protein–protein interaction (Shi & Xu 2003). Also, it would be beneficial to introduce new model systems analyzing endogenous genes where transcription and splicing are known to be hormone-dependent. Clearly, we are just beginning to understand the role of corepressors in coupling transcription and RNA processing, and more studies will certainly be done in the near future.

Other mechanisms

Other known or proposed mechanisms through which repressors could influence ERα activity involve inhibition of ERα dimerization (TR2, SHP) (Johansson et al. 1999, Hu et al. 2002) and DNA binding (SHP, TR2, p53) (Johansson et al. 1999, Liu et al. 2001, Hu et al. 2002), effects on ERα stability (BRCA1, NEDD8) (Brzovic et al. 2003, Fan et al. 2003), sequestration of ERα away from its place of action (MTA1s) (Kumar et al. 2002), or simply serving as a scaffold for the recruitment of a multi-protein complex (SHARP) (Shi et al. 2001).

We have outlined a model for the main mechanisms utilized by different ERα corepressors in Fig. 1. It is likely that synergy between different pathways cooperates to fully inhibit ERα transcriptional activity, and that the presence of different mechanisms controlling ERα creates an integrated response to a variety of different cellular signaling pathways. A major challenge is to unravel how these diverse mechanisms cooperate, and how different binding of the repressors and formation of multiprotein complexes could provide promoter and cell type-specific responses.

Biological role of corepressors

In this section, we present arguments, mostly resulting from studies in tissue culture, which strongly implicate a crucial role for ERα corepressors in the regulation of ERα activities. These activities (schematically illustrated in Fig. 2) include involvement in antiestrogen-mediated inhibition of ERα, control of the magnitude of the estrogen response, repression of apo-ERα (in the absence of the ligand), and downregulation of genes upon estrogen treatment. Finally, we will speculate on a role of corepressors in modulating non-nuclear ERα activities.

Role in mediating antiestrogen action

Currently, antiestrogens such as tamoxifen are the most effective and commonly prescribed treatments for patients with ERα-positive breast cancer. There is increasing evidence that antiestrogen-mediated inhibition of ERα is not only a passive process resulting from repositioning of helix 12 and thereby blocking the coactivator binding (Brzozowski et al. 1997, Shiau et al. 1998), but rather involves the active recruitment of corepressors to form an inactive or repressive ERα complex.

Interaction studies showed that various corepressors including NCoR/SMRT (Lavinsky et al. 1998), REA (Montano et al. 1999), RTA (Norris et al. 2002), SAFB1 (Oesterreich et al. 2000), and Smad4 (Wu et al. 2003) bind more strongly to ERα in the presence of tamoxifen. To some extent, details of these interactions are the subject of disagreement in the literature (Smith et al. 1997, Zhang et al. 1998). Differences in experimental results may depend on the choice of cell lines, constructs, hormone concentrations, etc., but it is also worthwhile mentioning that such studies are inherently difficult to perform and to interpret for several reasons. For example, GST-pulldown experiments do not consider the involvement of additional factors necessary for interaction, and the use of deletion mutants (either intended or as a result of degradation in the test tube) can obviously result in misfolding. Common precipitation studies in cell lines in the absence and presence of different ligands are hampered by the rapid effects on ERα levels as a result of proteasome-mediated degradation (Navaz et al. 1999).

A number of studies showed that overexpression of corepressors (SAFB1, REA, RTA) resulted in increased antagonist activities of antiestrogens (Montano et al. 1999, Oesterreich et al. 2000, Norris et al. 2002), whereas deletion of the corepressor led to loss of the antagonist activity (Lavinsky et al. 1998). Intriguingly, a dominant-negative RTA isoform converted both tamoxifen and the ‘pure’ anties-
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Confer Repression of ER-Downregulated Genes

e.g. DP97

Determine Magnitude of Estrogen Response

e.g. MTA1, MTA1s, DP97, BRCA1

Corepressor Regulation of ER in Breast Cancer Cells

Mediate Antiestrogen Effects

e.g. NCoR/SMRT, REA, RTA, SAFB1

Repress Unliganded ER

e.g. BRCA1, SAFB1

Figure 2 Role of corepressors in regulating a variety of ERα functions in breast cancer. Examples of corepressors that have been shown to mediate these effects are given.

trogen, ICI 182,780, into powerful agonists (Norris et al. 2002). Likewise, disruption of the NEDD8 pathway resulted in ICI 182,780 resistance (Fan et al. 2003). Interestingly, these cells still responded to tamoxifen supporting the role of NEDD8 in ICI 182,780-mediated degradation of ERα.

Further direct evidence for a critical role of corepressors in antiestrogen action came from the Brown laboratory, which utilized chromatin immunoprecipitation (ChIP) assays to demonstrate that, in the presence of tamoxifen, ERα recruits corepressors to estrogen-responsive promoters (Shang et al. 2000). The same laboratory went on to show that this active recruitment does not occur in cells in which tamoxifen functions as an agonist (Shang & Brown 2002), implicating the necessity of corepressor recruitment for tamoxifen’s antagonist activities. Consistent with these models, overexpression of RTA (Norris et al. 2002), SHP (Klinge et al. 2002), SAFB1 (Oesterreich et al. 2000), and NCoR/SMRT (Jackson et al. 1997, Smith et al. 1997, Lavinsky et al. 1998), can reverse tamoxifen’s agonistic activity. Of note, however, are the recent findings by Morrison et al. (2003) who failed to detect any effects of a dominant-negative NCoR construct on ERα suggesting that more studies are needed to clarify the role of NCoR in ERα action.

A finding that corepressors regulate the activity of tamoxifen-bound ER could obviously have important consequences in the clinical management of breast cancer, explaining the tissue-dependent ability of antiestrogens to either inhibit or activate ERα-mediated transactivation, and the development of antiestrogen resistance (discussed later). Clearly, more studies are needed on this critical issue of coregulator action that may have important clinical importance.

Role in controlling the magnitude of estrogen response

By definition, ERα corepressors can affect ERα in a way that ultimately leads to decreased transcriptional readout. This is primarily assayed in transient transfections using estrogen-responsive reporter constructs (ERE-Tk-Luc). Realizing the limitations of these experimental conditions, investigators have begun to study the effects of cofactors on the expression of endogenous estrogen-regulated genes. For example, MTA1 (Mazumdar et al. 2001) and MTA1s (Kumar et al.
Overexpression of an ERα-L372R mutant which is unable to interact with CoRNR box-containing peptides, but which can bind NR motif-containing peptides, results in a dramatic increase in the estrogen-mediated transcriptional activity when compared with wild-type ERα (Huang et al. 2002). These data suggest that binding of corepressors such as NCoR and SMRT in the presence of estrogen can attenuate the estrogen response. In support of this notion, Fernandez et al. (2003) have shown that the ERα corepressor LCoR specifically recognizes agonist-bound ERα, and the authors have accordingly proposed that LCoR is involved in reducing hormone-induced receptor function.

Finally, it is worthwhile mentioning that a number of cofactors themselves are under estrogen regulation. For example, SHARP expression is increased (Shi et al. 2001) and expression of the coactivator AIB1 (amplified in breast cancer 1) is decreased (Lauritzen et al. 2002) after estrogen treatment. Such estrogen regulation could represent a counterbalance to the increased estrogen-induced transactivation, and a potential mechanism employed by the cell to control hormonal response.

Together, these data imply that corepressors play a fundamental role in the regulation of ERα transcription and that their deregulation could lead to dramatic alterations in the estrogen response. A powerful tool to give weight to this hypothesis is the detailed analysis of mouse models. To date, knockout mice have been reported for the following genes which have all been implicated in repression of ERα activity: NCoR (Jepsen et al. 2000), RIP140 (White et al. 2000), BRCA1 (Deng 2002, Moynahan 2002), NSD1 (Rayasam et al. 2003), p53 (Donehower et al. 1992), COUP-TFI/II (Qiu et al. 1997, Pereira et al. 1999), and DAX-1 (Yu et al. 1998a). NCoR-deficient embryos die by day 15.5 of gestation, exhibiting defects in erythrocytes, thymocytes, and neural development. Mouse embryo fibroblasts (MEFs) transfected with ERα were utilized to study the response to estrogens and antiestrogens. Interestingly, tamoxifen’s antagonist activity was abolished in NCoR<sup>−/−</sup>MEFs; however, at least under the experimental conditions tested, there was no effect on ligand-independent or estrogen-mediated activation of ERα. Rather unexpectedly, estrogen-induction of ERα was diminished in BRCA1<sup>−/−</sup>MEFs (Zheng et al. 2001), while ligand-independent activation of ERα was dramatically increased. To get around the embryonic lethality observed in the BRCA1 germine knockout mice, hypomorphic and Cre-mediated mammary gland-specific BRCA1 deletions have been generated (Brodie & Deng 2001, Deng & Brodie 2001). Mammary glands from these mice display a variety of abnormalities during development, and exhibit genetic instability associated with increased tumor susceptibility (Xu et al. 1999). More studies are needed to decipher which, if any, of these phenotypes is associated with BRCA1’s function as an ERα corepressor. Therefore, it will be necessary to perform detailed studies of the mammary epithelial cells from +/+ and −/− mice, to manipulate the hormonal milieu, or to intercross with ERα-deficient mice. Studies with RIP140 knockout mice revealed its involvement in ovulation – RIP140 deficiency results in defects in oocyte release leading to female infertility (White et al. 2000). Similar, although less severe phenotypes were observed in the heterozygous mice, suggesting that even small alterations in the absolute levels of RIP140 may cause considerable changes in hormone response. The generation of additional mouse models for ERα corepressors is ongoing, and without doubt will provide additional decisive evidence for ERα corepressors and their role in hormone responses.

### Role in repressing apo-ERα

The classical model of ERα action involves the following sequences of events: interaction of ERα monomers with chaperones, dissociation of chaperones and formation of homodimers upon estrogen binding to the ERα LBD, and subsequently DNA binding and initiation of transcription. Over the last decade, significant progress has been made in our understanding of these steps, and this has challenged the initial view thatapo-ERα (i.e. unliganded ERα) is not bound to DNA. A number of in vitro studies have shown that unliganded ERα can bind to ERE-containing DNA (Brown & Sharp 1990, Reese & Katzenellenbogen 1992). More recently, footprinting and ChIP studies have revealed the association (Kim et al. 2000, Shang et al. 2000) and cyclic recruitment (Reid et al. 2003) of ERα to estrogen-responsive promoters in the absence of ligand. These findings parallel those of Belmont’s group who, reconstituting and visualizing transcriptional regulation and chromatin structure, has found that the apo-ERα was able to condense chromatin (Nye et al. 2002). In this experimental system, decondensation of large-scale chromatin was independent of helix 12 and did not require transcriptional activation by ERα, ligand-induced coactivator binding, or histone hyperacetylation.

The importance of apo-ERα is supported by recent evolutionary studies in which both phylogenetic and functional data strongly argue for a late and independent gain of ligand binding by the different NRs during evolution (Escriva et al. 2000). This more recently established model is in contrast to the classical view which suggests that orphan receptors evolved as liganded molecules, which through gene duplication reached the current diversity (Moore 1990). The new and attractive model implies that ancestral orphan receptors were regulated by conformational changes induced by post-translational modifications and by protein–protein interaction, i.e. with NR cofactors.

Although additional studies using promoters of different
estrogen-regulated genes need to be conducted before final conclusions can be made, we would like to propose that there is indeed an important physiological rationale for unliganded ERα occupancy of some (but probably not all) target gene promoters – these genes could be rapidly activated upon estrogen treatment, being in a ‘competent’ state. In this situation, do corepressors keep the unliganded DNA-bound ERα in check, i.e. preventing promoter activation by the ERα’s AF-1? Decisive evidence is lacking. Nevertheless, a number of corepressors including BRCA1 (Fan et al. 2001) and SAFB1 (Oesterreich et al. 2000) have been shown to interact weakly with unliganded ERα, and recent studies suggest that they are indeed involved in repression of DNA-bound apo-ERα. BRCA1+/−MEFs showed increased ligand-independent ERα activity when compared with wildtype MEFs (Zheng et al. 2001). Our laboratory has generated a truncated SAFB1 which is deficient in the autonomous repression domain. This mutant functions as a dominant-negative, i.e. it activates ERα not only in the presence but also in the absence of ligand, implicating SAFB1 in ligand-independent repression of ERα (SM Townson, RL Kang, AV Lee & S Oesterreich, unpublished observations).

It is necessary to dissect mechanistic details of DNA-binding of apo-ERα which is actively repressing, and DNA-binding of unliganded ERα which is activated by crosstalk with other signaling pathways, for example by mitogen-activated protein kinase (MAPK) phosphorylation (Kato et al. 1998). The further analysis is more complicated by the recent findings that activation and repression do not represent two separate events but are intimately connected, and active and repressed receptors exist in a flexible equilibrium (Schulman et al. 1996). This idea is supported by the discovery that corepressors can be found in complexes with coactivators, e.g. the ERα cofactor AIB1 interacts with the corepressor NCoR (Li et al. 2002b).

Role in conferring repression of ERα-downregulated genes

Although the biological role of estrogen-mediated activation of genes is well established, the significance of repression has only recently begun to be appreciated. A number of genes have been shown to be repressed by estrogen, among them vascular epithelial growth factor (VEGF) (Stoner et al. 2000), retinoblastoma (Rb) (Gottardis et al. 1995), AIB1 (Lauritzen et al. 2002), and Her2 (Read et al. 1990). Significant technological advances such as SAGE, and utilization of cDNA and oligonucleotide arrays, have led to dramatic improvements in gene expression analysis. Recent gene profiling studies of estrogen-treated breast cancer cell lines, and of tissue from estrogen-treated ovariectomized mice (Charpentier et al. 2000, Watanabe et al. 2002, Hodges et al. 2003) have provided tangible evidence that estrogen can clearly repress a significant subset of its target genes. Intriguingly, there seem to be as many genes downregulated as there are induced! We, as breast cancer researchers, can certainly expect some surprises in the near future, since our knowledge vacuum concerning which estrogen-regulated genes confer the estrogen effect might finally be filled. There is no doubt that this list of genes will include many estrogen-repressed genes, and indeed, our laboratory has recently identified E-cadherin as an estrogen-repressed gene (Oesterreich et al. 2003). E-cadherin plays a role in cell–cell adhesion, and its loss leads to the invasive growth of epithelial tumors. Intriguingly, ERα has also been indirectly connected to E-cadherin expression – absence of MTA3 in ERα-negative cells led to expression of the transcriptional repressor Snail, which in turn repressed E-cadherin (Fujita et al. 2003). It is therefore interesting to speculate that different members of the MTA family impart unique properties to ERα actions by directly inhibiting ERα (MTA1, MTA1s) and by indirectly regulating ERα target genes (MTA3).

Earlier studies have provided circumstantial evidence that ERα coregulators are involved in the ERα-AP-1-mediated downregulation of genes (Jakacka et al. 2001). The first direct evidence for corepressors being directly involved in estrogen repression came from the Katzenellenbogen laboratory, which showed that depletion of DP97 attenuated the repression of erbB2 (Rajendran et al. 2003). Similarly, overexpression of SMRT resulted in enhanced estrogen-ERα repression of the folate receptor FR-α, whereas none of the tested ERα coactivators altered FR-α repression (Kelley et al. 2003). Using ChIP analysis, we have shown that NCoR and SAFB1 can be found bound to the E-cadherin promoter, the activity of which is repressed in the presence of estrogen (Oesterreich et al. 2003). These results clearly show that an ERα-corepressor complex is directly involved in gene regulation, and that repression is not an indirect effect of cell cycle changes induced by estrogen-treatment. It is likely that estrogen-mediated repression of genes and the critical involvement of corepressors in this process will gain a lot of attention in the next years in basic, translational, and clinical research.

Role in regulation of non-nuclear ERα

For years there have been sporadic reports of a membrane-bound ERα responsible for certain very rapid effects of estrogen in cells including breast cancer cells. Several lines of evidence suggest that such ‘non-genomic’ ERα actions are involved in estrogen’s effects on the brain (Dhandapani & Brann 2002), vascular system (Cid et al. 2002, Mendelsohn 2002a), and cardiac tissue (Mendelsohn 2002b). For breast cancer, the field has been very controversial (Valverde & Parker 2002), and decisive evidence has been lacking. Recent studies, however, leave little doubt that ERα can indeed interact with important cytoplasmic signaling molecules such...
as phosphatidylinositol 3-kinase (PI3K) (Levin 2002), but more studies are needed to finally understand the relevance of these findings.

If indeed membrane-bound (and/or cytoplasmic) ERα plays an important role in mediating the estrogen response, one could imagine that there would be a similar need for its regulation as for nuclear ERα. How this regulation might be achieved is so far unclear. It has been proposed that the ERα coactivator PELP1/MNAR (proline-, glutamic acid-, and leucine-rich protein-1/modulator of non-genomic activity of ERα) can regulate ERα’s activity by increasing its interaction with members of the src tyrosine kinase family (Wong et al. 2002), and the overexpression of PELP1/MNAR resulted in estradiol hypersensitivity of breast cancer cells (Balasenthil & Vadlamudi 2003).

Interestingly, a common feature of several of the diverse corepressor proteins described earlier is that they exist in multiple isoforms which differ in their subcellular localization. For example, MTA1 is mainly localized in the nucleus whereas MTA1s, a naturally occurring short form of MTA1, is found in the cytoplasm (Kumar et al. 2002). MTA1s sequesters ERα in the cytoplasm and prevents ligand-induced nuclear translocation, ultimately resulting in breast tumors with low or no nuclear ERα activity. Similarly, the ERα corepressor SAFB has at least two family members, SAFB1 and SAFB2. While SAFB1 is only localized in the nucleus, SAFB2 is found also in the cytoplasm (Townson et al. 2003). In vitro experiments have shown that both proteins can interact with and repress ERα, and future studies will offer insights into the potential role of SAFB2 in regulating cytoplasmic ERα.

**Breast tumor development and progression – a role for corepressors?**

The biology of breast cancer is very complex (Keen & Davidson 2003), but there is no doubt that estrogen and ERα play a central role (Osborne et al. 2001b, Powles 2002, Santen 2002). Both molecular and epidemiological studies have highlighted estrogen’s role as a potent mitogen, promoting the G1/S phase transition and stimulating cell proliferation in hormone-responsive tissues and estrogen-dependent breast cancer. Although higher ERα levels lead to higher hormone sensitivity and might predispose to malignant transformation, they also confer a higher success rate to antiestrogen treatment. About 70% of breast cancer patients are ERα-positive upon initial diagnosis, and in the majority of those cancers ERα status serves as a valuable predictive marker for probable response to antiestrogen therapy. The role of corepressors in the common phenomenon of antiestrogen resistance, and their potential role in breast tumorigenesis, will be discussed below.

**ERα corepressors and breast cancer – results from cell line and mouse studies**

An important question that scientists are now facing is the significance of ERα corepressors in vivo. As discussed above, there is compelling evidence from a number of laboratories that corepressors are involved in a multitude of ERα functions. How does this translate to the biology of breast cancer cells? Not surprisingly, several ERα corepressors (such as SAFB1 and BRCA1) are able to block cell cycle progression (Townson et al. 2000, Venkitaraman 2002, Somasundaram 2003). Also, SAFB1 overexpression significantly inhibits both anchorage-dependent and -independent growth of breast cancer cell lines (Townson et al. 2000 and Oesterreich et al., unpublished results). Several members of the MTA family have been shown to be involved in breast tumorigenesis. Intriguingly, expression of MTA1 is regulated by growth factors, and overexpression of MTA1 and MTA1s in breast cancer cell lines enhances the ability of the cells to invade and to grow in an anchorage-independent manner (Mazumdar et al. 2001, Kumar et al. 2002). Taken together, these data imply a role for MTA1 in the formation of hormone-independent breast cancer. Indeed, the same laboratory was able to show that MTA1s expression is increased in ERα-negative human breast cancer, and MTA1s-overexpressing MCF-7 cells display a more tumorigenic phenotype in nude mice in the absence of estrogen treatment (Kumar et al. 2002).

To date, there are only a limited number of mouse models in which genes, which also have ERα corepressor activities, have been inactivated (see also ‘Role in controlling the magnitude of estrogen response’). With the exception of BRCA1 conditional knockout mice (Xu et al. 1999), no corepressor knockout mice display an obvious mammary gland phenotype. One possible explanation is the presence of other cofactors that can partially compensate for their loss and function in the mammary gland. Alternatively, early lethality might not allow analysis of the mammary glands, or exciting and dominant phenotypes in other organs may divert attention from the mammary gland. Therefore, on the basis of our current knowledge, it is impossible to conclude much about ERα corepressor function in the mouse mammary gland, but more studies utilizing both existing and novel knockout models will certainly be carried out in the near future.

**Expression of ERα corepressors in human breast tumors**

To date, very few studies have addressed the question of ERα corepressor levels and their associations with other biomarkers in breast cancer. Kurebayashi et al. (2000) showed that SMRT and NCoR were upregulated in intraductal carcinomas as compared with normal mammary glands.

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Subsequently, during progression from intraductal \((n = 6)\) to invasive ductal carcinomas \((n = 22)\), both ER\(\alpha\) and NCoR expression were simultaneously downregulated. Although the numbers were small, these data suggest that loss of ER\(\alpha\) and NCoR might mark the selection of a more aggressive and hormone-unresponsive cancer. Similar studies performed by the Murphy laboratory showed that REA levels were lower in high-grade tumors \((n = 23)\) as compared with low-grade tumors \((n = 16)\) (Simon et al. 2000), although they did not detect any difference in REA expression between tumors and normal tissues \((n = 19)\) (Murphy et al. 2000). Analyzing SAFB1/2 expression in 117 invasive breast cancers, we found a significant correlation of low SAFB1/2 levels with shorter overall survival of node-positive breast cancer patients (Oesterreich et al. 2002).

It is of interest to note that the studies described above and by others (Bautista et al. 1998) have shown that cofactor levels are highly correlated with ER\(\alpha\) levels. Such coordinated expression could potentially be achieved through estrogen-mediated regulation. Indeed, as mentioned earlier, a number of cofactors, among them RIP140 (Thenot et al. 1999), AIB1 (Lauritzen et al. 2002), and SHARP (Shi et al. 2001), are regulated by estrogen.

While these findings suggest that corepressors correlate with important biomarkers and with breast cancer progression, it is essential to perform additional studies. In order to obtain data which allow final conclusions, we must clearly define the analyzed patient subsets, and we should concurrently analyze a series of ER\(\alpha\) cofactors.

**ER\(\alpha\) corepressors in the development of antiestrogen resistance**

Antiestrogen resistance is a significant problem in the treatment of ER\(\alpha\)-positive breast cancer. Approximately 50% of ER\(\alpha\)-positive breast cancers are innately resistant to tamoxifen. Almost all of those who do respond will eventually become unresponsive despite the continued presence of both the antiestrogen and functional receptor. While the precise mechanism of resistance is largely unknown, it is clear that it results from an imbalance between antiestrogens’ agonist and antagonist actions. Also, resistance is not caused by a single event but rather by a combination including the activation of growth factor-related pathways, and possibly altered levels and/or activity of ER\(\alpha\) cofactors (Schiff et al. 2003).

A number of groups have shown that, in cell lines, overexpression of corepressors results in increased antagonist activity and reversal of tamoxifen’s agonist properties (see above). The Arteaga laboratory has studied MCF-7 breast cancer cells stably transfected with the growth factor receptor HER2 – cells which lost their sensitivity to estrogen (Benz et al. 1992). Intriguingly, levels of NCoR are not altered in these cells when compared with their parental cells, but its binding with ER\(\alpha\) in the presence of tamoxifen was significantly decreased (Kurokawa et al. 2000). Blockade of HER2 and MAPK restored the ER\(\alpha\)–NCoR interaction, providing evidence that increased growth factor signaling and subsequent alterations of ER\(\alpha\)–corepressor interactions contribute to tamoxifen resistance.

In mouse models (Osborne et al. 1995), corepressor levels have been shown to correlate with antiestrogen resistance. For instance, in MCF-7 xenografts which have become resistant after prolonged tamoxifen treatment, NCoR (Lavinsky et al. 1998) and SAFB1/2 (our own unpublished data) levels are substantially decreased. Additionally, fibroblasts from mice deficient in NCoR (Jepsen et al. 2000) are resistant to tamoxifen’s antiestrogenic actions.

There are only a few studies analyzing whether corepressor levels are associated with clinical tamoxifen resistance. As often with limited numbers of studies using different study populations and limited numbers of tumor specimens, the results do not, as yet, allow solid conclusions. RIP140 and SMRT were measured in a cohort of 19 tamoxifen-resistant tumors, and there was no significant difference compared with tamoxifen-treated \((n = 6)\) or untreated \((n = 21)\) tumors (not selected for resistance) (Chan et al. 1999). Another study analyzed the expression of SRA and AIB1 relative to REA as a function of resistance, and no significant differences were found (Murphy et al. 2002). In contrast, a recent study by Girault et al. (2003) reported a strong association of NCoR levels with tamoxifen response – analyzing 99 postmenopausal patients who only received tamoxifen as adjuvant therapy, the authors determined that NCoR levels showed prognostic value that remained significant in multivariate analysis, suggesting that NCoR could be a promising predictor of tamoxifen responsiveness in patients with ER\(\alpha\)-positive breast tumors.

Clearly, more studies are needed in order to determine whether corepressors are important in antiestrogen resistance. It has been suggested by a number of groups that the ratio of multiple coactivators to corepressors rather than the expression of a single player is altered in resistant tumors. Also, as mentioned earlier (Kurokawa et al. 2000), not only total levels but also posttranslational modifications of cofactors determine the interaction with ER\(\alpha\), and the response to antiestrogen. This model has recently been substantiated in a clinical study in which levels of the ER\(\alpha\) coactivator AIB1 (but not NCoR) and HER2 were found to be associated with tamoxifen response (Osborne et al. 2003). It is our opinion that only a collaborative effort of a number of investigators using a wide range of suitable antibodies and precious tumor material would make it possible to answer the question whether ER\(\alpha\) corepressors are a link to or a cause of antiestrogen resistance.

**ER\(\alpha\) corepressors as tumor suppressor genes – a direct or indirect connection?**

There are several previously unanticipated roles of ER\(\alpha\) corepressors in breast cancer, among them their potential direct...
involvement in tumor suppression and repair mechanisms. This idea is supported by several lines of evidence. First of all, to date at least four proteins with diverse roles in DNA repair have been assigned ER$_{\alpha}$ corepressor functions. These are the O$^{6}$-methylguanine-DNA methyltransferase (MGMT) (Teo 2001), the 3-methyladenine DNA glycosylase (MPG) (Likhite et al. 2003), and, as mentioned earlier, p53 (Yu et al. 1997) and BRCA1 (Fan et al. 1999). BRCA1 and p53 are not only involved in repair, but are ‘classical’ tumor suppressor genes (Wahl & Carr 2001, Venkitaraman 2002). Our laboratory has discovered that SAFB1 and SAFB2 map, adjacent to each other, to a locus of extremely high loss of heterozygosity in breast cancer specimens (Oesterreich et al. 2001, Townson et al. 2003), and we are currently analyzing whether the SAFBs are also true breast tumor suppressor genes.

Why would there be a need to couple ER$_{\alpha}$ transcription and repair? It is proposed that an increased proliferation rate reduces the time available for DNA repair. Along with the fact that the single-stranded DNA presented during DNA replication is more susceptible to damage than double-stranded DNA, an increased mutation rate is expected in estrogen-responsive tissues. It has also been suggested that estrogen can directly cause mutations, since its metabolites can form oxygen free radicals, quinines, and DNA adducts (Cavalieri & Rogan 2002, Santen 2002). The spatial and temporal coupling of ER$_{\alpha}$ repression and DNA repair could provide timely suppression of estrogen-mediated cell proliferation when DNA damage induces repair enzymes (that also function as ER$_{\alpha}$ corepressors), and inactivation of genes which are involved in this process would result in increased genomic instability.

A major challenge in this area is to prove a direct connection between ER$_{\alpha}$ corepressor function and tumor suppression. Many of the above described proteins are large and have multiple domains, which could confer tumor suppression in a completely ER$_{\alpha}$-independent manner. Is there any evidence that, for example, BRCA1’s involvement in ER$_{\alpha}$ repression has anything to do with its function as a tumor suppressor gene in human breast cancer? Yes, indeed there is. Elegant studies by the Rosen laboratory (Fan et al. 2001) have shown that tumor-associated BRCA1 mutants failed to suppress estrogen-stimulated expression of endogenous pS2 in T47D breast cancer cells. Further elucidation of naturally occurring mutants, along with manipulation of mouse models, will help us to answer this provocative question.

**Future challenges**

The biological activity and significance of ER$_{\alpha}$ signaling pathways are much more complex than originally predicted, and the identification of most, if not all, cofactors is necessary before we can fully understand their combinatorial role in regulating steroid receptor action.

To gain more insights into roles that different ER$_{\alpha}$ corepressors play, a combination of cell lines, animal models, and human sample studies are needed. The *in vitro* experiments might include inactivation of corepressors through iRNA to decrease endogenous protein levels, and ChIP assays to further study the mechanism of sequential recruitment of ER$_{\alpha}$-containing protein complexes to estrogen-target promoters, and to generate libraries of corepressor-bound promoters. Results from ChIP studies are beginning to reveal the dynamics of the ER$_{\alpha}$ complex, the ChIP assay is, however, a freeze-frame snapshot of a multitude of unsynchronized cells that could be highly heterogeneous, and more studies are needed to understand the relevance of the ER$_{\alpha}$ complex cycling onto promoters in relation to its transcriptional activity.

One of the major challenges that will need to be overcome is the limitation of the artificial systems and the need to test hypotheses based on reductionist models of ER$_{\alpha}$ action. For example, transient transfections using overexpression of coregulators may lead to aberrant responses that are more related to non-specific squelching mechanisms than to direct responses. Additionally, the well-known cell- and promoter-specific responses of ER$_{\alpha}$ make interpretation and integration of the literature fraught with difficulties. Complicating the already obvious confounding variable of cell-type responses is the use of immortalized and transformed cell lines grown on plastic. These immortalized and transformed cells already have several genetic abnormalities that can affect the results independently of the gene being studied. In addition, it is clear that cell attachment to the extracellular matrix is a dominant regulator not only of ER$_{\alpha}$ gene expression but also of ER$_{\alpha}$ action.

Therefore, the ultimate proof that ER$_{\alpha}$ corepressors play a role in breast cancer development will obviously come from *in vivo* studies involving animal models and human tissue. We need to generate additional knockout and transgenic animals which will allow assessment of the consequences of loss or gain of corepressor function, and it is expected that these animals will show phenotypes in the mammary gland and other estrogen-regulated tissues. Although it is true that it can be difficult to translate results from animal models to humans, this is essential for our understanding of human breast cancer. Finally, there is no doubt that all these studies will need to be corroborated by the analysis of human tissue.

Breast cancer is very heterogeneous in its molecular and clinical phenotype, as well as in its therapeutic sensitivity, which presents a major challenge for both researchers and clinicians. To prove that corepressors truly play a role in breast tumorigenesis and antioestrogen resistance will require larger and better integrated efforts of basic and translational researchers. One outstanding example is the generation of the
In this review we have described the large, growing family of ERα corepressors and shown that this is a diverse set of proteins that repress ERα via a number of different mechanisms. It is naive to assume that any protein has only one function, and this is true for ERα corepressors, which seem to have multiple functions many of which are independent of ERα. More studies, and in particular new models, are needed that incorporate the new emerging understanding of the mechanisms of ERα action, and may start to account for the ability of corepressors to regulate ERα action.

Given the importance of ERα in breast cancer, and the success of breast cancer prevention and treatment with anti-estrogens, one of the highest priorities must be to better understand the molecular mechanism of ERα action. It is easy to predict that loss of ERα corepressors plays an important role in breast cancer progression, but the evidence supporting this hypothesis is limited (cell culture) or virtually non-existent (human breast cancer patients).

We are only now starting to understand the mechanisms of action of ERα corepressors. The last couple of years have shed light on the importance that they play in the biology of normal and cancer cells, but it is also true that the more we learn, the more we need to understand. Besides basic research, more clinical investigation into the biological significance of ERα corepressors is needed, so that knowledge gained at the bench will lead to a more accurate and effective management of breast cancer and endocrine resistance.

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Conclusions

In this review we have described the large, growing family of ERα corepressors and shown that this is a diverse set of proteins that repress ERα via a number of different mechanisms. It is naive to assume that any protein has only one function, and this is true for ERα corepressors, which seem to have multiple functions many of which are independent of ERα. More studies, and in particular new models, are needed that incorporate the new emerging understanding of the mechanisms of ERα action, and may start to account for the ability of corepressors to regulate ERα action.

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