MINIREVIEW

Nuclear Receptor Coactivators and Corepressors

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The nuclear receptors belong to a superfamily of proteins, many of which are ligand-regulated, that bind to specific DNA sequences and control specific gene transcription. Recent data show that, in addition to contacting the basal transcription machinery directly, nuclear receptors inhibit or enhance transcription by recruiting an array of coactivator or corepressor proteins to the transcription complex. In this review we define the properties of these putative coregulatory factors; we describe the basal and coregulatory factors that are currently known to interact with nuclear receptors; we suggest various mechanisms by which coactivators and corepressors act; we discuss issues that are raised by the presence of multiple, perhaps competing, coregulatory factors; and we speculate how these additional regulatory layers may explain the heterogeneity of hormone responses that are observed in normal and malignant tissues. (Molecular Endocrinology 10: 1167–1177, 1996)

INTRODUCTION

For many years, the mechanisms of steroid receptor action seemed quite simple. The hormone somehow found itself in the cell nucleus, where inactive proteins —the receptors— awaited it. The hormonal signal then activated the receptors, which bound to specific DNA sequences and regulated transcription. Simple. We congratulated ourselves on the fact that we could study transcription directly and could avoid the baffling kinase-kinase-kinase cascades that our cell-surface receptor colleagues had to contend with, before their favorite signaling hormone-activated transcription.

It now seems that the congratulations were premature. Things began to unravel when it became clear that steroid receptors are only a subset of a larger family of receptors whose ligands are not only nonsteroidal but, in many cases, unknown; receptors that bind to one ligand, yet are expressed as multiple structural variants and isoforms; receptors that do not sit idly in the nucleus waiting to be activated, but are active even without ligand; receptors that pair with partners differing from themselves in a bewildering array of heterodimers and DNA-binding sites; receptors that can control transcription without (heresy!) binding to DNA; and, most humbling, receptors that— far from being direct controllers of transcription— interact with a complex array of coregulatory proteins, which function as signaling intermediates between the receptors and the general transcriptional machinery (Refs. 1–3 and references therein). Shades of kinase-kinase-kinase! At the Keystone Meeting on Nuclear Receptors held in Lake Tahoe, California, on March 17–22, coactivators and corepressors dominated the discussions and the pace of discoveries is quickening. Arguably, the major impetus for these discoveries has come from recent technological advances in protein-protein interaction-screening methods (Ref. 4, for example), and the concurrent ability to rapidly clone and characterize the unknown protein partner of the receptor bait. As a result an impressive number of coregulatory factors and basal transcription factors that interact with receptors have already been described, and these are certain to represent only the tip of the iceberg.

BASAL TRANSCRIPTION FACTORS

Gene transcription by RNA polymerase II requires assembly at the TATA box of multiple initiation complex proteins. Binding of transcription factor IID (TFIID)— itself a complex of the TATA-binding protein (TBP) and more than 10 other TBP-associated factors (TAFs)—is the first step in this assembly. TFIIB, which serves a bridging function between TBP and polymerase II, is then recruited to TFIID. Other general transcription

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factors, designated TFIIA to TFIIJ, join to complete the preinitiation complex (Refs. 5 and 6 and references therein).

There is considerable evidence that nuclear receptors can contact some of the basal factors of the preinitiation complex directly, without the need for intermediary coregulatory proteins. For example, the yeast two-hybrid assay (4) detects a specific ligand-dependent interaction between TBP and the hormone binding domain (HBD) of retinoid X receptors (RXR) that is dependent on a functional receptor activation domain (AF) (7). Apparently this interaction does not require that any of the reactants be bound to DNA. Similarly, both the N-terminal AF1 activation domain and the C-terminal AF2 activation domain of estrogen receptors (ER) bind to TBP *in vitro* (8). This binding is not restricted by promoter structure, however, since TBP overexpression enhances estrogen-induced transcription and relieves ER-induced self-squelching, despite varying distances between the estrogen response elements and the TATA box (8). This is somewhat surprising, since binding among other proteins of the initiation complex is constrained by DNA distance effects. For example, binding of TFIIB at the TATA box defines the maximum linear DNA distance allowable between the TATA box and polymerase II binding at the transcription start site, approximately 20 bp downstream (9, 10). If similar constraints apply to hormone response elements (HRE), then the long distances between the TATA box and many upstream HREs could be accommodated by looping out of the intervening DNA. Interestingly, it may be the binding of receptors to their HREs that drives assembly of proteins at the TATA box. Klein and Struhl (11) propose, for example, that accessibility of TBP to the chromatin template is the rate-limiting reaction of transcription, and that the activation domains of transactivators, like nuclear receptors, function in part to increase the rate of TBP recruitment to the promoter.

However, while TBP is necessary, it is not sufficient to mediate RNA polymerase II-dependent transcription in response to transactivators (12). This and other observations led to the hypothesis that additional factors, termed "coactivators," are also required for efficient transcription. Since, unlike TBP, the TFIID complex alone can support transcription regulated by upstream activators (10), it was concluded that coactivator functions are provided by TAFs associated with TFIID (Refs. 10 and 12 and references therein), and indeed interactions between nuclear receptors and TAFs have also been described. For example, ER self-squelching and the activities of its AF1 and AF2 domains are due in part to ER interactions with components of the TFIID complex that are chromatographically separable from TBP (13). One such TFIID-associated factor, $TAF_{II}30$, interacts specifically with the AF1 but not the AF2 activation domain of ER (14). These and other data suggest that there are functionally distinct populations of TFIID complexes, composed both of comm ı core TAFs and of unique TAF subunits, and that the unique TAFs are responsible for the specificity of interactions between TFIID and different classes of activation domains (14–17). However, even regions outside the activation domains of receptors can bind TAFs, as is the case for the interaction between the DNA-binding domain (DBD) of progesterone receptors (PR) and *Drosophila* $TAF_{II}110$ (18). RXR and thyroid hormone receptors (TR) also interact with $TAF_{II}110$ in a ligand-dependent manner, and for RXR at least, this interaction persists even if the receptors have been rendered transcriptionally defective by mutations in AF2 located in the HBD (7).

Transcription factors also form contacts with TFIIB (19). They induce conformational changes in TFIIB that disrupt its inactivating intramolecular interactions and expose new binding sites between TFIIB and other basal factors that stabilize assembly of the preinitiation complex (20). TFIIB is also a target of nuclear receptor binding. Several members of the nuclear receptor superfamily, including COUP-TF, ER, and PR (21), TRβ (22) and TRα (23), and vitamin D (VDR) and retinoic acid receptors [RARα, (24)] interact with TFIIB. However, the functional domains of the receptors involved in TFIIB binding differ, so that contact is variably formed between TFIIB and the AF-2 of ER (21); an AF-1/DBD construct of hPR (21); both an N- and a C-terminal contact site of TRβ (22); an N-terminal fragment of TRα lacking activation function (23); and the proximal HBD of VDR (24). There is no explanation for this variability at present, nor is there much information about the hormone dependence of these interactions in intact cells, other than for TRβ, where the switch between N- and C-terminal binding is controlled by ligand (22).

If the binding of nuclear receptors to components of the basal transcription apparatus is not absolutely dependent on receptor activation domains, does this mean that the AFs are not involved in protein-protein interactions? Or, are there additional nuclear factors whose binding to receptors is more specific for AFs? Evidence for additional factors is supported by observations that different classes of receptors can interfere with one another's transcriptional activity by squelching limiting factors that are not components of the basal transcription machinery (25–27). These factors are also termed "coactivators" if the liganded but non-DNA-bound interfering receptor can squelch transcription by a promoter-bound receptor, without lowering the levels of basal transcription, or inhibiting transcription from other promoters (25, 26). Such coactivators are the major subject of this review. However, it is clear from the preceding, and will be reinforced by the following, that the term "coactivator" is, at present, very loosely defined. Perhaps in the future, the terminology will be refined on the basis of discrete functional characteristics, as it becomes evident that receptors can form multiprotein complexes by binding to a variety of different coactivators, to other transcription and regulatory proteins, and to multiple basal factors.

COACTIVATORS

If the interactions between receptors and basal transcription factors are necessary but not sufficient for accurate and efficient hormone-dependent transcriptional control, then at least a third category of factors must also be involved. Such an oligomeric complex—involving activator proteins (*i.e.* the receptors), coactivators, and basal factors—is believed to be necessary to stabilize the interactants and provide transcriptional specificity (28). For example, to achieve efficient transcription, RARβ2 has been shown to interact not only with TFIID, but with an additional E1A-like factor (29). This model postulates the existence of one or more bridging factors present in limiting amounts, interposed between the receptors and basal factors.

Surprisingly then, the first nuclear receptor coactivators to be described are components of the pol II holoenzyme and probably serve another function. In yeast, the SWI/SNF family of proteins are required for transcription of differentially regulated genes, in part because SWI/SNF reverse the repressive effects of chromatin components (30–33). Yoshinaga *et al.* (34) reported that transcriptional activation by glucocorticoid receptors (GR) or ER in yeast is dependent on SWI1, SWI2, and SWI3 function. Human homologs of these proteins, termed hSNF2α, hSNF2β (31), or hbrm (30, 31), enhance transcription by ER, RAR (31), and GR (30, 31) in transfection assays and require an intact receptor DBD (30). The molecular mechanisms by which hSNF2 or hbrm enhance transcription are still unclear. However SWI/SNF are integral components of the polymerase II holoenzyme, and one model holds that the transactivator recruits pol II by binding to a subset of its components, and that SWI/SNF then enhance the stability of activator/DNA binding by destabilizing nucleosomes (35). A highly intriguing observation suggesting other possible mechanisms is that GR activation by hbrm is further enhanced by cotransfection of the retinoblastoma protein (Rb), which interacts directly with hbrm (32).

These studies raise additional questions about the definition of a coactivator. Is a coactivator any limiting factor that enhances the transcriptional activity of a nuclear receptor without altering basal transcription? Is direct protein-protein contact between the factor and the receptor a requirement? For example, while Rb enhances GR-dependent transcription, it does so without binding directly to the receptors, and strictly speaking, is therefore not a coactivator. Is ligand regulation of the receptor-coactivator interaction an additional requirement? What about a requirement for direct contact not only between the coactivator and the receptors, but also between the coactivator and basal factors, which the bridging model would predict? It is not clear at present whether SNF2/hbrm fulfill even the first of these criteria.

On the other hand, some recently described factors meet several of them (Table 1). Two members of a family of related proteins, a 160-kDa protein called ERAP 160 or p160 (36) and a 140-kDa nuclear protein called RIP 140 (37, 38) were recently identified by *in vitro* protein-protein interaction assays using the HBD of ER as bait. Binding of both factors to ER is stabilized by estrogens and destabilized by antiestrogens, suggesting that the coactivator-receptor interactions are ligand regulated and that they require a conformationally active HBD.

RIP 140 is AF2-specific since, in HeLa cells, it enhances the transcriptional activity of a construct containing a Gal4 DBD fused to a wild type ER AF2 domain, but not of a similar construct fused to a transcriptionally defective AF2 mutant (37). RIP 140 therefore meets three criteria for designation as a coactivator. However, unlike ER that bind to both TBP and TFIIB (8, 21, 38) in a ligand-independent manner (38), RIP 140 interacts with neither of these basal factors (38). Perhaps contact by either (or both) partner in the receptor/coactivator complex is sufficient to activate the basal transcriptional apparatus? Such a model would attenuate the bridging function for a coactivator, in favor of other functions.

In addition to binding ER, ERAP 160 binds two other members of the nuclear receptor family, namely RARβ and RXRα (36). This promiscuity is a recurrent theme for several coactivators isolated to date, which, together with the fact that these nuclear receptor coactivators are found in most tissues, suggests that they play a generic role in transcription that is not restricted to nuclear receptors. If they exist, coactivators that interact uniquely only with members of the nuclear receptor family have not yet been characterized.

Several additional factors that interact with nuclear receptors in a ligand-dependent manner have now been described, some of which may be members of a large family of related proteins:

1. SRC-1 was isolated by a yeast two-hybrid screen from a human cDNA library using the HBD of hPR as bait (39). Its predicted molecular mass is 125 kDa. *In vitro*, it interacts with agonist- but not antagonist-occupied hPR. In HeLa cells, SRC-1 enhances hPR-mediated transcription in the presence of the agonist R5020 but not the antagonist RU486, and it does not alter basal promoter activity. Its mRNA is present in all cells tested. SRC-1 also enhances *in vivo* transcription by GR, ER, TR, and RXR (39), and its overexpression reverses ER-induced squelching of PR-regulated transcription. The latter is an important test when defining a coactivator and confirms that, *in vivo*, the same or a similar factor is regulating the activities of both ER and PR. Additional studies with ER show that SRC-1 inactivates ER occupied by pure antiestrogens, but it enhances the activity of ligands that have mixed agonist/antagonist properties, and it is also involved in ligand-independent ER activation (3).

Recent data indicate that SRC-1 and ERAP 160 (p160) are variants of the same family of coactivators. Kamei *et al.* (40) have isolated mouse p160 as a ternary complex with liganded nuclear receptors and a 300-kDa protein related to the cAMP response ele-

Table 1. Nuclear Receptor Coregulators—1996

	Synonyms[a]	Splice Variants	Species Homologs[b]	Related Proteins[c]	Comments
COACTIVATORS					
ERAP160 (36, 40)[d]	hSRC-1 (39), p160 (36)	SRC-1b, 1c, 1d	mSRC-1 (40)	mGRIP1 (42) hTIF2 (87)	ERAPs identified biochemically (36); SRC-1 (39) is gene that encodes ERAP160/p160 (40)
ERAP140 (36)[d]	p140				
RIP160 (37)[d]	p160				Differs from ERAP160
RIP140 (37, 38)[d]	p140				Differs from ERAP140
Trip1 (45, 46)	p45, Mss1 CAD (43, 47)		ySUG1, mSUG1 (45, 46, 47, 47a)		26S Proteasome subunit
ySPT6 (43, 44)			ceSPT6, hSPT6 (43)		Interacts with histones
ySWI1/SNF (33, 34)			hSWI1/SNF (33, 34)		Multimeric protein complex (33, 34) Chromatin reorganization
ySWI2 (30)	ySNF2 (30)	hbrm (30), hSNFα (31) hSNFβ (31), BRG1 (31)	dbrm (brahma)		Same as SWI1/SNF
ARA_{70} (49)	RFG (49)				
hRPF1 (50, 51)			yRSP5 (51) mNEDD-4 (51)	E6-AP (50), Trip12 (45)	Ubiquitin protein-ligase
RAP46 (53)					
TIF1 (46a, 48)					Partial identity to T18 oncogene (48)
COREPRESSORS					
TRUP (65)	surf-3, L7a, PLA-X (65)				Blocks DNA binding
Calreticulin (63, 64)					Blocks DNA binding
ySsn6/Tup1 (67–69)					Interact with histones; yeast repressor complex
mN-CoR (72, 73)		RIP13WT, RIP13Δ1[e]	hN-CoR[f]	SMRT (74)	
SMRT (74, 79a)	TRAC-2 (78)	TRAC-1 (78)		N-CoR, RIP13WT	
COINTEGRATORS					
CBP (40, 80a, b)				p300 (40, 80a, b)	

Numbers in parentheses correspond to references in text. y, Yeast; m, mouse; h, human; d, *Drosophila*, ce, *C. elegans*.
[a] The same protein referred to by another name.
[b] The same protein reflecting species divergence.
[c] The same protein family regardless of species.
[d] ERAP160 and RIP160 are different proteins but both are commonly referred to as p160. Similarly ERAP140 and RIP140 are different proteins but both are referred to as p140.
[e] Unpublished, personal communication, D. Moore.
[f] T. A. Jackson, J. K. Richer, and K. B. Horwitz, unpublished.

ment-binding protein, CBP (41). The C terminus of p160 has 88% identity with SRC-1, and its N-terminal extensions predict proteins of 159 and 152 kDa. Other N-terminal (SRC-1b) or C-terminal (SRC-1c, SRC-1d) splice variants have also been detected (40).

GRIP1 is an 86-kDa mouse protein with homology to SRC-1. It interacts with the HBD of GR, ER, and androgen receptors (AR) in a ligand-regulated manner in yeast and *in vitro*, and it functions as a coactivator for steroid receptor HBDs in yeast (42). In addition to a receptor interaction domain, GRIP1 also has a strong endogenous activation function, and it inhibits basal transcription. Based on this basal squelching property, the authors infer that GRIP 1 can contact the basal transcriptional machinery in addition to the receptors, as would be expected of a true bridging protein (42).

2. SPT6 is a yeast protein involved in the regulation of several yeast genes. It is capable of transferring histones onto DNA to form nucleosomes and is postulated to be active in regions undergoing extensive chromatin reorganization such as those that occur at

highly regulated genes (43). SPT6 binds to the HBD of ER *in vitro*, and it enhances ER-mediated transcription in yeast and in mammalian cells (44). A mammalian homolog has not yet been described. Whether this is a true coactivator, or a protein in the SNF/SWI class, is still unclear.

3. Trip1, a homolog of the yeast transcriptional mediator Sug1, binds full-length TRβ1 and RXR *in vitro* (45) in a ligand-independent manner (46). Preliminary studies using TR domain fragments indicate that the TR N terminus binds Trip1 constitutively, while the TR HBD binds Trip1 in a ligand-dependent manner (46), suggesting that Trip1 can influence both AF1 and AF2. The mouse Trip1 homolog, mSug1, has recently been cloned and shown to differ from Trip1 by only three amino acids (46a). Mouse Sug1 is widely expressed in tissues and it interacts with ER, VDR, RXRα, RARα, and TRα in a ligand- and AF-2-dependent manner. These data, in conjunction with preliminary data showing that yeast Sug1 interacts with TBP (47), suggested that Sug1/Trip1 are subunits of the RNA pol II holoenzyme complex and serve a bridging function between transactivators and the basal transcription complex. More recent data suggest, however, that Sug1 is a subunit of the 26S proteasome complex that catalyzes the degradation of ubiquitin-conjugated proteins (47a). It may therefore affect transcription ony indirectly, by controlling the turnover rates of receptors or other regulatory factors. If so, Sug1/Trip1 may not be coactivators as defined above.

4. TIF1 is a mouse protein that has a RING finger domain and a bromodomain analogous to that of many other nuclear regulatory proteins (48). It interacts with the conserved AF2 motif in the HBD of RXRγ, RXRα, RARα1, VDR, PR, and ER *in vitro* and in yeast, and *in vivo* with ER in COS-1 cells, in a ligand-dependent manner. TIF1 does not bind the basal factors TBP, TFIIB, TFIID, or $TAF_{II}30$, nor does it bind receptors that have a mutated AF2 motif. However, despite these characteristics of a coactivator, TIF1 interferes with, rather than enables, transactivation by RXRα in the presence of 9-*cis*-retinoic acid. It also inhibits ligand-dependent transcription by RAR and ER. The authors suggest that TIF1 sequesters a limiting factor (but not a basal factor) which functions downstream of TIF1 (48) — adding at least one more factor to the putative ternary regulatory complex.

5. ARA_{70} is a 70-kDa human protein isolated with the hAR HBD as bait (49). It interacts with AR in an androgen-dependent manner *in vitro* and does not bind antiandrogen-occupied receptors. In cotransfection assays, ARA_{70} enhances AR-dependent transcription 10-fold and GR-, PR-, and ER-dependent transcription 2-fold.

6. hRPF1 is the human homolog of yeast RSP5, a protein related to a family of ubiquitin-protein lipases (50, 51). In mammalian cells, coexpression of hRPF1 enhances the hormone-dependent transcriptional efficiency of PR and GR, but not ER, without altering basal transcription (51). Additionally, yeast genetics data suggest that hRPF1/RSP5 operates in the same regulatory pathway as a homolog of the human TBP-associated factor $TAF_{II}18$, but it is not yet known whether hRPF1 can interact directly with either $TAF_{II}18$ or the receptors. Of interest is the fact that, *in vivo*, only the B isoform, and not the A isoform, of PR is activated by coexpression of hRPF1. B Receptors contain an activation domain (AF3) that A receptors lack (52), while their AF1 and AF2 domains are identical. Perhaps hRPF1 mediates the unique properties of AF3?

7. RAP46 is a 46-kDa protein that was isolated from a human cDNA library using an *in vitro* interaction screen (53). Like other factors for which this is known, RAP46 is expressed in all tissues. It binds promiscuously to GR, PR, ER, and TRβ *in vitro* whether the receptors are unliganded, liganded with agonists or antagonists, or activated by salt/heat treatment. The binding to ER is lost if the N-terminal A/B domain of the receptors is deleted. No functional data have been reported for RAP46 to date, so that its coactivator status remains unsettled.

COREPRESSORS

The search for coactivators is a logical outgrowth of the fact that all of the known natural ligands of nuclear receptors are agonists that activate transcription. However, there are two conditions under which the presence or absence of ligand inhibits transcription. One occurs when synthetically produced steroid hormone antagonists compete successfully with the natural steroidal agonists for binding to receptors and inhibit agonist-induced transcription. The other is a key property of some members of the nonsteroidal subfamily of nuclear receptors, which bind to DNA in the absence of ligand and actively repress basal transcription (54–56). Under similar conditions, unliganded steroid receptors cannot bind DNA, because they are sequestered by a complex coating of heat shock and other proteins (57). By what mechanisms do antagonist-occupied steroid receptors or unliganded RAR/TR repress transcription?

Transcriptional repression of eukaryotic promoters has been studied extensively in the last few years (see Refs. 58–60 for reviews). For the most part, this work has focused on DNA-binding proteins (including some nuclear receptors) under conditions in which they repress transcription by three mechanisms: 1) the repressor binds to the same, or an overlapping, DNA binding site as the activator and competitively blocks access of the activator to DNA; 2) both proteins bind to DNA at nonoverlapping sites, but the repressor interferes with, or quenches the activity of the activator; 3) the repressor binds to DNA and silences the basal transcription machinery directly. Nuclear receptors have been implicated in all of these mechanisms. Thus, the inhibitory effects of steroid antagonists, or of GR at the negative glucocorticoid response elements

of the PRL promoter, are thought to involve mechanism 1 (61); the mutual inhibition by AP1 and GR of the composite glucocorticoid response element on the proliferin promoter are thought to involve mechanism 2 (62); and the inhibitory effects of unliganded TR on TFIIB are thought to involve mechanism 3 (22). Other inhibitory mechanisms have also been described. For example, 4) transactivation by nuclear receptors is inhibited by factors such as the Ca^{2+}-binding protein, calreticulin (63, 64), or a ribosomal and nuclear protein termed TRUP [also known as surf-3, L7a, or PLA-X, (65)] that interfere with the binding of receptors to DNA. Calreticulin interacts with the DBD of AR, RAR, and GR and inhibits their ligand-dependent transcription *in vivo*, while TRUP interacts with the hinge region and N-terminal HBD of TR and RAR and blocks their ability to bind DNA and thus their ligand-dependent transcription as well. The interaction of TRUP with TR and RAR is ligand-independent, but it appears to have some specificity since TRUP does not inhibit the DNA binding of ER, RXR, or the DBD of GAL-4 (65).

Can any of the four inhibitory activities described above be attributed to the actions of corepressors? By analogy with coactivators, we define corepressors as limiting factors that inhibit transcription after being tethered to a promoter by DNA-bound receptors; factors whose binding to receptors is ligand-regulated; and factors whose inhibitory effects can be relieved by squelching. Using this definition, none of the four examples above represent actions of corepressors, despite the occasional use of this term in such cases (see Ref. 66, for example).

An early example of a steroid receptor corepressor is the yeast repressor Ssn 6. This protein effects glucose-dependent gene repression when it is tethered to a promoter by its interaction with a specific DNA-binding protein. Of interest is the fact that Ssn 6 requires a partner, Tup 1, for full repressor function (67, 68). McDonnell *et al*. (69) have identified Ssn 6 as a transcriptional repressor of ER and PR in yeast. Deletion of Ssn 6 strongly enhances the transcriptional effects of an ER AF1 fragment; enhances the agonist effects of estradiol and progesterone; allows the antiestrogens ICI164,384 and nafoxidine to behave as more potent ER agonists; and allows the antiprogestin RU486 to become a more potent PR agonist.

The interesting properties of Ssn6/Tup1 in yeast suggest a more global mechanism for the actions of some corepressors than an ability to, somehow, directly inhibit the basal transcription machinery. Edmondson *et al*. (68) have shown that the repression domain of Tup1 interacts specifically with histones H3 and H4. Mutations that interfere with Tup1/histone interactions compromise Tup1-mediated repression. The authors argue that Tup1 serves as a bridge between the DNA-bound transcription factor and neighboring nucleosomes, repositioning the nucleosome array downstream and physically blocking access of basal factors at the TATA box. Of course, coactivators (44) could act through related mechanisms, since they too can interact with histones (43).

More recently, the hunt for corepressors has focused on the transcriptional inhibitory activities of the unliganded TR/RAR/RXR subfamily (70, 71). In mammalian cells, the inhibitory activity of unliganded TRβ can be reversed (or squelched) by cotransfection of the C terminus of v-*erbA* or full-length unliganded RAR, suggesting the existence of a cellular corepressor present in limiting amounts. Basal promoter activity was not significantly altered in these studies. Binding of the putative corepressor maps to the hinge and N-terminal HBD region of TRβ. In the presence of thyroid hormone, the HBD of TRβ fails to compete for the corepressor, however, indicating that liganded TR do not bind this factor. Instead, the receptors appear to bind a coactivator at an activation domain (τ4) located in the far C terminus of the HBD, since deletion of τ4 produces a TRβ that is inhibitory even in the presence of ligand (71).

Two candidate corepressors for the TR/RAR/RXR subfamily have recently been isolated: a 270-kDa mouse protein termed N-CoR (nuclear receptor corepressor) (72, 73) and a 168-kDa human protein termed SMRT (silencing mediator for retinoid and thyroid hormone receptors) (74), both of which have similar properties. They interact with unliganded members of the TR and RAR family and their RXR partners on DNA, but the binding is destabilized by ligand.

N-CoR was isolated with a yeast two-hybrid screen using unliganded TRβ as bait. It is a nuclear protein (72), which appears to be widely expressed in tissues and cell lines (72, 73). N-CoR contains one (72) or more (74a) interaction domains (ID) at its C terminus that contact the hinge region and proximal HBD of the receptors, the same region postulated to bind a corepressor by Baniahmad *et al*. (71). Two transferable repressor domains map to the N terminus of N-CoR. Unliganded TRβ1/RXR heterodimers bind N-CoR *in vitro*, and the thyroid hormone analog, TRIAC, dissociates this binding. The N-CoR ID also interacts with unliganded TRα and RARα (72, 73) and, through an adjacent but distinct ID, with RevErb (74a). It does not interact with unliganded RXRα, RXRγ, VDR, ER, or GR (72). Of interest is the fact that when full-length N-CoR is used, its binding to TR and RAR is not ligand-regulated unless the receptors are bound to DNA. Similarly, activation of transcription by RAR/RXR heterodimers on a DR+5 element (74, 75) in response to ligand involves both the dissociation of N-CoR and the concomitant recruitment of coactivators that have the same molecular weight as ERAP 160 and RIP 140 (73). However, if the receptors occupy a DR+1 element (74, 75) N-CoR cannot be dissociated by ligand, and the coactivators do not bind. These data indicate that in addition to the presence or absence of ligand, allosteric structural changes imposed on the receptors by DNA binding, and even by the DNA sequence, influence the nature and the affinity of corepressor binding to the receptors.

SMRT was isolated by yeast two-hybrid screening using an unliganded hRXRα HBD fusion protein as bait (74). It too is a widely expressed nuclear protein with an N-terminal repressor domain and a C-terminal ID. SMRT interacts strongly with unliganded full-length RAR, and the HBD of RAR and TR, and this binding is released by ligand. It interacts with unliganded RAR/RXR or TR/RXR heterodimers on DR+5 or TR-response elements, and ligand also disrupts this ternary complex. Full-length SMRT suppresses the basal activity of promoters containing either RAR or TR response elements through an N-terminal repression domain. That cells contain limiting amounts of endogenous corepressors related to SMRT is shown by the ability of overexpressed v-*erbA* (74) or the TRβ HBD (76) to reverse (or squelch) the transcriptional inhibition produced by unliganded RAR or TR and by the ability of SMRT overexpression to restore this inhibition (74).

Since the ID of SMRT shares approximately 48% identity (74) with the ID of N-CoR [also known as RIP13; (45, 77)], the two corepressors appear to be related and to be members of a larger family of dominant-negative proteins (78–79a) whose binding to receptors is inhibited by ligand (45). Indeed the TRACs (78) also belong to this family, one of which, TRAC-2, is identical to SMRT. In addition to having multiple IDs (74a), recently discovered N-CoR isoforms lack the currently defined repressor domains (D. Moore, unpublished). They also reportedly (78) bind to receptors, such as the peroxisome proliferator-activated receptor (PPAR), that have no known repressor function, raising the possibility that these corepressors have a more complex biological role.

SMRT, N-CoR, and their homologs represent a new class of transcriptional mediators for nuclear receptors that actively silence basal transcription (79). Such corepressors would be expected to interact with receptors—such as TR and RAR—that bind to DNA when they are unliganded and constitutively repress transcription. These corepressors would not be expected to bind the class of receptors that activate transcription after ligand-dependent DNA binding, and indeed, the corepressors fail to bind unliganded or agonist-occupied VDR, ER, or GR. However, steroid receptors are inhibitory when they are occupied by synthetic antagonists. Is it possible that corepressors are involved in this inhibition? We have data suggesting that RU486-occupied PR or tamoxifen-occupied ER can attract corepressors to the steroid receptor/antagonist complex. If this is the case, then steroid receptor/antagonist complexes may not simply block activation passively, by out-competing receptor/agonist complexes for binding to HREs (analogous to mechanism 1, above), but they actively repress transcription by recruiting corepressors to the transcription complex (T. A. Jackson and K. B. Horwitz, unpublished). Such a mechanism would also explain puzzling studies (*i.e.* Ref. 80) showing that steroid antagonists can be inhibitory in the absence of agonists.

As with coactivators, the receptor/corepressor dyad may be a subunit of an even larger inhibitory complex, since there is evidence that the repressive effects of unliganded TR result not only from binding a corepressor, but also from the ability of TR to interact with and sequester or inactivate TFIIB directly (22). This is brought about through a ternary complex in which an upstream domain of the TR HBD (amino acids 168–259) binds the corepressor, and a downstream C-terminal domain of the TR HBD (amino acids 260-C-terminus) contacts and silences TFIIB (71). Whether corepressors can contact components of the basal transcription machinery directly is not yet known.

COINTEGRATORS?

Recent data hint at even greater complexities than the ternary model indicates. The involvement of Rb in the GR/hbrm complex (32) is one example, and the involvement of downstream factors in the receptor/TIF1 complex (48) is another. Several groups (40, 80a, 80b) have described a multiprotein complex involving the HBDs of nuclear receptors: the p160 variant (ERAP 160) of the coactivator SRC-1; and the CBP/p300 coactivator of the cAMP-response element binding protein. Fibroblasts microinjected with neutralizing anti-CBP antibodies cannot support RAR or GR-dependent transcription, demonstrating the involvement of CBP in nuclear receptor signaling *in vivo* (80b). Consistent with its role as a coactivator, CBP/p300 also interacts with TFIIB (41). The authors propose (40, 80a, 80b) that CBP/p300 coordinates the transcriptional effects of simultaneous signals emanating from cell surface receptors and from nuclear receptors and that it acts as a cointegrator for multiple competing and perhaps conflicting signals that can impact one promoter.

CONCLUDING COMMENTS

When the dust settles, this initial flurry of publications will undoubtedly be followed by detailed studies confirming that some (but perhaps not all?) of these factors are *bona-fide* coactivators and corepressors. What caveats should be of concern?

First, factor-binding sites that are accessible on receptor fragments may be inaccessible in the context of full-length, native receptors. Moreover, recombinant receptors may lack the companion accessory proteins (heat shock proteins for example), or covalent modifications found on receptors intracellularly, that could block or modify receptor access to coregulatory factors. Of course, the same caveats apply to the coregulatory proteins. If, for example, they are shown to be phosphoproteins, or to be subunits of multiprotein complexes, or to be sequestered within cellular organelles, then their *in vivo* accessibility to the recep-

tors may be more limited than current assays assume. To address issues like these, Le Douarin *et al.* (48) used double-labeling immunofluorescence to show that TIF1 can move a nuclear localization-deficient ER from the cytoplasm into the nucleus in intact cells, confirming that there is a direct TIF1/ER interaction *in vivo*—at least when both partners are overexpressed.

Second, *in vitro* interaction studies generally use fusion proteins of GST coupled to full-length receptors, or to receptor fragments, in pull-down assays. It is unknown whether in this state, the receptors are presented to the putative coregulatory proteins as dimers—their usual condition when bound to DNA. This may be important as shown for the *Drosophila* transcription factor, Krüppel, which as a monomer specifically interacts with TFIIB, but as a dimer does not. Instead, the dimer binds the β-subunit of TFIIE, with which the monomer cannot interact. This switch converts Krüppel from an activator (as monomer) to a repressor (as dimer) (81). Additionally, these pull-down assays rarely include receptor constructs containing a DBD plus the cognate HREs, which ignores the fact that DNA-induced structural changes can influence receptor dimerization and folding (82–84).

Third, no studies have yet addressed the physiological relevance of these factors, particularly their multiplicity and promiscuity. Even the preliminary data indicate that each receptor can interact with many factors and that multiple factors are present in each cell and tissue. For example, this review lists at least 10 coactivators or basal factors that can interact with ER. How do multiple factors present in one cell compete with one another for binding, not only to receptors, but to other transcription factors? How far from physiological reality are current assays, which are based on *in vitro* interactions, or on cellular overexpression of one receptor and one factor? Does overexpression force proteins together artifactually? How does the structure of the promoter influence these interactions? Does the affinity of interacting partners for each other allow physiologically relevant interactions, given their intracellular concentrations? [Refer to Estojak *et al.* (85) for discussion of these issues.] It is likely that in the future, knockout and other genetic studies will address the overlapping or unique properties of each factor and will reveal functional redundancies, if any.

It also seems possible that both coactivators and corepressors can bind simultaneously to nuclear receptors under appropriate conditions. In that case, the continuum of activity that ranges from complete transcriptional inhibition, on the one hand, to maximal transcriptional activation, on the other, may be controlled by the ratio of coactivators to corepressors that are bound to receptors under specific conditions. That ratio could be controlled by, among other things, the type of ligand (agonist, antagonist, or none), the structure of the promoter, and tissue-specific differences in the levels and types of endogenous coactivators and corepressors. Observations that the antiestrogen tamoxifen is an antagonist in the breast, but an agonist in the uterus, could be explained by a model in which the stoichiometry of the receptor-bound factors shifts from an excess of corepressors to an excess of coactivators. Similarly, in breast cancers, mutations responsible for anomalous over- or underproduction of corepressors or coactivators during tumor progression could explain the tendency of tamoxifen-sensitive tumors to convert to tamoxifen-resistant states (86).

Finally, is it too late to gain control over the burgeoning alphabet soup of factor names and the tendency to rename existing factors, or are we already trapped by K. Yamamoto's dictum that "scientists would rather share toothbrushes than nomenclature"?

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Note Added in Proof

After submission of this paper (Fondell *et al., Proc Natl Acad Sci USA* 93:8329–8333, 1996) reported that TRα, in the presence of T_3, associates *in vivo* with a group of at least nine nuclear proteins termed TRAPs (thyroid hormone receptor-associated proteins). In an *in vitro* system reconstituted with general transcription factors, the liganded TRα/TRAP multiprotein complex enhances transcription from a promoter containing TR response elements above the intrinsic levels of ligand-occupied TRα alone.

REFERENCES

1. Mangelsdorf DJ, Evans RM 1995 The RXR heterodimers and orphan receptors. Cell 83:841–850
2. Kastner P, Mark M, Chambon P 1995 Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? Cell 83:859–869
3. Katzenellenbogen JA, O'Malley BW, Katzenellenbogen BS 1996 Tripartite steroid hormone receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. Mol Endocrinol 10:119–131
4. Chien C-T, Barte PL, Sternglanz R, Fields S 1991 The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest. Proc Natl Acad Sci USA 88:9578–9582
5. Tjian R, Maniatis T 1994 Transcriptional activation: a complex puzzle with few easy pieces. Cell 77:5–8
6. Buratowski S 1994 The basics of basal transcription by RNA polymerase II. Cell 77:1–3
7. Schulman IG, Chakravarti D, Juguilon H, Romo A, Evans RM 1995 Interactions between the retinoid X receptor

and a conserved region of the TATA-binding protein mediate hormone-dependent transactivation. Proc Natl Acad Sci USA 92:8288–8292
8. Sadovsky Y, Webb P, Lopez G, Baxter JD, Fitzpatrick PM, Gizang-Ginsberg E, Cavailles V, Parker MG, Kushner PJ 1995 Transcriptional activators differ in their responses to overexpression of TATA-box-binding protein. Mol Cell Biol 15:1554–1563
9. Leuther KK, Bushnell DA, Kornberg RD 1996 Two-dimensional crystallography of TFIIB- and IIE-RNA polymerase II complexes: implications for start site selection and initiation complex formation. Cell 85:773–779
10. Lewin B 1990 Commitment and activation at Pol II promoters: a tail of protein-protein interactions. Cell 61:1161–1164
11. Klein C, Struhl K 1994 Increased recruitment of TATA-binding protein to the promoter by transcriptional activation domains *in vivo*. Science 266:280–282
12. Hernandez N 1993 TBP, a universal eukaryotic transcription factor? Genes Dev 7:1291–1308
13. Brou C, Wu J, Ali S, Scheer E, Lang C, Davidson I, Chambon P, Tora L 1993 Different TBP-associated factors are required for mediating the stimulation of transcription *in vitro* by the acidic transactivator GAL-VP16 and the two nonacidic activation functions of the estrogen receptor. Nucleic Acids Res 21:5–12
14. Jacq X, Brou C, Lutz Y, Davidson I, Chambon P, Tora L 1994 Human $TAF_{II}30$ is present in a distinct TFIID complex and is required for transcriptional activation by the estrogen receptor. Cell 79:107–117
15. Chen J-L, Attardi DL, Verrijzer CP, Yokomori K, Tjian R 1994 Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. Cell 79:93–105
16. Sauer F, Hansen SK, Tjian R 1995 Multiple TAF_{II}s directing synergistic activation of transcription. Science 270:1783–1788
17. Chiang C-M, Roeder RG 1995 Cloning of an intrinsic human TFIID subunit that interacts with multiple transcriptional activators. Science 267:531–536
18. Schwerk C, Klotzbucher M, Sachs M, Ulber V, Klein-Hitpass L 1995 Identification of a transactivation function in the progesterone receptor that interacts with the $TAF_{II}110$ subunit of the TFIID complex. J Biol Chem 270:21331–21338
19. Choy B, Green MR 1993 Eukaryotic activators function during multiple steps of preinitiation complex assembly. Nature 366:531–536
20. Roberts SGE, Green MR 1994 Activator-induced conformational change in general transcription factor TFIIB. Nature 371:717–720
21. Ing NH, Beekman JM, Tsai SY, Tsai M-J, O'Malley BW 1992 Members of the steroid hormone receptor superfamily interact with TFIIB (S300-II). J Biol Chem 267:17617–17623
22. Baniahmad A, Ha I, Reinberg D, Tsai SY, Tsai M-J, O'Malley BW 1993 Interaction of human thyroid hormone receptor beta with transcription factor TFIIB may mediate target gene derepression and activation by thyroid hormone. Proc Natl Acad Sci USA 90:8832–8836
23. Hadzic E, Desai-Yajnik V, Helmer E, Guo S, Wu S, Koudinova N, Casanova J, Raaka BM, Samuels H 1995 A 10-amino-acid sequence in the N-terminal A/B domain of thyroid hormone receptor a is essential for transcriptional activation and interaction with the general transcription factor TFIIB. Mol Cell Biol 15:4507–4517
24. Blanco JCG, Wang I-M, Tsai SY, Tsai M-J, O'Malley BW, Jurutka PW, Haussler MR, Ozato K 1995 Transcription factor TFIIB and the vitamin D receptor cooperatively activate ligand-dependent transcription. Proc Natl Acad Sci USA 92:1535–1539
25. Meyer M-E, Gronemeyer H, Turcotte B, Bocquel M-T, Tasset D, Chambon P 1989 Steroid hormone receptors compete for factors that mediate their enhancer function. Cell 57:433–442
26. Shemshedini L, Ji J, Brou C, Chambon P, Gronemeyer H 1992 *In vitro* activity of the transcription activation functions of the progesterone receptor. J Biol Chem 267:1834–1839
27. Meyer M-E, Quirin-Stricker C, Lerouge T, Bocquel M-T, Gronemeyer H 1992 A limiting factor mediates the differential activation of promoters by the human progesterone receptor isoforms. J Biol Chem 267:10882–10887
28. Goodrich JA, Hoey T, Thut CJ, Admon A, Tjian R 1993 *Drosophila* $TAF_{II}40$ interacts with both a VP16 activation domain and the basal transcription factor TFIIB. Cell 75:519–530
29. Berkenstam A, Ruiz d MV, Barettino D, Horikoshi M, Stunnenberg HG 1992 Cooperativity in transactivation between retinoic acid receptor and TFIID requires an activity analogous to E1A. Cell 69:401–412
30. Muchardt C, Yaniv M 1993 A human homologue of *Saccharomyces cerevisiae SNF2/SWI2* and *Drosophila brm* genes potentiates transcriptional activation by the glucocorticoid receptor. EMBO J 12:4279–4290
31. Chiba H, Muramatsu M, Nomoto A, Kato H 1994 Two human homologues of *Saccharomyces cerevisiae SWI2/SNF2* and *Drosophila brahma* are transcriptional coactivators cooperating with the estrogen receptor and the retinoic acid receptor. Nucleic Acids Res 22:1815–1820
32. Singh P, Coe J, Hong W 1995 A role for retinoblastoma protein in potentiating transcriptional activation by the glucocorticoid receptor. Nature 374:562–565
33. Kwon H, Imbalzano AN, Khavari PA, Kingston RE, Green MR 1994 Nucleosome disruption and enhancement of activator binding by a human SWI/SNF complex. Nature 370:477–481
34. Yoshinaga SK, Peterson CL, Herskowitz I, Yamamoto KR 1992 Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. Science 258:1598–1604
35. Wilson CJ, Chao DM, Imbalzano AN, Schnitzler GR, Kingston RE, Young RA 1996 RNA polymerase II holoenzyme contains SWI/SNF regulators involved in chromatin remodeling. Cell 84:235–244
36. Halachmi S, Marden E, Martin G, MacKay H, Abbondanza C, Brown M 1994 Estrogen receptor-associated proteins: possible mediators of hormone-induced transcription. Science 264:1455–1458
37. Cavaillès V, Dauvois S, Danielian PS, Parker MG 1994 Interaction of proteins with transcriptionally active estrogen receptors. Proc Natl Acad Sci USA 91:10009–10013
38. Cavaillès V, Dauvois S, L'Horset F, Lopez G, Hoare S, Kushner PJ, Parker MG 1995 Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. EMBO J 14:3741–3751
39. Oñate SA, Tsai SY, Tsai M-J, O'Malley BW 1995 Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270: 1354–1357
40. Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, Lin S-C, Heyman RA, Rose DW, Glass CK, Rosenfeld MG 1996 A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 85:403–414
41. Kwok RPS, Lundblad JR, Chrivia JC, Richards JP, Bächinger HP, Brennan RG, Roberts SGE, Green MR, Goodman RH 1994 Nuclear protein CBP is a coactivator for the transcription factor CREB. Nature 370:223–226
42. Hong H, Kohli K, Trivedi A, Johnson DL, Stallcup MR 1996 GRIP1, a novel mouse protein that serves as a transcriptional coactivator in yeast for the hormone binding domains of steroid receptors. Proc Natl Acad Sci USA 93:4948–4952
43. Bortvin A, Winston F 1996 Evidence that Spt6p controls chromatin structure by a direct interaction with histones.

Science 272:1473–1476
44. Baniahmad C, Nawaz Z, Baniahmad A, Gleeson MAG, Tsai M-J, O'Malley BW 1995 Enhancement of human estrogen receptor activity by SPT6: a potential coactivator. Mol Endocrinol 9:34–43
45. Lee JW, Choi H-S, Gyuris J, Brent R, Moore DD 1995 Two classes of proteins dependent on either the presence or absence of thyroid hormone for interaction with the thyroid hormone receptor. Mol Endocrinol 9:243–254
46. Lee JW, Ryan F, Swaffield JC, Johnston SA, Moore DD 1995 Interaction of thyroid-hormone receptor with a conserved transcriptional mediator. Nature 374:91–94
46a. Bauer EV, Zechel C, Heery D, Heine MJS, Garnier JM, Vivat V, Le Douarin B, Gronemeyer H, Chambon P, Losson R 1996 Differential ligand-dependent interactions between the AF-2 activating domain of nuclear receptors and the putative transcriptional intermediary factors mSUG1 and TIF1. EMBO J 15:110–124
47. Swaffield JC, Melcher K, Johnston SA 1995 A highly conserved ATPase protein as a mediator between acidic activation domains and the TATA-binding protein. Nature 374:88–91
47a. Rubin DM, Coux O, Wefex I, Hengartner C, Young RA, Goldberg AL, Finley D 1996 Identification of the *gal4* suppressor Sug1 as a subunit of the yeast 26S proteasome. Nature 379:655–657
48. Le Douarin B, Zechel C, Garnier J-M, Lutz Y, Tora L, Pierrat B, Heery D, Gronemeyer H, Chambon P, Losson R 1995 The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors, is fused to B-raf in the oncogenic protein T18. EMBO J 14:2020–2033
49. Yeh S, Chang C 1996 Cloning and characterization of a specific coactivator, ARA_{70}, for the androgen receptor in human prostate cells. Proc Natl Acad Sci USA 93:5517–5521
50. Huibregtse JM, Scheffner M, Beaudenon S, Howley PM 1995 A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. Proc Natl Acad Sci USA 92:2563–2567
51. Imhof MO, McDonnell DP 1996 Yeast RSP5 and its human homolog hRPF1 potentiate hormone-dependent activation of transcription by human progsterone and glucocorticoid receptors. Mol Cell Biol 16:2594–2605
52. Sartorius CA, Melville MY, Hovland AR, Tung L, Takimoto GS, Horwitz KB 1994 A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform. Mol Endocrinol 8:1347–1360
53. Zeiner M, Gehring U 1995 A protein that interacts with members of the nuclear hormone receptor family: identification and cDNA cloning. Proc Natl Acad Sci USA 92:11465–11469
54. Baniahmad A, Steiner C, Köhne AC, Renkawitz R 1990 Modular structure of a chicken lysozyme silencer: involvement of an unusual thyroid hormone receptor binding site. Cell 61:505–514
55. Damm K, Thompson CC, Evans RM 1989 Protein encoded by v-Erb A functions as a thyroid hormone receptor antagonist. Nature 339:593–597
56. Sap J, Munoz J, Schmitt H, Stunnenberg H, Vennstrom B 1989 Repression of transcription mediated by a thyroid hormone response element by the v-Erb A oncogene product. Nature 340:242–244
57. Smith DF, Toft DO 1993 Steroid receptors and their associated proteins. Mol Endocrinol 7:4–11
58. Levine M, Manley JL 1989 Transcriptional repression of eukaryotic promoters. Cell 59:405–408
59. Renkawitz R 1990 Transcriptional repression in eukaryotes. Trends Genet 6:192–197
60. Johnson AD 1995 The price of repression. Cell 81:655–658
61. Sakai DD, Helms S, Carlstedt-Duke J, Gustafsson J-Å, Rottman FM, Yamamoto KR 1988 Hormone-mediated repression: a negative glucocorticoid response element from the bovine prolactin gene. Genes Dev 2: 1144–1154
62. Diamond MI, Miner JN, Yoshinaga SK, Yamamoto KR 1990 Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. Science 249:1266–1272
63. Burns K, Duggan B, Atkinson EA, Famulski KS, Nemer M, Bleackley RC, Michalak M 1994 Modulation of gene expression by calreticulin binding to the glucocorticoid receptor. Nature 367:476–480
64. Dedhar S, Rennie PS, Shago M, Hagesteijn C-YL, Yang H, Filmus J, Hawley RG, Bruchovsky N, Cheng H, Matusik RJ, Giguère V 1994 Inhibition of nuclear hormone receptor activity by calreticulin. Nature 367:480–483
65. Burris TP, Nawaz Z, Tsai M-J, O'Malley BW 1995 A nuclear hormone receptor-associated protein that inhibits transactivation by the thyroid hormone and retinoic acid receptors. Proc Natl Acad Sci USA 92:9525–9529
66. Kirov N, Zhelnin L, Shah J, Rushlow C 1993 Conversion of a silencer into an enhancer: evidence for a co-repressor in *dorsal*-mediated repression in *Drosophila*. EMBO J 12:3193–3199
67. Keleher CA, Redd MJ, Schultz J, Carlson M, Johnson AD 1992 Ssn6-Tup1 is a general repressor of transcription in yeast. Cell 68:709–719
68. Edmondson DG, Smith MM, Roth SY 1996 Repression domain of the yeast global repressor Tup1 interacts directly with histones H3 and H4. Genes Dev 10:1247–1258
69. McDonnell DP, Vegeto E, O'Malley BW 1992 Identification of a negative regulatory function for steroid receptors. Proc Natl Acad Sci USA 89:10563–10567
70. Lazar MA 1993 Thyroid hormone receptors: multiple forms, multiple possibilities. Mol Endocrinol 86:3494–3498
71. Baniahmad A, Leng X, Burris TP, Tsai SY, Tsai M-J, O'Malley BW 1995 The t4 activation domain of the thyroid hormone receptor is required for release of a putative corepressor(s) necessary for transcriptional silencing. Mol Cell Biol 15:76–86
72. Hörlein AJ, Näär AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Söderström M, Glass CK, Rosenfeld MG 1995 Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature 377:397–403
73. Kurokawa R, Söderström M, Hörlein 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
74. Chen JD, Evans RM 1995 A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature 377:454–457
74a. Zamir I, Harding HP, Atkins GB, Hörlein A, Glass CK, Rosenfeld MG, Lazar MA 1996 A nuclear hormone receptor corepressor mediates transcriptional silencing by receptors with distinct repression domains. Mol Cell Biol, in press
75. Kurokawa R, DiRenzo J, Boehm M, Sugarman J, Gloss B, Rosenfeld MG, Heyman RA, Glass CK 1994 Regulation of retinoid signalling by receptor polarity and allosteric control of ligand binding. Nature 371:528–531
76. Tong G-X, Jeyakumar M, Tanen MR, Bagchi MK 1996 Transcriptional silencing by unliganded thyroid hormone receptor b requires a soluble copressor that interacts with the ligand-binding domain of the receptor. Mol Cell Biol 16:1909–1920
77. Seol W, Choi H-S, Moore DD 1995 Isolation of proteins that interact specifically with the retinoic × receptor: two novel orphan receptors. Mol Endocrinol 9:72–85
78. Sande S, Privalsky ML 1996 Identification of TRACs, a family of co-factors that associate with, and modulate

the activity of nuclear hormone receptors. Mol Endocrinol 10:813–825
79. Perlmann T, Vennström B 1995 The sound of silence. Nature 377:387–388
79a. Chen JD, Umesono K, Evans RM 1996 SMRT isoforms mediate repression and anti-repression of nuclear receptor heterodimers. Proc Natl Acad Sci USA 93: in press
80. Thomas M, Monet J-D 1992 Combined effects of RU486 and tamoxifen on the growth and cell cycle phases of the MCF-7 cell line. J Clin Endocrinol Metab 75:865–870
80a. Hanstein B, Eckner R, DiRenzo J, Halachmi S, Liu H, Searcy B, Brown M 1996 p300 is a component of an estrogen receptor coactivator complex. Proc Natl Acad Sci USA, in press
80b. Chakravarti D, LaMorte VJ, Nelson MC, Nakajima T, Schulman IG, Jugullon H, Montminy M, Evans RM 1996 Role of CBP/P300 in nuclear receptor signalling. Nature, in press
81. Sauer F, Fondell JD, Ohkuma Y, Roeder RG, Jäckle H 1995 Control of transcription by Krüppel through interactions with TFIIB and TFIIEb. Nature 375:162–164
82. Petersen JM, Skalicky JJ, Donaldson LW, McIntosh LP, Alber T, Graves BJ 1995 Modulation of transcription factor Ets-1 DNA binding: DNA-induced unfolding of an a helix. Science 269:1866–1869
83. Rastinejad F, Perlmann T, Evans RM, Sigler PB 1995 Structural determinants of nuclear receptor assembly on DNA direct repeats. Nature 375:203–211
84. Gronemeyer H, Moras D 1995 How to finger DNA. Nature 375:190–191
85. Estojak J, Brent R, Golemis EA 1995 Correlation of two-hybrid affinity data with *in vitro* measurements. Mol Cell Biol 15:5820–5829
86. Horwitz KB 1995 Editorial: when tamoxifen turns bad. Endocrinology 136:821–823
87. Voegel JJ, Heine MJS, Zechel C, Chambon P, Gronemeyer H 1996 TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. EMBO J 15:3667–3675