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Nuclear Receptor Coregulators Are New Players in Nervous System Development and Function

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Abstract

Steroid/thyroid hormones and their cognate nuclear receptors (NRs) play important roles in nervous system development and function. The spatial and temporal gene expression that is regulated by NRs in the nervous system requires transcriptional intermediary coregulators, designated as coactivators and corepressors. These coregulators enhance or repress transcriptional activity of NRs and modulate their target gene transcription. Recent progress has largely advanced our understanding of the molecular mechanisms by which NR coregulators function in the nervous system. This article summarizes our current knowledge about the molecular mechanisms, expression patterns, and biological functions of NR coactivators, such as the p160 steroid receptor coactivator family, CBP, p300, BRG1, TRAP220, PGC-1α, ERAP140, NIX1, and E6-AP, as well as corepressors such as NCoR and SMRT. Accumulated findings suggest that the functional spectrum of NR coregulators is much broader than was initially speculated, and these coregulators likely contribute to many physiological aspects of nervous system development and function.

Index Entries: Brain; gene expression; nuclear receptor; coactivator; corepressor; SRC.

Functional Nuclear Receptors in the Nervous System

Nuclear receptors (NRs) comprise a superfamily in mammals of approx 50 members. About half are termed classical receptors, which include receptors for estrogen (ER), progesterone (PR), glucocorticoid (GR), mineralocorticoid (MR), androgen (AR), thyroid hormones (TRα and TRβ), retinoic acids (RAR and RXR), and vitamin D (VDR). Almost all of these receptors are present in the brain and play important physiological functions through regulation of gene expression by binding to

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hormone response elements (HREs) in the presence or absence of hormones *(1).*

The gonadal steroids regulate various neuroendocrine features, including reproductive and aggressive behaviors, through influence of the central nervous system *(2).* Female ERα knockout (ERα–/–) mice, but not ERβ–/– mice, lack estradiol- and progesterone-induced lordosis, a female sexual behavior correlated with functional PR levels in the ventromedial nucleus of the hypothalamus *(3,4).* It has also been reported that female ERα–/– mice exhibit elevated inclination to infanticide and are more aggressive, whereas the male ERα–/– mice are less aggressive *(3,5).*

In the brain, testosterone is converted into estradiol by aromatase. Testosterone and estradiol activate AR and ERα, respectively, and regulate aggressive behaviors. The male and female brain regions that regulate aggressive behaviors exhibit different responses to testosterone and estradiol. In male mice, both testosterone and estradiol can induce aggressive behaviors. However, only testosterone, and not estradiol, effectively induces aggressive behaviors in female mice *(6,7).* Testosterone levels are elevated in the brain of both male and female ERα–/– mice. The elevated testosterone more efficiently activates AR in the female brain of ERα–/– mice and, therefore, causes more aggressive behavior in female ERα–/– mice than wild-type females. Although the elevated testosterone in the brain of male ERα–/– mice also activates AR, estradiol-mediated aggressive behaviors through ERα are diminished in male ERα–/– mice, causing an overall decrease in aggressive male behavior. These findings suggest that a single NR, such as ERα, can have different gender-dependent roles in the same tissues.

Stress-responsive hormones, including corticosterone, are released during learning and are required for establishment of enduring memory in the hippocampus throughout life *(8–10).* Although both MR and GR are expressed in the hippocampus, MR binds corticosterone with 10-fold higher affinity than GR *(11).* The balanced MR- and GR-mediated effects in the hippocampus are important to the cognitive process *(12–14).* Treatments with antagonists that are specific to MR or GR demonstrate that GR is required for retaining memory after a learning process in spatial and avoidance tasks, whereas MR is required for ongoing behavior in these tasks *(15).* Mice that lack GR also exhibit impaired long-term spatial memory when tested in the Morris water maze, suggesting that the MR-mediated ongoing behavior is affected in GR–/– mice *(16).* These observations indicate that both MR and GR play important roles in the regulation of hippocampal functions.

Hypothyroidism is accompanied by a significant reduction in dendritic arborization and synaptogenesis of the Purkinje cells as well as by retarded migration of the granular cells from the external granular layer to the internal granular layer in the mouse cerebellum *(17).* However, individual or combinatorial mutant mice of TR genes exhibit normal cerebellar morphology and function *(18,19).* This discrepancy may be credited to the existence of TRα1, an isoform of TRα with 80% of thyroid hormone (TH) binding capacity in the cerebellum. TRα1 represses gene expression in the absence of TH and affects development of granular and Purkinje cells. Therefore, TRα1 deficiency releases its repression and permits normal granular cell migration and Purkinje cell differentiation under hypothyroid conditions *(20).*

Another class of NRs, with unknown ligands, contains the orphan NRs. Orphan NRs also play critical roles in the regulation of gene expression in the nervous system *(1).* Multiple defects in brain development and function have been observed in mice that lack individual orphan NRs, including RAR-related orphan receptor α(RORα), Rev-erbAα, chick ovalbumin upstream promoter-transcription factor I (COUP-TFI), and Nurr1 *(21–27).* RORα binds its HRE and activates transcription as a monomer *(21).* Beginning at embryonic d 14, the RORα gene is expressed at high levels in the Purkinje cell precursors of the mouse cerebellum. RORα mutant mice display developmental problems of the Purkinje cells, including small cell sizes, reduced cell numbers,

and dendritic atrophy. Additionally, RORα mutant mice exhibit a cerebellar dysfunction, such as tremor and body imbalance, followed by early death after weaning *(22,23).* Rev-erbAα is homologous to TRα and serves as a transcription repressor to silence target gene expression. The Rev-erbAα gene is highly expressed in the Purkinje cells and the internal granular layer beginning at postnatal d 11 and postnatal d 14, respectively, in the mouse cerebellum *(24).* In contrast to RORα–/– mice, Rev-erbAα–/– mice are viable and have a normal number of Purkinje cells and no cerebellar ataxia. However, Purkinje cells in Rev-erbAα–/– mice exhibit less elaborated dendritic trees and a delay in migration of the external granular cells toward the internal layers *(24).* Although Rev-erbAα binds the same HREs as RORα, its expression is detected after birth, whereas RORα expression starts at embryonic d 14. These findings indicate that normal Purkinje cell development and function require functional crosstalks between Rev-erbAα and RORα.

COUP-TFI was first identified as a homodimer that bound to the direct repeat regulatory element of the chicken ovalbumin gene promoter *(12).* COUP-TFI can function as either a transrepressor or a transactivator to regulate target gene expression *(25).* In mice, COUP-TFI is expressed in the developing central and peripheral nervous systems beginning at embryonic d 7.5 and its expression declines prior to birth *(26).* COUP-TFI–/– mice lack the cortical layer IV and exhibit abnormal thalamocortical projection and innervation caused by excessive death of immature neurons in this region *(27).* These morphological defects cause a disability to receive peripheral information and result in a hearing disturbance *(27).* In the peripheral neurons of COUP-TFI–/– mice, the glossopharyngeal ganglions and nerves are defective, causing difficulties in suckling and swallowing and early death after birth *(26).* Nurr1 is expressed in the dopamine neurons of the developing midbrain, the loss of which causes Parkinson's disease *(28).* Although mice that lack other dopamine neuron survival factors, such as the glial cell line- and the brain-derived neurotrophic factor, have normal development of dopamine neurons, Nurr1–/– mice are unable to generate dopaminergic neurons in the midbrain and exhibit hypoactivity and early postnatal death *(28).*

An Overview of NR Coregulators

The biological functions of NRs summarized previously clearly indicate that the precise activation and repression of gene expression by NRs contributes to normal growth and differentiation of the nervous system. The precise spatial and temporal regulation of transcription by NRs requires the recruitment of intermediary factors, which are characterized as coregulators *(29,30).* These factors modulate transcriptional initiation at regulated promoters by modifying chromatin structures and/or assembling transcriptional initiation complexes *(31).* NR coregulators can be divided into two groups: (a) coactivators, which promote NR-dependent transcriptional activation in the presence of hormones or hormone agonists, and (b) corepressors, which facilitate NR-dependent transcriptional silencing in the absence of ligands or the presence of NR antagonists (Fig. 1).

NRs contain two trans-activation functions (AFs): an N-terminal AF (AF-1) and a C-terminal AF (AF-2). A distinct set of coactivators, such as the RNA coactivator SRA and the RNA binding DEAD-box protein p72/p68, are associated with AF-1 *(32,33).* Ligand-dependent recruitments of NR coactivators are dependent on AF-2 in the ligand-binding domain (LBD) *(34,35).* These AF-2 coactivators appear to function as large multiprotein complexes for remodeling the chromatin or facilitating the access of basal transcription machinery to the promoters of target genes (Fig. 1). The formation of coactivator complexes may provide modifiable platforms for fine control of gene expression through integration of regulatory signals from different pathways *(36,37).* These functional NR coactivators consist of several factors, including the BRG1 complex, the p160

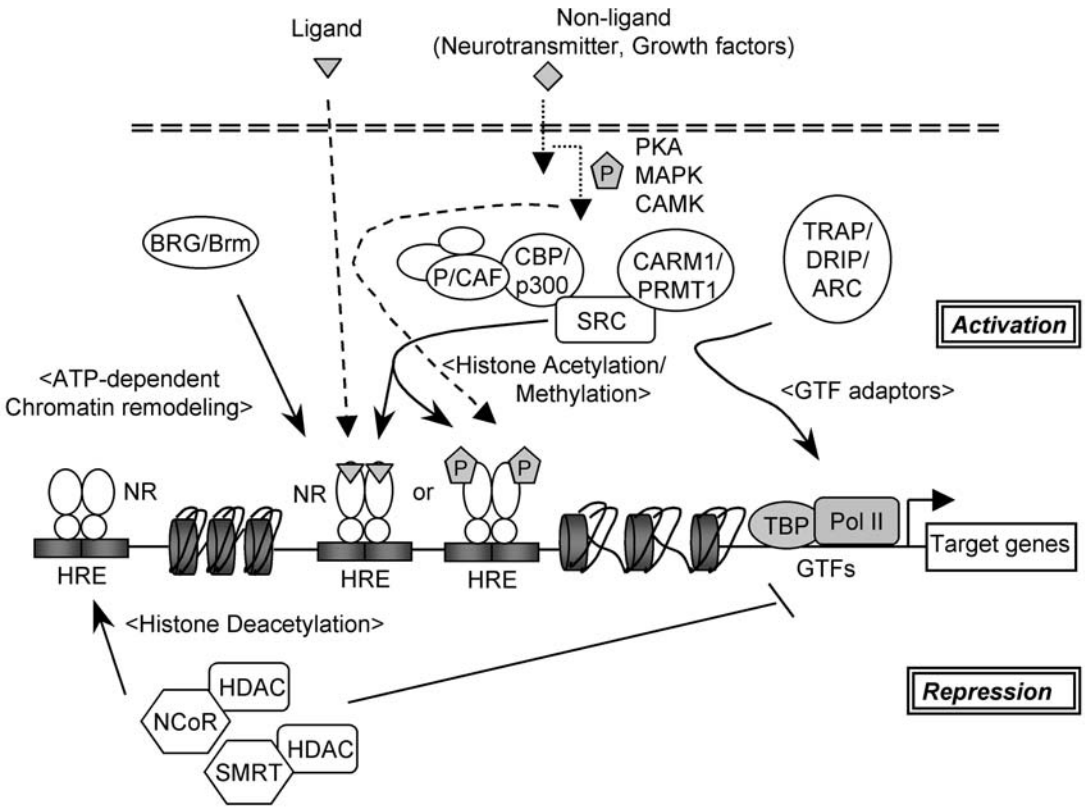

Fig. 1. Roles for corepressor and coactivator complexes in NR-mediated gene transcription. Certain NRs such as RAR and TR in the absence of ligands as well as tamoxifen-bound ER bind HREs, recruit corepressors (NCoR or SMRT), and histone deacetylases (HDACs), thereby causing histone deacetylation and repress gene transcription (the bottom part of the figure). On the other hand, activation of NR-dependent transcription can be induced by ligand binding such as binding to hormones or hormonal antagonists or by ligand-independent pathways such as neurotransmitters or growth factor-activated PKA, MAPK, and cAMK. Activated NRs bind HREs, dissociate corepressors, recruit coactivator complexes for chromatin remodeling, and general transcription factor (GTF) assembling, thereby enhancing gene transcription. The BRG–Brm complex possesses adenosine triphosphate-dependent chromatin remodeling activities. The SRC complex contains protein acetyltransferases CBP, p300, and p/CAF as well as protein methyltransferases CARM1 and PRMT1. The TRAP–DRIP-ARC complex facilitates the recruitment of GFTs such as TBP and poI II. Additionally, other signaling pathways such as neurotransmitter or growth factor-regulated protein kinases can modulate the function of individual coactivators.

steroid receptor coactivator (SRC) family, CBP/p300, and the TRAP/DRIP/ARC complex (reviewed in refs. *34* and *35*). Mammalian homologs of *Drosophila* SWI/SNF complex members, such as BRG1/hBrm, facilitate NR-dependent transcription through adenosine triphosphate-dependent histone acetylation (reviewed in ref. *35*). Members of the p160 SRC family, which were initially identified as ligand-dependent NR coactivators *(38),* function as adaptor molecules that recruit CBP and/or p300 for local chromatin modification *(39).* The SRC complexes contain acetyltransferases (including CBP, p300, and the p300/CBP-associated factor [p/CAF]) and methyltransferases (including CARM1 and PRMT1) *(39–42).* These

chromatin-remodeling enzymes (acetyltransferases and methyltransferases) are recruited to promoters in a ligand-dependent manner through interaction between NRs and p160 SRC coactivators. TRAP/DRIP/ARC coactivator complexes consist of more than a dozen proteins and interact with NRs in a ligand-dependent manner via a single component referred to as PBP/TRAP220/DRIP205 (reviewed in ref. *35*). The NR-recruited TRAP/DRIP/ARC complex directly interacts with general transcription factors to coactivate target gene transcription (reviewed in ref. *35*).

In contrast to coactivators, corepressors recruited by NRs repress gene transcription. The nuclear receptor corepressor (NCoR) *(43)* and its closely related protein, the silencing mediator for retinoid and thyroid hormone receptor (SMRT) *(44),* were identified based on the repression functions of TR and RAR in the absence of ligand. NCoR and SMRT contain a NR interaction domain that interacts with a NR region between the DNA-binding domain and the LBD (reviewed in ref. *34*). Recruitment of NCoR and SMRT not only appears to be essential for the repression of gene transcription by NRs such as TR and RAR in the absence of ligands but also appears to be essential for the antagonistic activities of ER and PR antagonists (reviewed in ref. *35*). Moreover, NCoR and SMRT interact with orphan NRs, including Rev-erbAα *(45)* and COUP-TFI *(46),* which serve as corepressors. Although NCoR and SMRT have no intrinsic enzyme activities for chromatin modification, their N-terminal domains can interact with other corepressors, such as Sin3 and histone deacetylases, which can change a chromatin structure into an inactive state for transcriptional silencing (Fig. 1) (reviewed in ref. *35*).

During the past decade, there has been a major focus on identification, cloning, and characterization of NR coregulators by using in vitro and in vivo approaches *(34).* Both coactivators and corepressors likely are involved in spatiotemporal control of gene expression through the interaction with NRs. Although our knowledge about the molecular mechanisms that are responsible for numerous NR coactivators and corepressors in vitro is rapidly accumulating, only a few physiological features regulated by NR coregulators have been examined in hormonally responsive tissues, including prostate, mammary gland, uterus, and brain in vivo *(36,37,47).* In vitro studies have demonstrated that transcriptional interference (termed as "squelching") occurs when one NR represses the transcriptional activity of another NR by sequestering coactivators required by both NRs *(38).* This transcriptional interference can be reversed by additional coactivator expression. Therefore, the concentration of coregulators in cells is believed to be a limiting factor in the transcription of target genes at a given level of hormone. The in vivo expression levels of coactivators are also likely to represent some functional properties in specific brain regions. Generally, coregulators are widely expressed in various tissues. However, their expression levels vary significantly among different tissues and cell types *(48).* We believe that the brain region-specific expression levels of NRs and their coregulators as well as their cell type-specific transcriptional activities, which are modulated by multiple signal transduction pathways such as posttranslational modification and interaction with other proteins, are among the critical determinants that regulate normal brain development and function from embryonic stages to adulthood. Consistent with this hypothesis, knockout mouse lines that lack individual coregulators exhibit different neurological phenotypes (Table 1). Here, we review several NR coregulators in more detail regarding their expression patterns and neurological functions and dysfunctions in brain development and differentiation.

The SRC p160 Family in the Nervous System

Among all of the reported NR coactivators, members of the SRC p160 family are the best characterized coactivators regarding their spatiotemporal expression patterns and their roles

Table 1
Neurological Phenotypes of Coregulator Knockout Mice

Coregulators	Phenotypes	References
SRC-1	Delay in Purkinje cell development Moderate motor dysfunction	*58*
CBP	Embryonic lethality Neuronal tube defect Locomoter and memory dysfunction	*82,83*
p300	Embryonic lethality Neural tube defect	*92*
BRG1	Embryonic lethality Exencephaly	*94*
TRAP220	Embryonic lethality Neural tube defect	*97*
E6-AP	Motor dysfunction Inducible seizures Context-dependent learning deficit	*109*
NCoR	Embryonic lethality Small thalamic nucleus Abnormal differentiation of neocortex Glial differentiation of neural stem cells	*111,113*

in brain development and function. The SRC family contains three homologous members that have an overall similarity of 40% in their amino acid sequences, including SRC-1 *(38)*, TIF2 (GRIP-1/SRC-2) *(49,50)* and p/CIP (RAC3/AIB1/ACTR/TRAM-1/SRC-3) *(40,51–55)*. All members of the SRC p160 family are discovered as ligand-dependent coactivators for multiple NRs, although their coactivation functions to different NRs do not completely overlap *(34)*.

In the rodent brain, SRC-1 is most abundantly expressed member of the SRC family through all developmental stages. In mouse embryos, SRC-1 transcript is expressed ubiquitously on embryonic d 8.5 and is particularly high in immature neuronal cells around embryonic d 11. High SRC-1 expression is detected in the olfactory epithelium, neocortex, hippocampus, hypothalamus, cerebellum, and anterior pituitary at embryonic d 14 *(56)*. Relative expression levels of SRC-1 are quite different in these brain regions at subsequent development stages. Although strong SRC-1 expression is detected in the embryonic neocortex, its expression in this brain region decreases rapidly after birth. In contrast, SRC-1 expression in the hypothalamus exhibits a prominent peak during the peripubertal period *(57)*. In the adult stage, SRC-1 is preferentially expressed in specific brain regions, including the olfactory bulb, piriform cortex, hippocampus, amygdala complex, cerebellar Purkinje cells, hypothalamus, and brainstem *(58,59)*. These expression patterns of SRC-1 in the mouse brain are very similar to those observed in the rat brain *(60)*. The SRC-1 protein is detected in the nucleus of neuronal, rather than glial, cells *(58)*. Both isoforms of SRC-1, the full-length SRC-1a and the C-terminal truncated SRC-1e, are differentially expressed in the rat brain *(60)*. In vitro experiments have demonstrated that NRs have selective interactions with SRC-1a or SRC-1e in the presence of cognate ligands for NRs, suggesting that alternative splicing may differentially confer SRC-1 function in specific brain regions.

Furthermore, SRC-1 expression is hormonally modulated in specific brain regions. In the anterior pituitary, TH stimulates SRC-1 expression, whereas estradiol decreases SRC-1 expression *(61)*. Although SRC-1 expression in the fetal cortex is not affected by TH or TH antagonists, pups that are treated with TH antagonists exhibit significantly lower expression of SRC-1 in the cortex and hippocampus *(62)*. Estrogen depletion causes downregulation of SRC-1 expression in the hypothalamus but not in the cortex *(57)*. Under restraint stress, which elevates the levels of glucocorticoids, SRC-1 expression levels differ between male and female brains in a region-specific manner *(63)*. The changes of SRC-1 expression levels and spatiotemporal expression patterns in accordance with steroid hormone status

may more precisely coordinate the hormone-induced gene transcription by NRs. However, the exact mechanisms by which steroid hormones act remain to be clarified, because currently, little is known about how the SRC-1 promoter is regulated.

SRC-1 participates in the regulation of sexual behaviors in the rat brain via steroid receptors *(64).* At perinatal stages in the male rat brain, testosterone is metabolized into estradiol by aromatase. Estradiol activates ER and causes defeminization, therefore, the lack of high-level testosterone affects defeminization of the male rat brain as a result of poor ER activation. However, testosterone causes masculinization through activation of AR. The blockade of SRC-1 function by injection of SRC-1 antisense oligodeoxynucleotides (ODNs) into the hypothalamus on the day of birth interferes with the defeminization, but not the masculinization, of male rats. The disruption of defeminization that is caused by using SRC-1 ODNs in male rats is similar to that caused by using ERα ODNs *(64).* Interestingly, a recent study showed that SRC-1 was not required for AR-dependent spinal cord development in mice *(65).* These findings suggest that SRC-1 is required for defeminization through mediation of ER function but is not required for the masculinizing action of AR during sexual differentiation of the nervous system.

To address the issue of whether SRC-1 is required for normal brain development and function, we generated SRC-1–/– mice *(66)* and performed a variety of experiments, including hormonal and histological examinations, developmental analysis, and a battery of behavior tests *(58,66).* SRC-1–/– mice are viable, but partially resistant to steroid/thyroid hormones in several tissues, including the pituitary *(66,67).* During embryonic development, SRC-1 is the only member of the SRC family that is expressed in the cerebellar primordium. Disruption of the *SRC-1* gene in mice results in a significant delay (embryonic d 10.5 to 12.5) in the generation of Purkinje cell precursors *(58).* Consequently, the calbindin-positive Purkinje cells are not observed in the cerebellum of SRC-1–/– mice at postnatal d 0 but are clearly detected in the cerebellum of wild-type mice at the same stage. Although the morphology of SRC-1–/– Purkinje cells develops to the same extent as wild-type Purkinje cells by postnatal d 10, adult SRC-1–/– mice still exhibit a moderate motor dysfunction. For example, the motor function of SRC-1–/– mice is partially impaired in hanging wire tests, rotarod tests, and swim speed tests *(58).* These results suggest that the abnormal development of Purkinje cells at early stages as a result of SRC-1 deficiency may have a negative impact on the cerebellar function in adulthood. Because TRs and ROR-α play important roles in Purkinje cell development *(17,68)* of these NRs in in vitro assays, SRC-1 may be required for normal function of TRs and ROR-α in Purkinje cell development and function.

Brain regions that express SRC-1 also coexpress multiple NRs in addition to ROR-α and TR, suggesting that SRC-1 may function with multiple NR partners in the brain *(1,69,70).* However, in SRC-1–/– mice, no structural and functional abnormalities can be detected by histological examination and behavioral tests, with the exception of the delay in the development of Purkinje cells during prenatal and neonatal stages and the moderate motor dysfunction in adulthood, *(58).* One way to explain the subtle phenotype observed in the brain structure and function of SRC-1–/– mice is the partial overlapping and compensatory expression patterns of SRC family members during development. The SRC-2 messenger RNA is expressed at relatively lower levels in almost all of the brain regions (with the exception of the brainstem) where SRC-1 is expressed *(58).* SRC-3 expression is only detectable in the hippocampus and olfactory bulbs of the mouse brain *(58,71).* During postnatal development, both SRC-1 and SRC-2 expression in the cerebellum are elevated, and their peak levels are reached by postnatal d 30, but SRC-3 expression in the same region is very low and stays unchanged through these developmental stages *(72).* To date, neither SRC-2- nor SRC-3-deficient mice have been reported to exhibit abnormal phenotypes in the nervous

system *(71,73,74).* These expression and functional profiles allow us to propose that SRC-1 may be a major coactivator in the SRC family for support of Purkinje cell development in early stages and that SRC-2 may functionally compensate for the loss of SRC-1 function in Purkinje cells. Several lines of evidence support the possibility that a partial functional redundancy exists among SRC family members in the brain. First, the expression levels of SRC-2, but not SRC-3, are elevated in the brain of SRC-1–/– mice *(58,66).* Second, the recovery of Purkinje cell development in SRC-1–/– mice by postnatal d 10 correlates with an earlier and higher expression of SRC-2 *(58).* Third, coexpression of all three SRCs in the hippocampus of wild-type mice may at least partly explain, why SRC-1–/– mice can still maintain their normal hippocampal functions in the learning and memory tests *(58).*

Because SRCs were initially identified and characterized by molecular cloning and biochemical approaches, no inherent human genetic disease of the nervous system has been linked to any SRC mutations. However, genetic changes of SRC genes have been reported in other human diseases. It has been demonstrated that translocation of the human SRC-1 locus 2p23 and neighboring bands is involved in various hematological malignancies *(75).* Fusions have been found between the *SRC-2* and *moz* genes at 8q13 and 8p1 in human acute myeloid leukemia *(76,77).* The *SRC-3* gene is highly amplified and overexpressed in a proportion of human breast and ovarian cancers *(51).* A significant correlation has also been shown between the expression of SRC-1 and SRC-2 and PR expression in brain tumors *(78).*

Other NR Coactivators in the Brain

In addition to the SRC family previously discussed, several other coactivators may also play important roles in the nervous system. However, functional insights of these coactivators in the nervous system are less well-understood when compared with the information that is known about the SRC family. The cAMP response element-binding protein (CREB)-binding protein (CBP), which was originally identified as a coactivator for CREB *(79,80),* interacts with and coactivates a number of transcription factors, including NRs. In the adult rat brain, high levels of CBP expression are found in the amygdala, cortex, thalamus, hypothalamus, hippocampus, and cerebellum *(81).* CBP–/– mice are lethal in the uterus and exhibit defects in cranial neural tube closure, exencephaly, and hemorrhage in neural tissues at embryonic d 11 *(82).* Another line of CBP mutant mice, which harbor a heterozygous allele encoding a truncated CBP that acts as a dominant negative form, can survive but exhibit a phenotype similar to the clinical features of Rubinstein-Taybi Syndrome. Patients with Rubinstein-Taybi Syndrome have craniofacial abnormalities and severe mental retardation. Mice that express dominant negative CBP mutants possess normal brain structure and short-term memory, but they exhibit abnormal neurotube development after birth as well as deficits in locomotor activity and long-term memory *(83).*

Treating the hippocampal neurons with estradiol in culture increases the level of CBP expression and the density of neuronal dendritic spines, suggesting that CBP is regulated by estrogen in these neurons *(84,85).* Interestingly, knockdown of CBP in the brain of neonatal rats using ODNs interferes with the defeminization, but not the masculinization, of effects of testosterone on behavior, suggesting that CBP is involved in the regulation of sexual differentiation in the brain in a way that is similar to SRC-1 *(86).* In agreement with this phenotypic similarity, CBP and SRC-1 synergistically coactivate NR function in cultured cells *(34),* and combined treatment of the female rat brain with SRC-1 and CBP ODNs decreases PR expression and lordosis frequency *(87).* These findings suggest that CBP is required for normal neural development and function, including locomotor activity, long-term memory, and sexual behavior.

p300 is a key cellular protein targeted by the adenovirus E1A oncoprotein *(88).* p300 and CBP

are homologous proteins, and both interact with a similar set of cellular factors *(89,90).* In the rat brain, p300 is highly expressed in the cerebrum, cerebellum, and hypothalamus *(61).* p300 and SRC-1 are also coexpressed in the nucleus of hippocampus *(91).* Both p300–/– and +/– mice exhibit embryonic lethality. The compound heterozygous mutant mice of p300 and CBP also die in the uterus *(92).* These results indicate that both CBP and p300 are essential coactivators for normal development, and their haploinsufficiencies suggest that these coactivators are rate-limiting factors for gene transcription in vivo.

BRG1, the mammalian homolog of *Drosophila* SWI/SNF, is highly expressed in the brain and spinal cord in mouse embryos *(93).* BRG1–/– mice die at embryonic d 6.5 and 15 to 30% of BRG1+/– mice exhibit exencephaly *(94).* Additionally, the expression of a dominant-negative form of the *Drosophila* BRM, a component of the BRG1 complex in flies causes defects in the peripheral nerve system and decreases cell viability *(95),* suggesting that BRG1 complex is an essential coactivator for development of the nervous system.

TRAP220 is a key component of the TRAP/DRIP/ARC coactivator complex, which interacts with NRs and basal transcription machinery (reviewed in ref. *35*). TRAP220 is expressed beginning at embryonic d 9 in mouse and beginning at embryonic d 12 in rat. In the adult rat brain, TRAP220 expression is more restricted to specific regions, including the granular layer of cerebellar cortex, the piriform cortex, and the hippocampus, and is predominantly localized in neurons in these brain regions *(96).* TRAP220–/– mice die in uteri by embryonic d 11 and exhibit smaller and disproportional head morphologies and a much thinner neuroepithelial layer with cortical neurons when compared to the wild-type embryos *(97).* These phenotypes are probably caused by a decrease in neuroepithelial cell proliferation and an increase in neuronal apoptosis after TRAP220 is disrupted *(97).* These findings indicate that TRAP220 is required for normal organogenesis of the central nervous system.

The peroxisome proliferator-activated receptor (PPAR) coactivator-1α (PGC-1α) is one of the coactivators that modulate NR activities and gene expression patterns in a tissue and cell type-specific manner. PGC-1α coactivates PPAR and contributes to adaptive thermogenesis in the fat tissue *(34).* Cold exposure induces PGC-1α expression in the skeletal muscle and brown fat *(98),* and starvation induces PGC-1α expression in the heart and liver *(99).* PGC-1α is also expressed in many brain regions, including the olfactory bulb, cerebral cortex, piriform cortex, medial septal nucleus, diagonal band of Broca, striatum, globus, reticular thalamic nucleus, substantia nigra, mesencephalic nucleus of the trigeminal nerve, cochlear nucleus, and superior olivary complex *(100).* Interestingly, PGC-1α expression in the brain is not affected by cold exposure or starvation *(100),* suggesting that the role of PGC-1α in the brain may be different from that in peripheral adaptive thermogenic tissues. Consistent with this speculation, PGC-1α also has been demonstrated to interact with other NRs such as TR, ER, and RXR *(34).*

Recently, ERAP140 was identified as a coactivator that interacts with ER in a ligand-dependent manner. ERAP140 expression is especially abundant in brain regions, including neurons of the cerebral cortex, thalamus, hypothalamus, hippocampus, cerebellum, striatum, and choroid plexus *(101).* Because high levels of ER are coexpressed in these brain regions *(70),* it is possible that ERAP140 may regulate ER function in the brain.

The neuron interacting factor X (NIX1) was discovered as a neuronal-specific cofactor that is exclusively expressed in the brain *(102).* NIX1 expression can be observed in neurons of the dentate gyrus, amygdala, thalamus, hypothalamus, and several brainstem nuclei. NIX1 interacts with a subgroup of NRs (e.g., RAR and TR) in a ligand-dependent manner but does not interact with RXR and steroid hormone receptors *(102).* Although NIX1 can interact with the AF-2 domains of NRs in a ligand-dependent manner, its role is to inhibit NR-mediated gene transcription, similarly to the function of the

corepressor RIP140 *(103).* The brain-specific expression pattern of NIX1 and its unique mechanism to repress NR-dependent transcription suggest that NIX1 may play an important feedback role in the protection of NR target genes from overexpression upon strong hormonal stimulation in specific brain regions.

The E6-associated protein (E6-AP), which was originally identified as an E3 ubiquitin ligase *(104,105),* also serves as a coactivator for multiple nuclear receptors such as ER, PR, AR, GR, RAR, and TR in a ligand-dependent manner *(106).* Loss of the functional maternal E6-AP allele causes Angelman Syndrome—a human genetic disorder characterized by mental retardation, seizures, inappropriate laughter, and severe motor dysfunction. Imprinting of the maternal expression is also observed in human brains with the Angelman Syndrome *(107,108).* Indeed, E6-AP expression is diminished in the hippocampus and cerebellum of heterozygous mice that lack the maternal E6-AP allele; these mice exhibit a number of neurological defects, such as motor dysfunction, inducible seizures, and deficient context-dependent learning *(109).* Interestingly, it has been demonstrated that the ligase activity of E6-AP is not required for its transcriptional coactivation function for nuclear receptors, and disruption of the *E6-AP* gene in mice causes tissue-specific influences on steroid hormone action in certain hormonal target tissues *(106,110).* Although the coactivator function of E6-AP is affected in certain mutant forms of E6-AP identified in patients with Angelman Syndrome, more insights are needed to understand the relationship between the coactivator function of E6-AP and the Angelman Syndrome-related neurological defects in the brain.

Expression and Functions of Corepressors in the Brain

Corepressors bind to unliganded or antagonist-bound nuclear receptors and repress gene expression. NCoR and SMRT are two of the well-characterized corepressors that mediate repression functions for certain NRs and some other classes of transcription factors (reviewed in refs. *34* and *35*).

Recent studies have demonstrated that long-term repression of specific gene expression by these corepressors is critical for normal development and differentiation of both central and peripheral nervous systems. NCoR and SMRT are homologous proteins that are distributed in similar regions of the nervous system during embryonic development, although NCoR expression is much higher in the thalamus and neocortex than SMRT *(111).* In NCoR–/– mouse embryos, the thalamic nucleus is smaller at embryonic d 12.5 and the neocortex neurons are more differentiated at embryonic d 14.5, suggesting that NCoR is required for normal neural development at specific embryonic stages *(111).* NCoR deficiency in non-neuronal cells such as mouse embryonic fibroblasts (MEFs) diminishes the ligand-independent gene silencing by RAR and TR as well as the effect of ER antagonists on ER-dependent gene transcription.

Interestingly, the loss of NCoR repression function permits non-neuronal peripheral tissues to express neuron-specific genes, probably as a result of an impaired repression function of the neuron restrictive silencer factor (NRSF/REST) *(111).* NRSF is a long-term silencer that is bound to many gene promoters and that silences gene expression in non-neuronal cells *(112).* In agreement with the diminished repressive function of NRSF in NCoR–/– MEFs, the class III β-tubulin (a neuronal-specific marker) is ectopically expressed in NCoR–/– MEFs *(111).* Consistent with the expression of NCoR in undifferentiated neural progenitor cells in the ventricular zones of the mouse embryonic brains *(113),* the forebrains of NCoR–/– mouse embryos exhibit a decrease in the expression of nestin, a marker for neural progenitors. Furthermore, more than 70% of their brain regions at developmental stages as early as embryonic d 14.5 display a prominent expression of the glial fibrillary astrocytic protein (GFAP), which is a marker for astroglia differentiation. In contrast, the brains of wild-type mouse embryos at embryonic d 14.5 never

express GFAP *(111,113).* These results suggest that disruption of NCoR reduces the number of neural progenitor cells and promotes astroglia differentiation.

GFAP expression normally occurs in response to the stimulation of the ciliary neurotrophic factor (CNTF), which activates the Akt1 protein kinase. In turn, the phosphorylation of NCoR by the activated Akt1 leads to its translocation from the nucleus to cytoplasm, where NCoR is unable to exert its repression function *(113).* Because NCoR is directly recruited to the repressor region of the GFAP promoter and is required for the repression of GFAP transcription, inactivation of NCoR is expected to cause overexpression of GFAP even in the absence of CNTF, as in the case of NCoR–/– brains in which the differentiation of neural stem cells into astroglia cells is significantly enhanced. In support of these findings, overexpression of NCoR inhibits the CNTF-induced astroglial differentiation *(113).* However, CBP or p300 is recruited to the promoters of glial-specific genes for transcriptional activation *(114).* In turn, the products of these genes participate in the astrocyte lineage determination. In summary, one critical function of NCoR is to suppress the initiation of neural stem cell differentiation into glial cells through long-term repression (Table 1).

Roles of NR Coregulators in the Ligand-Independent Activation of NRs and the Action of Non-NR Transcription Factors

Generally, NRs are defined as ligand-induced transactivators. Most NRs bind molecular chaperones or corepressors in the absence of ligands and either are not transcriptionally active or posses gene-silencing functions. However, activation of NRs by ligand-independent pathways has been demonstrated to play important roles in the nervous system *(115).* For example, neurotransmitters such as dopamine or growth factors such as epidermal growth factor (EGF) bind to their cognate receptors and activate protein kinase A (PKA), mitogen-activated protein kinase, or calmodulin kinase *(116,117).* Certain NRs, such as ER and PR, in the brain can be phosphorylated by these kinases. The phosphorylated NRs can bind DNA and recruit coactivators to activate gene transcription (Fig. 1) *(31,115).* It has been demonstrated that SRC-1 and/or CBP can enhance transcriptional activities of ERα, ERβ, and PR in a ligand-independent manner through interaction with their AF-1 domains *(118–120).*

In the pituitary cells, the neurotransmitter-cAMP-PKA pathway causes phosphorylation of ER and activates ER transcriptional function, and the ER transcriptional activity can be further enhanced by SRC-1 *(121).* Interestingly, the EGF-induced phosphorylation sites in ER are different between neural and non-neural cells, suggesting that ligand-independent ER activation by growth factor-activated kinases is cell type-specific *(122).* Compared to the ligand-dependent activation of NRs, which results in rapid degradation of NR proteins, the ligand-independent activation of NRs has less effect on NR degradation, which facilitates the maintenance of NR levels as well as persistent transcriptional activation *(121).* Therefore, the ligand-independent activation of NRs is particularly suitable for regulation of biological functions that require gene expression over a relatively long period. However, the exact molecular mechanisms by which NRs and coregulators interact with each other and participate in the regulation of brain functions still need to be characterized. For example, the neurotransmitter dopamine or EGF induces ER and PR phosphorylation and female sexual behavior (lordosis), whereas the contribution of individual coactivators to transcription by the phosphorylated ER and PR in the absence of ligand remains unclear *(123–125).*

Certain orphan NRs also require coregulators for their biological functions in the nervous system. It has been demonstrated that RORα interacts with GRIP1 (SRC-2) for transcriptional activation *(126),* and RORβ interacts with NIX1

to activate transcription in a ligand-independent manner *(102).* In the cerebellum, RORα, SRC1, and GRIP1 are coexpressed in the Purkinje cells, and both RORα and SRC-1 are required for normal Purkinje cell development *(58).* Additionally, Rev-erbAα and COUP-TFI interact with NCoR and SMRT in the absence of ligands and repress gene expression, although their direct target genes have not been defined in the nervous system *(45,46).*

NR coregulators may be involved in neurodegenerative diseases through interaction and function with certain non-NR transcription factors. CBP and p300 are generally known coactivators for many non-NR transcription factors, including p53 and nuclear factor (NF)κB *(34,35).* Members of the SRC family interact with several non-NR transcription factors, including NFκB, c-fos, c-jun, and IκK *(127–129).* Cerebral ischemia manifests various neurological symptoms that result from degenerative and dead neurons, which is a process that is counter-regulated by p53 and NFκB target genes in focal or global brain regions *(130).* Activation of p53 induces apoptosis, whereas activation of NFκB enhances cell survival. Therefore, the role of p53 in blocking the antiapoptotic function of NFκB is critical in p53-mediated cell death. Interestingly, the pifithrin-α, a p53 inhibitor that promotes NFκB to interact with p300, can preserve the antiapoptotic activity of NFκB, thereby protecting neural degeneration *(131).* The hippocampus is a brain region that is very sensitive to ischemic damage. Because NFκB, SRCs, CBP, and p300 are coexpressed in the hippocampus *(58,81, 91,131),* they may be involved in prevention of neurodegenerative progress induced by neurotoxic products. Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by a mutation in the huntingtin (Htt) protein *(132).* NCoR has been shown to bind the mutated Htt in the yeast-two-hybrid analysis *(133).* Mutant Htt enhances the ability of NCoR to repress TR-mediated transcription in the absence of ligand *(134).* Furthermore, mutant Htt expression interferes with CBP-mediated transcription, whereas overexpression of CBP neutralizes the mutant Htt-induced neuronal toxicity *(135),* suggesting that a functional balance is required between coactivators and corepressors to maintain normal gene expression and control the progress of HD.

Conclusions and Future Directions

Individual NR coregulators are expressed in various brain regions with spatially and temporally controlled patterns and function with NRs and non-NR transcription factors. Studies using genetically manipulated mouse models have demonstrated that many coregulators are required for normal development of the nervous system and for survival of the mouse embryos (Table 1). Several NR coregulators are particularly important in the process of early neurogenesis. NCoR is essential for maintenance of neural stem cells to prevent glial differentiation *(113).* In contrast, CBP and p300 play a role in induction of glial differentiation *(114).* However, CBP and p300 are mainly localized in neurons, rather than in glial cells *(91),* suggesting that these coactivators are involved in paracrine regulations among different cell types in the nervous system. The unsolved issue steals with which genes are regulated by specific coregulators and their NR or non-NR partners and the mechanism by these coregulator activities are modulated by different signaling pathways to regulate precise neuronal development and function.

Another important feature of NR coregulator function is the compensatory mechanisms among homologous family members. SRC-1 is expressed in both the cerebellum and the hippocampus, but SRC-1–/– mice only exhibit problems in the development of cerebellar Purkinje cells, because other members of the SRC family are expressed at low levels in the cerebellum but at high levels in the hippocampus *(58).* Similarly, NCoR–/– mice exhibit abnormal morphologies only in brain structures where the SMRT is not expressed or is expressed at a low level *(111).* Although the functional redundancy

among these coregulators is a mechanism of life security, it burdens our analyses of individual coregulator functions in vivo. Generation and characterization of combinatorial and tissue-specific mutant mouse models for multiple coregulators will be extremely useful to define the in vivo function of individual coregulators in the nervous system.

Overaction or dysfunction of several NR coregulators is related to certain human neural diseases such as brain tumors or neurodegenerative diseases including Rubinstein-Taybi Syndrome and HD. Detailed knowledge regarding the expression patterns, functional profiles, and molecular mechanisms of NR coregulators will help explain the pathological basis of these diseases and will eventually lead to control of these neural diseases. There is no doubt that these coregulators are involved in many aspects of numerous neurobiological events, and the information we currently know about these coregulators is just the beginning of the exploration of a new world. Future studies will identify more coregulators in the nervous system and will further establish NR coregulators as important players in neural development, function, and diseases.

Collectively, NR coregulators are involved in a much broader spectrum of neurobiological processes than was initially speculated through crosstalks with various signaling pathways. Further studies on the functional molecular mechanisms of coregulators in the nervous system under physiological and pathological conditions will lead to better understanding of the nervous system development and function as well as better control of many diseases of the nervous system.

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