Chapter 3
Nuclear Receptor Coactivators

Ioannis Zalachoras and Onno C. Meijer

Abstract The effects that steroid hormones exert on gene expression via their nuclear receptors (NRs) must be tightly regulated, in particular because of their pleiotropic effects in many tissues. To that end, regulation of receptor activity takes place at multiple levels, which include ligand availability, epigenetic modifications of chromatin around tissue-specific target genes, expression levels of the receptor, and the presence or absence of other NRs in the same cell. One of the levels of transcriptional control is that of the NR coregulators, proteins that can interact with NRs and modulate their function. Coregulators can interact with multiple NRs and NRs can interact with multiple coregulators. As a consequence, coregulator expression in certain cell types may play the roles of hubs and bottleneck that offers gene target, cell type, or context specificity. Below we offer an overview of NR coregulator function, highlighting the best-described coregulators in the brain, as well as possibilities for the manipulation of NR–coregulator interactions for therapeutic or experimental purposes.

Keywords Sex steroids • Glucocorticoids • Gene transcription • Selective modulators • Brain

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3.1 Introduction

Steroid hormones exert their effects in both the brain and the periphery, orchestrating a wide range of behavioral and physiological responses. Given the nature of neuroendocrine regulation, steroids not only act as final signaling molecules of neuroendocrine axes but they also shape the activity of these axes via direct negative feedback actions and more complex indirect feedback on the brain. Their effects are mediated by their respective nuclear receptors (NRs). NRs act in large measure as transcription factors that modulate gene expression and chromatin structure. They show a wide distribution pattern in peripheral target organs and different cell types in brain and pituitary (Gofflot et al. 2007). Given the wide range of possible steroid actions and the broad expression pattern of their receptors, it is important that their effects are regulated at various levels. Such regulation can take place at the level of the ligand availability, type and local concentration (Awasthi and Simons Jr 2012; Yang and Fuller 2012), the expression levels and posttranslational modifications of the receptor (Noguchi et al. 2010; Nicolaides et al. 2010), interactions with molecular chaperones in the cytoplasm (Hartmann et al. 2012; Touma et al. 2011), dimerization and translocation to the nucleus (Fitzsimons et al. 2008), the presence or affinity of multiple receptor types for the same ligand in the same cell (de Kloet et al. 2005), the presence and activity of kinases such as SGK-1 (Anacker et al. 2013), and, once the receptor is in the nucleus, the chromatin landscape and many interactions with proteins that interact with or compete for nuclear receptors (de Kloet et al. 2009). The latter can be divided into other – non-receptor – transcription factors and nuclear receptor coregulators.

3.2 Nuclear Receptors

All nuclear receptors consist of functional domains that can be directly coupled to their function as transcription factors and indirect chromatin modifiers. The relationship between the structure and the function of the nuclear receptors has been extensively studied and described (Mittelstadt and Ashwell 2003; Giguere et al. 1986). In short, the NR proteins are composed of three critical modular domains: a poorly conserved N-terminal domain that harbors the hormone-independent activation function 1 (AF1), a central DNA-binding domain (DBD) that shows extensive homology between related NR family members, and a C-terminal ligand-binding domain (LBD) that also forms the hormone-dependent activation function 2 (AF2) domain that is activated allosterically upon ligand binding (Fig. 3.1) (Mittelstadt and Ashwell 2003; Danielian et al. 1992; Giguere et al. 1986; Stanišić et al. 2010).

In the absence of ligand, NRs are bound to chaperone protein complexes in the cytoplasm, such as FKBP5 and HSP90 (Menke et al. 2013; Klengel et al. 2013; Picard et al. 1990). Upon ligand binding, a conformational change takes place that leads to the dimerization of the nuclear receptor and its translocation to the nucleus. There, the receptor binds to the DNA, either directly via its DBD or indirectly via

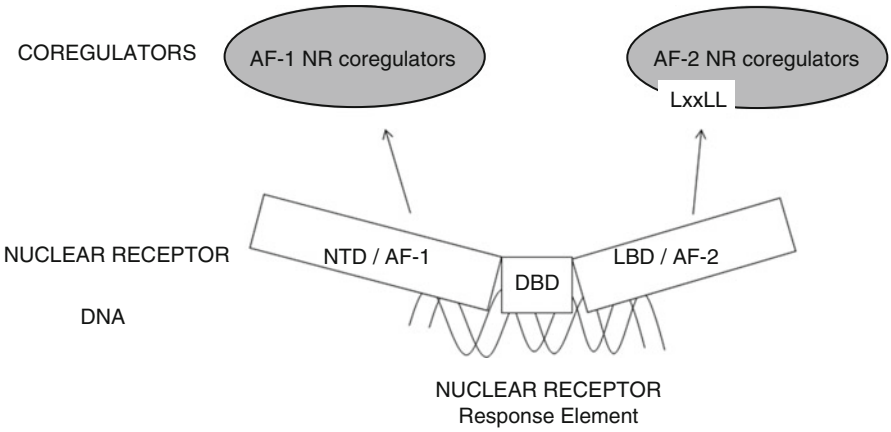

Fig. 3.1 The domain structure of nuclear receptors and their modes of coregulator interactions. The central DNA-binding domain (DBD) is flanked by the N-terminal domain (NTD) and the ligand-binding domain (LBD). The LBD has a well-conserved structure consisting of 12 alpha helices. These form activation function 2 (AF2) that contacts AF2 NR coregulators via their LxxLL motifs, or "NR-boxes." AF2 NR coregulators are shared between many NRs, and interactions can readily be identified using in vitro protein assays, such as MARCoNI. AF1 lies in the NTD – it is intrinsically unstructured, and in general, the AF1 NR coregulators are more specific to a particular receptor and more difficult to identify

interaction with other transcription factors. In the direct DNA-binding mode, molecular interactions with a host of transcriptionally active proteins may take place via the AF1 and AF2 domains. Direct DNA binding occurs at nuclear receptor responsive elements (NREs), specific nucleotide sequences for each NR linked to activation or repression of specific genes. There may be hundreds of thousands of NRE-like sequences in mammalian genomes (Datson et al. 2011), but chromatin structure and the demand of associated binding partners limit actual binding to only a couple of thousand detectable binding loci per cell type. However, these show a very substantial cell specificity (John et al. 2011), and even strong evolutionary conservation of response elements does not automatically imply responsiveness in a particular tissue or cell type (Datson et al. 2011). The receptors are thought to mainly form homodimers, act as monomers in conjunction with other non-receptor or transcription factors, or heterodimerize with other steroid receptors (Pearce 1994; Chen et al. 1997; Presman et al. 2014; Trapp et al. 1994). Of note, although initial promoter-directed study revealed a substantial number of response elements within hundreds of base pairs of transcription start sites, genome-wide approaches have revealed that NREs can be localized many kilobases away from the genes they regulate and may act *in cis* over very long ranges.

Other transcription factors can interact with NRs during DNA binding. Some of the identified transcription factors will bring the receptors to the DNA by way of "tethering" mechanisms, like those involved in classic transrepression in the immune system (De Bosscher et al. 2008). There are also those transcription factors that bind in the vicinity (within hundreds of base pairs) of the steroid receptors and

are in some way involved in modulating their function, also by chromatin and DNA modifications (Biddie et al. 2011).

3.3 Coregulators' Mode of Action

Nuclear receptor coregulators are proteins that interact with NRs but not the DNA (they are not transcription factors) (Zalachoras et al. 2013c). They have three main functions: (i) they can recruit other transcriptionally active proteins, (ii) they have histone acetyltransferase or methyltransferase activity and/or they can recruit histone acetyl- or methyltransferase, and (iii) they stabilize the transcriptional machinery (Tetel et al. 2009). In direct DNA-binding mode, the rate of transcriptional stimulation at a given ligand concentration is thought to be limited by any of the transcriptional coregulators of the receptors, in a gene-specific manner (Ong et al. 2010). In a general sense, the coregulator repertoire that is assembled at a particular locus on the DNA determines the magnitude as well as the nature of the transcriptional response.

The activities of the AF1 and AF2 output domains of NRs by definition depend on interactions with coregulators (Fig. 3.1). AF1 coregulators are difficult to predict and often relatively unique for particular NRs, based on the low degree of homology between the different receptors and the intrinsic unordered nature of the domain. AF2 domains are much more structured and conserved, and their coregulators share structural domains called NR-boxes. These contain specific motifs containing the amino acid sequence LXXLL. The AF2–NR-box interactions have revealed detailed structural information, which can be coupled to the conformational change of the receptor after binding to agonists or antagonists (Huang et al. 2010). In addition, the interactions may be screened for in vitro based on binding of the (recombinant) receptor protein to NR-box-containing peptide fragments from many different coregulators (Zalachoras et al. 2013a).

The coregulators that are recruited after hormone binding tend to be coactivators rather than corepressors. In this respect the classical steroid receptors differ from other classes of nuclear receptors, which may be DNA-bound in absence of ligand, but transcriptionally inactive based on corepressor binding. Interestingly, some steroid receptor antagonists, such as the mixed AR/GR/PR antagonist RU486/mifepristone, induce recruitment of corepressors rather than coactivators (Zhang et al. 1998). As discussed later in this chapter, this screening opens the possibility to find new steroid receptor ligands with intermediate coregulator recruitment profiles which consequently combine agonistic and antagonistic properties – so-called selective receptor modulators (Zalachoras et al. 2013b).

Cell-type-specific chromatin organization and coregulator repertoire (Meijer et al. 2000) (www.nursa.org) interact as the recruitment of coregulators by nuclear receptors may take place in a cell-type- and locus-specific manner (Trousson et al. 2007). Coactivators tend to either be direct modifiers of histones via acetyltransferase or methyltransferase activity or recruit other coactivators that subsequently change histone posttranslational modifications, such as CREB-binding protein (CBP) and many

others (Won Jeong et al. 2012). The ensuing histone marks may act as epigenetic determinants of cell fate of future cell behavior. This model indicates that coregulators do not act in isolation but in protein complexes that may involve transcription factors, coregulator–coregulator interactions, and RNA molecules (Tetel et al. 2009).

Steroid hormone treatment may also lead to epigenetic changes at the level of CpG methylation on the DNA (Auger et al. 2011; Yu et al. 2013; Zhao et al. 2010; Sharma et al. 2013). There is however not much evidence for direct recruitment of DNA methyltransferases by NR coregulators, although this would constitute a mechanism for gene-specific regulation of CpG islands. On the other hand, DNA methylation at specific loci may determine which coregulators are recruited by specific nuclear receptors (Ceschin et al. 2011).

With tissue- and cell-specific expression patterns, as well as their promiscuity (coregulators can often interact with multiple NRs, mostly based on common interactions with the relatively homologous LBD/AF2 domains of the receptors), coregulators can create an additional level of regulation that drives the pleiotropic effects of NRs toward cell-specific transcriptional changes, as well as to NR-specific expression programs when competition for coregulators occurs. Moreover, given their potential to induce chromatin modifications, coregulators may be the link between NR function and the appropriate epigenetic changes in response to stimuli (Hunter 2012). Below, we describe examples of interactions between NRs and coregulators that highlight the importance of coregulators for the steroid receptor family members with focus on brain function.

Estimates of the number of nuclear receptor coregulators are as high as >350 different proteins (Stanisić et al. 2010), but the importance of most of these for individual steroid receptors is unknown and not every coregulator interacts with every receptor type. It is also clear that there is a pronounced differential distribution of different coregulators per brain region (www.brain-map.org). Neuroendocrine relevance of individual coregulators discussed below, based on interactions found in cell lines or other organ systems, clearly depends on actual coexpression with steroid receptors in the brain and/or pituitary.

3.3.1 Sex Steroid Receptor Coregulators

AR plays important roles in the brain, most pronounced in regulating male sexual behavior. However, given therapeutic urgency, coregulator function in AR action has been extensively studied in relation to the development and progression of prostate cancer. For example, E6-associated protein (E6-AP) is a coregulator that interacts with AR during the development of the prostate gland, and it plays an important role in development of the brain, but it is not clear whether it is important for AR function in the adult brain.

In neuroendocrine setting, steroid receptor coactivator-1 (SRC-1) is the best-characterized AR coregulator (Feng and O'Malley 2014), and it has been shown to be necessary for regulation of the androgen-induced behavior and plasticity in Japanese quail (Charlier et al. 2006a). Blockade of SRC-1 expression in the brain

led to abrogation of testosterone-dependent sexual behaviors, as well as the testosterone-dependent growth of the preoptic medial nucleus, an area of the quail brain involved in sexual behavior (Charlier et al. 2006b). Interestingly, both AR and SRC-1 expression are regulated by photoperiod and testosterone treatment, indicating the significance of parallel regulation of these two components for signaling (Charlier et al. 2006a).

SRC-1 was the first of the classical coregulators to be described (Oñate et al. 1995) and belongs to the so-called p160 family. Other members such as SRC-2 and SRC-3 can also be involved in AR signaling, although their involvement has not been studied as extensively in the brain. Nevertheless, they may show some redundancy with SRC-1, as SRC-2 overexpression is a known compensatory mechanism in the absence of SRC-1 (Apostolakis et al. 2002), or offer differential regulation in certain brain areas (i.e., SRC-3 shows a very distinct pattern in the brain with high expression only in the hippocampus).

Estrogen receptors are expressed in two subtypes ERα and ERβ which are coded for by two different genes. The effects of the interactions between ER and coregulators have been broadly studied in relation to breast cancer, sexual behavior, and cognitive function. In the same context epigenetic regulation of the expression of ER-dependent genes has been shown to be relevant as well, in rather complex cascades of events. For instance, estradiol can control the expression of enhancer of zeste homolog 2 (EZH2), a methyltransferase specific for lysine 27 of histone 3, overexpressed in breast cancer, together with mixed lineage leukemia (a coregulator) and CBP/p300 (Bhan et al. 2014).

Next to many studies in relation to sexual function and differentiation, considerable work has been done on the effects of estrogens on stress responses and susceptibility (Calmarza-Font et al. 2012; Shansky and Lipps 2013). The fact that early life treatment with estrogen results in altered later life stress responses indicates that epigenetic mechanisms may be at play (Panagiotidou et al. 2014), something that is backed up by observation after manipulation of downstream DNA methylation factor expression (Wang et al. 2013b). The exact effects of estrogens (e.g., anxiolytic or anxiogenic) differ, depending on the age of treatment, the sex of the animals, and, more importantly, treatment with other steroid hormones or the expression levels of other steroid receptors.

In relation to specific coregulators that interact with ER, again most data collected indicate the involvement of members of the p160 family. They are largely coexpressed with ER in the rodent brain, interact with ER (Yore et al. 2010), and often follow the seasonal or age-dependent expression patterns of ER (Tetel et al. 2007; Tognoni et al. 2011). Moreover, SRC-1 expression varies in the hypothalamus of cycling female rats with a nadir during diestrus and a zenith during proestrus and estrus (Charlier et al. 2010). Furthermore, in the brains of aged female mice, SRC-1 has lower expression, indicating a reduction in hormone sensitivity. Experimental deletion of SRC-1 and SRC-2 expression in the brain resulted in loss of sensitivity to estrogens leading to aberrant hormone-induced sexual behavior in female rats, which was not recovered even after high doses of estradiol (Apostolakis et al. 2002), even if other studies reported similar but much less striking effects (Molenda et al. 2002). Also in male Japanese quail, intracerebroventricular injections of antisense

oligonucleotides targeting SRC-1 blocked the estrogen-dependent sexual behaviors (Charlier et al. 2006b).

Another interesting ER coregulator is ribosomal protein L7 (RPL7). This protein is a selective coactivator of the ER involved in mRNA translation, and in avian species, it is highly expressed in the brain and particularly in the regions involved in song control such as HVC, RA, and area X (Duncan et al. 2009). Sexual dimorphism in its expression in the brain has been reported. It is believed that its upregulation at a certain age may play an additional role in the sexual differentiation of the avian brain in response to estrogens (Duncan and Carruth 2011). Interestingly, in vivo knockdown of RPL7 in the zebra finch brain resulted in altered morphology in song control regions, without however any differences in song learning and singing behavior, possibly related to other coregulators that can take over RPL7's function in its absence.

Recently, data collected postmortem from the brains of patients with autism spectrum disorders have shown that ERβ, together with SRC-1, CBP, and P/CAF, has reduced expression in the medial frontal gyrus compared to controls (Crider et al. 2014). Combined with data showing the effects of the AR and ER together with coregulator NCOA5 on the retinoic acid receptor-related orphan receptor A (RORA) promoter may suggest that sex hormones may be relevant for autism spectrum disorders, as well as for the sex bias in the development of such disorders (Sarachana and Hu 2013a, b). Models of RORA insufficiency show behavioral patterns similar to autism spectrum disorders such as spatial learning deficits and reduced object exploration (Sarachana et al. 2011).

3.3.2 GR and MR Coregulators

Among other functions, GR and MR orchestrate the expression of responses to stressors, which involves the coordination of multiple systems in the brain and the periphery (Myers et al. 2013; Herman 2013; Rodrigues et al. 2009; de Kloet et al. 2005). The HPA axis plays a central role in the regulation of stress responses via control of glucocorticoid hormone levels. Glucocorticoids, in turn, exert a wide range of effects, including effects on memory, behavior, and metabolism, that are mediated by their receptors MR and GR. Importantly, glucocorticoids can block the expression and release of CRH in the PVN and *POMC*/ACTH in the pituitary, thus creating a negative feedback loop (Kovács 2013; Laryea et al. 2013). Of interest, there is at least one coregulator – the SRC-1 splice variant SRC-1a – that is enriched in the hypothalamus and pituitary and may mediate transcriptional effects that are part of negative feedback actions (Meijer et al. 2000). Many coregulators are shared between MR and GR, even if some functional differences exist (Meijer et al. 2005). Specific coregulators likely act via the poorly conserved AF1. Recently, Gemin4 has been shown to function as an MR coregulator, as well as 11–19 lysine-rich leukemia (ELL) (Yang et al. 2015; Yang and Young 2009; Pascual-Le Tallec et al. 2005). Within the brain, MR- and GR-specific coregulator pathways are basically unknown.

In the multiple GR- and MR-dependent neuromodulatory pathways, some of which result in epigenetic changes (Hunter et al. 2014), several coregulators take part. Many studies have been conducted on the coregulators of GR and to a lesser extent MR in relation to brain function, particularly for learning and memory and stress responses. Apart from members of the p160 family, one dominant example is the coregulators of CREB CBP/p300, which via p160 interaction are secondary coregulators of steroid receptors. Other examples are p300/CBP-associated factor (pCAF), members of the CREB-regulated transcription coactivator (CRTC) family, and the coregulators of steroid hormone receptors RIP-140 and Ube3a (Barrett et al. 2011; Malvaez et al. 2011; Oliveira et al. 2007; Jeanneteau et al. 2012; Ch'ng et al. 2012; Augereau et al. 2006; Duclot et al. 2010, 2012; Maurice et al. 2007; Godavarthi et al. 2012; Mardirossian et al. 2009; Wallace et al. 2012; Weeber et al. 2003; Engel and Yamamoto 2011). Not surprisingly, mutations or deletions of these coregulators often result in impairments in learning and memory, decreased neuronal plasticity, inappropriate regulation of stress responses, or abnormal brain morphology (Zalachoras et al. 2013c).

SRC-1 and the other p160 family members are arguably the best-characterized GR coregulators. SRC-1 has been shown to be crucial for GR-dependent regulation of CRH expression in the PVN and the central nucleus of the amygdala (Lachize et al. 2009). SRC-1 is expressed in two splice variants, SRC-1a and SRC-1e, which have differential distribution in the brain and opposite activities on the *crh* promoter (Fig. 3.2) (van der Laan et al. 2008; Meijer et al. 2000, 2005). SRC-1a downregulates *crh* expression and is highly expressed in the PVN, while SRC-1e lacks repressive activity and shows high expression in the CeA. In SRC-1 KO animals, *crh* expression in the PVN and the CeA is largely resistant to regulation by glucocorticoids, as well as POMC expression in the anterior pituitary, while *crh* expression in the CeA is

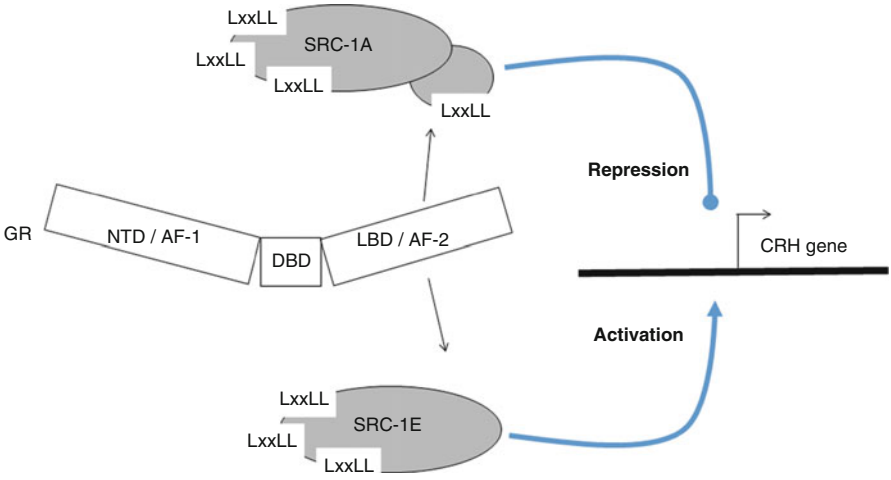

Fig. 3.2 Proposed model of SRC-1 splice variant differential action. SRC-1a has a longer C-terminal domain that contains an additional LxxLL NR-box, as well as a repressor function. Its recruitment by GR can lead to repression of the *CRH* gene, whereas SRC-1e may act as a stimulatory coactivator

decreased (Lachize et al. 2009; Winnay et al. 2006). The HPA activity and behavioral responses to stress are close to normal in these animals, despite the transcriptional phenotype. This can be partly attributed to the compensatory developmental upregulation of SRC-2 expression in the absence of SRC-1 (Nishihara et al. 2003), and definitive conclusions await transient or inducible genetic experiments. Despite the extensive study of the SRC-1 splice variant function in vitro, only recently have there been attempts to study their function in vivo utilizing exon skipping methods to shift the expression ratio of SRC-1 and SRC-1e in the mouse brain (Zalachoras et al. 2013a). On the other hand, apart from the compensatory effects of SRC-2 in the absence of SRC-1, deletion of SRC-2 results in impaired adrenocortical output at the level of the adrenal, thus increasing the HPA axis in response to stress. Interestingly, the deletion of any of SRC-1, SRC-2, and SRC-3 was shown to have effects on anxiety behavior, which were often sex-dependent (Stashi et al. 2013).

CREB-binding protein (CBP) is a potential brain GR (Conway-Campbell et al. 2011) and MR coregulator (Kitagawa et al. 2002). CBP is a HAT that can be recruited by SRC-1 and therefore likely is a secondary coregulator to all steroid receptors. CBP and its homolog p300 (Barrett et al. 2011) are also downstream transcriptional modulators of CREB and AP1. Since gene transcription is essential for many processes such as learning and memory and stress responses, as well as a key mode of action of steroid receptors, the broad involvement of CBP/p300 in such processes is not surprising (Maurice et al. 2007; Malvaez et al. 2011). GR effects on learning and memory may be to some extent CBP/p300 dependent (Roozendaal et al. 2010) either via LxxLL-dependent GR-CBP/p300 direct interactions or via recruitment by SRC-1. CBP/p300 has different LxxLL interaction domains for GR, one KIX domain for interaction with CREB, and one SRC-1 interaction domain; thus, combinatorial binding may be possible (Wang et al. 2013a; Waters et al. 2006; Chan and La Thangue 2001). Thus, a model has been proposed in which glucocorticoids can functionally interact with CBP and alter gene expression both by direct binding and promoter transactivation and by histone modifications (Roozendaal et al. 2010). Lack of CBP results in decreased histone methylation, together with impairments in long- and short-term memory (Barrett et al. 2011; Chen et al. 2010), while similar results have been observed after deletion of p300 (Oliveira et al. 2007, 2011). Interestingly, in the absence of CBP, p300 is not always upregulated and cannot take over all CBP-dependent functions, thus indicating a certain degree of non-redundancy of the functions of the two proteins (Barrett et al. 2011). Thus, CBP and p300 have significant involvement in learning and memory, and despite their homology, they have possibly non-redundant roles in these processes, although their roles may also be brain region dependent (Marek et al. 2011). The functional "integrator" CBP may well be one of the direct substrates of the close cross talk between GR and CREB pathways in neuronal plasticity.

CREB-regulated transcription coactivators (CRTCs) are primarily known as transcriptional coregulators of CREB. Upon cAMP and calcium exposure, they are dephosphorylated and translocate into the nucleus where they can interact with CREB over relevant promoters controlling the function of NRs (Liu et al. 2010, 2011, 2012; Altarejos and Montminy 2011). Jeanneteau et al. studied how BDNF, GR, and CREB

regulate *crh* expression (Jeanneteau et al. 2012). It had been known that BDNF can upregulate *crh* expression in the PVN (Givalois et al. 2004), whereas GR activation (e.g., after treatment with glucocorticoids) represses *crh* expression in the PVN (Makino et al. 1994). Jeanneteau et al. showed with a combination of loss- and gain-of-function techniques that there is a cross talk between GR and BDNF and its receptor TrkB through interactions with CREB, and mediation of the CRTC2 may activate the *crh* promoter while glucocorticoids through the GR may target phosphorylation and nuclear localization of CRTC2 and repress the *crh* promoter (Jeanneteau et al. 2012).

Another HAT involved in learning and memory that interacts both directly and indirectly (via p160 family members) with GR is p300/CBP-associated factor (PCAF) (He et al. 2002; Szapary et al. 2008; Blanco et al. 1998; Li et al. 2003), which can also acetylate other transcriptional regulators (Pérez-Luna et al. 2012). It has been found to be upregulated together with increased histone acetylation in the rat hippocampus during memory consolidation (Bousiges et al. 2010). Lack of PCAF resulted in impaired memory function, exaggerated stress responses, anatomical differences in their hippocampus, and decreased synaptic plasticity (Maurice et al. 2007), while its blockade in the infralimbic prefrontal cortex impaired fear memory extinction (Wei et al. 2012).

Ube3a is a transcriptional coactivator of steroid hormone receptors. Repression of its expression is one of the causes of Angelman syndrome (Sutcliffe et al. 1997). Lack of Ube3a resulted in cognitive and memory impairments, deficits in hippocampal plasticity, seizures, decrease of CaMKII activity, altered adult hippocampal neurogenesis, increased stress and anxiety, and differences in neuronal morphology (Jiang et al. 2010; Mardirossian et al. 2009; Godavarthi et al. 2012; Sato and Stryker 2010; Wallace et al. 2012; Weeber et al. 2003). These phenotypes may be related to defective GR signaling leading to increased stress and anxiety as shown by the fact that mice lacking Ube3a have higher morning corticosterone levels and poor scores in a novel object recognition test and spend more time in the dark (anxiety behavior) in a light/dark test than their wild-type or paternal copy-deficient ube3a mice (Godavarthi et al. 2012). However, as for other coregulators, direct evidence for Ube3a as a mediator of MR and/or GR effects is lacking, and we are at a stage where interactions may be likely, but unproven.

3.4 Discovery of Novel NR Coregulators

Given the effects of coregulators on gene expression and the additional regulation levels they generate, it becomes increasingly more interesting to i) discover new NR coregulators and ii) develop pharmacological agents that can selectively manipulate NR–coregulator interactions.

Finding or predicting new NR coregulators is the first important step, as there is relatively little known about which coregulators interact with which NR. Moreover, even for those coregulators/NRs whose interactions are well documented, little is known regarding their in vivo function in the brain, since the majority of the data comes from in vitro studies. Important data regarding putative interactions between

coregulators and NR may come from the Allen Brain Atlas, where the expression patterns of all NRs and coregulators in the brain have been studied. Furthermore, correlations between the expression of NRs, coregulators, and target genes can take place, providing first hints toward the interactions and involvement of both NRs and coregulators in specific pathways. A second tool to identify putative coregulators with relevance for a particular NR is the MARCoNI peptide array in which receptor–coactivator interactions can be predicted based on NR-box interactions (Desmet et al. 2014; Koppen et al. 2009). With this system, not only the NR–coregulator interactions induced by different ligands can be quantified, but the interactions between coregulators and mutant or recombinant NRs or even the behavior of NRs derived from different in vivo contexts (Houtman et al. 2012). The MARCoNI assay profiles have been previously corroborated in a battery of in vivo tests ranging from stress-related behavior to target gene expression (Zalachoras et al. 2013b). Finally, tools like the MARCoNI assay can also be used in the early stages of drug development to select the better candidates for in vivo use.

For lack of open biochemical approaches based on molecular interactions in small tissues (York et al. 2013), combing data coming from the MARCoNI assay with tools like the Allen Brain Atlas can play an important role in the discovery of novel NR coregulators, predict the behavior and properties of novel NR ligands, and study the properties of NR mutations or modifications.

3.5 Making Use of Coregulator Diversity: Selective Nuclear Receptor Modulators (SNRMs)

Endogenous or exogenous steroids may combine beneficial and disadvantageous effects. Ever since it became clear that there are multiple mechanisms by with the receptors signal, there has been the notion to dissociate such mechanisms with drugs that allow one signaling mechanism, but not others. Such “dissociated compounds” or “selective receptor modulators” that have tissue- or pathway-specific effects may work by several mechanisms, including selective recruitment of coregulators by the receptors (Fig. 3.3). Accordingly, many attempts have been made to develop new drugs with the potential to induce or block selective interactions between NRs and coregulators. Hence, these drugs should induce such an NR-ligand conformation that will make the complex accessible only to a subset of the available coregulators (Martinkovich et al. 2014; Højfeldt et al. 2014).

Most work done on selective androgen receptor coregulators is related to ligands that can target the brain, the bone, or the muscle without affecting prostate tissue with oncogenic potential (Akita et al. 2013). Age-related androgen depletion is a risk factor for sarcopenia, osteoporosis, and accumulation of β-amyloid protein and development of Alzheimer’s disease. Androgen replacement therapies are not always effective due to side effects. The selective androgen receptor modulator NEP28 was shown to increase the expression of an enzyme that breaks down β-amyloid plaques in the brain and was effective in the muscle and bone, without prostate-related adverse effects (Akita et al. 2013). Similar results were also

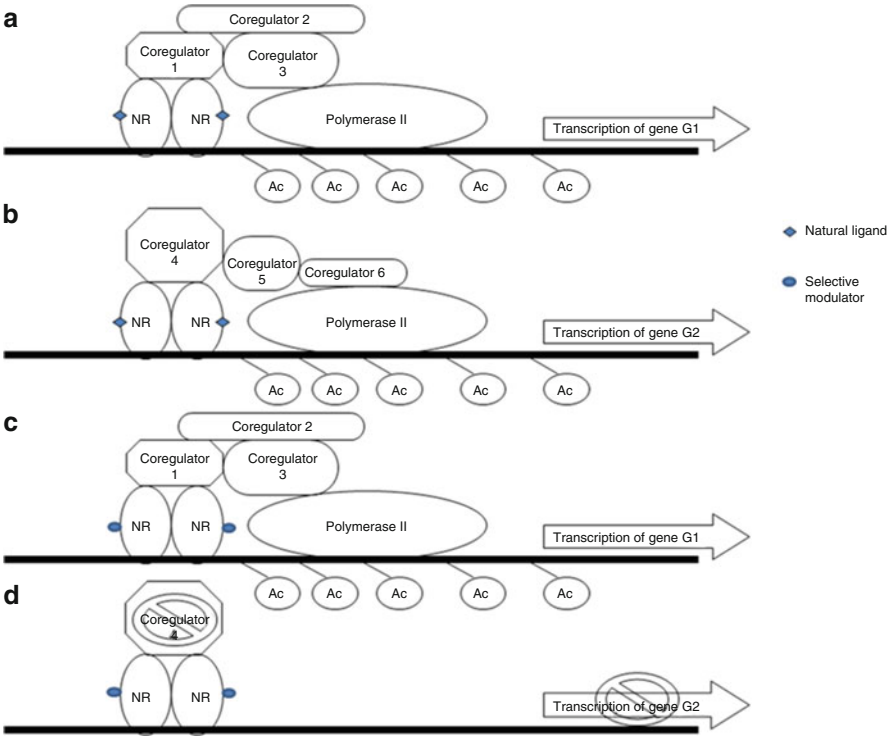

Fig. 3.3 Proposed model of the function of selective modulators. (**a**–**b**). The nuclear receptor is bound to its natural ligand, dimerized, and on chromatin. It can recruit a number of different coregulators that interact directly with it (1,4), which can, in turn, recruit other coregulators (2,3,5, and 6). These NR–coregulator complexes can then stabilize the transcriptional machinery, acetylate histones, and activate the transcription of genes G1 and G2. (**c**–**d**). When NR binds a selective modulator, it only induces/allows interaction with coregulator 1, but not 4. Therefore, only transcription of G1 takes place, while the transcription of G2 is blocked

observed after use of another selective androgen receptor modulator, 3beta,19-NA (Page et al. 2008). Yet another selective AR modulator, A-262536, also showed high selectivity for muscle and bone, in contrast to prostate (Piu et al. 2008). The mechanism of action of these compounds is not fully known; however, at least some of them may induce different AR–coregulator interactions compared to testosterone, while others may capitalize on partial agonist effects or differential penetration of different tissues.

Drugs targeting the ER have a variety of uses including menopausal symptoms, fertility agents or oral contraceptives, and breast cancer treatments (Wardell et al. 2014). Due to the pleiotropic effects of ER in the periphery and the brain, it is important to find agents that have selective action on specific pathways. ER was the prototype target for selective steroid receptor modulators, with tamoxifen, which acts as agonist in the bone and endometrium but as antagonist in the breast (tumors). This selective action was mainly attributed to the selective profile of interactions

between ER and coregulators it can induce, also taking advantage of local expression differences of ER coregulators. Since the development of tamoxifen, additional selective estrogen receptor modulators have been developed with lower side effects and variable ER–coregulator interaction profiles (Feng and O'Malley 2014; Evers et al. 2014; Gottardis et al. 1988). Important for the directionality of the effects of tamoxifen in different tissues are the expression levels of p160 coregulators. Interestingly, increased levels of SRC-1, SRC-3, or other coregulator expression are associated with tamoxifen resistance in breast cancer (Feng and O'Malley 2014; Kumar et al. 2009). Other compounds similar to tamoxifen (nonsteroidal triphenylethylene) are toremifene, droloxifene, and idoxifene all with chemical structure variations in attempts to find the balance between side effects and potency (Martinkovich et al. 2014).

Most attention regarding selective GR modulators has been drawn by GR ligands that have anti-inflammatory efficacy, but no effects on metabolism or osteoporosis (Rauch et al. 2011; van Lierop et al. 2012). However, given the pleiotropic actions of glucocorticoids in the brain, it may be beneficial to distinguish between different effects of glucocorticoids. Blocking detrimental effects of chronically elevated glucocorticoid exposure with full antagonists such as mifepristone can lead to disinhibition of the HPA axis and counteract efficient antagonism. Moreover, blocking all effects of GR on emotional and cognitive processes may not be optimal in order to counteract the negative effects of stress. Similarly, induction in the brain of a pro-inflammatory state by pharmacological blockade of GR in astrocytes and/or microglia may not be desirable. Selective GR or MR modulators may be beneficial in stress-related psychopathology and an interesting and useful tool to distinguish different GR-dependent pathways in experimental settings (Zalachoras et al. 2013c).

First attempts tried to base selective GR modulation on the dissociation of effects that depend on DNA binding by the receptor and classical transrepressive effects directly on pro-inflammatory transcription factors NF-κB and AP1 (De Bosscher et al. 2003). Such an example is the GR ligand "compound A" which induces inhibition of NF-κB-dependent pro-inflammatory transcription, but not DNA binding of GR (De Bosscher et al. 2005; Reber et al. 2012). However, part of the anti-inflammatory effects mediated by GR does depend on binding by GR to classical GREs (Beaulieu and Morand 2011). Coghlan et al. (2003) showed a GR ligand that retained anti-inflammatory effects while preventing the GR effects on glucose metabolism and impact on bones. This study showed that the specific behavior of the compound arose from the GR–coregulator interaction profile it induces. An arylpyrazole type of GR ligand was shown to exert selective agonism on hippocampal neurogenesis without affecting skeletal muscle protein synthesis, bone or skin collagen synthesis, or splenic lymphocyte counts (Roohk et al. 2010) and had transcriptional effects on few target genes in cell lines (Wang et al. 2006). This proves the point that GR effects relevant for modulation of brain may be quite selectively targeted with selective modulator types of drugs.

Recently, a novel selective GR modulator has been studied, C108297. It has been shown that it is more specific for GR than mifepristone and can induce a number of GR–coregulator interactions while preventing others. Moreover, it was

shown to have mixed agonist and antagonist properties in stress-related circuits in the brain. For instance, it had agonist effects on the consolidation of fear-related memory, antagonist effects on stress-induced *crh* expression in the CeA, and gene expression in the hippocampus, without inducing HPA axis disinhibition (Zalachoras et al. 2013b). It was also shown that it can counteract the neuroendocrine effects of stress that are induced by glucocorticoid excess (Solomon et al. 2014), as well as prevent the weight uptake as a result of high-fat diet (Asagami et al. 2011). Finally, the same ligand showed strong antagonism that improved the phenotype in animal models of Alzheimer's disease and ALS (Meyer et al. 2014; Baglietto-Vargas et al. 2013).

Selective receptor modulators for MR have not been studied in depth, as plain MR antagonism has been a major clinical goal in cardiovascular disease. However, MR agonism in the brain may be of benefit in relation to particular psychiatric disorders, such as depression (Klok et al. 2011), where its expression has been shown to be decreased in several brain areas (Qi et al. 2012). The development in selective MR modulators is currently taking place, and it will be exciting to see what the potential of such ligands will be (Yang et al. 2011).

3.6 Conclusions

Due to the pleiotropic effects of NRs, modulation of NR-dependent pathways is relevant in a number of conditions. NR coregulators are important for immediate and long-term tissue-, cell-, and target gene-dependent effects of NRs. Therefore, better understanding of NR–coregulator interactions and the development of more selective ligands capable of manipulating those interactions to a desirable direction may be decisive in the treatment of a number of conditions. Although our knowledge has advanced during the past 20 years, there are outstanding questions regarding the gene targets of each coregulator and which protein cocktail is recruited to each particular context. Thus, knowledge of coregulator recruitment to the promoters of certain genes may assist the development of ligands that can affect the expression of genes with high specificity depending on cellular context.

Finally, coregulators can be involved in epigenetic regulation of gene expression either via own activity or via recruitment of appropriate proteins. Thus, studying their epigenetic effects in relation to the changes that appear after a number of environmental stimuli (Elliott et al. 2010; Yehuda et al. 2013; Suderman et al. 2012; Gräff et al. 2014) may reveal new level of regulation and possibilities for intervention.

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