

Estrogen receptor corepressors – a role in human breast cancer?

K M Dobrzycka, S M Townson, S Jiang and S Oesterreich

Baylor Breast Center, Department of Medicine and Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, BCM 600, N1110 Houston, Texas 77030, USA

(Requests for offprints should be addressed to Steffi Oesterreich; Email: stefio@breastcenter.tmc.edu)

Abstract

Estrogen receptor α (ER α) has an established role in promoting breast cancer. Transcriptional activation by ER α is a complex and multistep process, and it is influenced by coactivator and corepressor proteins that can either positively or negatively modulate ER α -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 ER α -mediated action, and that their loss could promote breast cancer development and resistance to endocrine therapy. We propose that ER α corepressors play an important biological role by controlling the magnitude of the estrogen response, mediating antiestrogen inhibition of ER α , repressing DNA-bound ER α in the absence of the ligand, and conferring active repression of ER α -downregulated genes. Different ER α 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 ER α homodimerization, alteration of ER α stability, sequestration of ER α in the cytoplasm, and effects on RNA processing. Most ER α 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 ER α 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 ER α 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.

Endocrine-Related Cancer (2003) 10 517–536

Introduction

The estrogen receptor (ER α) is a transcription factor that regulates genes involved in development, reproduction, differentiation and transformation (Osborne *et al.* 2001a). ER α 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)), ER α can bind to non-perfect or half ERE sequences, particularly in the context of appropriate flanking sequences. ER α 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).

ER α 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 ER α activity is both cell type- and promoter-specific.

Recently, a second ER has been discovered, termed ER β (Kuiper *et al.* 1996). ER α and ER β are encoded by different genes and clearly have both overlapping and different functions (Paech *et al.* 1997). Our review will focus on ER α , since ER β was discovered more recently, and thus few

studies have addressed ER β –corepressor interactions. As suggested by recent studies there will be some corepressors shared between ER α and ER β (Montano *et al.* 1999), and there will be others which have different effects on ER α and ER β (Seol *et al.* 1998). Interestingly, *in vitro* and *in vivo* data show that ER α is negatively regulated by ER β (Hall & McDonnell 1999, Weihua *et al.* 2000). It is clear that additional studies need to be conducted to understand the role of ER β 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 ER α is well established, the importance of corepressors is still somewhat controversial. This controversy mainly arises from the dogma that ER α '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 (Baniahmad *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 ER α only binds to DNA in the presence of ligand, eliminating the perceived need for corepressors. However, an increasing number of ER α 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 ER α -mediated actions, and (b) their loss could be involved in breast cancer development and resistance to endocrine breast cancer treatment.

ER α corepressors

Definition of ER α 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 ER α and repress its action. For example, this includes proteins that cannot affect transcription themselves, but can repress ER α via competition with coactivators or with DNA binding. As our understanding of ER α 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 ER α 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 ER α corepressors

Given the broad definition of ER α 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 ER α can also interact with these corepressors in the presence of antagonist (Xu *et al.* 1996, Jackson *et al.* 1997, Smith *et al.* 1997, Lavinsky *et al.* 1998).

Yeast two-hybrid screens have often been applied to identify ER α corepressors, such as the repressor of ER α 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 antiestrogen 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 ER α (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 ER α actions.

Table 1 Estrogen receptor corepressors. The binding sites of corepressors in ER α 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

Name	ER binding site	Mechanisms of repression	Other cellular functions	References
NCoR	LBD	HDACs	Repression of other of transcription factors	Lavinsky <i>et al.</i> (1998)
SMRT	LBD	HDACs	Repression of other of transcription factors	Smith <i>et al.</i> (1997)
SHARP	(SMRT)	HDACs, competition with SRA		Shi <i>et al.</i> (2001)
SAFB1	DBD/hinge	HDAC-dependent and independent	RNA and S/MAR binding, inhibits cell growth	Oesterreich <i>et al.</i> (2000)
SAFB2	ND	ND	Inhibition of cell growth	Townson <i>et al.</i> (2003)
RIP140	LBD	HDACs, CtBP, competition with coactivators	Interaction with other NRs	Cavaillès <i>et al.</i> (1995)
LCoR	LBD	HDAC-dependent and independent		Fernandes <i>et al.</i> (2003)
SHP	AF-2	Competition with coactivators, interference with DNA binding	Inhibition of bile acid synthesis	Johansson <i>et al.</i> (1999)
DAX-1	AF-2	Competition with coactivators, inhibition of ER α dimerization, competition for ERE	Cofactor for SF-1	Zhang <i>et al.</i> (2000)
COUP-TF	ND	Inhibition of ER α DNA binding	Negative regulation of a range of NRs	Klinge <i>et al.</i> (1997)
DP97	LBD/AF-2	ND	ATP-dependent RNA helicase	Rajendran <i>et al.</i> (2003)
NSD1	LBD	ND	HMTase activity	Huang <i>et al.</i> (1998)
BRCA1	LBD/AF-2	CtIP interaction, p300 down regulation	DNA repair, recombination transcription	Fan <i>et al.</i> (1999, 2001)
MTA1	AF-2	HDACs	Increase in metastasis, member of NURD complex	Mazumdar <i>et al.</i> (2001)
MTA1s	AF-1, DBD, AF-2	Sequestration of ER α in cytoplasm		Kumar <i>et al.</i> (2002)
RTA	Af-1	HDAC-independent	RNA binding	Norris <i>et al.</i> (2002)
REA	LBD	Competition with coactivators		Montano <i>et al.</i> (1999)
FKHR	LBD	ND	Transcription factor that regulates apoptosis and cell cycle	Zhao <i>et al.</i> (2001)
TR2	DBD/hinge LBD/AF-2	Inhibition of ER α dimerization	Transcription factor, interaction with HDACs	Hu <i>et al.</i> (2002)
NEDD8	ND	Proteolysis of ER α	Modification of cullins	Fan <i>et al.</i> (2003)
TAF-I β	DBD/hinge F domain	Decrease of ER α acetylation Increase of ER α -DNA binding	Decrease of histone acetylation and modulation chromatin structure	Loven <i>et al.</i> (2003b)
Smad4	Af-1	ND	Transcriptional regulator	Wu <i>et al.</i> (2003)
p53	ND	Inhibition of ER α -ERE binding	DNA repair, inhibition of apoptosis and cell growth	Yu <i>et al.</i> (1997)

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

RIP140 (or nuclear receptor interacting protein 1, Nrip1) was originally identified as an ER α coactivator by expression cloning using the ER α AF-2 in the presence of estrogen (Cavaillès *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 ER α corepressor (Lee *et al.* 1998, Treuter *et al.* 1998, Lee & Wei 1999). There are a number of ER α cofactors which have

been proposed to function as both coactivators and corepressors, including the FKHR (forkhead homolog in rhabdomyosarcoma) (Schoor *et al.* 2001, Zhao *et al.* 2001), ERR α (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 ER α . 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 ER α . 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 ER α by glutathione *S*-transferase (GST)-pulldown and in mammalian two-hybrid assays, and to repress ER α '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 ER α corepressor.

Our laboratory is studying the ER α 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 ERE-like elements, we began to consider a potential role of SAFB1 in ER α '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 ER α and repress its activity.

As with coactivators, the list of ER α corepressors is growing, and other recently described ER α corepressors include TERP-1 (truncated estrogen receptor product-1) (Resnick *et al.* 2000), the POU transcription factor Brn-3a (Budhram-Mahadeo *et al.* 1998), NEDD8 (neural precursor cell-expressed developmentally downregulated) (Fan *et al.* 2002), TAF-I β (template-activating factor-I β) (Loven *et al.* 2003b), pp32 (Loven *et al.* 2003a), and Smad4 (Wu *et al.* 2003). It is likely that cofactors which have been described to repress other NRs, such as Alien (Dressel *et al.* 1999, Polly *et al.* 2000), PSF (polypyrimidine tract-binding protein) (Mathur *et al.* 2001), and SUN-CoR (small unique nuclear receptor corepressor) (Zamir *et al.* 1997), might also modulate ER α activity.

Corepressor-interaction domains in ER α

To date, corepressors have been identified that interact with ER α in AF-1, DBD/hinge, and LBD/AF-2 regions (see also

Table 1). The majority of reported ER α 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.

ER α -interaction domains in corepressors

ER α coactivators and corepressors differ in their interaction with ER α . In contrast to ER α 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 ER α 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). This motif includes a L/IXXI/VI sequence called the CoRNR box (Hu & Lazar 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 ER α (Qin & Schiff, unpublished observations). Other corepressors such as REA (Delage-Mourroux *et al.* 2000), and DP97 (Rajendran *et al.* 2003) harbor novel ER α interaction domains. Likewise, SAFB1 mediates ER α 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 ER α and corepressors

The interaction of NR boxes with the ER α 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 ER α agonism and antagonism. The big questions, however, are these: if only the antiestrogen-occupied receptor conformation allows corepressor recruitment, are corepressors

a pharmacological anomaly? Or are we going to find novel natural ligands with structural similarity to antagonists? Or do ER α corepressors play other important roles in the regulation of ER α , independent of antiestrogen binding? While we will provide evidence for the latter possibility in the following section, it is clear that to fully understand ER α –corepressor interactions, we need to know the structure of full-length ER α (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 ER α and thus affects interaction with cofactors (Hall *et al.* 2002), suggesting that co-crystals of ER α 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 ER α 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 ER α 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 ER α , 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 ER α -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 ER α 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 ER α 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 ER α corepressor BRCA1 interacts with CtIP, a protein originally identified on the basis of its association with the C-terminal binding protein CtBP (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 ER α 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.

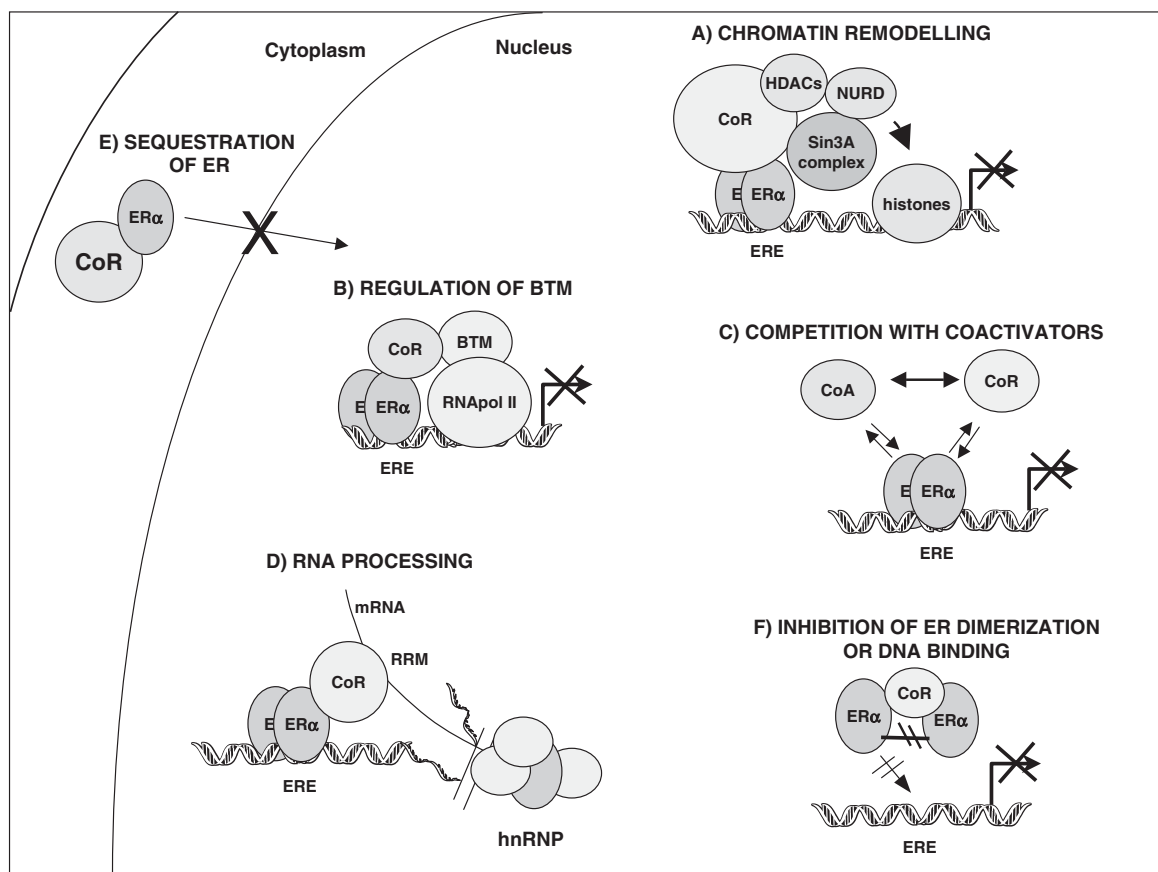


Figure 1 Mechanisms of ER α corepressor action include (A) recruitment of histone deacetylase and nucleosome remodeling complexes, (B) interaction with the basal transcription machinery, (C) competition with coactivators, (D) interference with RNA processing, (E) sequestration of ER α in the cytoplasm, and (F) interference with ER α dimerization and DNA binding. Potentially more than one mechanism can be employed by the same corepressors, and other novel mechanisms are still being elucidated. CoR, corepressor; CoA, coactivator; RRM, RNA recognition motif; BTM, basal transcription machine; hnRNP, heterogenous nuclear ribonucleoprotein.

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 TFIIB, TAF_{II}32, and TAF_{II}70 (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 ER α binding sites, and can reverse coactivator-mediated enhancement of ER α 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 ER α 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 ER α 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 ER α coactivators, less is known about ER α 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 ER α corepression in the case of RTA (Norris *et al.* 2002). For SAFB1 (SM Townson, 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. RRM is 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 ER α activity involve inhibition of ER α 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 ER α stability (BRCA1, NEDD8) (Brzovic *et al.* 2003, Fan *et al.* 2003), sequestration of ER α 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 ER α corepressors in Fig. 1. It is likely that synergy between different pathways cooperates to fully inhibit ER α transcriptional activity, and that the presence of different mechanisms controlling ER α creates an integrated response to a variety of different cellular signaling pathways. A major challenge is to unravel how these diverse mecha-

nisms 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 ER α corepressors in the regulation of ER α activities. These activities (schematically illustrated in Fig. 2) include involvement in antiestrogen-mediated inhibition of ER α , control of the magnitude of the estrogen response, repression of apo-ER α (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 ER α activities.

Role in mediating antiestrogen action

Currently, antiestrogens such as tamoxifen are the most effective and commonly prescribed treatments for patients with ER α -positive breast cancer. There is increasing evidence that antiestrogen-mediated inhibition of ER α 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 ER α 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 ER α 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. Coimmunoprecipitation studies in cell lines in the absence and presence of different ligands are hampered by the rapid effects on ER α levels as a result of proteasome-mediated degradation (Nawaz *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-

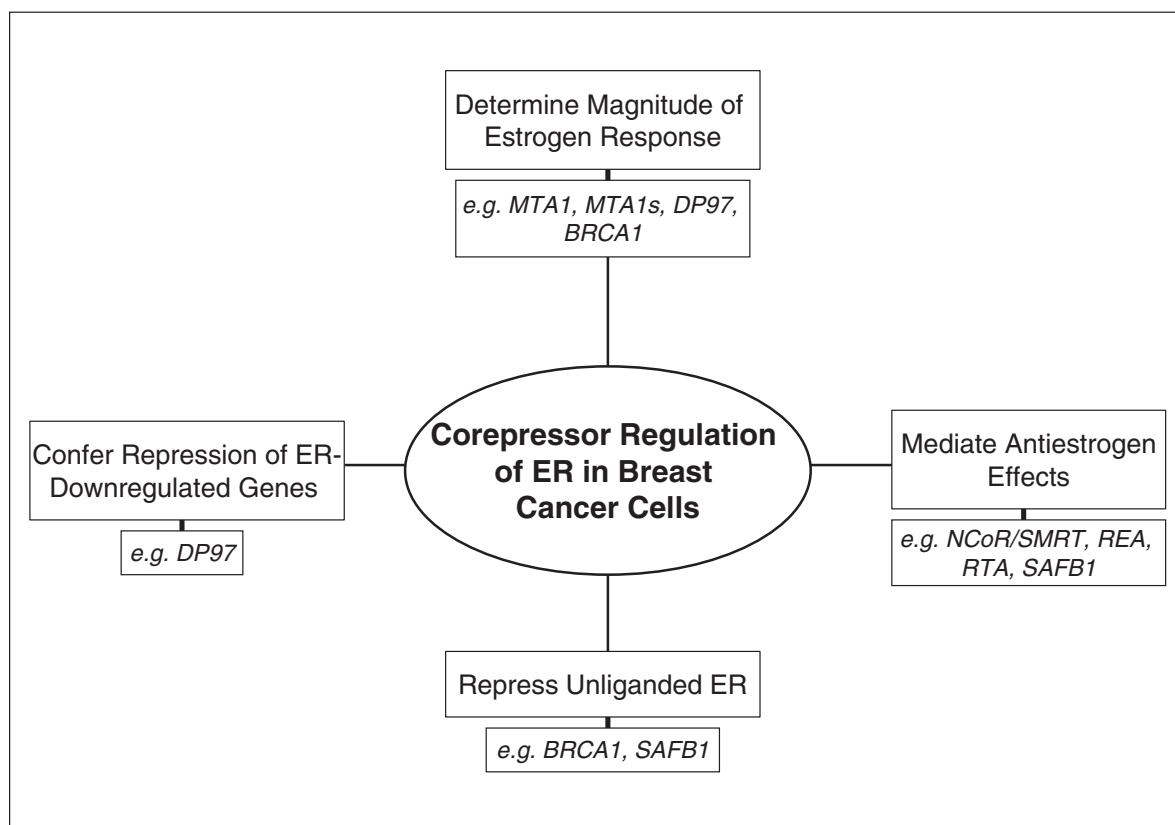


Figure 2 Role of corepressors in regulating a variety of ER α functions in breast cancer. Examples of corepressors that have been shown to mediate these effects are given.

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 ER α .

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, ER α 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 ER α suggesting that more

studies are needed to clarify the role of NCoR in ER α 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 ER α -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, ER α corepressors can affect ER α 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.*

2002) overexpression leads to decreased expression of the estrogen-induced genes *c-myc* and *pS2*, and siRNA-mediated depletion of *DP97* results in increased estrogen induction of *pS2* and *WISP2* (Rajendran *et al.* 2003).

Overexpression of an ER α -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 ER α (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, Fernandes *et al.* (2003) have shown that the ER α corepressor LCoR specifically recognizes agonist-bound ER α , 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 (Lauritsen *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 ER α 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 ER α 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 ER α were utilized to study the response to estrogens and antiestrogens. Interestingly, tamoxifen's antagonist activity was abolished in NCoR^{-/-}MEFs; however, at least under the experimental conditions tested, there was no effect on ligand-independent or estrogen-mediated activation of ER α . Rather unexpectedly, estrogen-induction of ER α was diminished in BRCA1^{-/-}MEFs (Zheng *et al.* 2001), while ligand-independent activation of ER α was dramatically increased. To get around the embryonic lethality observed in the BRCA1 germline 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 ER α 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 ER α -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 ER α corepressors is ongoing, and without doubt will provide additional decisive evidence for ER α corepressors and their role in hormone responses.

Role in repressing apo-ER α

The classical model of ER α action involves the following sequences of events: interaction of ER α monomers with chaperones, dissociation of chaperones and formation of homodimers upon estrogen binding to the ER α 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 that apo-ER α (i.e. unliganded ER α) is not bound to DNA. A number of *in vitro* studies have shown that unliganded ER α 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 ER α to estrogen-responsive promoters in the absence of ligand. These findings parallel those of Belmont's group who, reconstructing and visualizing transcriptional regulation and chromatin structure, has found that the apo-ER α was able to decondense 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 ER α , ligand-induced coactivator binding, or histone hyperacetylation.

The importance of apo-ER α 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 ER α 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 ER α in check, i.e. preventing promoter activation by the ER α ’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 ER α , and recent studies suggest that they are indeed involved in repression of DNA-bound apo-ER α . BRCA1^{-/-}MEFs show increased ligand-independent ER α 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 ER α not only in the presence but also in the absence of ligand, implicating SAFB1 in ligand-independent repression of ER α (SM Townson, RL Kang, AV Lee & S Oesterreich, unpublished observations).

It is necessary to dissect mechanistic details of DNA-binding of apo-ER α which is actively repressing, and DNA-binding of unliganded ER α 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 ER α cofactor AIB1 interacts with the corepressor NCoR (Li *et al.* 2002b).

Role in conferring repression of ER α -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 (Lauritsen *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, ER α has also been indirectly connected to E-cadherin expression – absence of MTA3 in ER α -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 ER α action by directly inhibiting ER α (MTA1, MTA1s) and by indirectly regulating ER α target genes (MTA3).

Earlier studies have provided circumstantial evidence that ER α coregulators are involved in the ER α -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-ER α repression of the folate receptor FR- α , whereas none of the tested ER α 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 ER α -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 ER α

For years there have been sporadic reports of a membrane-bound ER α responsible for certain very rapid effects of estrogen in cells including breast cancer cells. Several lines of evidence suggest that such ‘non-genomic’ ER α 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 ER α 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) ER α 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 ER α . How this regulation might be achieved is so far unclear. It has been proposed that the ER α coactivator PELP1/MNAR (proline-, glutamic acid-, and leucine-rich protein-1/modulator of non-genomic activity of ER α) can regulate ER α '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 ER α in the cytoplasm and prevents ligand-induced nuclear translocation, ultimately resulting in breast tumors with low or no nuclear ER α activity. Similarly, the ER α 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 ER α , and future studies will offer insights into the potential role of SAFB2 in regulating cytoplasmic ER α .

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 ER α 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 ER α 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 ER α -positive upon initial diagnosis, and in the majority of those cancers ER α 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.

ER α corepressors and breast cancer – results from cell line and mouse studies

An important question that scientists are now facing is the significance of ER α corepressors *in vivo*. As discussed above, there is compelling evidence from a number of laboratories that corepressors are involved in a multitude of ER α functions. How does this translate to the biology of breast cancer cells? Not surprisingly, several ER α 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 ER α -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 ER α 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 ER α 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 ER α corepressors in human breast tumors

To date, very few studies have addressed the question of ER α 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.

Subsequently, during progression from intraductal ($n = 6$) to invasive ductal carcinomas ($n = 22$), both ER α and NCoR expression were simultaneously downregulated. Although the numbers were small, these data suggest that loss of ER α 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 α 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 (Lauritsen *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 α cofactors.

ER α corepressors in the development of antiestrogen resistance

Antiestrogen resistance is a significant problem in the treatment of ER α -positive breast cancer. Approximately 50% of ER α -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 α 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 α in the presence of tamoxifen was significantly decreased (Kurokawa *et al.* 2000). Blockade of HER2

and MAPK restored the ER α -NCoR interaction, providing evidence that increased growth factor signaling and subsequent alterations of ER α -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 α -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 α , and the response to antiestrogen. This model has recently been substantiated in a clinical study in which levels of the ER α 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 α corepressors are a link to or a cause of antiestrogen resistance.

ER α corepressors as tumor suppressor genes – a direct or indirect connection?

There are several previously unanticipated roles of ER α 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 α corepressor functions. These are the O⁶-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 α 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 α repression and DNA repair could provide timely suppression of estrogen-mediated cell proliferation when DNA damage induces repair enzymes (that also function as ER α 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 α 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 α -independent manner. Is there any evidence that, for example, BRCA1’s involvement in ER α 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 α 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 α 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 α -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 α 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 α 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 α 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 α 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 α gene expression but also of ER α action.

Therefore, the ultimate proof that ER α 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 antiestrogen resistance will require larger and better integrated efforts of basic and translational researchers. One outstanding example is the generation of the

Nuclear Receptor Signaling Atlas (NURSA) (<http://www.nursa.org>), a web-based 'resource within which data in all areas of this discipline can be freely accessed, shared and evaluated by the entire community'. This unique resource should foster a synergistic and multidisciplinary approach not only to common intellectual problems but also to clinical applications.

Conclusions

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

Given the importance of ER α 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 ER α action. It is easy to predict that loss of ER α 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 ER α 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 ER α 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.

Acknowledgements

We sincerely apologize to all authors for the many outstanding papers that could not be referenced due to space limitations. S O is supported by an NIH grant (R01 CA97213), and is the recipient of a Women's Health Research Award (Eli Lilly). S M T and S J are supported by postdoctoral fellowships from the Department of Defense (DAMD 17-01-0146 and DAMD 17-03-01-0323). We thank Drs Adrian Lee and Gary Chamness for critical comments on this review article.

References

Alland L, Muhle R, Hou H Jr, Potes J, Chin L, Schreiber-Agus N & DePinho RA 1997 Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. *Nature* **387** 49–55.

- Auboeuf D, Honig A, Berget SM & O'Malley BW 2002 Coordinate regulation of transcription and splicing by steroid receptor coregulators. *Science* **298** 416–419.
- Balaseshthil S & Vadlamudi RK 2003 Functional interactions between the estrogen receptor coactivator PELP1/MNAR and retinoblastoma protein. *Journal of Biological Chemistry* **278** 22119–22127.
- Baniahmad A, Kohne AC & Renkawitz R 1992 A transferable silencing domain is present in the thyroid hormone receptor, in the v-erbA oncogene product and in the retinoic acid receptor. *EMBO Journal* **11** 1015–1023.
- Bautista S, Valles H, Walker RL, Anzick S, Zeillinger R, Meltzer P & Theillet C 1998 In breast cancer, amplification of the steroid receptor coactivator gene AIB1 is correlated with estrogen and progesterone receptor positivity. *Clinical Cancer Research* **4** 2925–2929.
- Benz CC, Scott GK, Sarup JC, Johnson RM, Tripathy D, Coronado E, Shepard HM & Osborne CK 1992 Estrogen-dependant, tamoxifen-resistant tumorigenic growth of MCF-7 cells transfected with HER2/neu. *Breast Cancer Research and Treatment* **24** 85–95.
- Brodie SG & Deng CX 2001 BRCA1-associated tumorigenesis: what have we learned from knockout mice? *Trends in Genetics* **17** S18–S22.
- Brown M & Sharp PA 1990 Human estrogen receptor forms multiple protein–DNA complexes. *Journal of Biological Chemistry* **265** 11238–11243.
- Brzovic PS, Keffe JR, Nishikawa H, Miyamoto K, Fox D 3rd, Fukuda M, Ohta T & Klevit R 2003 Binding and recognition in the assembly of an active BRCA1/BARD1 ubiquitin-ligase complex. *PNAS* **100** 5646–5651.
- Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson JA & Carlquist M 1997 Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **389** 753–758.
- Budhram-Mahadeo V, Parker M & Latchman DS 1998 POU transcription factors Brn-3a and Brn-3b interact with the estrogen receptor and differentially regulate transcriptional activity via an estrogen response element. *Molecular and Cellular Biology* **18** 1029–1041.
- Cavailles V, Dauvois S, L'Horsset F, Lopez G, Hoare S, Kushner PJ & Parker MG 1995 Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. *EMBO Journal* **14** 3741–3751.
- Cavaliere EL & Rogan EG 2002 A unified mechanism in the initiation of cancer. *Annals of the New York Academy of Sciences* **959** 341–354.
- Chan CM, Lykkesfeldt AE, Parker MG & Dowsett M 1999 Expression of nuclear receptor interacting proteins TIF-1, SUG-1, receptor interacting protein 140, and corepressor SMRT in tamoxifen-resistant breast cancer. *Clinical Cancer Research* **5** 3460–3467.
- Charpentier AH, Bednarek AK, Daniel RL, Hawkins KA, Laffin KJ, Gaddis S, MacLeod MC & Aldaz CM 2000 Effects of estrogen on global gene expression: identification of novel targets of estrogen action. *Cancer Research* **60** 5977–5983.
- Chen JD & Evans RM 1995 A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* **377** 454–457.
- Chinnadurai G 2002 CtBP, an unconventional transcriptional corepressor in development and oncogenesis. *Molecular Cell* **9** 213–224.

- Chong MT & Lippman ME 1982 Effects of RNA and ribonuclease on the binding of estrogen and glucocorticoid receptors from MCF-7 cells to DNA-cellulose. *Journal of Biological Chemistry* **257** 2996–3002.
- Cid MC, Schnaper HW & Kleinman HK 2002 Estrogens and the vascular endothelium. *Annals of the New York Academy of Sciences* **966** 143–157.
- Deckert J & Struhl K 2001 Histone acetylation at promoters is differentially affected by specific activators and repressors. *Molecular and Cellular Biology* **21** 2726–2735.
- Delage-Mourroux R, Martini PG, Choi I, Kraichely DM, Hoeksema J & Katzenellenbogen BS 2000 Analysis of estrogen receptor interaction with a repressor of estrogen receptor activity (REA) and the regulation of estrogen receptor transcriptional activity by REA. *Journal of Biological Chemistry* **275** 35848–35856.
- Deng CX 2002 Tumor formation in Brca1 conditional mutant mice. *Environmental and Molecular Mutagenesis* **39** 171–177.
- Deng CX & Brodie SG 2001 Knockout mouse models and mammary tumorigenesis. *Seminars in Cancer Biology* **11** 387–394.
- Dhandapani KM & Brann DW 2002 Protective effects of estrogen and selective estrogen receptor modulators in the brain. *Biology of Reproduction* **67** 1379–1385.
- Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, Butel JS & Bradley A 1992 Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356** 215–221.
- Dressel U, Thormeyer D, Altincicek B, Paululat A, Eggert M, Schneider S, Tenbaum SP, Renkawitz R & Baniahmad A 1999 Alien, a highly conserved protein with characteristics of a corepressor for members of the nuclear hormone receptor superfamily. *Molecular and Cellular Biology* **19** 3383–3394.
- Escriva H, Delaunay F & Laudet V 2000 Ligand binding and nuclear receptor evolution. *BioEssays* **22** 717–727.
- Fan M, Bigsby RM & Nephew KP 1999 BRCA1 inhibition of estrogen receptor signaling in transfected cells. *Science* **284** 1354–1356.
- Fan M, Long X, Bailey JA, Reed CA, Osborne E, Gize EA, Kirk EA, Bigsby RM & Nephew KP 2001 Role of direct interaction in BRCA1 inhibition of estrogen receptor activity. *Oncogene* **20** 77–87.
- Fan S, Ma YX, Wang C, Yuan RQ, Meng Q, Wang JA, Erdos M, Goldberg ID, Webb P, Kushner PJ *et al.* 2002 The activating enzyme of NEDD8 inhibits steroid receptor function. *Molecular Endocrinology* **16** 315–330.
- Fan M, Bigsby RM & Nephew KP 2003 The NEDD8 pathway is required for proteasome-mediated degradation of human estrogen receptor (ER)-alpha and essential for the antiproliferative activity of ICI 182,780 in ERalpha-positive breast cancer cells. *Molecular Endocrinology* **17** 356–365.
- Fernandes I, Bastien Y, Wai T, Nygard K, Lin R, Cormier O, Lee HS, Eng F, Bertos NR, Pelletier N *et al.* 2003 Ligand-dependent nuclear receptor corepressor LCoR functions by histone deacetylase-dependent and -independent mechanisms. *Molecular Cell* **11** 139–150.
- Franco PJ, Farooqui M, Seto E & Wei LN 2001 The orphan nuclear receptor TR2 interacts directly with both class I and class II histone deacetylases. *Molecular Endocrinology* **15** 1318–1328.
- Fujita N, Jaye DL, Kajita M, Geigerman C, Moreno CS & Wade PA 2003 MTA3, a Mi-2/NuRD complex subunit, regulates an invasive growth pathway in breast cancer. *Cell* **113** 207–219.
- Girault I, Lerebours F, Amarir S, Tozlu S, Tubiana-Hulin M, Lidereau R & Bieche I 2003 Expression analysis of estrogen receptor alpha coregulators in breast carcinoma: evidence that NCOR1 expression is predictive of the response to tamoxifen. *Clinical Cancer Research* **9** 1259–1266.
- Glass CK, Rose DW & Rosenfeld MG 1997 Nuclear receptor coactivators. *Current Opinion in Cell Biology* **2** 222–232.
- Gottardis MM, Saceda M, Garcia-Morales P, Fung YK, Solomon H, Sholler PF, Lippman ME & Martin MB 1995 Regulation of retinoblastoma gene expression in hormone-dependent breast cancer. *Endocrinology* **136** 5659–5665.
- Guenther MG, Lane WS, Fischle W, Verdin E, Lazar MA & Shiekhata R 2000 A core SMRT corepressor complex containing HDAC3 and TBL1, a WD40-repeat protein linked to deafness. *Genes and Development* **14** 1048–1057.
- Hall JM & McDonnell DP 1999 The estrogen receptor beta-isoform (ERbeta) of the human estrogen receptor modulates ERalpha transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. *Endocrinology* **140** 5566–5578.
- Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B & King MC 1990 Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* **250** 1684–1689.
- Hall JM, McDonnell DP & Korach KS 2002 Allosteric regulation of estrogen receptor structure, function, and coactivator recruitment by different estrogen response elements. *Molecular Endocrinology* **16** 469–486.
- Heery DM, Hoare S, Hussain S, Parker MG & Sheppard H 2001 Core LXXLL motif sequences in CREB-binding protein, SRC1, and RIP140 define affinity and selectivity for steroid and retinoid receptors. *Journal of Biological Chemistry* **276** 6695–6702.
- Heinzel T, Lavinsky RM, Mullen TM, Soderstrom M, Laherty CD, Torchia J, Yang WM, Brard G, Ngo SD, Davie JR *et al.* 1997 A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. *Nature* **387** 43–48.
- Hermanson O, Glass CK & Rosenfeld MG 2002 Nuclear receptor coregulators: multiple modes of modification. *Trends in Endocrinology and Metabolism* **13** 55–60.
- Hodges LC, Cook JD, Lobenhofer EK, Li L, Bennett L, Bushel PR, Aldaz CM, Afshari CA & Walker CL 2003 Tamoxifen functions as a molecular agonist inducing cell cycle-associated genes in breast cancer cells. *Molecular Cancer Research* **1** 300–311.
- Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK *et al.* 1995 Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* **377** 397–404.
- Hu X & Lazar MA 1999 The CoNR motif controls the recruitment of corepressors by nuclear hormone receptors. *Nature* **402** 93–96.
- Hu X, Li Y & Lazar MA 2001 Determinants of CoNR-dependent repression complex assembly on nuclear hormone receptors. *Molecular and Cellular Biology* **21** 1747–1758.
- Hu YC, Shyr CR, Che W, Mu XM, Kim E & Chang C 2002 Suppression of estrogen receptor-mediated transcription and cell growth by interaction with TR2 orphan receptor. *Journal of Biological Chemistry* **277** 33571–33579.
- Huang EY, Zhang J, Miska EA, Guenther MG, Kouzarides T & Lazar MA 2000 Nuclear receptor corepressors partner with class II histone deacetylases in a Sin3-independent repression pathway. *Genes and Development* **14** 45–54.

- Huang HJ, Norris JD & McDonnell DP 2002 Identification of a negative regulatory surface within estrogen receptor alpha provides evidence in support of a role for corepressors in regulating cellular responses to agonists and antagonists. *Molecular Endocrinology* **16** 1778–1792.
- Huang N, vom Baur E, Garnier JM, Lerouge T, Vonesch JL, Lutz Y, Chambon P & Losson R 1998 Two distinct nuclear receptor interaction domains in NSD1, a novel SET protein that exhibits characteristics of both corepressors and coactivators. *EMBO Journal* **17** 3398–3412.
- Inoue S, Orimo A, Hosoi T, Kondo S, Toyoshima H, Kondo T, Ikegami A, Ouchi Y, Orimo H & Muramatsu M 1993 Genomic binding-site cloning reveals an estrogen-responsive gene that encodes a RING finger protein. *PNAS* **90** 11117–11121.
- Jackson TA, Richer JK, Bain DL, Takimoto GS, Tung L & Horwitz KB 1997 The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. *Molecular Endocrinology* **11** 693–705.
- Jakacka M, Ito M, Weiss J, Chien PY, Gehm BD & Jameson JL 2001 Estrogen receptor binding to DNA is not required for its activity through the nonclassical API pathway. *Journal of Biological Chemistry* **276** 13615–13621.
- Jepsen K & Rosenfeld MG 2002 Biological roles and mechanistic actions of co-repressor complexes. *Journal of Cell Science* **115** 689–698.
- Jepsen K, Hermanson O, Onami TM, Gleiberman AS, Lunyak V, McEville RJ, Kurokawa R, Kumar V, Liu F, Seto E et al. 2000 Combinatorial roles of the nuclear receptor corepressor in transcription and development. *Cell* **102** 753–763.
- Johansson L, Thomsen JS, Damdimopoulos AE, Spyrou G, Gustafsson JA & Treuter E 1999 The orphan nuclear receptor SHP inhibits agonist-dependent transcriptional activity of estrogen receptor alpha and beta. *Journal of Biological Chemistry* **274** 345–353.
- Johansson L, Bavner A, Thomsen JS, Farnegardh M, Gustafsson JA & Treuter E 2000 The orphan nuclear receptor SHP utilizes conserved LXXLL-related motifs for interactions with ligand-activated estrogen receptors. *Molecular and Cellular Biology* **20** 1124–1133.
- Kao HY, Downes M, Ordentlich P & Evans RM 2000 Isolation of a novel histone deacetylase reveals that class I and class II deacetylases promote SMRT-mediated repression. *Genes and Development* **14** 55–66.
- Kato S, Kitamoto T, Masuhiro Y & Yanagisawa J 1998 Molecular mechanism of a cross-talk between estrogen and growth-factor signaling pathways. *Oncology* **55** 5–10.
- Keen JC & Davidson NE 2003 The biology of breast carcinoma. *Cancer* **97** 825–833.
- Kelley KM, Rowan BG & Ratnam M 2003 Modulation of the folate receptor alpha gene by the estrogen receptor: mechanism and implications in tumor targeting. *Cancer Research* **63** 2820–2828.
- Kim J, Petz LN, Ziegler YS, Wood JR, Potthoff SJ & Nardulli AM 2000 Regulation of the estrogen-responsive pS2 gene in MCF-7 human breast cancer cells. *Journal of Steroid Biochemistry and Molecular Biology* **74** 157–168.
- Klinge CM 2000 Estrogen receptor interaction with co-activators and co-repressors. *Steroids* **65** 227–251.
- Klinge CM 2001 Estrogen receptor interaction with estrogen response elements. *Nucleic Acids Research* **29** 2905–2919.
- Klinge CM, Silver BF, Driscoll MD, Sathya G, Bambara RA & Hilf R 1997 Chicken ovalbumin upstream promoter-transcription factor interacts with estrogen receptor, binds to estrogen response elements and half-sites, and inhibits estrogen-induced gene expression. *Journal of Biological Chemistry* **272** 31465–31474.
- Klinge CM, Jernigan SC & Risinger KE 2002 The agonist activity of tamoxifen is inhibited by the short heterodimer partner orphan nuclear receptor in human endometrial cancer cells. *Endocrinology* **143** 853–867.
- Kokura K, Kaul SC, Wadhwa R, Nomura T, Khan MM, Shinagawa T, Yasukawa T, Colmenares C & Ishii S 2001 The Ski protein family is required for MeCP2-mediated transcriptional repression. *Journal of Biological Chemistry* **276** 34115–34121.
- Kraus RJ, Ariazi EA, Farrell ML & Mertz JE 2002 Estrogen-related receptor alpha 1 actively antagonizes estrogen receptor-regulated transcription in MCF-7 mammary cells. *Journal of Biological Chemistry* **277** 24826–24834.
- Kuiper GG, Enmark E, Peltö-Huikko M, Nilsson S & Gustafsson JA 1996 Cloning of a novel receptor expressed in rat prostate and ovary. *PNAS* **93** 5925–5930.
- Kumar R, Wang RA, Mazumdar A, Talukder AH, Mandal M, Yang Z, Bagheri-Yarmand R, Sahin A, Hortobagyi G, Adam L et al. 2002 A naturally occurring MTA1 variant sequesters oestrogen receptor-alpha in the cytoplasm. *Nature* **418** 654–657.
- Kurebayashi J, Otsuki T, Kunisue H, Tanaka K, Yamamoto S & Sonoo H 2000 Expression levels of estrogen receptor-alpha, estrogen receptor-beta, coactivators, and corepressors in breast cancer. *Clinical Cancer Research* **6** 512–518.
- Kurokawa H, Lenferink A, Simpson J, Pisacane P, Sliwkowski M, Forbes J & Arteaga C 2000 Inhibition of HER2/neu (erbB-2) and mitogen-activated protein kinases enhances tamoxifen action against HER2-overexpressing, tamoxifen-resistant breast cancer cells. *Cancer Research* **60** 5887–5894.
- Kurokawa R, Soderstrom M, Horlein A, Halachmi S, Brown M, Rosenfeld MG & Glass CK 1995 Polarity-specific activities of retinoic acid receptors determined by a co-repressor. *Nature* **377** 451–454.
- Kushner PJ, Agard DA, Greene GL, Scanlan TS, Shiau AK, Uht RM & Webb P 2000 Estrogen receptor pathways to AP-1. *Journal of Steroid Biochemistry and Molecular Biology* **74** 311–317.
- Lanz RB, McKenna NJ, Onate SA, Albrecht U, Wong J, Tsai SY, Tsai MJ & O'Malley BW 1999 A steroid receptor coactivator, SRA, functions as an RNA and is present in an SRC-1 complex. *Cell* **97** 17–27.
- Lauritsen KJ, List HJ, Reiter R, Wellstein A & Riegel AT 2002 A role for TGF-beta in estrogen and retinoid mediated regulation of the nuclear receptor coactivator AIB1 in MCF-7 breast cancer cells. *Oncogene* **21** 7147–7155.
- Lavinsky RM, Jepsen K, Heinzl T, Torchia J, Mullen TM, Schiff R, Del-Rio AL, Ricote M, Ngo S, Gemsch J et al. 1998 Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. *PNAS* **95** 2920–2925.
- Lee CH & Wei LN 1999 Characterization of receptor-interacting protein 140 in retinoid receptor activities. *Journal of Biological Chemistry* **274** 31320–31326.
- Lee CH, Chinpaisal C & Wei LN 1998 Cloning and characterization of mouse RIP140, a corepressor for nuclear orphan receptor TR2. *Molecular and Cellular Biology* **18** 6745–6755.

- Levin ER 2002 Cellular functions of plasma membrane estrogen receptors. *Steroids* **67** 471–475.
- Levine AJ 1997 p53, the cellular gatekeeper for growth and division. *Cell* **88** 323–331.
- Li J, Wang J, Nawaz Z, Liu JM, Qin J & Wong J 2000 Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. *EMBO Journal* **19** 4342–4350.
- Li J, Lin Q, Wang W, Wade P & Wong J 2002a Specific targeting and constitutive association of histone deacetylase complexes during transcriptional repression. *Genes and Development* **16** 687–692.
- Li X, Kimbrel EA, Kenan DJ & McDonnell DP 2002b Direct interactions between corepressors and coactivators permit the integration of nuclear receptor-mediated repression and activation. *Molecular Endocrinology* **16** 1482–1491.
- Likhite VS, Kass EI, Nardulli AM, Anderson SD & Yates JR 2003 A novel interaction between estrogen receptor and 3-methyladenine DNA glycosylase. *The Endocrine Society's 85th Annual Meeting*, Philadelphia, PA, USA.
- Liu G, Schwartz JA & Brooks SC 1999 p53 down-regulates ER-responsive genes by interfering with the binding of ER to ERE. *Biochemical and Biophysical Research Communications* **264** 359–364.
- Liu Y, Asch H & Kulesz-Martin MF 2001 Functional quantification of DNA-binding proteins p53 and estrogen receptor in cells and tumor tissues by DNA affinity immunoblotting. *Cancer Research* **61** 5402–5406.
- Loven MA, Muster N, Yates JR & Nardulli AM 2003a A novel ER-associated protein represses ER-mediated transcription and alters ER-DNA interactions. *The Endocrine Society's 85th Annual Meeting*, Philadelphia, PA, USA.
- Loven MA, Muster N, Yates JR & Nardulli AM 2003b A novel estrogen receptor alpha-associated protein, template-activating factor I beta, inhibits acetylation and transactivation. *Molecular Endocrinology* **17** 67–78.
- McDonnell DP & Norris JD 2002 Connections and regulation of the human estrogen receptor. *Science* **296** 1642–1644.
- McKenna NJ & O'Malley BW 2002 Minireview: nuclear receptor coactivators – an update. *Endocrinology* **143** 2461–2465.
- McKenna NJ, Lanz RB & O'Malley BW 1999 Nuclear receptor coregulators: cellular and molecular biology. *Endocrine Reviews* **20** 321–344.
- Martens JH, Verlaan M, Kalkhoven E, Dorsman JC & Zantema A 2002 Scaffold/matrix attachment region elements interact with a p300-scaffold attachment factor A complex and are bound by acetylated nucleosomes. *Molecular and Cellular Biology* **22** 2598–2606.
- Martin MD, Fischbach K, Osborne CK, Mohsin SK, Allred DC & O'Connell P 2001 Loss of heterozygosity events impeding breast cancer metastasis contain the MTA1 gene. *Cancer Research* **61** 3578–3580.
- Mathur M, Tucker PW & Samuels HH 2001 PSF is a novel corepressor that mediates its effect through Sin3A and the DNA binding domain of nuclear hormone receptors. *Molecular and Cellular Biology* **21** 2298–2311.
- Mazumdar A, Wang RA, Mishra SK, Adam L, Bagheri-Yarmand R, Mandal M, Vadlamudi RK & Kumar R 2001 Transcriptional repression of oestrogen receptor by metastasis-associated protein 1 corepressor. *Nature Cell Biology* **3** 30–37.
- Mendelsohn ME 2002a Genomic and nongenomic effects of estrogen in the vasculature. *American Journal of Cardiology* **90** 3F–6F.
- Mendelsohn ME 2002b Protective effects of estrogen on the cardiovascular system. *American Journal of Cardiology* **89** 12E–17E; discussion 17E–18E.
- Miki Y, Swensen J, Shattuck-Eidens D *et al.* 1994 A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* **266** 66–71.
- Monsalve M, Wu Z, Adelmant G, Puigserver P, Fan M & Spiegelman BM 2000 Direct coupling of transcription and mRNA processing through the thermogenic coactivator PGC-1. *Molecular Cell* **6** 307–316.
- Montano MM, Ekena K, Delage-Mourroux R, Chang W, Martini P & Katzenellenbogen BS 1999 An estrogen receptor-selective coregulator that potentiates the effectiveness of antiestrogens and represses the activity of estrogens. *PNAS* **96** 6947–6952.
- Monteiro AN 2000 BRCA1: exploring the links to transcription. *Trends in Biochemical Sciences* **25** 469–474.
- Moore DD 1990 Diversity and unity in the nuclear hormone receptors: a terpenoid receptor superfamily. *New Biology* **2** 100–105.
- Morrison AJ, Herrera RE, Heinsohn EC, Schiff R & Osborne CK 2003 Dominant negative N-CoR relieves transcriptional inhibition of retinoic acid receptor but does not alter the agonist/antagonist activities of the tamoxifen-bound estrogen receptor. *Molecular Endocrinology* **17** 1534–1554.
- Moynahan ME 2002 The cancer connection: BRCA1 and BRCA2 tumor suppression in mice and humans. *Oncogene* **21** 8994–9007.
- Murphy LC, Simon SL, Parkes A, Leygue E, Dotzlaw H, Snell L, Troup S, Adeyinka A & Watson PH 2000 Altered expression of estrogen receptor coregulators during human breast tumorigenesis. *Cancer Research* **60** 6266–6271.
- Murphy LC, Leygue E, Niu Y, Snell L, Ho SM & Watson PH 2002 Relationship of coregulator and oestrogen receptor isoform expression to *de novo* tamoxifen resistance in human breast cancer. *British Journal of Cancer* **87** 1411–1416.
- Muscatt GE, Burke LJ & Downes M 1998 The corepressor N-CoR and its variants RIP13a and RIP13Delta directly interact with the basal transcription factors TFIIB, TAFII32 and TAFII70. *Nucleic Acids Research* **26** 2899–2907.
- Nagy L, Kao H-Y, Chakravarti D, Lin R, Hassig C, Ayer D, Schreiber S & Evans R 1997 Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell* **69** 373–380.
- Nawaz Z, Lonard DM, Dennis AP, Smith CL & O'Malley BW 1999 Proteasome-dependent degradation of the human estrogen receptor. *PNAS* **96** 1858–1862.
- Nayler O, Stratling W, Bourquin JP, Stajlgjar I, Lindemann L, Jasper H, Hartmann AM, Fackelmayer FO, Ullrich A & Stamm S 1998 SAF-B protein couples transcription and pre-mRNA splicing to SAR/MAR elements. *Nucleic Acids Research* **26** 3542–3549.
- Newman SP, Bates NP, Vernimmen D, Parker MG & Hurst HC 2000 Cofactor competition between the ligand-bound oestrogen receptor and an intron 1 enhancer leads to oestrogen repression of ERBB2 expression in breast cancer. *Oncogene* **19** 490–497.
- Nicolson GL, Nawa A, Toh Y, Taniguchi S, Nishimori K & Moustafa A 2003 Tumor metastasis-associated human MTA1 gene and its MTA1 protein product: role in epithelial cancer cell invasion, proliferation and nuclear regulation. *Clinical and Experimental Metastasis* **20** 19–24.
- Norris JD, Fan D, Sherk A & McDonnell DP 2002 A negative coregulator for the human ER. *Molecular Endocrinology* **16** 459–468.

- Nye AC, Rajendran RR, Stenoien DL, Mancini MA, Katzenellenbogen BS & Belmont AS 2002 Alteration of large-scale chromatin structure by estrogen receptor. *Molecular and Cellular Biology* **22** 3437–3449.
- Oesterreich S, Lee AV, Sullivan TM, Samuel SK, Davie JR & Fuqua SA 1997 Novel nuclear matrix protein HET binds to and influences activity of the HSP27 promoter in human breast cancer cells. *Journal of Cellular Biochemistry* **67** 275–286.
- Oesterreich S, Zhang Q, Hopp T, Fuqua SA, Michaelis M, Zhao HH, Davie JR, Osborne CK & Lee AV 2000 Tamoxifen-bound estrogen receptor (ER) strongly interacts with the nuclear matrix protein HET/SAF-B, a novel inhibitor of ER-mediated transactivation. *Molecular Endocrinology* **14** 369–381.
- Oesterreich S, Allred DC, Mohsin SK, Zhang Q, Wong H, Lee AV, Osborne CK & O’Connell P 2001 High rates of loss of heterozygosity on chromosome 19p13 in human breast cancer. *British Journal of Cancer* **84** 493–498.
- Oesterreich S, Kang K, Townson S, Clark GM, Hilsenbeck SG, Osborne C & Bardou V 2002 Critical balance of scaffold attachment factor SAFB levels plays important role in breast tumor suppression. *25th Annual San Antonio Breast Cancer Symposium*, San Antonio, TX, USA.
- Oesterreich S, Deng W, Jiang S, Cui X, Ivanova M, Schiff R, Kang K, Hadsell D, Behrens J & Lee AV 2003 Estrogen-mediated downregulation of E-cadherin in breast cancer cells. *Cancer Research* **63** 5203–5208.
- Ordentlich P, Downes M, Xie W, Genin A, Spinner NB & Evans RM 1999 Unique forms of human and mouse nuclear receptor corepressor SMRT. *PNAS* **96** 2639–2644.
- Osborne CK, Coronado-Heinsohn EB, Hilsenbeck SG, McCue BL, Wakeling AE, McClelland RA, Manning DL & Nicholson RI 1995 Comparison of the effects of a pure steroidal antiestrogen with those of tamoxifen in a model of human breast cancer. *Journal of the National Cancer Institute* **87** 746–750.
- Osborne CK, Schiff R, Fuqua SA & Shou J 2001 Estrogen receptor: current understanding of its activation and modulation. *Clinical Cancer Research* **7** 4338s–4342s; discussion 4411s–4412s.
- Osborne CK, Bardou V, Hopp TA, Chamness GC, Hilsenbeck SG, Fuqua SA, Wong J, Allred DC, Clark GM & Schiff R 2003 Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. *Journal of the National Cancer Institute* **95** 353–361.
- Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J, Kushner PJ & Scanlan TS 1997 Differential ligand activation of estrogen receptors ERalpha and ERbeta at AP1 sites. *Science* **277** 1508–1510.
- Palmieri C, Cheng GJ, Saji S, Zelada-Hedman M, Warri A, Weihua Z, Van Noorden S, Wahlstrom T, Coombes RC, Warner M et al. 2002 Estrogen receptor beta in breast cancer. *Endocrine Related Cancer* **9** 1–13.
- Parker M, Leonardsson G, White R, Steel J & Milligan S 2003 Identification of RIP140 as a nuclear receptor cofactor with a role in female reproduction. *FEBS Letters* **546** 149–153.
- Pereira FA, Qiu Y, Zhou G, Tsai MJ & Tsai SY 1999 The orphan nuclear receptor COUP-TFII is required for angiogenesis and heart development. *Genes and Development* **13** 1037–1049.
- Perissi V, Staszewski LM, McInerney EM, Kurokawa R, Krone A, Rose DW, Lambert MH, Milburn MV, Glass CK & Rosenfeld MG 1999 Molecular determinants of nuclear receptor-corepressor interaction. *Genes and Development* **13** 3198–3208.
- Polly P, Herdick M, Moehren U, Baniahmad A, Heinzel T & Carlberg C 2000 VDR-Alien: a novel, DNA-selective vitamin D(3) receptor-corepressor partnership. *FASEB Journal* **14** 1455–1463.
- Powles TJ 2002 Anti-oestrogenic prevention of breast cancer—the make or break point. *Nature Reviews Cancer* **2** 787–794.
- Qiu Y, Pereira FA, DeMayo FJ, Lydon JP, Tsai SY & Tsai MJ 1997 Null mutation of mCOUP-TFI results in defects in morphogenesis of the glossopharyngeal ganglion, axonal projection, and arborization. *Genes and Development* **11** 1925–1937.
- Rajendran RR, Nye AC, Frasier J, Balsara RD, Martini PG & Katzenellenbogen BS 2003 Regulation of nuclear receptor transcriptional activity by a novel DEAD box RNA helicase (DP97). *Journal of Biological Chemistry* **278** 4628–4638.
- Rayasam GV, Wendling O, Angrand PO, Mark M, Niederreither K, Song L, Lerouge T, Hager GL, Chambon P & Losson R 2003 NSD1 is essential for early post-implantation development and has a catalytically active SET domain. *EMBO Journal* **22** 3153–3163.
- Read LD, Keith D Jr, Slamon DJ & Katzenellenbogen BS 1990 Hormonal modulation of HER-2/neu protooncogene messenger ribonucleic acid and p185 protein expression in human breast cancer cell lines. *Cancer Research* **50** 3947–3951.
- Reese JC & Katzenellenbogen BS 1992 Examination of the DNA-binding ability of estrogen receptor in whole cells: implications for hormone-independent transactivation and the actions of antiestrogens. *Molecular and Cellular Biology* **12** 4531–4538.
- Reid G, Hubner MR, Metivier R, Brand H, Denger S, Manu D, Beaudouin J, Ellenberg J & Gannon F 2003 Cyclic, proteasome-mediated turnover of unliganded and liganded ERalpha on responsive promoters is an integral feature of estrogen signaling. *Molecular Cell* **11** 695–707.
- Renz A & Fackelmayr FO 1996 Purification and molecular cloning of the scaffold attachment factor B (SAF-B), a novel human nuclear protein that specifically binds to S/MAR-DNA. *Nucleic Acids Research* **24** 843–849.
- Resnick EM, Schreihofer DA, Periasamy A & Shupnik MA 2000 Truncated estrogen receptor product-1 suppresses estrogen receptor transactivation by dimerization with estrogen receptors alpha and beta. *Journal of Biological Chemistry* **275** 7158–7166.
- Safe S 2001 Transcriptional activation of genes by 17 beta-estradiol through estrogen receptor-Sp1 interactions. *Vitamins and Hormones* **62** 231–252.
- Santen RJ 2002 To block estrogen’s synthesis or action: that is the question. *Journal of Clinical Endocrinology and Metabolism* **87** 3007–3012.
- Schiff R, Massarweh S, Shou J & Osborne CK 2003 Breast cancer endocrine resistance: how growth factor signaling and estrogen receptor coregulators modulate response. *Clinical Cancer Research* **9** 447S–454S.
- Schulman IG, Juguilon H & Evans RM 1996 Activation and repression by nuclear hormone receptors: hormone modulates an equilibrium between active and repressive states. *Molecular and Cellular Biology* **16** 3807–3813.
- Schuur ER, Loktev AV, Sharma M, Sun Z, Roth RA & Weigel RJ 2001 Ligand-dependent interaction of estrogen receptor-alpha with members of the forkhead transcription factor family. *Journal of Biological Chemistry* **276** 33554–33560.
- Seol W, Choi HS & Moore DD 1996 An orphan nuclear hormone receptor that lacks a DNA binding domain and heterodimerizes with other receptors. *Science* **272** 1336–1339.

- Seol W, Hanstein B, Brown M & Moore DD 1998 Inhibition of estrogen receptor action by the orphan receptor SHP (short heterodimer partner). *Molecular Endocrinology* **12** 1551–1557.
- Shang Y & Brown M 2002 Molecular determinants for the tissue specificity of SERMs. *Science* **295** 2465–2468.
- Shang Y, Hu X, DiRenzo J, Lazar MA & Brown M 2000 Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell* **103** 843–852.
- Shi H & Xu RM 2003 Crystal structure of the *Drosophila* Mago nashi-Y14 complex. *Genes and Development* **17** 971–976.
- Shi Y, Downes M, Xie W, Kao HY, Ordentlich P, Tsai CC, Hon M & Evans RM 2001 Sharp, an inducible cofactor that integrates nuclear receptor repression and activation. *Genes and Development* **15** 1140–1151.
- Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA & Greene GL 1998 The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* **95** 927–937.
- Simon SL, Parkes A, Leygue E, Dotzlaw H, Snell L, Troup S, Adeyinka A, Watson PH & Murphy LC 2000 Expression of a repressor of estrogen receptor activity in human breast tumors: relationship to some known prognostic markers. *Cancer Research* **60** 2796–2799.
- Smith CL, Nawaz Z & O'Malley BW 1997 Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. *Molecular Endocrinology* **11** 657–666.
- Somasundaram K 2003 Breast cancer gene 1 (BRCA1): role in cell cycle regulation and DNA repair – perhaps through transcription. *Journal of Cellular Biochemistry* **88** 1084–1091.
- Stoner M, Wang F, Wormke M, Nguyen T, Samudio I, Vyhlihal C, Marme D, Finkenzerler G & Safe S 2000 Inhibition of vascular endothelial growth factor expression in HEC1A endometrial cancer cells through interactions of estrogen receptor alpha and Sp3 proteins. *Journal of Biological Chemistry* **275** 22769–22779.
- Teo AK, Oh HK, Ali RB & Li BF 2001 The modified human DNA repair enzyme O(6)-methylguanine-DNA methyl transferase is a negative regulator of estrogen receptor mediated transcription upon alkylation DNA damage. *Molecular and Cellular Biology* **21** 7105–7114.
- Teyssier C, Belguise K, Galtier F, Cavailles V & Chabos D 2003 Receptor-interacting protein 140 binds c-jun and inhibits estradiol-induced activator protein-1 activity by reversing glucocorticoid receptor-interacting protein 1 effect. *Molecular Endocrinology* **17** 287–299.
- Thenot S, Charpin M, Bonnet S & Cavailles V 1999 Estrogen receptor cofactors expression in breast and endometrial human cancer cells. *Molecular and Cellular Endocrinology* **156** 85–93.
- Toh Y, Pencil SD & Nicolson GL 1994 A novel candidate metastasis-associated gene, mta1, differentially expressed in highly metastatic mammary adenocarcinoma cell lines. cDNA cloning, expression, and protein analyses. *Journal of Biological Chemistry* **269** 22958–22963.
- Townson SM, Sullivan T, Zhang Q, Clark GM, Osborne CK, Lee AV & Oesterreich S 2000 HET/SAF-B overexpression causes growth arrest and multinuclearity and is associated with aneuploidy in human breast cancer. *Clinical Cancer Research* **6** 3788–3796.
- Townson SM, Dobrzycka KM, Lee AV, Air M, Deng W, Kang K, Jiang S, Kioka N, Michaelis K & Oesterreich S 2003 SAFB2, a new scaffold attachment factor homolog and estrogen receptor corepressor. *Journal of Biological Chemistry* **278** 20059–20068.
- Tremblay GB & Giguere V 2002 Coregulators of estrogen receptor action. *Critical Reviews in Eukaryotic Gene Expression* **12** 1–22.
- Treuter E, Albrechtsen T, Johansson L, Leers J & Gustafsson J-A 1998 A regulatory role for RIP140 in nuclear receptor activation. *Molecular Endocrinology* **12** 864–881.
- Ueki N & Hayman MJ 2003 Signal-dependent N-CoR requirement for repression by the Ski oncoprotein. *Journal of Biological Chemistry* **278** 24858–24864.
- Valverde MA & Parker MG 2002 Classical and novel steroid actions: a unified but complex view. *Trends in Biochemical Sciences* **27** 172–173.
- Vanacker JM, Bonnelye E, Delmarre C & Laudet V 1998 Activation of the thyroid hormone receptor alpha gene promoter by the orphan nuclear receptor ERR alpha. *Oncogene* **17** 2429–2435.
- Vanacker JM, Bonnelye E, Chopin-Delannoy S, Delmarre C, Cavailles V & Laudet V 1999 Transcriptional activities of the orphan nuclear receptor ERR alpha (estrogen receptor-related receptor-alpha). *Molecular Endocrinology* **13** 764–773.
- Venkiteswaran AR 2002 Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell* **108** 171–182.
- Wahl GM & Carr AM 2001 The evolution of diverse biological responses to DNA damage: insights from yeast and p53. *Nature Cell Biology* **3** E277–286.
- Watanabe H, Suzuki A, Mizutani T, Khono S, Lubahn DB, Handa H & Iguchi T 2002 Genome-wide analysis of changes in early gene expression induced by oestrogen. *Genes Cells* **7** 497–507.
- Watanabe T, Inoue S, Hiroi H, Orimo A, Kawashima H & Muramatsu M 1998 Isolation of estrogen-responsive genes with a CpG island library. *Molecular and Cellular Biology* **18** 442–449.
- Wei LN, Hu X, Chandra D, Seto E & Farooqui M 2000 Receptor-interacting protein 140 directly recruits histone deacetylases for gene silencing. *Journal of Biological Chemistry* **275** 40782–40787.
- Weighardt F, Cobianchi F, Cartegni L, Chiodi I, Villa A, Riva S & Biamonti G 1999 A novel hnRNP protein (HAP/SAF-B) enters a subset of hnRNP complexes and relocates in nuclear granules in response to heat shock. *Journal of Cell Science* **112** 1465–1476.
- Weihua Z, Saji S, Makinen S, Cheng G, Jensen EV, Warner M & Gustafsson JA 2000 Estrogen receptor (ER) beta, a modulator of ER alpha in the uterus. *PNAS* **97** 5936–5941.
- White R, Leonardsson G, Rosewell I, Ann Jacobs M, Milligan S & Parker M 2000 The nuclear receptor co-repressor nr1p1 (RIP140) is essential for female fertility. *Nature Medicine* **6** 1368–1374.
- Wong AK, Ormonde PA, Pero R, Chen Y, Lian L, Salada G, Berry S, Lawrence Q, Dayananth P, Ha P *et al.* 1998 Characterization of a carboxy-terminal BRCA1 interacting protein. *Oncogene* **17** 2279–2285.
- Wong CW, McNally C, Nickbarg E, Komm BS & Cheskis BJ 2002 Estrogen receptor-interacting protein that modulates its nongenomic activity-crosstalk with Src/Erk phosphorylation cascade. *PNAS* **99** 14783–14788.
- Wu L, Wu Y, Gathings B, Wan M, Li X, Grizzle W, Liu Z, Lu C, Mao Z & Cao X 2003 Smad4 as a transcription corepressor for estrogen receptor alpha. *Journal of Biological Chemistry* **278** 15192–15200.
- Xu HE, Stanley TB, Montana VG, Lambert MH, Shearer BG, Cobb JE, McKee DD, Galardi CM, Plunket KD, Nolte RT *et al.* 2002 Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPARalpha. *Nature* **415** 813–817.
- Xu J, Nawaz Z, Tsai SY, Tsai MJ & O'Malley BW 1996 The extreme C terminus of progesterone receptor contains a

- transcriptional repressor domain that functions through a putative corepressor. *PNAS* **93** 12195–12199.
- Xu X, Wagner KU, Larson D, Weaver Z, Li C, Ried T, Hennighausen L, Wynshaw-Boris A & Deng CX 1999 Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. *Nature Genetics* **22** 37–43.
- Yang A, Kaghad M, Caput D & McKeon F 2002 On the shoulders of giants: p63, p73 and the rise of p53. *Trends in Genetics* **18** 90–95.
- Yu CL, Driggers P, Barrera-Hernandez G, Nunez SB, Segars JH & Cheng S 1997 The tumor suppressor p53 is a negative regulator of estrogen receptor signaling pathways. *Biochemical and Biophysical Research Communications* **239** 617–620.
- Yu RN, Ito M, Saunders TL, Camper SA & Jameson JL 1998a Role of Ahc in gonadal development and gametogenesis. *Nature Genetics* **20** 353–357.
- Yu X, Wu LC, Bowcock AM, Aronheim A & Baer R 1998b The C-terminal (BRCT) domains of BRCA1 interact *in vivo* with CtIP, a protein implicated in the CtBP pathway of transcriptional repression. *Journal of Biological Chemistry* **273** 25388–25392.
- Zamir I, Dawson J, Lavinsky RM, Glass CK, Rosenfeld MG & Lazar MA 1997 Cloning and characterization of a corepressor and potential component of the nuclear hormone receptor repression complex. *PNAS* **94** 14400–14405.
- Zhang H, Thomsen JS, Johansson L, Gustafsson JA & Treuter E 2000 DAX-1 functions as an LXXLL-containing corepressor for activated estrogen receptors. *Journal of Biological Chemistry* **275** 39855–39859.
- Zhang X, Jeyakumar M, Petukhov S & Bagchi MK 1998 A nuclear receptor corepressor modulates transcriptional activity of antagonist-occupied steroid hormone receptor. *Molecular Endocrinology* **12** 513–524.
- Zhao HH, Herrera RE, Coronado-Heinsohn E, Yang MC, Ludes-Meyers JH, Seybold-Tilson KJ, Nawaz Z, Yee D, Barr FG, Diab SG *et al.* 2001 Forkhead homologue in rhabdomyosarcoma functions as a bifunctional nuclear receptor-interacting protein with both coactivator and corepressor functions. *Journal of Biological Chemistry* **276** 27907–27912.
- Zheng L, Annab LA, Afshari CA, Lee WH & Boyer TG 2001 BRCA1 mediates ligand-independent transcriptional repression of the estrogen receptor. *PNAS* **98** 9587–9592.