CONTROLLING NUCLEAR RECEPTORS: THE CIRCULAR LOGIC OF COFACTOR CYCLES

Valentina Perissi and Michael G. Rosenfeld

Abstract | Nuclear receptors regulate many biologically important processes in development and homeostasis by their bimodal function as repressors and activators of gene transcription. A finely tuned modulation of the transcriptional activities of nuclear receptors is crucial for determining highly specific and diversified programmes of gene expression. Recent studies have provided insights into the molecular mechanisms that are required to switch between repression and activation functions, the combinatorial roles of the multiple cofactor complexes that are required for mediating transcriptional regulation, and the central question of how several different signalling pathways can be integrated at the nuclear level to achieve specific profiles of gene expression.

ORPHAN RECEPTOR
A subclass of nuclear receptors that were originally identified as orphans because the ligand was unknown.

Howard Hughes Medical Institute, Department of Medicine, University of California San Diego, 9500 Gilman Drive, La Jolla, California 92093-064, USA.
e-mails: vperissi@ucsd.edu; mrosenfeld@ucsd.edu
doi:10.1038/nrm1680

Precise spatial and temporal patterns of gene expression are crucial for the normal development of all organisms. Orchestrating these patterns requires the coordination of numerous regulatory events and mechanisms that mediate both the repression and activation of specific target genes at specific times and places during development. DNA-binding transcription factors, non-DNA-binding coregulators and general components of the basal RNA polymerase machinery are essential to regulate transcription and achieve the correct patterns of gene expression. Although there are many variations in their functions and in the highly specific strategies of regulation, several general themes and common rules have emerged.

Nuclear receptors provide an interesting model to study the specific, as well as the more general, mechanisms of transcriptional regulation; they are highly regulated DNA-binding transcription factors that are directly modulated by ligand binding and can both activate and repress gene expression. Here, we review several recent advances in our understanding of nuclear-receptor-mediated transcriptional regulation by focusing on the dedicated mechanisms that regulate the switch from gene repression to gene activation and that further modulate transcriptional activity. The complexity of regulatory proteins and the many ways of modulating their recruitment and their activity tightly regulates this apparently simple, but fascinating, recruitment event.

The nuclear receptor superfamily: an overview

The mammalian nuclear receptor superfamily comprises more than 45 transcription factors, many of which regulate gene expression in a ligand-dependent manner. Members of the nuclear receptor superfamily include: receptors for steroid hormones, such as the oestrogen receptor (ER), the androgen receptor (AR) and the glucocorticoid receptor (GR); receptors for non-steroidal ligands, such as the thyroid hormone receptor (TR) and the retinoic acid receptor (RAR); as well as receptors that bind diverse products of lipid metabolism such as fatty acids and prostaglandins (peroxisome proliferator activated receptors (PPARs) and liver X receptors (LXRs))[1,2]. The nuclear receptor superfamily also includes many so-called ORPHAN RECEPTORS for which regulatory ligands are still unknown or for which candidates have only recently been identified by screening strategies that

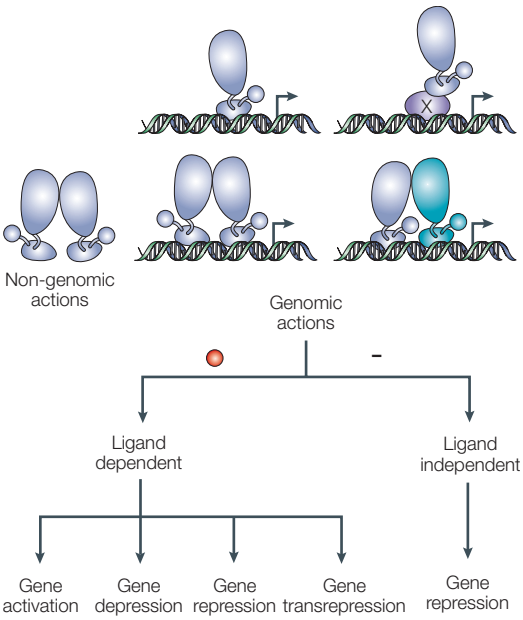


Figure 1 | **Mechanisms of transcriptional regulation by nuclear receptors.** Ligand-inducible nuclear receptors modulate transcription through several distinct mechanisms, which include both activation and repression activities. These activities can be genomic or non-genomic (a description of rapid steroid actions that activate signalling pathways and that do not directly influence gene expression can be found in REF. 144), ligand dependent or independent, and can mediate gene repression, the release of gene repression, gene activation, or gene transrepression (when they negatively regulate transcription indirectly through another transcription factor 'X'). Furthermore, nuclear receptors can bind to their DNA-response elements as a single unit (monomer; for example, steroidogenic factor-1 (SF1)), or associated with another identical receptor (homodimer; for example, ER–ER), or with a receptor of a different type (heterodimer; for example, RAR–RXR). ER, oestrogen receptor; RAR, retinoic acid receptor; RXR, retinoic X receptor.

have uncovered physiological and pharmacological ligands[3].

Remarkably, the sequence of the *Caenorhabditis elegans* genome has revealed that the nuclear receptor family, with more than 200 members, constitutes the largest family of transcriptional regulators encoded by this genome, which indicates that these proteins have an important role in the environmental adaptation of nematodes[4]. The sequencing of the human genome has so far led to the identification of 48 nuclear receptors, which have diverse, crucial roles in the regulation of growth, development and homeostasis. Comparative analysis among human, nematode and insect genome sequences has shown that divergent evolutionary pathways have defined separate phylogenetic lineages so that most of the *C. elegans* nuclear receptors diverge from those found in humans and flies[5].

Nuclear receptor proteins have a characteristic, modular structure, which includes a highly conserved DNA-binding domain (DBD) and ligand-binding domain (LBD)[1,2]. However, despite the structural conservation, members of the nuclear receptor family are functionally extremely flexible in regulating transcription. For example, they can bind directly to specific response elements in DNA, either as a monomer or as homo- and heterodimers. Or, they can regulate transcription indirectly through other classes of DNA-bound transcription factors. Also, a subset of receptors, which includes TR and RAR, can actively repress target genes in the absence of ligand binding, whereas on ligand binding, all nuclear receptors usually become potent transcriptional activators. Conversely, several nuclear receptors have been shown to inhibit transcription in a ligand-dependent manner — either by binding to negative response elements or by antagonizing the transcriptional activities of other classes of transcription factors, an effect known as transrepression[1,2,6,7] (FIG. 1).

Coregulators: corepressor and coactivators

Nuclear receptors carry out their many different transcriptional functions through the recruitment of a host of positive and negative regulatory proteins, referred to as coactivators or corepressors, respectively[8] (FIG. 2). These regulatory cofactors are not exclusive to nuclear receptors, and are used in a similar manner by numerous other DNA-binding transcription factors. The molecular mechanism that regulates the alternative interactions of the nuclear receptor with either class of cofactors has been decoded by crystallographic studies[9]. For a detailed description of how these alternative interactions are directly regulated by specific changes in the receptor conformation, see BOX 1.

In general, unliganded nuclear receptors preferentially interact with corepressors to mediate repression, whereas liganded receptors are transcriptional activators owing to their ability to recruit coactivator proteins. However, a few exceptions have been identified, as some corepressors, such as LCoR (ligand-dependent nuclear-receptor corepressor), RIP140 (receptor-interacting protein-140) and REA (repressor of oestrogen-receptor activity)[10,11], can bind to nuclear receptors in a ligand-dependent manner and compete with coactivators by displacing them. These findings indicate the existence of specific regulatory mechanisms that use similar, but reverse, approaches to attenuate the function of agonist-bound receptors. Furthermore, there are coregulatory factors, such as the ATP-dependent chromatin remodelling complexes SWI/SNF, which have been associated with the regulation of both gene activation and gene repression[12–16]. Therefore, it is important to consider that the role of a given coregulatory protein can be context dependent and, hence, it is not always possible to assign a functional outcome on the basis of the recruitment of a specific cofactor.

Alternatively, coregulators can also be classified into two main groups according to their mode of action. The first group contains factors that covalently modify histones (for example, by ACETYLATION/deacetylation, METHYLATION/demethylation, phosphorylation/dephosphorylation or UBIQUITYLATION/deubiquitylation), a process that follows a precise and combinatorial code.

ACETYLATION
The enzymatic process of adding an acetyl group to a Lys residue on histone tails or on other proteins.

METHYLATION
The enzymatic process of adding a methyl group to a Lys or an Arg residue on histone tails or other proteins. Alternatively, methyl groups can be added to DNA itself on cytosine bases.

UBIQUITYLATION
A three-step enzymatic process that covalently conjugates the small protein ubiquitin to a Lys residue on a protein substrate. Ubiquitin can be added as a monomer or as a polymer to form long chains.

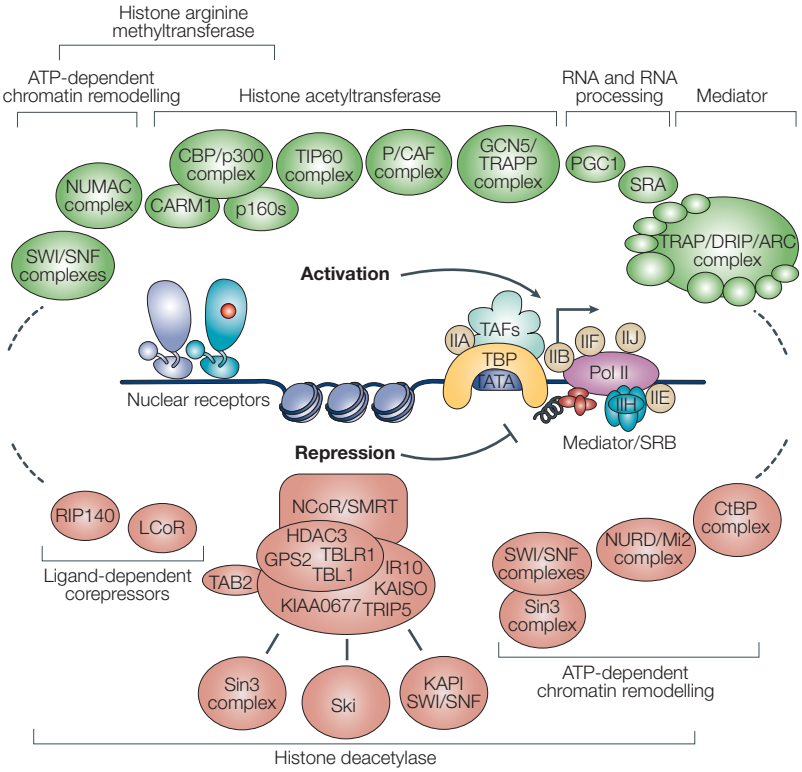


Figure 2 | **Coactivator and corepressor complexes are required for nuclear-receptor-mediated transcriptional regulation.** The regulation of a general transcription unit by nuclear receptors requires a vast number of coregulatory complexes that have various functions and enzymatic activities. Coactivator complexes (green) include factors that contain ATP-dependent chromatin remodelling activity[14,38,52], histone arginine methyltransferases[145–147], histone acetyltransferases[103,148,149], as well as factors that are involved in RNA processing[20,150] and components of the so-called Mediator complex that mediate the interaction with the RNA polymerase II (pol II) machinery[151–154]. Conversely, corepressors (red) include ATP-dependent chromatin remodelling complexes[21,155–159], basal corepressors, such as NCoR and SMRT, which function as platforms for the recruitment of several subcomplexes that often contain histone deacetylase activity[13,27,88,122,123], and specific corepressors, such as LCoR and RIP140, which are surprisingly able to recruit general corepressors on ligand induction[10]. This schematic representation is useful to underline the numerous regulatory complexes that are involved in nuclear-receptor-mediated regulation; however, it is important to keep in mind that it does not illustrate the dynamics of their recruitment to the regulated transcription unit (see FIG. 3). IIA, IIB, IIE, IIF, IIH, IIJ, general transcription factors A, B, E, F, H, J; HDAC, histone deacetylase; LCoR, ligand-dependent nuclear-receptor corepressor; NCoR, nuclear-receptor corepressor; RIP140, receptor-interacting protein-140; SMRT, silencing mediator of retinoic acid and thyroid hormone receptors; TAF, TBP-associated factor; TBP, TATA-binding protein.

The second group includes ATP-dependent chromatin remodelling factors that modulate promoter accessibility to transcription factors and to the basal transcriptional machinery[17–19]. However, this broad division is not fully inclusive as there are numerous cofactors that do not have any known direct effects on chromatin structure or modification, but instead function in the assembly, the recruitment or the release of coregulatory complexes.

Furthermore, a precise classification is complicated by the fact that new coregulators are continually being discovered and these include factors that were not expected to serve such functions, as exemplified by the RNA transcript for the steroid-receptor-RNA activator-1 (SRA1) coactivator[20], the NAD/NADH sensor CtBP (C-terminal binding protein of E1A)[21–23], and several ACTIN-binding proteins[24–29]. This indicates that transcriptional regulation cannot be considered as an independent, chromatin-based process, but rather should be considered as coupled to many other cellular events that are carried out by several distinct groups of factors. For an exhaustive list of known coregulators, see REFS 30–33.

In conclusion, the general concept that emerges from the uncovering of this complicated network of coregulators is that an unexpectedly large array of proteins and enzymatic activities converges at promoters, defining what could be considered a 'cofactor code', which is characterized by distinct patterns of cofactor recruitment and by their regulated enzymatic activities. The HISTONE CODE, which is determined by specific combinations of covalent histone modifications[18], is a consequence as well as a determinant of this cofactor code, as histones are crucial targets for the enzymatic activities of cofactors, but also have a key role in specifying cofactor recruitment on the basis of the 'reading' of the histone code by specific chromatin-binding domains.

Coregulators in multiprotein complexes

The ability of corepressors and coactivators to associate in several different diverse complexes further increases the complexity of their transcriptional regulatory roles and allows a temporal- and tissue-specific modulation of the combinatorial cofactor and histone codes for specific, single genes. The existence of numerous platform coregulatory proteins, each capable of several interactions, has a fundamental role in determining this flexibility, as these interactions can be effectively modulated by covalent modifications, protein levels and competition between proteins, and they provide a conceptual basis for the exquisite control of specific gene expression.

Furthermore, many coregulators share functions and enzymatic activities with other, related coregulators that can exert partly redundant functions in certain biological settings, but that have unique functions in others. An example is provided by the mammalian homologues of SWI/SNF2, Brahma and BRG1, which cannot genetically compensate for each other, and which associate with different BRG1-associated factor (BAF) chromatin remodelling complexes[34–37] that are required by different nuclear receptors[14,38,39].

FIGURE 2 shows a generic nuclear-receptor-regulated transcription unit with a subset of the cohorts of coactivator and corepressor complexes that can participate in its control — the transition from repression to activation results from ligand binding to the receptor and from the exchange of corepressors for coactivators. Although this representation is useful to illustrate the multiplicity of coregulatory complexes, this type of model has turned out to be erroneously static and oversimplified. The switch between gene repression and gene activation, for example, can hardly be defined by the simple alternative recruitment of two different regulatory complexes. Rather, it is achieved by a series of sequential events that are mediated by multiple enzymatic activities that

ACTIN
An abundant protein that forms filaments that are the main constituents of the cytoskeleton of all eukaryotic cells: the monomeric form is known as G-actin and the polymeric, filamentous form as F-actin.

can be factor, promoter and cell-type specific, as will be discussed in more detail below[17,32,40,41].

The idea of transcriptional regulation as a highly dynamic event also raises the interesting issue of whether transcription complexes are temporarily recruited to each active gene for cofactor exchange to occur, or whether a crucial component of transcriptional activation might involve the migration of the promoters themselves to physiological 'stations' of preassembled transcription 'factories' [42–44].

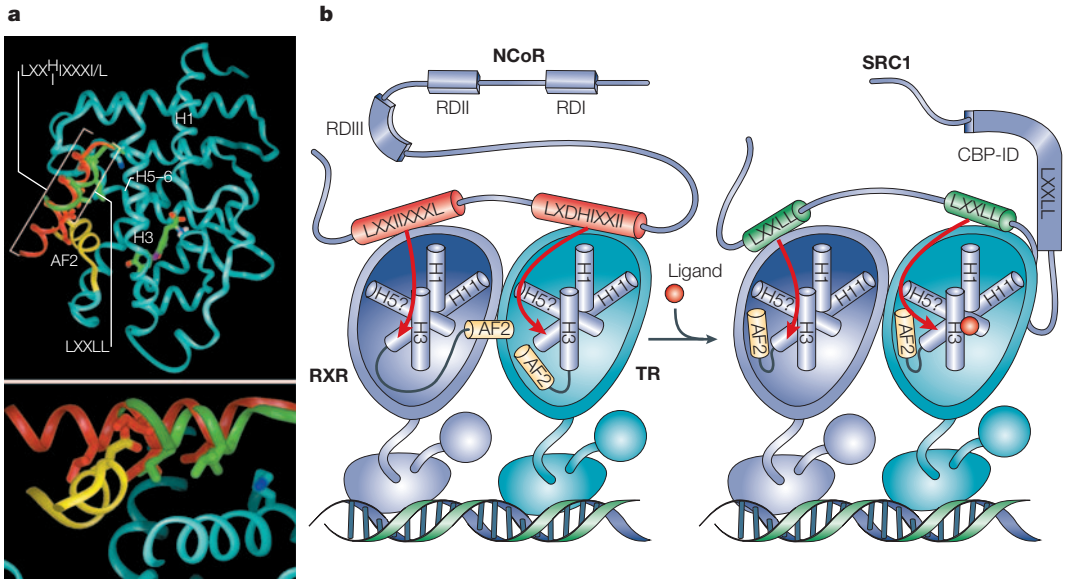
Interestingly, many studies have indicated a role for nuclear actin in regulating gene transcription[45,46], and therefore it is intriguing to consider that actin-binding proteins and actin itself, as detected in various biochemically purified cofactors complexes, might be important for mediating these nuclear relocalization events. For more detail on the functional architecture of the nucleus and the spatial localization of active domains of transcription, see REFS 47–49.

Box 1 | Nuclear-receptor-coregulator interaction motifs

Crystal structures of the ligand-binding domains (LBDs) of several nuclear receptors have revealed a three-layered, anti-parallel α-HELICAL sandwich, with a central core layer of three helices packed between two additional layers of helices to form a ligand-binding cavity. In the unliganded retinoic X receptor (RXR) structure, helix 12, which contains the transactivation domain AF2, extends away from the LBD[131], whereas in the agonist-bound retinoic acid receptor (RAR)α, thyroid hormone receptor (TR)β, oestrogen receptor (ER) and peroxisome proliferator activated receptor (PPAR)γ structures, the AF2 helix (shown in yellow in part a of the figure) is tightly packed against the LBD and makes direct contact with the ligand[132–137]. This spatial organization indicated that ligand-dependent changes in the conformation of the AF2 helix could form a novel surface that might facilitate coactivator interactions. In the presence of ligand, numerous coactivators, such as steroid receptor coregulator (SRC1 or NCoA1) interact with nuclear receptors through helical motifs that contain an LXXLL consensus sequence (see figure, part b)[138,139]. Interestingly, co-crystal structures of nuclear receptors with LXXLL-interacting motifs from different coactivators indicate that the LXXLL helix fits into a defined pocket (indicated by red arrow), and that two conserved crucial residues of the receptor, a Glu in the AF2 helix and a Lys in the binding pocket, form a 'charge clamp' that stabilizes the interaction[133,136,137].

Similarly, corepressor proteins, such as nuclear-receptor corepressor (NCoR) and SMRT, interact with unliganded RAR and TR and with antagonist-bound steroid hormone receptors using two nuclear-receptor-interaction domains that share a core consensus motif: LXX I/H IXXX I/L or corepressor–nuclear-receptor (CoRNR) box (see figure, part b)[140–142]. Interestingly, this motif is highly related to the coactivator-interacting motif, and can be visualized as an N-terminally extended helix (shown in red in part a of the figure) when compared to the shorter, LXXLL-containing helix (shown in green in part a of the figure). As the crucial determinants of corepressor binding reside in the same pocket that is required for coactivator binding, it was proposed that ligand-dependent exchange between corepressors and coactivators is caused by the difference in length of the interacting motifs that can be accommodated in the binding pocket in the two conformations[141].

Screening of a phage-display library of CoRNR box motifs allowed the identification of several peptides that specifically bind to the antagonist-bound ER, which confirms that the receptor uses overlapping surfaces for binding to corepressors and coactivators. It also showed that it is possible to identify mutations that differentiate between the two classes of regulators[143]. CBP-ID, CREB-binding protein (CBP)-interaction domain; RDI, RDII, RDIII, repressor domains 1–3; H1–H12, α helices 1–12.



HISTONE CODE
The post-translational modifications of histone tails usually in characteristic clusters, which include acetylation, phosphorylation, ubiquitylation, methylation and ADP-ribosylation. The modified histone tails combine to create an epigenetic code for the regulation of gene expression.

A HELIX
An element of protein secondary structure in which hydrogen bonds along the backbone of a single polypeptide cause the chain to form a right-handed helix.

CHROMATIN IMMUNOPRECIPITATION (ChIP). A technique that is used to specifically immunoprecipitate complexes of DNA with associated proteins. The use of antibodies specific for histone modifications, DNA-binding transcription factors or coregulators has allowed the study of promoter occupancy by different factors and the state of chromatin modification.

Ordered recruitment of coregulator complexes

Given the diversity of coregulatory complexes and their often mutually exclusive mode of interaction with transcription factors, it was important to establish whether these series of transcription-factor–coactivator interactions occur randomly or in a sequential and regulated fashion. Indeed, although the cell- and time-specific expression patterns of some cofactors can produce distinct modulations of nuclear receptor transcriptional activity owing to differences in the relative corepressor and coactivator protein levels[50], it is true that many other cofactors are coexpressed in the same cell type at relatively similar, high levels, which raises the possibility of their concomitant recruitment to a specific promoter.

In an elegant pioneering study, Cosma and colleagues[51] presented the first unambiguous example of the ordered recruitment of different coregulatory complexes to a regulated transcription unit. Using CHROMATIN IMMUNOPRECIPITATION (ChiP) analysis to study the transcriptional activation of the *HO* gene in budding yeast, they found that the recruitment of the transcription factors Swi5 and SBF, the SWI/SNF and the SAGA coactivator complexes, and the Ash1 repressor followed a precise pattern in which every step was precisely timed and necessary for the subsequent event[51].

This first observation presaged numerous studies in mammalian systems, often related to nuclear-receptor-regulated genes that have established a conceptually similar pattern of recruitment and release of cohorts of coregulatory complexes[52–56]. A good example is provided by the examination of ER-mediated transcriptional regulation of either the cathepsin D (*CATD*)[53] or the trefoil factor-1 (*TFF1*/*pS2*) promoter[16,55] in breast cancer cells. These analyses revealed detailed and coordinated patterns of cofactor recruitment and preferential selectivity for factors that have similar enzymatic activities[16,55]. In the case of the *pS2* promoter, for example, different classes of cofactors could be identified, which are each defined by distinct enzymatic activities and recruited to inducible promoters by the DNA-bound transcription factor in a factor-specific, precisely timed and sequential fashion[16,32] (FIG. 3). Interestingly, in this experimental setting, some coregulators seemed to be redundant, and different family members were equally capable of being recruited alternatively to the promoter (that is, the nuclear-receptor coactivators NCoA1/SRC1 and NCoA3/pCIP or the coactivator-associated arginine methyltransferases CARM1 and PRMT1 never co-occupied the *pS2* promoter)[16]. By contrast, preferential interactions between a nuclear receptor and a specific histone acetyltransferase (HAT)-containing enzyme or a specific member of the NCoA family of coactivators have also been observed[57–59].

A similar scenario of coordinated recruitment of dedicated corepressor complexes is also emerging for transcriptional repression. For example, 4-hydroxy-tamoxifen (4-OHT)-bound ERα mediates the sequential recruitment of two different complexes, NCoR–TBL1–HDAC3 and the chromatin-remodelling complexes known as nucleosome remodelling and histone deacetylation (NURD), which each harbour distinct histone deacetylase (HDAC) activities[60]. The N-terminal truncated ERα isoform (apo-ERα-46kD) also engages these two complexes, but in this case, unliganded ER recruits them in a combinatorial fashion and no sequential recruitment was observed[61]. Interestingly, the specific recruitment of diverse corepressor complexes could reflect a different specificity for the deacetylation of histone tail residues[62], and this might be important to define a histone code for repression.

In conclusion, several recent studies have begun to depict a fascinating picture of the ordered recruitment of different complexes of cofactors to regulated transcription units. The challenge now is to extrapolate these single-factor-, single-gene-, single-cell-based observations to a more general level to understand the rules that govern the deposition of this cofactor code and the strategies for its modulation. An interesting aspect, for example, is that the promoter-specific DNA-binding sequence by itself could represent an allosteric regulator of receptor activity and cofactor recruitment[63–65]. Similarly, the mode of interaction with DNA, either directly or through other transcription factors, could be an important regulator of specificity. Shang and Brown, for example, have reported differences in the regulation of the *Myc* and *CATD* genes by ERα that is bound to the antagonist 4-OHT, which might depend on the different organization of the two promoters analysed — which either contain or lack a classic oestrogen response element (ERE)[50]. Furthermore, Rogatsky and colleagues adopted a genome-wide approach to

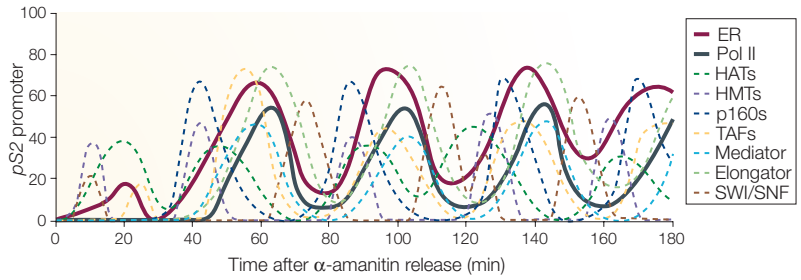


Figure 3 | **Ordered cofactor recruitment by the ER.** The diagram summarizes the observations of Metivier and colleagues on the kinetics of cofactor recruitment to the *pS2* promoter. In this kinetic ChIP analysis, the *pS2* promoter was stimulated with 10 nM oestradiol, and transcription by RNA polymerase II (pol II) was inhibited by treatment with α-amanitin[16]. For purposes of clarity, different factors that have the same enzymatic activities have been grouped together. However, this simplification might hide differences among proteins that belong to the same enzymatic class and that are represented as a single unit. For example, the histone acetyltransferase (HAT) GCN5 is always recruited slightly after the other family members, shown by the green curve. Also, in the first cycle, only p300, the HAT TIP60 and the histone methyltransferase (HMT) PRMT1 are engaged, whereas the other HATs and HMTs are not yet recruited. However, it is unclear whether this initial, non-productive cycle exists under physiological conditions, as the use of α-amanitin, although very important to distinguish the kinetics of ER-dependent recruitment from other basal activation events of the promoter, creates an artificial situation in which transcription has been completely shut off. The *y* axis represents the amount of immunoprecipitated *pS2* promoter expressed as a percentage of the input[16]. p160 proteins belong to the NCoA family of coactivators, components of the Mediator complex mediate pol II transcription, elongator factors are involved in transcription elongation and SWI/SNF are ATP-dependent chromatin remodelling factors. TAFs, TBP-associated factors.

identify GR targets and to determine differences in their regulation. This permitted the identification of several distinct patterns of use of specific GR domains, which indicates that a single transcription factor might perform differently at specific gene locations because diverse surfaces and different interacting cofactors can be engaged depending on the promoter context[66].

For further discussions about the models of cooperation between different complexes that regulate transcription and their ordered recruitment to specific gene promoters by transcription factors other than nuclear receptors, see REFS 32,67.

Kinetics of promoter occupancy

The dynamic recruitment of different coregulator complexes is associated with an equally dynamic binding of the nuclear receptor itself to the promoter. Indeed, in the past few years, the conventional view that receptors remain stably bound to their DNA response elements has been challenged. ChIP analyses of promoter occupancy by nuclear receptors have shown that the binding of the receptor to DNA is characterized by cycles of recruitment and release [53–55] and, for example, in the case of ERα binding to the *pS2* promoter, the duration of each cycle is 15–20 minutes[55]. Furthermore, photobleaching techniques, which were used to study GR turnover on synthetic promoters, have uncovered a much more rapid exchange of receptor molecules on DNA, which can be measured in seconds[68,69]. Although these potential differences might have arisen from distinctions between the nuclear receptors analysed and from the comparison of artificial response elements with endogenous promoters, we suggest that the ChIP data accurately reflect the temporal differences of promoter occupancy and non-occupancy by the receptor. Superimposed on this, an ultra-rapid exchange of each individual receptor molecule on DNA, as identified by photobleaching analysis, might represent the 'breathing' of this highly dynamic system.

Interestingly, cycles of recruitment and release have been observed for different nuclear receptors and for nuclear factor-κB (NF-κB), which indicates that the ordered and cyclic recruitment of various components of transcription complexes might be a common feature of promoters that are activated by inducible transcription factors[16,53–55,70]. AR-mediated transcriptional regulation, for example, involves a rapid and cyclic assembly of the AR transcription complex on the prostate-specific antigen (*PSA*) and kallikrein-2 promoters[54]. This kinetic behaviour of inducible promoters is particularly interesting because the continual cycling of nuclear receptors and their associated complexes on inducible promoters might represent a way to monitor the environment, which allows a tighter control of the expression of genes that are under hormonal stimulation. Cycling of the receptor on the promoter would indeed allow regular clearance phases between each successive cycle. It is reasonable to think that these clearance phases are potential regulatory checkpoints during which gene activation can be rapidly reversed to a default repressed state unless the hormonal stimulation continues[55,70,71]. It is interesting that the SWI/SNF complexes are initially directly engaged on the *pS2* promoter after oestrogen activation, which induces chromatin remodelling and promotes an open chromatin conformation that is permissive for transcription initiation. But later, after the first cycle of nuclear receptor binding, the SWI/SNF complexes are again recruited to the promoter during each clearance phase, where they associate with HDACs and with the NURD complex[16] . This indicates that the cyclic recruitment of chromatin remodelling factors might play a part in resetting the permissiveness of the promoter for transcription.

Furthermore, nuclear receptor cycling might not be exclusively confined to the phase when the receptor is activated by ligand binding. Metivier and colleagues recently proposed the interesting hypothesis that the unliganded ERα can also cycle on the promoter, and that this unexpected nuclear role of the aporeceptor is important to keep the target gene 'ready' for activation when ligand stimulation occurs[61] . This challenges, or at least increases, the complexity of the classic model, in which steroid receptors are kept inactive in the cytoplasm until ligand stimulation triggers their dissociation from inhibitory complexes and their subsequent nuclear translocation.

Further studies, which include the investigation of nuclear receptors that bind constitutively to the promoter and are responsible for active repression, such as RAR or TR, will be required to understand more completely the rules that underlie the dynamics of nuclear receptor binding to DNA.

Nuclear receptor turnover and transcription

The cyclic turnover of nuclear receptors on regulated promoters seems to correlate with PROTEASOME-dependent degradation activity, chaperone activity and chromatin remodelling events[55,69,72]. Accordingly, the degradation of transcriptional activators is often required for gene activation[54,55,73–77], and nuclear receptors, as well as many of their coregulators, can be ubiquitylated and regulated by protein degradation[78–83].

The significance of an association between transcriptional activation and proteolysis of the activator is not completely clear and is somehow counterintuitive, as one might expect the removal of activators to correlate with the negative control of gene transcription. However, as briefly discussed above, the cyclic clearance of nuclear receptors might be crucial, because it allows a continuous reassessment of the 'state of the cell' — each cycle would overcome the default programme of transcriptional repression only if the activating stimulus was still present. Furthermore, the site of ubiquitylation and the transcriptional activation domain overlap in many transcription factors. This raises the intriguing possibility that an ubiquitylation event is required to license the function of transcription factors, thereby linking their activity to their destruction[73]. However, there are also cases in which proteasome inhibition has been reported to enhance transcriptional activation[84–86], which indicates that the role of protein degradation in transcriptional

PROTEASOME
A large protein complex that degrades intracellular proteins that have been tagged for destruction by the addition of ubiquitin.

regulation could be cell, nuclear-receptor and even promoter specific[86].

Finally, the inhibition of proteasome activity might affect many cellular processes and, therefore, the interpretation of the data obtained by using inhibitor reagents with respect to specific biochemical events can be misleading. More focused experiments will be crucial to examine the role of specific ubiquitylation events and of specific components of the ubiquitin–proteasome machinery.

From repression to activation

The possibility of switching gene expression from 'off' to 'on' and vice versa in living organisms emerged as a revolutionary concept in biology less than 50 years ago. Seminal work by Jacob and Monod showed that bacteria could respond to changes in the growth medium by the activation or repression of METABOLIC OPERONS that are important for using alternative carbon sources[87]. The basic strategy that regulates this crucial switch has been conserved in higher eukaryotes, but the molecular mechanisms in mammalian systems have evolved further layers of complexity, which include several regulatory strategies that cooperate to impose the precise control of gene expression. These strategies include the use of different DNA-binding activators and repressors that compete for regulatory regions in promoters and/or ENHANCER regions; the modulation (inhibition or enhancement) of transcription factor activities; the regulation of the intracellular localization of transcription factors by external signals; and the allosteric regulation of the activity of transcription factors, such as the nuclear receptors, by ligands.

Corepressor–coactivator exchange. Ligand binding is the crucial molecular event that switches the function of nuclear receptors from active repression to transcriptional activation, at least in the case of heterodimeric receptors such as RAR or TR that are constitutively bound to DNA. A combination of elegant structural and molecular studies of the interactions between nuclear receptors and coregulators has provided the evidence that hormone binding induces a conformational change in the ligand-binding domain of the receptor, which results in reduced affinity for corepressors and, simultaneously, enhanced affinity for coactivators (see BOX 1 for references and details). Similarly, agonist binding to steroid receptors, such as ER, progesterone receptor (PR), GR or AR, also induces the adoption of a specific conformation that favours coactivator binding, whereas antagonist binding promotes the interaction with corepressors.

However, it recently became clear that there are additional and unexpected layers of regulation in the molecular events that modulate the nuclear receptor switch from repression to activation (FIG. 4). Indeed, recruitment of the ubiquitylation machinery and proteasome-dependent degradation of the repressors are required, at least in the case of the NCoR-containing corepressor complex, to fully promote the release of the corepressors in response to ligand binding[71]. Therefore, the activation of the nuclear corepressor exchange (NCoEx) factors could represent a further control level, which is imposed to maintain more robust transcriptional repression and to avoid undesirable gene expression. This level of regulation would increase the amplitude of transcriptional activation events by imposing a repression checkpoint. Interestingly, TBL1 and TBLR1 — the NCoEx factors that are required as adaptors for this function — are components of the NCoR and silencing mediator of retinoic acid and thyroid hormone receptors (SMRT) corepressor complexes and are required for the repression of specific transcription units[27,88,89]. This implies that, as a required component of this action, signals that promote gene induction must activate parallel pathways to activate the exchange machinery and release the repression checkpoint. Similar strategies are probably also used to regulate the release of the corepressor machinery by other transcription factors, as reported in the case of activator protein-1 (AP1) and NF-κB[70,71,90].

Obviously, there are many common elements and similarities between this proteasomal-dependent degradation of corepressors and the cyclic degradation of nuclear receptors, which indicates that, although they are distinct events, they might also be tightly linked. Indeed, if the receptor periodic cycles are important to allow a continuous assessment of the hormonal state of the cell, the re-establishment of a repression checkpoint at each clearance phase could also provide a tighter control on activation. This means that the ubiquitylation and the release of the corepressors would be crucial, not only during the first activating step, but at each cycle of receptor assembly on the promoter.

Nuclear integration of signalling pathways

If we want to appreciate fully the fine regulation of the transcriptional activity of nuclear receptors, we have to consider the fact that they are not only able to respond to hormonal stimulation, but they can also integrate information derived from a large variety of external stimuli and interact on promoters with DNA-binding transcription factors under independent regulation. Several signalling pathways that are activated by various developmental or physiological signals have been reported to cross-talk with nuclear-receptor-mediated responses through both direct and indirect mechanisms. The transcriptional activity of nuclear receptors is modulated by the induction of post-translational modification of the receptor itself or of its coregulatory proteins. Phosphorylation, acetylation, SUMOYLATION, ubiquitylation and methylation are among the modifications that have been reported to modulate the functions of nuclear receptors and that potentially constitute an important cellular integration mechanism. It has been suggested that these modifications influence cellular localization, enzymatic activity and stability of targeted proteins, and could also be important in modulating the timing of the sequential recruitment of the different classes of coregulators to a single transcription unit.

METABOLIC OPERON
A group of contiguous bacterial genes that are required for a metabolic function, and which are transcribed together in a single mRNA molecule.

ENHANCER
A DNA regulatory sequence that modulates the rate of transcription of a specific gene from a distance.

SUMOYLATION
The enzymatic process of adding the small SUMO protein to Lys residues on target proteins.

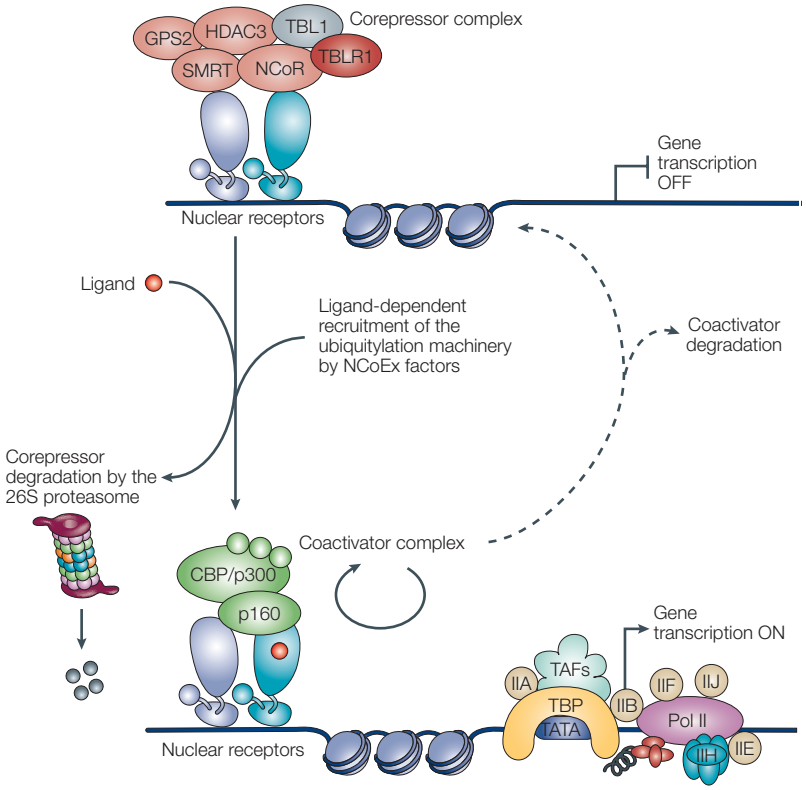


Figure 4 | **Ubiquitin-dependent exchange of corepressors for coactivators.** During the transition from gene repression to gene activation on ligand stimulation, there is a required exchange among cofactor complexes. This molecular switch is regulated by conformational changes in the nuclear receptor, which results in a different affinity for cofactor complexes, and by the recruitment of the ubiquitylation machinery, which has a fundamental role in the dismissal of the corepressor machinery. The diagram shows a generic, unliganded nuclear receptor heterodimer that recruits the NCoR and SMRT corepressor complexes to repress transcription. The nuclear corepressor exchange (NCoEx) factors TBL1 and TBLR1 are required to recruit the ubiquitylation machinery following ligand stimulation, thereby allowing the dismissal and degradation of the corepressor complex and the recruitment of coactivator complexes. Ubiquitin-dependent protein degradation events have also been associated with cycling of the receptor itself on the promoter and with coactivator turnover. IIA, IIB, IIE, IIF, IIH, IIJ, general transcription factors A, B, E, F, H, J; CBP/p300; the co-activators CREB-binding protein and p300; GPS2, G-protein pathway suppressor-2; HDAC, histone deacetylase; NCoR, nuclear-receptor corepressor; p160, a co-activator of the NCoA family; pol II, RNA polymerase II; SMRT, silencing mediator of retinoic acid and thyroid hormone receptors; TAF, TBP-associated factor; TBP, TATA-binding protein.

A detailed description of all these modifications is beyond the scope of this review (for relevant reviews, see REFS 30, 91–94). Here, we will describe three examples — a nuclear receptor (RAR), a coactivator (cAMP-response-element-binding protein (CREB)-binding protein (CBP)) and a corepressor (NCoR) — to illustrate how signalling pathways can be integrated into specific transcriptional responses (FIG. 5).

Nuclear receptor modifications: RAR. Three RAR family members — α, β and γ — regulate the transcriptional activation and repression of target genes by functioning as the heterodimeric partners for retinoic X receptors (RXRs) in response to all-*trans*-RA or to its 9-*cis* isomer (9-*cis* RA). Their ability to integrate the input from multiple signalling pathways has been studied extensively and multiple phosphorylation sites have been identified. Protein kinase A (PKA) activation, for example, exerts functional effects on the receptor because of the direct phosphorylation of a conserved Ser residue in the LBD[95], whereas the DBD can be phosphorylated by protein kinase C (PKC)[96], and both the N-terminal and the C-terminal RAR regions contain consensus phosphorylation sites for cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAPKs), and the Jun N-terminal kinases (JNKs)[77,97–99].

Phosphorylation of the N-terminus of RARα by cyclin H–CDK7, which is associated with the basal transcription factor TFIIH, is required for the function of the transactivation domain, AF1 (REF. 99). This indicates that phosphorylation of this region might be important for its interaction with either important coregulators — to mediate the AF1 transactivation function — or for its direct interaction with the basal transcriptional machinery. Defects in the RARα phosphorylation status due to mutations that affect the interaction between the receptor and TFIIH have been identified in patients with xeroderma pigmentosum[100], thereby providing genetic evidence of the importance of these regulatory mechanisms. RARγ also shows similar regulation by CDK7 kinase, but in this case, phosphorylation of a nearby residue by p38MAPK is also required for full transcriptional activity[77,98]. Interestingly, these phosphorylation events are important for the ubiquitylation and subsequent degradation of RARγ, although they do not seem to play any part in RARα ubiquitylation[77,101].

Coactivator modifications: CBP and p300. The transcriptional coactivators CBP and p300 are involved in numerous signal-regulated transcriptional events that are mediated by different DNA-binding transcription factors. CBP was originally identified as an interacting partner for the transcription factor CREB in response to cAMP signalling, and p300 was identified as a cellular-associated factor of the adenoviral protein E1A. Interestingly, CBP and p300 can promote transcriptional activation and integrate upstream signals through a series of different mechanisms, which includes not only direct chromatin remodelling activity by histone acetylation, but also acetylation of other factors, direct binding to components of the basal transcriptional machinery and recruitment of other coregulators, such as scaffold proteins[102,103].

Both CBP and p300 are well-known phosphoproteins[104]. Interestingly, their phosphorylation status seems to be under cell-cycle control; p300, for example, was shown to be phosphorylated by CDC2 and CDK2 kinases in a physiological process that could be targeted and blocked by the adenoviral protein E1A[105]. And the observation that the transcriptional function of p300 was negatively regulated by cyclin E–CDK2 allowed a further understanding of the functional implications of CBP and p300 phosphorylation by cell-cycle-dependent kinases[106]. An interesting possibility is that the enzymatic activities of CBP and p300 are directly

G1/S TRANSITION
The cell-cycle checkpoint at the transition between the Gap phase-1 (G1) and the beginning of DNA replication.

modulated as a result of the phosphorylation events that occur during cell-cycle progression. This was suggested by Ait-Si-Ali *et al.*[107], who reported that general HAT activity peaked during the G1/S TRANSITION, and that the HAT activity of CBP was enhanced by the C-terminal phosphorylation mediated by cyclin E–CDK2 as well as by the interaction with E1A. As E1A was reported to stimulate the phosphorylation of CBP and p300 (REF. 108), this suggests the interesting possibility that viral oncoproteins can mimic physiological signals that activate the HAT function of p300 and CBP. However, further studies are needed for a better understanding of the mechanisms of this regulation, as several reports have shown an opposite effect, in that the interaction with E1A inhibits the enzymatic activity of CBP[109–111].

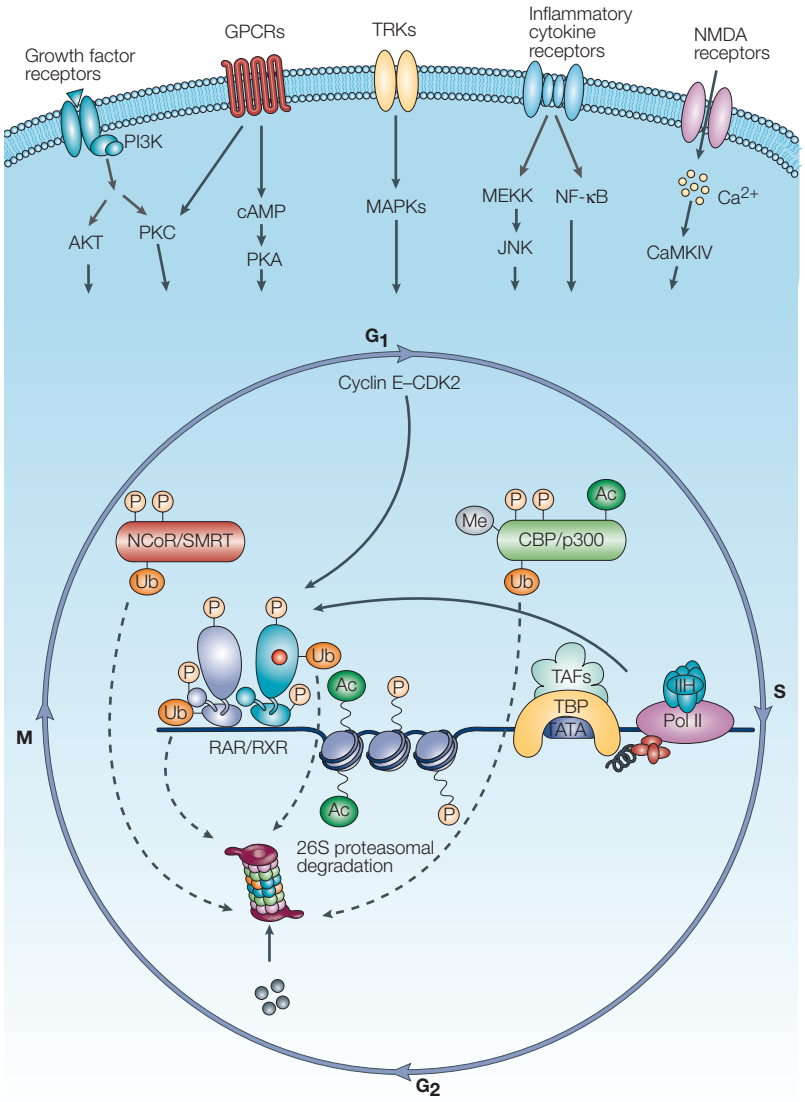


Figure 5 | **Integration of signalling pathways on nuclear-receptor-mediated transcriptional regulation.** Nuclear-receptor-mediated transcriptional regulation can be modulated by several signalling pathways that are activated by external or internal signals, including cell-cycle-dependent signals, growth factor stimulation, inflammatory signals and signals that activate G-protein-coupled receptors (GPCRs), tyrosine kinase receptors (TRKs) and ion channel receptors. The diagram shows retinoic acid receptor (RAR) as an example of nuclear receptors, the coactivator CREB-binding protein (CBP)/p300 and the corepressors NCoR and SMRT. For each, the activity and the ability to interact with other partners can be modulated by various mechanisms (see the main text for details and references), which include phosphorylation (P; yellow), methylation (Me; grey) and acetylation (Ac; green). Polyubiquitylation (Ub; orange) is often used to mark nuclear receptors and associated cofactors for proteasomal degradation. IIH, general transcription factor H; CaMKIV, Ca^{2+}/calmodulin-dependent kinase IV; CDK2, cyclin-dependent kinase-2; JNK, Jun N-terminal kinase; MAPK; mitogen-activated protein kinase; MEKK, MAPK-kinase kinase; NCoR, nuclear-receptor corepressor; NF-κB, nuclear factor-κB; NMDA, *N*-methyl-D aspartate; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; pol II, RNA polymerase II; RAR, retinoic acid receptor; RXR, retinoic X receptor; SMRT, silencing mediator of retinoic acid and thyroid hormone receptors; TAF, TBP-associated factor; TBP, TATA-binding protein.

Other kinases, which include PKA[112], Ca^{2+}/calmodulin-dependent kinase IV (CaMKIV)[113] and MAPK[114], can phosphorylate different CBP residues, thereby enhancing its transcriptional-activation activity. For example, phosphorylation by p44 MAPK has been reported to have a positive effect on the enzymatic activity of CBP[115]. By contrast, p300 phosphorylation by PKC represses transcriptional activity[116], perhaps consistent with the opposing activities of CBP and p300 on proliferation and the response to DNA damage.

Interestingly, detailed studies of the signalling pathways that are involved and exhaustive mapping analysis of the phosphorylation sites have uncovered a correlation between certain modifications and precise functional effects: for example, the phosphorylation of Ser301 of CBP by CaMKIV is required for the *N*-methyl-D aspartate (NMDA)-dependent activation of CREB-responsive genes in hippocampal neurons[117].

Phosphorylation is clearly not the only modification that is used to integrate signalling pathways in the regulation of coactivator functions. For example, methylation by CARM1 of a specific domain of CBP (the KIX domain), which interacts with the kinase-interacting domain (KID) of CREB, has been shown to be important for inducing the dissociation of CBP from CREB, and for inhibiting CREB-dependent transcriptional activation[118]. Furthermore, p300 is also ubiquitylated and degraded by the ubiquitin–proteasome pathway during F9 embryonal carcinoma cell differentiation. Interestingly, p300 shows different phosphorylation patterns in undifferentiated versus differentiated cells, and the changes in phosphorylation status that are promoted by PKA affect its HAT activity only during differentiation[119,120].

Corepressor modifications: NCoR. Although the nuclear-receptor corepressors NCoR and SMRT do not harbour intrinsic enzymatic activity, each recruits several other corepressors, which include many histone deacetylases[121]. Indeed, there are numerous distinct NCoR and SMRT complexes that have been biochemically purified[13,27,88,122,123], but most components probably assemble to form a holoenzyme complex.

Several kinases, including MAPKs, AKT/protein kinase B (PKB) and casein kinase-2 (CK2), have been shown to modify the NCoR and SMRT corepressors and to induce their relocalization from the nucleus to the cytoplasm[30, 124–126]. Interestingly, some of these modifications seem to be specific for either SMRT or NCoR, which introduces a fascinating level of specificity to their regulation. Jonas and Privalsky have recently shown that SMRT is negatively regulated by

CYTOKINE
A member of a large family of secreted proteins that interact with cellular receptors. Cytokine production results in the activation of an intracellular signalling cascade that commonly regulates processes such as immune function and inflammation.

SCF E3 LIGASE COMPLEX
A multisubunit E3 ubiquitin ligase complex that contains an F-box protein for specific substrate recognition.

MAPK signalling pathways in response to stress or growth factors, whereas NCoR is not affected by this regulation[127]. Similarly, previous studies indicate that NCoR phosphorylation status and/or nuclear localization are modulated in response to CYTOKINES, such as interleukin (IL)1β, or to differentiation factors, such as the ciliary neurotrophic factor (CNTF), whereas SMRT is not affected by them[30,124].

A recent paper by Hoberg and colleagues, who analysed the derepression of NF-κB target genes, reports the direct phosphorylation of SMRT by inhibitor of NF-κB (IκB) kinase α (IKKα) and its subsequent ubiquitylation, release from repressed promoters and nuclear export[70]. This is consistent with the observation that the ubiquitin-dependent dismissal and degradation of corepressors is required for the switch from gene repression to gene activation of nuclear receptors and other transcription factors[71], and also with the observation that protein phosphorylation is commonly used to mark proteins to be ubiquitylated by SCF E3 LIGASE COMPLEXES[128]. Furthermore, it is interesting to note that SMRT is recruited back to the promoter ~20–40 min after being released following phosphorylation and ubiquitylation, which indicates that ordered waves of corepressor recruitment also occur at NF-κB-regulated promoters[70]. This implies that, as discussed for nuclear receptors, there are transcriptional repression checkpoints that need to be periodically released by dedicated ubiquitylation-dependent strategies for each cycle of transcription to proceed. Interestingly, IKKα has also been shown to be important for the enhancement of the transcriptional activation of NF-κB-regulated genes on cytokine treatment, because it can phosphorylate histone H3 on Ser10 and can modulate the subsequent CBP-dependent acetylation of Lys14 (REFS 129,130). This represents a fascinating example of a signalling pathway that simultaneously regulates both the dismissal of corepressors and the enhancement of coactivators, which indicates that similar strategies are also likely to operate for nuclear-receptor-mediated transcription.

Finally, the possibility of regulating the localization of corepressors in the cell by nuclear export in response to specific signals raises the interesting question of whether the degradation of NCoR and SMRT occurs directly at the promoter level, or whether its release from the promoter following phosphorylation/ubiquitylation is coupled with a relocalization event. This relocalization event might be associated with chaperones such as the 14-3-3 proteins, which confine the degradation of NCoR and SMRT to the cytoplasm, or at least to a nuclear location that is not closely associated with the promoter.

Concluding remarks

The nucleus is a cellular compartment that is crucial for coordinating the responses to diverse signals. At the level of transcriptional regulation, this coordination resides in numerous coactivators, corepressors and chromatin remodelling complexes that are recruited to their target genes by DNA-binding transcription factors. As these complexes have several functional domains, each of which is subject to modifications and interactions that can activate, nullify or switch their function, and as there are so many — literally hundreds — of components that can participate to form these complexes, the vast number of possible combinations allows for highly specific and flexible responses.

Therefore, in this review, we have tried to show how this complexity translates into an exquisitely precise sequence of events that cooperate to achieve the precise regulation of gene transcription, which depends on promoter context, cell-cycle stage and regulatory signals. Indeed, each transcriptional activation event comprises a series of changes, such as DNA demethylation, histone modification, cofactor modification and so on, each of which alone might not be sufficient for full activation, but together converge to define a specific 'combinatorial code'. Furthermore, we have focused on the emergence of the rapid reprogramming of gene repression and activation as a major step in ligand-dependent gene regulatory events and have reviewed the most recent findings on the molecular mechanisms that mediate these events. Unfortunately, lack of space has not permitted a complete review of the phenotypic consequences of the deletion of single coregulators during development, as reported by several excellent *in vivo* analyses of mice models.

However, the more we learn about the regulation of nuclear receptor function, and as new and more powerful technologies become available to further study it, more novel, unexpected questions arise. For example, what distinguishes genes that respond to derepression alone from those requiring further ligand-dependent effects? How is the timing of the transcriptional response regulated? What are the molecular marks that permit the serial recruitment and release of distinct cohorts of coregulators? Which enzymatic components of coactivator and corepressor complexes function at transcriptional and post-transcriptional levels? How are these 'rules' of regulation applied on a genome-wide basis? Clearly, the stage is set for an exciting new season in which the secrets of nuclear receptor regulation will be further unravelled.

1. Aranda, A. & Pascual, A. Nuclear hormone receptors and gene expression. *Physiol. Rev.* **81**, 1269–1304 (2001).
2. Gronemeyer, H., Gustafsson, J. A. & Laudet, V. Principles for modulation of the nuclear receptor superfamily. *Nature Rev. Drug Discov.* **3**, 950–964 (2004).
3. Giguere, V. Orphan nuclear receptors: from gene to function. *Endocr. Rev.* **20**, 689–725 (1999).
4. Sluder, A. E., Mathews, S. W., Hough, D., Yin, V. P. & Maina, C. V. The nuclear receptor superfamily has undergone extensive proliferation and diversification in nematodes. *Genome Res.* **9**, 103–120 (1999).
5. Maglich, J. M. *et al.* Comparison of complete nuclear receptor sets from the human, *Caenorhabditis elegans* and *Drosophila* genomes. *Genome Biol.* **2**, RESEARCH0029 (2001).
6. Weston, A. D., Blumberg, B. & Underhill, T. M. Active repression by unliganded retinoid receptors in development: less is sometimes more. *J. Cell Biol.* **161**, 223–228 (2003).
7. Nettles, K. W. & Greene, G. L. Ligand control of coregulator recruitment of nuclear receptors. *Annu. Rev. Physiol.* **67**, 309–333 (2004).

References 7 and 9 provide comprehensive reviews of the molecular switch from transcriptional repression to activation, which focuses on the conformational changes in the receptor and

summarizes crystallographic studies of the interactions of cofactors with nuclear receptors.
8. McKenna, N. J., Lanz, R. B. & O'Malley, B. W. Nuclear receptor coregulators: cellular and molecular biology. *Endocr. Rev.* **20**, 321–344 (1999).
9. Nagy, L. & Schwabe, J. W. Mechanism of the nuclear receptor molecular switch. *Trends Biochem. Sci.* **29**, 317–324 (2004).
10. White, J. H., Fernandes, I., Mader, S. & Yang, X. J. Corepressor recruitment by agonist-bound nuclear receptors. *Vitam. Horm.* **68**, 123–143 (2004).
11. Delage-Mourroux, R. *et al.* Analysis of estrogen receptor interaction with a repressor of estrogen receptor activity (REA) and the regulation of estrogen receptor transcriptional activity by REA. *J. Biol. Chem.* **275**, 35848–35856 (2000).
12. Chiba, H., Muramatsu, M., Nomoto, A. & Kato, H. 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 (1994).
13. Underhill, C., Qutob, M. S., Yee, S. P. & Torchia, J. A novel nuclear receptor corepressor complex, N-CoR, contains components of the mammalian SWI/SNF complex and the corepressor KAP-1. *J. Biol. Chem.* **275**, 40463–40470 (2000).
14. Fryer, C. J. & Archer, T. K. Chromatin remodelling by the glucocorticoid receptor requires the BRG1 complex. *Nature* **393**, 88–91 (1998).
15. Sif, S., Saurin, A. J., Imbalzano, A. N. & Kingston, R. E. Purification and characterization of mSin3A-containing Brg1 and hBrm chromatin remodeling complexes. *Genes Dev.* **15**, 603–618 (2001).
16. Metivier, R. *et al.* Estrogen receptor-α directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. *Cell* **115**, 751–763 (2003).
Detailed description of the ordered and cyclical recruitment of several cofactor complexes to the oestrogen-regulated *pS2* promoter in MCF7 breast cancer cells.
17. Dilworth, F. J. & Chambon, P. Nuclear receptors coordinate the activities of chromatin remodeling complexes and coactivators to facilitate initiation of transcription. *Oncogene* **20**, 3047–3054 (2001).
18. Jenuwein, T. & Allis, C. D. Translating the histone code. *Science* **293**, 1074–1080 (2001).
19. Travers, A. An engine for nucleosome remodeling. *Cell* **96**, 311–314 (1999).
20. Lanz, R. B. *et al.* A steroid receptor coactivator, SRA, functions as an RNA and is present in an SRC-1 complex. *Cell* **97**, 17–27 (1999).
21. Kumar, R., Wang, R. A. & Barnes, C. J. Coregulators and chromatin remodeling in transcriptional control. *Mol. Carcinog.* **41**, 221–230 (2004).
22. Kumar, V. *et al.* Transcription corepressor CtBP is an NAD^+-regulated dehydrogenase. *Mol. Cell* **10**, 857–869 (2002).
23. Vo, N., Fjeld, C. & Goodman, R. H. Acetylation of nuclear hormone receptor-interacting protein RIP140 regulates binding of the transcriptional corepressor CtBP. *Mol. Cell. Biol.* **21**, 6181–6188 (2001).
First example of acetylation of a corepressor as a way to regulate its transcriptional activity by modulating protein–protein interactions.
24. Lee, Y. H., Campbell, H. D. & Stallcup, M. R. Developmentally essential protein flightless I is a nuclear receptor coactivator with actin binding activity. *Mol. Cell. Biol.* **24**, 2103–2117 (2004).
25. Huang, S. M., Huang, C. J., Wang, W. M., Kang, J. C. & Hsu, W. C. The enhancement of nuclear receptor transcriptional activation by a mouse actin-binding protein, α actinin 2. *J. Mol. Endocrinol.* **32**, 481–496 (2004).
26. Loy, C. J., Sim, K. S. & Yong, E. L. Filamin-A fragment localizes to the nucleus to regulate androgen receptor and coactivator functions. *Proc. Natl Acad. Sci. USA* **100**, 4562–4567 (2003).
27. Yoon, H. G. *et al.* Purification and functional characterization of the human N-CoR complex: the roles of HDAC3, TBL1 and TBLR1. *EMBO J.* **22**, 1336–1346 (2003).
28. Nishimura, K. *et al.* Modulation of androgen receptor transactivation by gelsolin: a newly identified androgen receptor coregulator. *Cancer Res.* **63**, 4888–4894 (2003).
29. Ting, H. J., Yeh, S., Nishimura, K. & Chang, C. Supervillin associates with androgen receptor and modulates its transcriptional activity. *Proc. Natl Acad. Sci. USA* **99**, 661–666 (2002).
30. Hermanson, O., Glass, C. K. & Rosenfeld, M. G. Nuclear receptor coregulators: multiple modes of modification. *Trends Endocrinol. Metab.* **13**, 55–60 (2002).
31. Privalsky, M. L. The role of corepressors in transcriptional regulation by nuclear hormone receptors. *Annu. Rev. Physiol.* **66**, 315–360 (2004).
32. Cosma, M. P. Ordered recruitment: gene-specific mechanism of transcription activation. *Mol. Cell* **10**, 227–236 (2002).
33. Kraus, W. L. & Wong, J. Nuclear receptor-dependent transcription with chromatin. Is it all about enzymes? *Eur. J. Biochem.* **269**, 2275–2283 (2002).
34. Bultman, S. *et al.* A *Brg1* null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. *Mol. Cell* **6**, 1287–1295 (2000).
35. Kadam, S. & Emerson, B. M. Transcriptional specificity of human SWI/SNF BRG1 and BRM chromatin remodeling complexes. *Mol. Cell* **11**, 377–389 (2003).
36. Reyes, J. C. *et al.* Altered control of cellular proliferation in the absence of mammalian brahma (SNF2α). *EMBO J.* **17**, 6979–6991 (1998).
37. Wang, W. *et al.* Purification and biochemical heterogeneity of the mammalian SWI–SNF complex. *EMBO J.* **15**, 5370–5382 (1996).
38. DiRenzo, J. *et al.* BRG-1 is recruited to estrogen-responsive promoters and cooperates with factors involved in histone acetylation. *Mol. Cell. Biol.* **20**, 7541–7549 (2000).
39. Lemon, B., Inouye, C., King, D. S. & Tjian, R. Selectivity of chromatin-remodelling cofactors for ligand-activated transcription. *Nature* **414**, 924–928 (2001).
40. Glass, C. K. & Rosenfeld, M. G. The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev.* **14**, 121–41 (2000).
41. McKenna, N. J. & O'Malley, B. W. Minireview: nuclear receptor coactivators — an update. *Endocrinology* **143**, 2461–2465 (2002).
42. Iborra, F. J., Pombo, A., Jackson, D. A. & Cook, P. R. Active RNA polymerases are localized within discrete transcription 'factories' in human nuclei. *J. Cell Sci.* **109**, 1427–1436 (1996).
43. Jackson, D. A., Hassan, A. B., Errington, R. J. & Cook, P. R. Visualization of focal sites of transcription within human nuclei. *EMBO J.* **12**, 1059–1065 (1993).
44. Osborne, C. S. *et al.* Active genes dynamically colocalize to shared sites of ongoing transcription. *Nature Genet.* **36**, 1065–1071 (2004).
Dynamic colocalization of distal active genes into discrete nuclear subcompartments or 'transcription factories'.
45. Bettinger, B. T., Gilbert, D. M. & Amberg, D. C. Actin up in the nucleus. *Nature Rev. Mol. Cell Biol.* **5**, 410–415 (2004).
46. Olave, I. A., Reck-Peterson, S. L. & Crabtree, G. R. Nuclear actin and actin-related proteins in chromatin remodeling. *Annu. Rev. Biochem.* **71**, 755–781 (2002).
47. Szentirmay, M. N. & Sawadogo, M. Spatial organization of RNA polymerase II transcription in the nucleus. *Nucleic Acids Res.* **28**, 2019–2025 (2000).
48. Cook, P. R. Nongenic transcription, gene regulation and action at a distance. *J. Cell Sci.* **116**, 4483–4491 (2003).
49. Stein, G. S. *et al.* Functional architecture of the nucleus: organizing the regulatory machinery for gene expression, replication and repair. *Trends Cell Biol.* **13**, 584–592 (2003).
50. Shang, Y. & Brown, M. Molecular determinants for the tissue specificity of SERMs. *Science* **295**, 2465–2468 (2002).
Promoter-specific and tissue-specific recruitment of coregulatory proteins by an antagonist-bound oestrogen receptor in uterine and breast cancer cells.
51. Cosma, M. P., Tanaka, T. & Nasmyth, K. Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. *Cell* **97**, 299–311 (1999).
First example of the ordered recruitment of cofactor complexes to a regulated transcription unit.
52. Dilworth, F. J., Fromental-Ramain, C., Yamamoto, K. & Chambon, P. ATP-driven chromatin remodeling activity and histone acetyltransferases act sequentially during transactivation by RAR/RXR *in vitro*. *Mol. Cell* **6**, 1049–1058 (2000).
53. Shang, Y., Hu, X., DiRenzo, J., Lazar, M. A. & Brown, M. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell* **103**, 843–852 (2000).
Analysis of the occupancy of the CATD promoter following ligand activation: first description of an ordered and cyclical recruitment of different cofactors by nuclear receptors in a mammalian system.
54. Kang, Z., Pirskanen, A., Janne, O. A. & Palvimo, J. J. Involvement of proteasome in the dynamic assembly of the androgen receptor transcription complex. *J. Biol. Chem.* **277**, 48366–48371 (2002).
55. Reid, G. *et al.* Cyclic, proteasome-mediated turnover of unliganded and liganded Erα on responsive promoters is an integral feature of estrogen signaling. *Mol. Cell* **11**, 695–707 (2003).
56. Sharma, D. & Fondell, J. D. Ordered recruitment of histone acetyltransferases and the TRAP/Mediator complex to thyroid hormone-responsive promoters *in vivo*. *Proc. Natl Acad. Sci. USA* **99**, 7934–7939 (2002).
57. Grenier, J. *et al.* Selective recruitment of p160 coactivators on glucocorticoid-regulated promoters in Schwann cells. *Mol. Endocrinol.* **18**, 2866–2879 (2004).
58. Suen, C. S. *et al.* A transcriptional coactivator, steroid receptor coactivator-3, selectively augments steroid receptor transcriptional activity. *J. Biol. Chem.* **273**, 27645–27653 (1998).
59. Li, X., Wong, J., Tsai, S. Y., Tsai, M. J. & O'Malley, B. W. Progesterone and glucocorticoid receptors recruit distinct coactivator complexes and promote distinct patterns of local chromatin modification. *Mol. Cell. Biol.* **23**, 3763–3773 (2003).
60. Liu, X. F. & Bagchi, M. K. Recruitment of distinct chromatin-modifying complexes by tamoxifen-complexed estrogen receptor at natural target gene promoters *in vivo*. *J. Biol. Chem.* **279**, 15050–15058 (2004).
61. Metivier, R. *et al.* Transcriptional complexes engaged by apo-estrogen receptor-α isoforms have divergent outcomes. *EMBO J.* **23**, 3653–3666 (2004).
62. Vermeulen, M. *et al. In vitro* targeting reveals intrinsic histone tail specificity of the Sin3/histone deacetylase and N-CoR/SMRT corepressor complexes. *Mol. Cell. Biol.* **24**, 2364–2372 (2004).
63. Kurokawa, R. *et al.* Polarity-specific activities of retinoic acid receptors determined by a co-repressor. *Nature* **377**, 451–454 (1995).
64. Klinge, C. M., Jernigan, S. C., Mattingly, K. A., Risinger, K. E. & Zhang, J. Estrogen response element-dependent regulation of transcriptional activation of estrogen receptors α and β by coactivators and corepressors. *J. Mol. Endocrinol.* **33**, 387–410 (2004).
65. Loven, M. A., Likhite, V. S., Choi, I. & Nardulli, A. M. Estrogen response elements alter coactivator recruitment through allosteric modulation of estrogen receptor β conformation. *J. Biol. Chem.* **276**, 45282–45288 (2001).
66. Rogatsky, I. *et al.* Target-specific utilization of transcriptional regulatory surfaces by the glucocorticoid receptor. *Proc. Natl Acad. Sci. USA* **100**, 13845–138450 (2003).
67. Narlikar, G. J., Fan, H. Y. & Kingston, R. E. Cooperation between complexes that regulate chromatin structure and transcription. *Cell* **108**, 475–487 (2002).
68. McNally, J. G., Muller, W. G., Walker, D., Wolford, R. & Hager, G. L. The glucocorticoid receptor: rapid exchange with regulatory sites in living cells. *Science* **287**, 1262–1265 (2000).
First paper to show nuclear receptor binding to a DNA response element in real time, in living cells, using fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP) techniques.
69. Nagaich, A. K., Walker, D. A., Wolford, R. & Hager, G. L. Rapid periodic binding and displacement of the glucocorticoid receptor during chromatin remodeling. *Mol. Cell* **14**, 163–174 (2004).
Novel UV-laser crosslinking technique used to analyse the ultra-rapid binding of GR and associated complexes on a multimerized array of the MMTV promoter.
70. Hoberg, J. E., Yeung, F. & Mayo, M. W. SMRT derepression by the IκB kinase α; a prerequisite to NF-κB transcription and survival. *Mol. Cell* **16**, 245–255 (2004).
Exchange of a corepressor for a coactivator on NF-κB requires SMRT phopshorylation by IKKα.
71. Perissi, V., Aggarwal, A., Glass, C. K., Rose, D. W. & Rosenfeld, M. G. A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. *Cell* **116**, 511–526 (2004).
Identification of an ubiquitylation/degradation event required to release the NCoR corepressor complex from activated nuclear receptors.
72. Freeman, B. C. & Yamamoto, K. R. Disassembly of transcriptional regulatory complexes by molecular chaperones. *Science* **296**, 2232–2235 (2002).
Involvement of chaperone proteins in transcriptional regulation by mediating promoter clearance.
73. Salghetti, S. E., Muratani, M., Wijnen, H., Futcher, B. & Tansey, W. P. Functional overlap of sequences that activate transcription and signal ubiquitin-mediated proteolysis. *Proc. Natl Acad. Sci. USA* **97**, 3118–3123 (2000).

74. Molinari, E., Gilman, M. & Natesan, S. Proteasome-mediated degradation of transcriptional activators correlates with activation domain potency *in vivo*. *EMBO J.* **18**, 6439–6447 (1999).
75. Lonard, D. M., Nawaz, Z., Smith, C. L. & O'Malley, B. W. The 26S proteasome is required for estrogen receptor-α and coactivator turnover and for efficient estrogen receptor-α transactivation. *Mol. Cell* **5**, 939–948 (2000).
76. Lin, H. K. *et al.* Proteasome activity is required for androgen receptor transcriptional activity via regulation of androgen receptor nuclear translocation and interaction with coregulators in prostate cancer cells. *J. Biol. Chem.* **277**, 36570–36576 (2002).
77. Gianni, M., Bauer, A., Garattini, E., Chambon, P. & Rochette-Egly, C. Phosphorylation by p38MAPK and recruitment of SUG-1 are required for RA-induced RAR γ degradation and transactivation. *EMBO J.* **21**, 3760–3769 (2002).
78. Alarid, E. T., Bakopoulos, N. & Solodin, N. Proteasome-mediated proteolysis of estrogen receptor: a novel component in autologous down-regulation. *Mol. Endocrinol.* **13**, 1522–1534 (1999).
79. Dace, A. *et al.* Hormone binding induces rapid proteasome-mediated degradation of thyroid hormone receptors. *Proc. Natl Acad. Sci. USA* **97**, 8985–8990 (2000).
80. Floyd, Z. E. & Stephens, J. M. Interferon-γ-mediated activation and ubiquitin-proteasome-dependent degradation of PPARγ in adipocytes. *J. Biol. Chem.* **277**, 4062–4068 (2002).
81. Nawaz, Z., Lonard, D. M., Dennis, A. P., Smith, C. L. & O'Malley, B. W. Proteasome-dependent degradation of the human estrogen receptor. *Proc. Natl Acad. Sci. USA* **96**, 1858–1862 (1999).
82. Zhu, J. *et al.* Retinoic acid induces proteasome-dependent degradation of retinoic acid receptor alpha (RARα) and oncogenic RARα fusion proteins. *Proc. Natl Acad. Sci. USA* **96**, 14807–14812 (1999).
83. Yan, F., Gao, X., Lonard, D. M. & Nawaz, Z. Specific ubiquitin-conjugating enzymes promote degradation of specific nuclear receptor coactivators. *Mol. Endocrinol.* **17**, 1315–1331 (2003).
84. Wallace, A. D. & Cidlowski, J. A. Proteasome-mediated glucocorticoid receptor degradation restricts transcriptional signaling by glucocorticoids. *J. Biol. Chem.* **276**, 42714–42721 (2001).
85. Boudjelal, M., Voorhees, J. J. & Fisher, G. J. Retinoid signaling is attenuated by proteasome-mediated degradation of retinoid receptors in human keratinocyte HaCaT cells. *Exp. Cell Res.* **274**, 130–137 (2002).
86. Fan, M., Nakshatri, H. & Nephew, K. P. Inhibiting proteasomal proteolysis sustains estrogen receptor-α activation. *Mol. Endocrinol.* **18**, 2603–2615 (2004).
87. Jacob, F. & Monod, J. Genetic regulatory mechanisms in the synthesis of proteins. *J. Mol. Biol.* **3**, 318–356 (1961).
88. Li, J. *et al.* Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. *EMBO J.* **19**, 4342–4350 (2000).
89. Tomita, A., Buchholz, D. R. & Shi, Y. B. Recruitment of N-CoR/SMRT–TBLR1 corepressor complex by unliganded thyroid hormone receptor for gene repression during frog development. *Mol. Cell. Biol.* **24**, 3337–3346 (2004).
90. Ogawa, S. *et al.* A nuclear receptor corepressor transcriptional checkpoint controlling activator protein 1-dependent gene networks required for macrophage activation. *Proc. Natl Acad. Sci. USA* **101**, 14461–14466 (2004).
91. McKenna, N. J. & O'Malley, B. W. Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell* **108**, 465–474 (2002).
92. Sengupta, N. & Seto, E. Regulation of histone deacetylase activities. *J. Cell. Biochem.* **93**, 57–67 (2004).
93. Baek, S. H. & Rosenfeld, M. G. Nuclear receptor coregulators: their modification codes and regulatory mechanism by translocation. *Biochem. Biophys. Res. Commun.* **319**, 707–714 (2004).
94. Fischle, W., Wang, Y. & Allis, C. D. Histone and chromatin cross-talk. *Curr. Opin. Cell Biol.* **15**, 172–183 (2003).
95. Rochette-Egly, C. *et al.* Phosphorylation of the retinoic acid receptor-α by protein kinase A. *Mol. Endocrinol.* **9**, 860–871 (1995).
96. Delmotte, M. H., Tahayato, A., Formstecher, P. & Lefebvre, P. Serine 157, a retinoic acid receptor α residue phosphorylated by protein kinase C *in vitro*, is involved in RXR•RARα heterodimerization and transcriptional activity. *J. Biol. Chem.* **274**, 38225–38231 (1999).
97. Adam-Stitah, S., Penna, L., Chambon, P. & Rochette-Egly, C. Hyperphosphorylation of the retinoid X receptor α by activated c-Jun NH2-terminal kinases. *J. Biol. Chem.* **274**, 18932–18941 (1999).
98. Bastien, J. *et al.* TFIIH interacts with the retinoic acid receptor γ and phosphorylates its AF-1-activating domain through cdk7. *J. Biol. Chem.* **275**, 21896–21904 (2000).
99. Rochette-Egly, C., Adam, S., Rossignol, M., Egly, J. M. & Chambon, P. Stimulation of RAR α activation function AF-1 through binding to the general transcription factor TFIIH and phosphorylation by CDK7. *Cell* **90**, 97–107 (1997).
100. Keriel, A., Stary, A., Sarasin, A., Rochette-Egly, C. & Egly, J. M. XPD mutations prevent TFIIH-dependent transactivation by nuclear receptors and phosphorylation of RARα. *Cell* **109**, 125–135 (2002).
Genetic significance of the regulation of RAR by phosphorylation.
101. Kopf, E. *et al.* Dimerization with retinoid X receptors and phosphorylation modulate the retinoic acid-induced degradation of retinoic acid receptors α and γ through the ubiquitin–proteasome pathway. *J. Biol. Chem.* **275**, 33280–33288 (2000).
102. Goodman, R. H. & Smolik, S. CBP/p300 in cell growth, transformation, and development. *Genes Dev.* **14**, 1553–1577 (2000).
103. Chan, H. M. & La Thangue, N. B. p300/CBP proteins: HATs for transcriptional bridges and scaffolds. *J. Cell Sci.* **114**, 2363–2373 (2001).
104. Yaciuk, P. & Moran, E. Analysis with specific polyclonal antiserum indicates that the E1A-associated 300-kDa product is a stable nuclear phosphoprotein that undergoes cell cycle phase-specific modification. *Mol. Cell. Biol.* **11**, 5389–5397 (1991).
105. Banerjee, A. C. *et al.* The adenovirus E1A 289R and 243R proteins inhibit the phosphorylation of p300. *Oncogene* **9**, 1733–1737 (1994).
106. Perkins, N. D. *et al.* Regulation of NF-κB by cyclin-dependent kinases associated with the p300 coactivator. *Science* **275**, 523–527 (1997).
107. Ait-Si-Ali, S. *et al.* Histone acetyltransferase activity of CBP is controlled by cycle-dependent kinases and oncoprotein E1A. *Nature* **396**, 184–186 (1998).
108. Kitabayashi, I. *et al.* Phosphorylation of the adenovirus E1A-associated 300 kDa protein in response to retinoic acid and E1A during the differentiation of F9 cells. *EMBO J.* **14**, 3496–3509 (1995).
109. Chakravarti, D. *et al.* A viral mechanism for inhibition of p300 and PCAF acetyltransferase activity. *Cell* **96**, 393–403 (1999).
110. Hamamori, Y. *et al.* Regulation of histone acetyltransferases p300 and PCAF by the bHLH protein twist and adenoviral oncoprotein E1A. *Cell* **96**, 405–413 (1999).
111. Perissi, V. *et al.* Factor-specific modulation of CREB-binding protein acetyltransferase activity. *Proc. Natl Acad. Sci. USA* **96**, 3652–3657 (1999).
112. Xu, L. *et al.* Signal-specific co-activator domain requirements for Pit-1 activation. *Nature* **395**, 301–306 (1998).
113. Chawla, S., Hardingham, G. E., Quinn, D. R. & Bading, H. CBP: a signal-regulated transcriptional coactivator controlled by nuclear calcium and CaM kinase IV. *Science* **281**, 1505–1509 (1998).
114. Janknecht, R. & Nordheim, A. MAP kinase-dependent transcriptional coactivation by Elk-1 and its cofactor CBP. *Biochem. Biophys. Res. Commun.* **228**, 831–837 (1996).
115. Ait-Si-Ali, S. *et al.* Phosphorylation by p44 MAP Kinase/ERK1 stimulates CBP histone acetyl transferase activity *in vitro*. *Biochem. Biophys. Res. Commun.* **262**, 157–162 (1999).
116. Yuan, L. W. & Gambee, J. E. Phosphorylation of p300 at serine 89 by protein kinase C. *J. Biol. Chem.* **275**, 40946–40951 (2000).
117. Impey, S. *et al.* Phosphorylation of CBP mediates transcriptional activation by neural activity and CaM kinase IV. *Neuron* **34**, 235–244 (2002).
Example of the physiological relevance of the regulation of CBP function by phosphorylation.
118. Xu, W. *et al.* A transcriptional switch mediated by cofactor methylation. *Science* **294**, 2507–2511 (2001).
119. Brouillard, F. & Cremisi, C. E. Concomitant increase of histone acetyltransferase activity and degradation of p300 during retinoic acid-induced differentiation of F9 cells. *J. Biol. Chem.* **278**, 39509–39516 (2003).
120. Iwao, K., Kawasaki, H., Taira, K. & Yokoyama, K. K. Ubiquitination of the transcriptional coactivator p300 during retinoic acid induced differentiation. *Nucleic Acids Symp. Ser.* **42**, 207–208 (1999).
121. Jones, P. L. & Shi, Y. B. N-CoR–HDAC corepressor complexes: roles in transcriptional regulation by nuclear hormone receptors. *Curr. Top. Microbiol. Immunol.* **274**, 237–268 (2003).
122. Zhang, J., Kalkum, M., Chait, B. T. & Roeder, R. G. The N-CoR–HDAC3 nuclear receptor corepressor complex inhibits the JNK pathway through the integral subunit GPS2. *Mol. Cell* **9**, 611–623 (2002).
123. Guenther, M. G., Barak, O. & Lazar, M. A. The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase 3. *Mol. Cell. Biol.* **21**, 6091–6101 (2001).
124. Baek, S. H. *et al.* Exchange of N-CoR corepressor and Tip60 coactivator complexes links gene expression by NF-κB and β-amyloid precursor protein. *Cell* **110**, 55–67 (2002).
125. Hong, S. H. & Privalsky, M. L. The SMRT corepressor is regulated by a MEK-1 kinase pathway: inhibition of corepressor function is associated with SMRT phosphorylation and nuclear export. *Mol. Cell. Biol.* **20**, 6612–6625 (2000).
126. Zhou, Y., Gross, W., Hong, S. H. & Privalsky, M. L. The SMRT corepressor is a target of phosphorylation by protein kinase CK2 (casein kinase II). *Mol. Cell. Biochem.* **220**, 1–13 (2001).
127. Jonas, B. A. & Privalsky, M. L. SMRT and N-CoR corepressors are regulated by distinct kinase signaling pathways. *J. Biol. Chem.* **279**, 54676–54686 (2004).
128. Cardozo, T. & Pagano, M. The SCF ubiquitin ligase: insights into a molecular machine. *Nature Rev. Mol. Cell Biol.* **5**, 739–751 (2004).
129. Anest, V. *et al.* A nucleosomal function for IκB kinase-α in NF-κB-dependent gene expression. *Nature* **423**, 659–663 (2003).
130. Yamamoto, Y., Verma, U. N., Prajapati, S., Kwak, Y. T. & Gaynor, R. B. Histone H3 phosphorylation by IKK-α is critical for cytokine-induced gene expression. *Nature* **423**, 655–659 (2003).
131. Bourguet, W., Ruff, M., Chambon, P., Gronemeyer, H. & Moras, D. Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-α. *Nature* **375**, 377–382 (1995).
132. Renaud, J. P. *et al.* Crystal structure of the RAR-γ ligand-binding domain bound to all-*trans* retinoic acid. *Nature* **378**, 681–689 (1995).
133. Darimont, B. D. *et al.* Structure and specificity of nuclear receptor–coactivator interactions. *Genes Dev*. **12**, 3343–3356 (1998).
134. Wagner, R. L. *et al.* A structural role for hormone in the thyroid hormone receptor. *Nature* **378**, 690–697 (1995).
135. Brzozowski, A. M. *et al.* Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **389**, 753–758 (1997).
136. Shiau, A. K. *et al.* The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* **95**, 927–937 (1998).
137. Nolte, R. T. *et al.* Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ. *Nature* **395**, 137–143 (1998).
138. Heery, D. M., Kalkhoven, E., Hoare, S. & Parker, M. G. A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature* **387**, 733–736 (1997).
139. Torchia, J. *et al.* The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature* **387**, 677–684 (1997).
140. Nagy, L. *et al.* Mechanism of corepressor binding and release from nuclear hormone receptors. *Genes Dev*. **13**, 3209–3216 (1999).
141. Perissi, V. *et al.* Molecular determinants of nuclear receptor-corepressor interaction. *Genes Dev.* **13**, 3198–3208 (1999).
142. Hu, X. & Lazar, M. A. The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors. *Nature* **402**, 93–96 (1999).
143. Huang, H. J., Norris, J. D. & McDonnell, D. P. Identification of a negative regulatory surface within estrogen receptor α provides evidence in support of a role for corepressors in regulating cellular responses to agonists and antagonists. *Mol. Endocrinol.* **16**, 1778–1792 (2002).
144. Losel, R. & Wehling, M. Nongenomic actions of steroid hormones. *Nature Rev. Mol. Cell Biol*. **4**, 46–56 (2003).
145. Koh, S. S., Chen, D., Lee, Y. H. & Stallcup, M. R. Synergistic enhancement of nuclear receptor function by p160 coactivators and two coactivators with protein methyltransferase activities. *J. Biol. Chem.* **276**, 1089–1098 (2001).
146. Wang, H. *et al.* Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. *Science* **293**, 853–857 (2001).

147. Xu, W. *et al.* A methylation-mediator complex in hormone signaling. *Genes Dev.* **18**, 144–156 (2004).
Biochemical purification of the CARM1 complex, which indicates a direct association between ATP-dependent chromatin remodelling complexes and arginine methyltransferases.
148. Brady, M. E. *et al.* Tip60 is a nuclear hormone receptor coactivator. *J. Biol. Chem.* **274**, 17599–17604 (1999).
149. Yanagisawa, J. *et al.* Nuclear receptor function requires a TFTC-type histone acetyl transferase complex. *Mol. Cell* **9**, 553–562 (2002).
150. Puigserver, P. *et al.* A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* **92**, 829–839 (1998).
151. Fondell, J. D., Ge, H. & Roeder, R. G. Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex. *Proc. Natl Acad. Sci. USA* **93**, 8329–8333 (1996).
152. Ito, M. *et al.* Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. *Mol. Cell* **3**, 361–370 (1999).
153. Naar, A. M. *et al.* Composite co-activator ARC mediates chromatin-directed transcriptional activation. *Nature* **398**, 828–832 (1999).
154. Rachez, C. *et al.* Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex. *Nature* **398**, 824–828 (1999).
155. Wade, P. A., Jones, P. L., Vermaak, D. & Wolffe, A. P. A multiple subunit Mi-2 histone deacetylase from *Xenopus laevis* cofractionates with an associated Snf2 superfamily ATPase. *Curr. Biol.* **8**, 843–846 (1998).
156. Zhang, Y., LeRoy, G., Seelig, H. P., Lane, W. S. & Reinberg, D. The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities. *Cell* **95**, 279–289 (1998).
157. Xue, Y. *et al.* NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. *Mol. Cell* **2**, 851–861(1998).
158. Tong, J. K., Hassig, C. A., Schnitzler, G. R., Kingston, R. E. & Schreiber, S. L. Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex. *Nature* **395**, 917–921 (1998).
159. Shi, Y. *et al.* Coordinated histone modifications mediated by a CtBP co-repressor complex. *Nature* **422**, 735–738(2003).

Acknowledgements
We sincerely thank I. Garcia Bassets and C. K. Glass for critical reading of this manuscript and for insightful discussions. We are also grateful to J. Hightower for preparation of the figures. We apologize to all our colleagues whose important and insightful findings could not be included in this review because of space limitations. M.G.R is an investigator with the Howard Hughes Medical Institute and work in the laboratory is supported by grants from the National Institutes of Health.

Competing interests statement
The authors declare no competing financial interests.

Online links

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
CATD | *Myc* | RAR | *TFF1*
Swiss-Prot: http://www.expasy.ch/
AP1 | AR | CARM1 | CBP | CNTF | CtBP | GR | NCoA1 | NCoA3 | p300 | PRMT1 | RIP140 | SMRT | SRA1 | TBL1| TBLR1
Saccharomyces Genome Database: http://www.yeastgenome.org
Ash1 | *HO* | Swi5

FURTHER INFORMATION
Michael Rosenfeld's laboratory: http://rosenfeldlab.ucsd.edu
NuReBaSe: http://www.ens-lyon.fr/LBMC/laudet/nurebase/nurebase.html
Nuclear Receptor Signaling Atlas: http://www.nursa.org/flash/gene/nuclearreceptor/start.html
Frank Gannon's laboratory website: http://www-db.embl.de/jss/EmblGroupsHD/per_862.html
Access to this interactive links box is free online